

# **Journal of Medicines Development Sciences**

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# Journal of Medicines Development Sciences

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# The Role of Gut Microbiota in Ischemic Stroke and Its Traditional Chinese Medicine Intervention

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**Abstract:** Ischemic stroke (IS) ranks among the leading causes of disability and mortality worldwide, imposing a significant burden upon society and families. Neuroprotection following ischemic stroke constitutes a vital therapeutic approach. In recent years, an increasing body of research has demonstrated that the gut microbiota and its regulation through traditional Chinese medicine may exert neuroprotective effects. This paper systematically reviews relevant studies, delving into the pathways and mechanisms by which traditional Chinese medicine counteracts cerebral ischaemic injury through modulating gut microbiota composition and metabolic functions. It aims to provide novel insights and theoretical foundations for the prevention and treatment of ischaemic stroke.

**Keywords:** IS; Gut Microbiota; Traditional Chinese Medicine

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## 1. Introduction

Ischemic stroke, as a prevalent cerebrovascular disorder, poses a significant global public health challenge due to its high incidence, disability rate, and mortality. This condition not only imposes a heavy burden on patients' families but also carries a substantial risk of recurrence. Its pathogenesis commences with intracranial arterial occlusion, subsequently triggering a complex cascade of pathophysiological responses. Core pathways encompass excitotoxicity, oxidative stress, neuroinflammation, and disruption of the blood-brain barrier. These interacting processes collectively culminate in parenchymal necrosis and neurological deficits. Research indicates that approximately half of stroke patients experience concomitant gastrointestinal symptoms, including constipation, gastrointestinal haemorrhage, and faecal incontinence<sup>[1]</sup>.

Modern research defines the human gut microbiota as a vast, diverse, and highly dynamic ecosystem, comprising bacteria, archaea, fungi, viruses (including bacteriophages), and protozoa. Alterations in the composition and balance of this gut flora constitute a key factor in triggering intestinal dysfunction and, through the gut-brain axis, influencing brain function and emotional behaviour. It is also closely associated with the progression of diseases such as cerebral ischaemia. This aligns remarkably with the principles of traditional Chinese medicine<sup>[2]</sup>.

Within the holistic framework of Traditional Chinese Medicine, the pivotal functions of the spleen and stomach are intrinsically linked to the generation and transformation of qi, blood, and body fluids. The proper functioning of

the spleen, stomach, and intestines in transporting and transforming the essence of food and drink forms the foundation for maintaining bodily health. Conversely, when this transport and transformation process is disrupted, it may lead to deficiencies in qi and blood or the accumulation of turbid toxins internally. Pathological by-products may then ascend to the cerebral vessels, resulting in stroke. Traditional Chinese medicine exerts its effects through multiple targets, pathways, and levels. An increasing body of research indicates that it can improve the prognosis of ischaemic stroke by regulating the gut microbiota. This paper reviews the relationship between the gut microbiota and ischaemic stroke, along with relevant aspects of its regulation by traditional Chinese medicine, with the aim of providing new insights and approaches for the prevention and treatment of ischaemic stroke using traditional Chinese medicine.

## 2. The Association Between Gut Microbiota and Ischemic Stroke

### 2.1. Gut Microbiota Dysbiosis and Ischemic Stroke

The normal gut microbiota maintains a dynamic equilibrium with its host through mutualistic symbiosis. This balance relies upon regulation via the bidirectional communication pathway known as the gut-brain axis, whilst the microbiota itself undergoes alterations in response to the host's disease state. Stroke disrupts the established equilibrium of the gut-brain axis. During the acute phase of cerebral ischaemia, the body's intense stress response activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, subsequently triggering reduced gastrointestinal blood flow, motility disorders, and increased permeability<sup>[3]</sup> with a highly dynamic pathological process. Post-stroke neuroinflammation, mediated by microglia, demonstrates a dual role in both injury and repair. The CX3CR1/CX3CL1 signaling axis, highly expressed in microglia, acts as a key regulator. This review examines the spatiotemporal dynamics of the axis across the stroke process and its involvement in neural repair. Crucially, this signaling pathway demonstrates stage-dependent functional duality: its cellular sources, receptor expression profiles, and functional consequences undergo temporally orchestrated shifts, manifesting coexisting or interconverting protective and damaging properties. Ignoring this dynamism compromises the therapeutic efficacy of targeted interventions. Thus, we propose a triple precision strategy of '\stroke phase-biomarker-targeted intervention'. It uses specific biomarkers for precise staging and designs interventions based on each phase's signaling characteristics. Despite challenges like biomarker validation, mechanistic exploration, and cross-species differences, integrating cutting-edge technologies such as spatial metabolomics and AI-driven dynamic modeling promises to shift stroke therapy toward personalized spatiotemporal programming. Temporally targeting CX3CR1 signaling may offer a key basis for developing next-generation precision neural repair strategies for stroke.

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### 2.2. The Intestinal Epithelial Barrier and Ischemic Stroke

An intact intestinal mucosal barrier serves as a crucial defence against pathogenic bacteria and harmful substances within the gut. However, dysbiosis induced by stroke exacerbates damage to this barrier, allowing intestinal endotoxins and

pathogens to enter the circulatory system. This subsequently triggers systemic secondary infections, ultimately becoming one of the significant contributing factors to mortality in stroke patients. To investigate the effects of stroke on the intestinal barrier, we established a rat model of middle cerebral artery occlusion (MCAO). Detection results revealed that the expression levels of key tight junction proteins—including occludin, claudin-5, and zonulin-1—were significantly downregulated in the colonic tissue of the model rats<sup>[4]</sup>. This finding reveals structural damage to the intestinal epithelial barrier and increased permeability following stroke. Concurrently, elevated plasma levels of diamine oxidase (DAO), lipopolysaccharide (LPS), and D-lactic acid indicate the occurrence of intestinal microbial translocation. Therefore, modulating gut probiotics to enhance the integrity and function of the intestinal epithelial barrier holds significant therapeutic potential for ischaemic stroke.

### **2.3. Gut Microbiota Dysbiosis and Ischemic Stroke**

Gut microbiota metabolites play a pivotal role in regulating bodily equilibrium, with short-chain fatty acids (SCFAs) representing a quintessential beneficial product. Their function extends beyond maintaining intestinal homeostasis; by influencing immune cells, they also promote motor recovery following stroke, demonstrating significant therapeutic potential. Gut microbiota metabolites serve as crucial messengers in microbe-host interactions<sup>[5]</sup>. Furthermore, stroke-induced dysbiosis of the gut microbiota—particularly the proliferation of opportunistic pathogens—leads to elevated levels of harmful metabolites, thereby activating the body's immune inflammatory response. These inflammatory mediators subsequently migrate to the ischaemic brain region, further exacerbating cerebral injury.

## **3. Traditional Chinese Medicine improves ischaemic stroke outcomes by regulating the gut microbiota**

Traditional Chinese Medicine exerts its effects through multi-targeted, multi-pathway, and multi-level mechanisms. By regulating the brain-gut axis through holistic intervention, it embodies the wisdom of treating different diseases with similar approaches and addressing upper-body ailments through lower-body interventions. An increasing body of research indicates that Traditional Chinese Medicine can improve outcomes in ischaemic stroke by modulating the gut microbiota. This section elaborates on the relevant findings.

### **3.1. Active constituents of traditional Chinese medicine**

Astragalus is a traditional Chinese medicinal herb renowned for its properties of tonifying qi and fortifying the exterior, expelling toxins and draining pus, promoting diuresis and tissue regeneration. It is lauded in the Compendium of Materia Medica as the 'superior tonic herb'. Modern pharmacological research further reveals that its active constituents primarily comprise astragaloside IV and astragalus polysaccharides, each exerting therapeutic effects through distinct mechanisms. On the one hand, astragaloside IV regulates intestinal microbiota; on the other, astragalus polysaccharides inhibit NO protein expression that induces apoptosis following cerebral ischaemia-reperfusion, thereby mitigating brain tissue damage and enhancing therapeutic outcomes<sup>[6]</sup>. Ginseng possesses potent effects in replenishing vital energy, benefiting the lungs and strengthening the spleen, generating fluids and nourishing the blood, as well as calming the spirit and enhancing intelligence. It may be employed in the treatment of stroke. Its primary active component, ginsenosides, exhibits low direct absorption rates in the human body, undergoing metabolic conversion primarily within the intestinal tract by gut microbiota. Research confirms that although ginsenosides exhibit species-specific metabolic pathways in humans and rats, they ultimately convert into metabolites entering the bloodstream. Consequently, this intestinal metabolic process is recognised as the key mechanism through which ginseng exerts its broad pharmacological effects<sup>[7]</sup>. Research indicates that the kudzu root-chuanxiong combination exhibits protective effects against cerebral ischaemic injury. This action may be closely associated with its regulation of the gut-brain axis, specifically through promoting the growth of beneficial bacteria and correcting post-stroke gut dysbiosis<sup>[8]</sup>. Targeted metabolomics studies indicate that *Gastrodia elata* may mitigate



cerebral ischaemia-reperfusion injury in rats by modulating gut microbiota-associated amino acid metabolic pathways. *Gastrodia* regulates tryptophan metabolism, balances glutamate/glutamine levels, and reduces phenylalanine and arginine content. This subsequently influences the expression of inflammatory mediators such as IL-6 and TNF- $\alpha$ , ultimately mitigating inflammatory damage to brain tissue<sup>[9]</sup>the mechanism of *G. elata* Blume in improving CIRI by regulating the intestinal flora has not been reported until now. This research aimed to comprehensively evaluate the mechanism of *G. elata* Blume in CIRI based on fecal metabolomics and 16S rDNA sequencing. The rat model with CIRI was created based on the Zea Longa method. Enzyme-linked immunosorbent assay (ELISA).

### 3.2. Compound Chinese Medicinal Preparations

In MCAO model mice, Xingqichengqi Decoction significantly modulated gut microbiota composition, such as altering the abundance of Bacteroidetes and Firmicutes phyla, and elevated  $\alpha$ -diversity indices including Chao1, Shannon, and Simpson indices, indicating its efficacy in enhancing gut microbial diversity. These beneficial microbial alterations were accompanied by improved neurological function in mice, suggesting its neuroprotective effects may be linked to regulation of the gut-brain axis<sup>[10]</sup>followed by behavioral evaluation, TTC and TUNEL staining. Additionally, to investigate the effects of gut microbiota on neurological function after stroke, C57BL/6 mice were treated with anti-biotic cocktails 14 days prior to ischemic stroke (IS). The Tongqiao Huoxue Decoction improves stroke outcomes by regulating post-stroke gut microbiota dysbiosis, repairing the intestinal barrier, and suppressing immune inflammatory responses. Experimental studies indicate that this formula increases bifidobacteria abundance in the intestines of MCAO model rats, promotes Treg cell expression, and inhibits the activation and migration of  $\gamma\delta$  T cells in the small intestine. Consequently, it reduces the secretion of the downstream pro-inflammatory factor IL-17 in the cerebral ischaemic area<sup>[11]</sup>the high throughput 16S ribosomal DNA (rDNA). Research indicates that An Gong Niu Huang Wan modulates multiple metabolic pathways in rats with middle cerebral artery occlusion (MCAO) and enhances uridine metabolism. Uridine is known to mitigate post-traumatic cerebral oedema and promote synaptic formation. Based on Spearman's correlation analysis, researchers hypothesise that uridine production may correlate with the formulation's regulation of gut microbiota, including genera such as *Bacteroides* and *Ruminococcus*<sup>[12]</sup>the mechanism of action of ANP in stroke treatment has rarely been reported. With increasing evidence for a mechanistic link between acute ischemic stroke and gut microbiota alterations, this study aimed to determine the mechanism of action of ANP in treating acute ischemic stroke from the perspective of the gut microbiota. A mouse model of acute ischemic stroke by middle cerebral artery occlusion (MCAO).

## 4. Conclusion and Outlook

Ischemic stroke is a prototypical network-based disorder, with pathological damage propagating along neural circuits to trigger complex secondary lesions, constituting the primary cause of poor prognosis. Consequently, neuroprotection targeting this process represents a key therapeutic strategy for improving stroke outcomes. The gut microbiota represents an emerging therapeutic avenue for ischaemic stroke, offering neuroprotective effects. However, its multi-targeted and complex mechanisms pose significant barriers to clinical translation. Traditional Chinese medicine, with its inherent advantages in stroke treatment through multi-targeted, multi-pathway, and multi-level effects, has been increasingly demonstrated to significantly correct post-stroke gut dysbiosis. This, in turn, regulates downstream metabolome and proteome expression, thereby improving outcomes for patients with ischaemic stroke.

Current research has primarily confirmed the phenomenon of traditional Chinese medicine regulating the microbiota and metabolites, yet the specific mechanisms underlying this remain a “black box”, particularly lacking in-depth exploration and validation of the causal relationship between microbiota and metabolites. In future, it will be necessary to integrate techniques such as metabolomics and metagenomics to more precisely elucidate the specific bacterial strains influenced by traditional Chinese medicine, along with the precise action targets and signalling pathways of their key metabolites (such as secondary bile acids and indole derivatives). Through experimental methods such as faecal microbiota



transplantation and germ-free animal models, it has been further demonstrated that alterations in the gut microbiota constitute both a ‘necessary condition’ and a ‘causal link’ for the efficacy of traditional Chinese medicine. Moreover, multicentre clinical trials may be conducted to expand the sample size for traditional Chinese medicine treatment of ischaemic stroke, establish standardised herbal intervention protocols, and incorporate metagenomics for dynamic monitoring of patient microbiota changes. This approach will provide high-level evidence-based medical evidence for the clinical application of traditional Chinese medicine. In summary, the gut microbiota and its medicinal interventions hold immense potential as novel therapeutic targets for future stroke management.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Yin X, Li S, Wang J, 2025, Research progress of active compounds from traditional chinese medicine in the treatment of stroke. *European Journal of Medicinal Chemistry*, 291: 117599.
- [2] Ren Y, Chen G, Hong Y, 2025, Novel insight into the modulatory effect of traditional chinese medicine on cerebral ischemia-reperfusion injury by targeting gut microbiota: a review. *Drug Design Development and Therapy*, 19: 185-200.
- [3] He Q, Zhou T, He Q, 2025, Targeting CX3CR1 signaling dynamics: a critical determinant in the temporal regulation of post-stroke neurorepair. *Brain Sciences*, 15(7): 759.
- [4] Chen R, Wu P, Cai Z, 2019, Puerariae lobatae radix with chuanxiong rhizoma for treatment of cerebral ischemic stroke by remodeling gut microbiota to regulate the brain-gut barriers. *Journal of Nutritional Biochemistry*, 65: 101-114.
- [5] Silva Y P, Bernardi A, Frozza R L, 2020, The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Frontiers in endocrinology*, 11: 25.
- [6] Kim Y S, Kim J J, Cho K H, 2008, Biotransformation of ginsenoside Rb1, crocin, amygdalin, geniposide, puerarin, ginsenoside Re, hesperidin, poncirin, glycyrrhizin, and baicalin by human fecal microflora and its relation to cytotoxicity against tumor cells. *Journal of Microbiology and Biotechnology*, 18(6): 1109-1114.
- [7] Trinh H T, Joh E H, Kwak H Y, 2010, Anti-pruritic effect of baicalin and its metabolites, baicalein and oroxylin a, in mice. *Acta Pharmacologica Sinica*, 31(6): 718-724.
- [8] Chen R, Wu P, Cai Z, 2019, Puerariae lobatae radix with chuanxiong rhizoma for treatment of cerebral ischemic stroke by remodeling gut microbiota to regulate the brain-gut barriers. *Journal of Nutritional Biochemistry*, 65: 101-114.
- [9] Ding X, Liu Z, Liu Y, 2022, Comprehensive evaluation of the mechanism of Gastrodia elata Blume in ameliorating cerebral ischemia-reperfusion injury based on integrating fecal metabonomics and 16S rDNA sequencing. *Frontiers in Cellular and Infection Microbiology*, 12: 1026627.
- [10] Gao Q, Han Z Y, Tian D F, 2021, Xinglou chengqi decoction improves neurological function in experimental stroke mice as evidenced by gut microbiota analysis and network pharmacology. *Chinese Journal of Natural Medicines*, 19(12): 881-899.
- [11] Zhang F, Zhai M, Wu Q, 2020, Protective Effect of Tong-Qiao-Huo-Xue Decoction on Inflammatory Injury Caused by Intestinal Microbial Disorders in Stroke Rats. *Biological & Pharmaceutical Bulletin*, 43(5): 788-800.
- [12] Zhang H, Hui X, Wang Y, 2022, Angong niuhuang pill ameliorates cerebral ischemia/reperfusion injury in mice partly by restoring gut microbiota dysbiosis. *Frontiers in Pharmacology*, 13: 1001422.

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# Application of Botulinum Toxin Type A after Plastic Surgery Incision and Its Impact on Patients' Complication Risks

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**Abstract:** Objective: To investigate the application effect of Botulinum Toxin Type A after plastic surgery incision and its impact on patients' complication risks. Methods: 104 patients who underwent plastic surgery incision in our hospital from February 2023 to June 2025 were selected and randomly divided into a control group (n=52) and an observation group (n=52) using a random number table method. Both groups underwent plastic surgery incision, while the observation group received additional application of Botulinum Toxin Type A. The treatment effects, skin conditions, complication rates, and patient satisfaction were compared between the two groups. Results: After intervention, the indicators of both groups improved compared to before treatment. The observation group showed significant advantages in various indicator improvements: In terms of treatment effect, the total clinical effective rate of the observation group (94.23%) was better than that of the control group (76.92%) ( $\chi^2=6.310$ ,  $P=0.000<0.001$ ); in terms of skin condition, the observation group showed more significant improvement ( $P=0.000<0.001$  for all); in terms of adverse reaction rate, the observation group (1.92%) was lower than the control group (15.38%) ( $\chi^2=4.379$ ,  $P=0.036<0.05$ ); in terms of patient satisfaction, the total satisfaction rate of the observation group (96.15%) was better than that of the control group (80.77%) ( $\chi^2=6.029$ ,  $P=0.014<0.01$ ). Conclusion: The application of Botulinum Toxin Type A after plastic surgery incision can improve treatment effects, skin conditions, and patient satisfaction. Although the complication rate increases slightly, the overall safety is acceptable, making it worthy of clinical promotion.

**Keywords:** Botulinum Toxin Type A; Plastic Surgery Incision; Complications; Clinical Efficacy; Skin Condition

**Online publication:** September 26, 2025

## 1. Introduction

In recent years, the field of aesthetic and plastic surgery in China has undergone significant development and garnered increasing attention. Aesthetic incision surgery is a commonly used cosmetic surgical method that effectively improves local appearance through precise incision design and suture counting, ultimately achieving aesthetic goals<sup>[1]</sup>. However, wound healing after surgery often encounters issues such as excessive scar growth<sup>[2]</sup>, uneven skin texture, abnormal skin color, etc., which not only detract from the aesthetic result but also increase the psychological burden on patients. Additionally, complications like incision non-healing and infection can affect patient recovery and satisfaction with

treatment. Botulinum toxin type A is a neurotoxin produced by *Clostridium botulinum* that exerts a muscle-relaxing effect by inhibiting the release of acetylcholine at the neuromuscular junction. In recent years, its application in aesthetic and plastic surgery has become increasingly widespread, including for face slimming, wrinkle removal, and improvement of masticatory muscle hypertrophy. Studies have found that botulinum toxin type A can reduce excessive muscle activity around the incision, lower incision tension, create a favorable environment for incision healing, and consequently minimize scar formation and improve skin appearance<sup>[3]</sup>. However, systematic research on its application after aesthetic incision surgery remains limited, and there is controversy regarding its impact on the risk of complications. Some studies suggest that botulinum toxin type A may affect local blood circulation and immune function at the incision site, increasing the risk of complications. Other studies indicate that the appropriate use of botulinum toxin type A does not significantly elevate the incidence of complications but can enhance overall treatment effectiveness by optimizing healing conditions<sup>[4]</sup>. Therefore, this study aims to conduct a prospective controlled investigation to explore the application effects of botulinum toxin type A after aesthetic incision surgery and its impact on the risk of complications. This will provide a scientific basis for clinical treatment plan selection, further improving the efficacy and safety of aesthetic and plastic surgeries and meeting patients' aesthetic needs.

## 2. Materials and Methods

### 2.1. General Information

This study is a prospective one, selecting 104 patients who underwent plastic surgery incision in our hospital from February 2023 to June 2025 as the research subjects. They were divided into a control group (n=52) and an observation group (n=52) using a random number table method.

Inclusion criteria: (1) Underwent plastic surgery incision on the face, neck, or trunk, with an incision length of  $\geq 2$ cm; (2) No history of allergy to type A botulinum toxin; (3) Did not receive similar cosmetic treatment within 3 months before surgery; (4) Patients and their families were informed of the study and signed an informed consent form.

Exclusion criteria: (1) Suffering from severe diseases of important organs such as heart, liver, and kidney; (2) Presence of coagulation dysfunction or immunocompromise; (3) Presence of infection, ulcer, or other lesions at the incision site; (4) Being in pregnancy or lactation; Suffering from mental illness and unable to cooperate with treatment and follow-up.

### 2.2. Methods

Both groups of patients underwent plastic surgery incision. The surgeries were performed by the same experienced team of doctors. The surgical procedure strictly followed the principle of sterile operation. Appropriate incisions were designed based on the surgical site and cosmetic needs. The skin and subcutaneous tissue were cut layer by layer. After completing the corresponding plastic surgery operations, cosmetic suturing techniques were used to close the incisions. Antibiotics were routinely administered postoperatively to prevent infection, and patients were guided on incision care.

The observation group received Botulinum Toxin Type A on this basis. Within 24 to 48 hours after surgery, Botulinum Toxin Type A was administered through multi-point microinjection around the incision site within a 1cm range, based on the length and location of the incision. The injection dosage was controlled, and the spacing between each injection point was 0.5 to 1cm. During the injection process, the depth and angle of the needle were strictly controlled to avoid damaging blood vessels and nerves.

Both groups of patients were followed up for 6 months after surgery, and the incision healing and changes in related indicators were regularly observed.

### 2.3. Observation indicators

(1) General information: average age, gender

- (2) Treatment effect: evaluated based on cosmetic results and patients' subjective feelings. Significant effect: good incision healing, no obvious scar hyperplasia, high skin smoothness, color close to normal, and patients are very satisfied with the results; Effective: better incision healing, slight scarring, acceptable skin smoothness, slightly abnormal color, and patients are basically satisfied with the results; Ineffective: poor incision healing, obvious scarring, poor skin smoothness, significantly abnormal color, and patients are not satisfied with the results. Total effective rate = (number of significant effects + number of effective cases) / total number of cases  $\times$  100%.
- (3) Skin condition: Skin elasticity, smoothness, and color around the incision were measured using a skin detector at 1 month, 3 months, and 6 months postoperatively. Elasticity is represented by the elastic modulus measured by the instrument, with higher values indicating better elasticity; Smoothness is represented by the surface roughness parameter of the skin, with lower values indicating better smoothness; Color is represented by the L\* value (brightness) measured by a skin colorimeter, with higher values indicating a color closer to normal.
- (4) Incidence of complications: The occurrence of scar hyperplasia, incision non-healing, and incision infection within 6 months after surgery in both groups was counted, and the total incidence of complications was calculated.
- (5) Patient satisfaction: Evaluated using a self-made satisfaction scale, divided into three levels: very satisfied, generally satisfied, and dissatisfied. Comprehensive satisfaction rate = (number of very satisfied cases + number of generally satisfied cases) / total number of cases  $\times$  100%.

## 2.4. Statistical Methods

Data were analyzed using SPSS 27.0 statistical software. Measurement data conforming to a normal distribution were expressed as ( $\bar{x} \pm s$ ), and paired t-tests were used for within-group comparisons. Count data were expressed as [n(%)], and chi-square tests were used for between-group comparisons. A P-value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Comparison of General Information Between the Two Groups

There were no statistically significant differences in age, gender, or disease type between the two groups ( $P > 0.05$ ). See **Table 1** for details.

**Table 1.** Comparison of General Information Between the Two Groups

Group	Age ( $\bar{x} \pm s$ , years)	Gender (Male/Female)	Disease Type			
			Nevus	Sebaceous Cyst	Scar	Protruding/Depressed Facial Lesions
Control (n=52)	30.21 $\pm$ 5.38	20/32	15	12	15	10
Observation (n=52)	31.25 $\pm$ 5.36	19/33	12	13	17	10
$\chi^2/t$ -value	0.988	0.041			0.498	
P-value	0.326	0.840			0.919	

### 3.2. Comparison of Treatment Effects Between the Two Groups

Compared with the control group, the clinical total effective rate in the observation group (94.23%) was significantly better than that in the control group (76.92%) ( $\chi^2 = 6.310$ ,  $P = 0.000 < 0.001$ ). See **Table 2** for details.

**Table 2.** Comparison of Clinical Efficacy Between the Two Groups

Group	Markedly Effective	Effective	Ineffective	Total Clinical Effectiveness Rate
Control (n=52)	12 (23.07%)	28 (53.85%)	12 (23.08%)	40 (76.92%)
Observation (n=52)	39 (75%)	10 (19.23%)	3 (5.77%)	49 (94.23%)
$\chi^2$ -value				6.310
p-value				0.000

### 3.3. Comparison of Skin Condition Between the Two Groups

After intervention, the skin condition of both groups improved, but the improvement was more significant in the observation group ( $P = 0.000 < 0.001$  for all comparisons). See **Table 3** for details.

**Table 3.** Comparison of Skin Condition Between the Two Groups

Group	Elasticity		Smoothness		Pigmentation	
	Before Tx	After Tx	Before Tx	After Tx	Before Tx	After Tx
Control (n=52)	4.58±0.33	6.21±0.48	4.02±0.15	5.25±0.42	4.10±0.16	5.21±0.32
Observation (n=52)	4.62±0.28	7.98±0.59	4.01±0.14	7.71±0.55	4.12±0.13	7.98±0.56
t-value	0.667	16.781	0.351	25.634	0.700	30.970
p-value	0.507	<0.001	0.726	<0.001	0.486	<0.001

### 3.4. Comparison of Complication Rates between the Two Groups

Compared with the control group, the total incidence of adverse reactions in the observation group (1.92%) was lower than that in the control group (15.38%) ( $\chi^2=4.379$ ,  $P=0.036<0.05$ ). See **Table 4**.

**Table 4.** Comparison of Adverse Reactions between the Two Groups

Group	Hypertrophic Scarring	Wound Infection	Delayed Wound Healing	Total Adverse Event Rate
Control (n=52)	2 (3.85%)	2 (3.85%)	4 (7.68%)	8 (15.38%)
Observation (n=52)	1 (1.92%)	0	0	1 (1.92%)
$\chi^2$ -value				4.379
p-value				0.036

### 3.5. Comparison of Patient Satisfaction between the Two Groups

Compared with the control group, the total satisfaction rate of nursing in the observation group (96.15%) was better than that in the control group (80.77%) ( $\chi^2=6.029$ ,  $P=0.014<0.01$ ). See **Table 5**.



**Table 5.** Comparison of Nursing Satisfaction between the Two Groups

Group	Dissatisfied	Moderately Satisfied	Highly Satisfied	Total Satisfaction Rate
Control (n=52)	10 (19.23%)	28 (53.85%)	14 (26.92%)	42 (80.77%)
Observation (n=52)	2 (3.85%)	12 (23.08%)	38 (73.07%)	50 (96.15%)
$\chi^2$ -value	-	-	-	6.029
p-value	-	-	-	0.014

## 4. Discussion

With the improvement of people's living standards and changes in aesthetic concepts, the demand for cosmetic surgery is increasing. As a common cosmetic procedure, the goal of plastic surgery incision is to improve appearance while minimizing postoperative adverse effects and enhancing patient satisfaction. However, postoperative scar formation, poor skin condition, and complications have always been important issues that trouble clinicians and patients<sup>[5]</sup>.

The application of Botulinum toxin type A in the field of cosmetic surgery provides a new approach to solve these problems. The results of this study showed that the total effective rate of treatment in the observation group was significantly higher than that in the control group, indicating that the application of Botulinum toxin type A after plastic surgery incision can significantly improve the treatment effect. This may be because Botulinum toxin type A can inhibit the contraction of muscles around the incision and reduce incision tension<sup>[6]</sup>. Incision tension is an important factor that affects scar formation, and excessive tension can lead to ischemia and hypoxia of the tissue at the incision edge, stimulating excessive proliferation of fibroblasts and resulting in obvious scarring. By relaxing the muscles, Botulinum toxin type A reduces this mechanical stimulation, creates a low-tension environment for incision healing, facilitates the orderly arrangement of collagen, reduces scar hyperplasia, and thereby improves the treatment effect<sup>[7,8]</sup>.

In terms of skin condition, the observation group showed better skin elasticity, smoothness, and color at various time points after surgery compared to the control group. The improvement in skin elasticity may be related to the reduction of muscle tension by Botulinum Toxin Type A, which promotes the normal repair of skin collagen and elastic fibers. The enhancement of smoothness benefits from the neat healing of incisions in a low-tension environment, reducing the unevenness of the skin surface. The improvement in skin color may be associated with the optimization of local blood circulation. Although Botulinum Toxin Type A can inhibit muscle activity, its reasonable application does not significantly affect skin blood supply. Instead, it may reduce blood vessel compression due to muscle relaxation, increase local blood flow, promote skin pigment metabolism, and make the color closer to normal.

Regarding the incidence of complications, the complication rate in the observation group was lower than that in the control group in this study, which is consistent with some research findings. The reasons for this can be analyzed as follows: On the one hand, Botulinum Toxin Type A reduces incision tension, decreases tissue stretching and irritation, and promotes incision healing, thereby lowering the incidence of incision non-healing and scar hyperplasia. On the other hand, the improved healing environment also reduces the chances of bacterial growth, lowering the risk of incision infection. However, it is important to note that the use of Botulinum Toxin Type A requires strict control of dosage and injection methods to avoid adverse reactions due to improper operation<sup>[9,10]</sup>.

Patient satisfaction is an important indicator for evaluating the effect of cosmetic surgery. The overall satisfaction rate of the observation group was significantly higher than that of the control group, which is closely related to the better treatment effect, better skin condition, and no significant increase in the incidence of complications in the observation group. Good cosmetic results and fewer adverse reactions can effectively reduce patients' psychological pressure and enhance their recognition and satisfaction with the treatment.

In summary, the application of Botulinum toxin type A after cosmetic incision surgery has important clinical value

and is worthy of clinical promotion and application. However, in practical application, it is necessary to strictly grasp the indications and contraindications, standardize the injection operation, reasonably control the dose, and strengthen postoperative care to ensure the safety and effectiveness of the treatment. In the future, large-sample, long-term follow-up studies can be further carried out to explore the effects of different doses and injection methods of Botulinum toxin type A on the postoperative effects and complication risks of cosmetic incision surgery, providing more precise treatment options for clinical practice.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] An X, Yang T, Huang J, 2025, Evaluation of the efficacy and safety of botulinum toxin in reducing postoperative tension after plastic surgery incision. *China Medical Cosmetology*, 15(03): 33-37.
- [2] Liu B, Cui X, Qin A, 2020, Experimental study on the improvement of wound healing quality after cosmetic suture with closed negative pressure therapy. *Shaanxi Medical Journal*, 49(05): 553-556.
- [3] Song L, Ye J, Lu M, 2020, Injection of botulinum toxin type A to prevent hypertrophic scars after facial trauma or surgery: a systematic review of efficacy and safety. *Chinese Journal of Tissue Engineering Research*, 24(29): 4744-4750.
- [4] Li H, 2020, Current status of the application of botulinum toxin in cosmetic surgery. *Military Surgeon in Southwest China*, 22(02): 144-146.
- [5] Yan X, Zhou J, Feng Y, 2025, Clinical application of modified external incision combined with botulinum toxin type A injection in nasal alar reduction plasty. *Chinese Journal of Aesthetic and Plastic Surgery*, 36(05): 283-284+319.
- [6] Wang J, Dong Y, Cao Z, 2018, Observation on the curative effect of keloid surgery combined with botulinum toxin type A and superficial X-ray. *Journal of Weifang Medical College*, 40(05): 334-336+348.
- [7] Che M, Zhang N, Yang R, 2021, Clinical study on the treatment of hypertrophic scars with botulinum toxin type A combined with triamcinolone acetonide. *China Medical Cosmetology*, 11(05): 13-16.
- [8] Tao J, Liu B, Wang Y, 2018, Clinical study on the reduction of forehead postoperative scar hyperplasia with botulinum toxin type A. *Shaanxi Medical Journal*, 47(08): 1011-1013.
- [9] Zhong Y, Liang F, 2020, Analysis of the cosmetic effect of botulinum toxin type A injection at fixed points of masseter muscle combined with hyaluronic acid injection for chin filling in reshaping female maxillofacial contour. *Chinese and Foreign Women's Health Research*, (13): 112-113.
- [10] Liu K, 2018, Analysis of the effect of botulinum toxin type A masseter injection combined with hyaluronic acid chin injection for filling micro-plastic surgery in reshaping the lower 1/3 contour of the face. *Guide of China Medicine*, 16(10): 70-71.

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# Research Progress on Chemical Constituents and Bioactivities of the Ice Plant (*Mesembryanthemum crystallinum* L.)

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**Abstract:** *Mesembryanthemum crystallinum* L. has unique physiological characteristics and is rich in nutrients, which is regarded as a potential functional food resource. Studies have found that the ice plant is rich in a variety of minerals, including five essential macroelement: sodium, potassium, calcium, magnesium, phosphorus and seven essential trace elements: iron, zinc, selenium, copper, molybdenum, chromium, cobalt; it rich in a variety of vitamins, mainly vitamin C and  $\beta$ -carotene; it contains 18 kinds of total amino acids, including 9 kinds of essential amino acids including histidine. And rich in dietary fiber. Ice plant also contains active ingredients such as flavonoids, polysaccharides, polyphenols and alkaloids, which have antioxidant, hypoglycemic, antibacterial, acetylcholinesterase inhibition, immune regulation and other functions. In this paper, the nutritional value, active ingredients and biological activity of ice vegetables were reviewed to provide reference for the development of new functional dietary resources and the further research and development of the ice plant.

**Keywords:** Ice plant; Chemical constituents; Nutritional components; Bioactivity

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## 1. Introduction

Ice plant (*Mesembryanthemum crystallinum* L.), also known as crystalline iceplant or African ice plant, is an annual or biennial herb belonging to the family Aizoaceae and genus *Mesembryanthemum*<sup>[1]</sup>. Its leaves are opposite, rhombic or ovate, succulent, smooth, thick, and light green. It possesses a well-developed fibrous root system, with lateral branches emerging from each leaf axil. Dense transparent bladder cells containing saline fluid cover the young leaves and stems. These cells reflect sunlight, giving the plant a glistening, crystalline appearance, hence the name “crystalline iceplant”<sup>\*,[2]</sup>.

The characteristic salty and juicy taste of ice plant arises from saline fluid stored within its epidermal bladder cells<sup>[2,3]</sup>. When mineral absorption (salts, etc.) from the roots exceeds its regulatory capacity, excess components are sequestered in



these specialized cells on the stems and leaves<sup>[3]</sup>. Consequently, ice plant is a rich natural source of inorganic elements like Na, K, and Ca<sup>[4]</sup>. It thrives in saline-alkali soils and arid conditions, originating from dry regions such as the Namib Desert in South Africa, and is now distributed worldwide<sup>[5]</sup>. It is cultivated in regions of China including Xinjiang, Shandong, and Tianjin. Successful pilot cultivation has been achieved in the Taiping Town area of Tianjin Binhai New Area, where plans are underway to utilize ice plant agriculture to support rural revitalization<sup>[6]</sup> (**Figure 1**).

In China, ice plant is primarily consumed raw in salads. Its full food value remains underutilized. Current research mainly focuses on cultivation conditions<sup>[7-9]</sup>, with fewer studies on its chemical composition, bioactivities, and product development. This review aims to summarize the nutritional composition, chemical constituents, and bioactive effects of ice plant to support its broader development and utilization.



**Figure 1.** Illustration of ice plant (*Mesembryanthemum crystallinum* L.)

## 2. Nutritional and Bioactive Components

Ice plant is a highly valuable health-promoting vegetable, primarily consumed for its tender, crystalline-coated stems and leaves. It boasts a rich nutritional profile, including inorganic elements, amino acids<sup>[4,10]</sup>, and polyols (e.g., myo-inositol, pinitol, ononitol)<sup>[11]</sup>. It has high moisture content (up to 95.7%) and ash content. Ice plant contains the seven essential nutrient groups for humans, with notably high levels of inorganic elements, vitamins, protein, and water compared to common vegetables. It contains lower amounts of insoluble dietary fiber and carbohydrates, and negligible fat content<sup>[4]</sup>.

### 2.1. Plant-derived Electrolytes

Minerals play crucial roles in vital physiological functions. As the human body cannot synthesize them, they must be obtained from the diet<sup>[12]</sup>. Ice plant contains various inorganic elements (**Table 1**), including sodium, potassium, and calcium, which show therapeutic potential for conditions like salt-sensitive hypertension, hyperlipidemia, diabetes, and cardiovascular diseases<sup>[13]</sup>. Its salty taste originates from natural plant-based sodium salts, which are beneficial to health<sup>[1]</sup>.

Mineral accumulation in ice plant primarily occurs within its epidermal bladder cells<sup>[2]</sup>. These structures are key to its salt tolerance. Studies show that as seawater concentration increases, salt content in both the whole plant and epidermal bladders rises proportionally. Epidermal bladders can sequester up to 31.059% of Na<sup>+</sup> and 35.527% of Cl<sup>-</sup> within the plant<sup>[14]</sup>.

Research indicates high levels of Na, K, Ca, and Mg in ice plant. Wang et al.<sup>[10]</sup> used inductively coupled plasma mass spectrometry (ICP-MS) to analyze 24 inorganic elements in introduced varieties, finding a total content of 12.709 mg/g. Levels of toxic heavy metals (As, Pb, Hg, Cd) were all  $\leq 0.06$  mg/kg, well below national safety limits. Jiao<sup>[4]</sup> detected 10 inorganic elements, including 5 macroelements (K, Na, Ca, Mg, P) and 5 microelements (Fe, Zn, Se, Mn, Cu), with Na, K, and Ca being particularly abundant. This confirms ice plant as an excellent mineral source, especially for Ca, Fe, Zn, and Mg. Its natural high Na and K content makes it suitable for formulating sports drinks to replenish electrolytes without added salts.

**Table 1.** Content of inorganic elements in ice plant (mg/100 g)

Element	Jiao et al. <sup>[4]</sup>	Wang et al. <sup>[10]</sup>	Adult Requirement (mg/d) <sup>[12]</sup>
Na	2965±49.0	587.00	1500
K	1825±21.21	499.80	2000
Ca	288.00±4.24	120.30	800
Mg	137±1.41	59.70	330
P	22.12±0.04	-	720
B	-	0.16	-
Al	-	0.591	-
Ti	-	0.116	-
V	-	0.0029	-
Cr	-	0.0078	30
Mn	1.40±0.00	1.090	4.5
Fe	17.6±1.13	1.090	M 12 F 20
Co	-	0.0024	-
Ni	-	0.0063	-
Cu	0.40±0.00	0.087	0.8
Zn	33.90±2.97	0.804	M 12.5 F 7.5
As	-	0.001	-
Se	0.29±0.00	0.0008	60
Mo	-	0.007	0.1
Cd	-	0.006	-
Ba	-	0.018	-
Hg	-	0.0002	-
Ti	-	0.0044	-
Pb	-	0.004	-

## 2.2. National Vitamins

Vitamins are essential micronutrients crucial for normal physiological function, growth, metabolism, and development. They are classified as fat-soluble (e.g., Vitamins A, D, E, K) or water-soluble (e.g., B vitamins, Vitamin C), differing in solubility, absorption, excretion, and deficiency symptom onset<sup>[15]</sup>. Ice plant primarily contains water-soluble vitamins like Vitamin C<sup>[16]</sup>, folate, pantothenic acid<sup>[4]</sup>, and Vitamin B4 (adenine)<sup>[17]</sup>. Excessive intake of water-soluble vitamins is generally non-toxic as excess is excreted in urine; however, deficiency symptoms manifest rapidly. Fat-soluble vitamins in ice plant include Vitamin A and  $\beta$ -carotene. Deficiency in Vitamin A can lead to clinical symptoms like night blindness, xerophthalmia, and conjunctival dryness, and can impair skin/mucous membrane integrity and growth.

Studies comparing ice plant to common vegetables show higher vitamin levels. Liu et al.<sup>[16]</sup> determined Vitamin C content using the molybdenum blue colorimetric method, finding  $15.42 \pm 0.15$  mg/100g, significantly higher than common lettuce. Jiao<sup>[4]</sup> compared vitamins in ice plant, romaine lettuce, spinach, and lettuce, finding ice plant had markedly higher

Vitamin C and  $\beta$ -carotene content, but the vitamin content of ice vegetables was also affected by its variety, producing area and planting environment. SHU et al.<sup>[17]</sup> isolated and identified Vitamin B4 (adenine) in ice plant ethanol extracts.

Ice plant is rich in polyols (e.g., myo-inositol, pinitol). Its polyol accumulation levels are comparable to cowpea, known for high polyol accumulation<sup>[7]</sup>. Myo-inositol, a vitamin-like compound, promotes fat metabolism, lowers cholesterol, and prevents fatty liver and atherosclerosis<sup>[1]</sup>. Under salt stress (400 mM NaCl), myo-inositol peaked at 0.7 mg g<sup>-1</sup> FW (Fresh Weight) in seedlings after 3 days but decreased over time. Control plants generally had higher inositol concentrations. In mature plants, highest concentrations (1.7, 2.8, 7.9 mg g<sup>-1</sup> FW) were found in control leaflets at 25, 35, and 45 days post-treatment initiation<sup>[11]</sup>.

Ice plant also contains natural folate, pantothenic acid (Vitamin B5), retinol<sup>[2]</sup>, and other vitamins less common in vegetables, making it a potential source for nutritional supplements.

**Table 2.** Composition and content of vitamins in ice plant

Category	Type	Content ((g/100g)	Reference	Adult Requirement/d <sup>[12]</sup>
Water-Soluble	Vitamin C	3000	[2]	100mg
		20800±0.11	[4]	
		15420±0.15	[16]	
	Folate	31	[2]	400mg
	Pantothenic acid	0.63	[2]	5mg
Fat-Soluble	Vitamin B4	-	[17]	-
	$\beta$ -Carotene	926	[2]	7mg
		406±4.15	[4]	
	Vitamin A (RAE)	6.20±0.05	[4]	M 800mg F 700mg
Vitamin-like	Myo-inositol	-	[4]	-

Note: RAE = Retinol Activity Equivalents; “-” indicates not detected or not specified.

### 2.3. Plant Protein

Proteins are fundamental macromolecules for life. Jiao<sup>[4]</sup> reported a protein content of  $1.53 \pm 0.01$  g/100g in ice plant, higher than lettuce and romaine, while crude fat content was negligible. This indicates ice plant is a high-protein, low-fat vegetable.

Amino acids are the building blocks of proteins. Studies have analyzed ice plant amino acid composition and content<sup>[4,10]</sup>. Oh et al.<sup>[18]</sup> isolated and identified eight compounds, including three amino acids: phenylalanine, tyrosine, and tryptophan. Wang et al.<sup>[10]</sup> used an amino acid analyzer on differently pre-treated samples. Homogenized samples contained 8 amino acids (total 0.090 mg/g), including 4 essential amino acids (73% of total). Manually ground samples contained 6 amino acids (total 0.099 mg/g), including 4 essential amino acids (78% of total). Jiao et al.<sup>[4]</sup> detected 16 amino acids (**Table 3**), with essential amino acids constituting 38% of total amino acids. Non-essential amino acids accounted for 60.80%, suggesting a relatively balanced profile. Variations in reported amino acid content likely stem from differences in origin, variety, cultivation conditions, sample preparation, extraction methods, and experimental error.

Humans must obtain essential amino acids (EAAs) from diet. There are eight EAAs for adults; histidine is also essential for infants, making nine in total<sup>[12]</sup>. Ice plant contains all nine EAAs (lysine, tryptophan, phenylalanine, methionine, threonine, isoleucine, leucine, valine, histidine), making it a good source for amino acid supplementation.

**Table 3.** Composition and content of amino acids in ice plant

Amino Acid	Wang et al. <sup>[10]</sup> (Homogenized)(mg/100g)	Wang et al. <sup>[10]</sup> (Manual) (mg/100g)	Jiao <sup>[4]</sup> (mg/100g)	Adult Requirement (mg/kg/d) <sup>[12]</sup>
Protein	-	-	1.53±0.01	M:65g ; F:55g
Threonine (Thr*)	1.185	1.388	50±0.35	7
Valine (Val*)	0.395	-	58±0.92	10
Methionine (Met*)	0.691	-	13±0.71	13 (Met+Cys)
Leucine (Leu*)	-	-	90±2.19	14
Lysine (Lys*)	4.345	4.957	74±0.99	12
Isoleucine (Ile*)	-	0.297	52±0.64	10
Tryptophan (Trp*)	-	-	-	3.5
Phenylalanine (Phe*)	-	1.091	60±0.64	8 (Phe+Tyr)
Histidine (His*)	-	-	66±1.84	12
Glutamic acid (Glu <sup>#</sup> )	0.987	-	130±0.71	256
Cystine (Cys <sup>#</sup> )	-	1.6 85	-	13(Met+Cys)
Serine(Ser <sup>#</sup> )	0.592	-	50±0.00	131
Glycine (Gly <sup>#</sup> )	0.099	-	59±0.00	52
Alanine(Ala <sup>#</sup> )	0.790	0.496	78±0.14	143
Tyrosine(Try <sup>#</sup> )	-	-	32±0.85	8 (Phe+Tyr)
Aspartic acid ((Asp <sup>#</sup> )	-	-	99±0.07	129
Arginine(Arg <sup>#</sup> )	-	-	80±1.98	117
Proline (Pro <sup>#</sup> )	-	-	59±3.68	66
Total Amino Acids(T)	9.084	9.914	10 50±15.70	-
Essential AA Total (E)	6.62	7.733	397	-
E/T(%)	73%	78%	38%	-

Note: \*, essential amino acids; #, non-essential amino acids; -, not detected. Adult requirements for sulfur AAs (Met+Cys) and aromatic AAs (Phe+Tyr) are combined.

## 2.4. Dietary Fiber

Dietary fiber comprises indigestible carbohydrates with significant health benefits. Recommended intake varies; Chinese adults (19-50 years) are advised to consume 25-39 g/d, with soluble fiber comprising 25%-30% and insoluble fiber 70%-75%<sup>[15]</sup>. Ice plant is rich in dietary fiber. Jiao<sup>[4]</sup> detected approximately 0.52 g of insoluble dietary fiber per 100g, comparable to levels in romaine lettuce and common lettuce.

## 3. Functional Bioactive Components

Ice plant contains diverse bioactive compounds, with research focused on flavonoids, polysaccharides, and polyphenols<sup>[18-21]</sup>. Its total flavonoids exhibit antioxidant<sup>[19,21]</sup>, hypoglycemic<sup>[22]</sup>, antitumor, and free radical scavenging activities. Polysaccharide and polyphenol extracts demonstrate antioxidant, antibacterial, and immuno-modulatory effects<sup>[23-25]</sup>.

### 3.1. Total Flavonoids

Flavonoids are representative antioxidant compounds found in plants, possessing various bioactivities like inhibiting lipid peroxidation and reactive oxygen species generation<sup>[25]</sup>. Recent studies have identified characteristic flavonoid structures in ice plant extracts.

Sun et al.<sup>[22]</sup> confirmed the C6-C3-C6 flavonoid backbone via UV-Vis spectroscopy. FT-IR spectra revealed vibration absorption peaks for functional groups like -OH, C-H, C=O, C=C, and phenolic hydroxyl, consistent with flavonoid structures. UPLC-MS/MS identified over 30 flavonoids in purified extracts, including tangeretin, nobiletin, farrerol, protocathechualdehyde, diosmin, chalconaringenin, sinensetin, naringenin, and rutin (**Table 4**). Tangeretin had the highest relative percentage ( $50.854\% \pm 0.089\%$ ). Duan et al.<sup>[9]</sup> found that total flavonoid content decreased with increasing seawater concentration; plants grown in 20% seawater had 5.500 g/100g. Kang et al.<sup>[25]</sup> reported flavonoid content (mg QE/g, Quercetin Equivalents) varied by organ: highest in cotyledons ( $1218.07 \pm 1.00$ ), followed by young stems ( $703.97 \pm 0.25$ ) and stems ( $671.29 \pm 0.63$ ). Variations are attributable to extraction methods, origin, etc. Analyzing flavonoid composition provides a theoretical basis for functional development.

Ultrasound-assisted extraction (UAE) is commonly used. Sun et al.<sup>[19]</sup> extracted total flavonoids using UAE, followed by sequential extraction with petroleum ether, ethyl acetate, and n-butanol. The n-butanol and ethyl acetate fractions contained the highest flavonoid levels (191.0 mg/g and 184.5 mg/g, respectively). Sun et al.<sup>[22]</sup> optimized UAE conditions: 60% ethanol, solid-liquid ratio 1:25 (mg/mL), temperature 45°C, time 120 min, power 250 W, achieving a yield of 2.776%. Wang et al.<sup>[21]</sup> also used UAE and orthogonal optimization, determining optimal conditions as 60% ethanol, ratio 1:15, temperature 50°C, time 120 min, yielding 4.91%. Ethanol concentration, solid-liquid ratio, temperature, time, and power significantly impact extraction efficiency, with ratio and temperature causing major yield variations.

Purification involves fractionation and macroporous resin adsorption. Sun et al.<sup>[22]</sup> found D101 resin effective. Optimal purification conditions were: crude extract concentration 0.30 g/L, pH 4, sample flow rate 60 mL/h, sample volume 56 mL, eluent 80% ethanol (pH 6), flow rate 60 mL/h, volume 128 mL. This increased flavonoid purity to 57.67%, a 2.98-fold improvement over the crude extract.

**Table 4.** Composition and relative content of purified flavonoids in ice plant<sup>[20]</sup>

NO.	Compound	Chemical Formula	Relative Percentage(%)
1	Tangeretin	C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	50.854±0.089
2	Nobiletin	C <sub>21</sub> H <sub>22</sub> O <sub>8</sub>	20.949±0.115
3	Farrerol	C <sub>17</sub> H <sub>16</sub> O <sub>5</sub>	8.781±0.072
4	Protocatechualdehyde	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	3.962±0.107
5	Diosmi	C <sub>28</sub> H <sub>32</sub> O <sub>15</sub>	2.875±0.079
6	Chalconaringenin	C <sub>27</sub> H <sub>34</sub> O <sub>14</sub>	2.523±0.054
7	sinensetin	C <sub>20</sub> H <sub>20</sub> O <sub>7</sub>	2.241±0.056
8	Naringenin	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	2.151±0.066
9	Rutin	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	1.723±0.052

### 3.2. Saccharides and Glycosides

Polysaccharides are natural polymers enhancing immunity. Ice plant contains diverse polysaccharides and glycosides (**Table 5**). Yang et al.<sup>[24]</sup> extracted polysaccharides using UAE under optimal conditions (ratio 1:20, 60°C, 60 min), yielding 11.21% (dry weight).

Several studies have isolated and identified saccharides and glycosides. Li<sup>[26]</sup> isolated 5 glycosides and one saccharide

derivative from ethanol extracts (**Table 5**). Further work identified 11 monomeric compounds from ethyl acetate and n-butanol fractions, including 5 glycosides, with coniferin being newly identified <sup>[27]</sup>(**Table 5**). Shu et al.<sup>[17]</sup> isolated 9 compounds, including three glycosides (**Table 5**). These studies provide references for extraction and identification, supporting functional food development.

**Table 5.** Saccharide and glycoside components in ice plant

NO.	Compound	Reference
1	3, 4, 5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl(E)-3-(4-hydroxy-3-methoxyphenyl)acrylate	[26,27]
2	Syringin	[17,26,27]
3	2, 4, 4-trimethyl-3-(((3, 4, 5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)cyclohex-2-en-1-one	[26]
4	6-(((1R, 2S)-2-hydroxy-4-((S, E)-3-hydroxybut-1-en-1-yl)-3, 5, 5-trimethylcyclohex-3-en-1-yl)oxy)-5-(hydroxymethyl)tetrahydro-2H-pyran-2, 3, 4-triol	[26,27]
5	2-ethoxy-5-(hydroxymethyl)tetrahydrofuran-3, 4-diol(	[26]
6	(E)-2-(hydroxymethyl)-6-(4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenoxy)tetrahydro-2H-pyran-3, 4, 5-triol	[27]
7	ixerol B	[17]
8	Jasmioside E	[17]
9	(E)-4-hydroxy-3, 5, 5-trimethyl-4-(3-(((3, 4, 5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)but-1-en-1-yl)cyclohex-2-en-1-one	[26,27]

### 3.3. Polyphenols

Wang et al.<sup>[28]</sup> optimized polyphenol extraction using UAE: 60% ethanol, ratio 1:10 (g:mL), 80°C, 120 min, achieving a yield of 1.42%. Kang et al.<sup>[25]</sup> identified 17 polyphenolic compounds via UHPLC-TOF/HRMS, primarily flavonoids and O-glycoside derivatives. Content varied by organ: highest in cotyledons ( $115.43 \pm 0.47$  mg GAE/g, Gallic Acid Equivalents), followed by young stems ( $78.90 \pm 0.27$  mg GAE/g) and stems ( $78.83 \pm 0.40$  mg GAE/g).

### 3.4. Other Chemical Components

Beyond flavonoids, polysaccharides, and polyphenols, studies have isolated alkaloids, terpenoids, and sterols. Li<sup>[26]</sup> identified 19 compounds from ethanol extracts, including 6 saccharides/glycosides (**Table 5**), 4 alkaloids, 2 terpenoids, 2 phenylpropanoids, 1 sterol, 1 ester, along with uridine and Vitamin B4. Further work identified 11 compounds from ethyl acetate and n-butanol fractions: 2 phenylpropanoids (cis-p-hydroxycinnamic acid, ferulic acid), 2 alkaloids (nicotinic acid, ethane-1,2-diyl dinicotinate), 5 glycosides, 1 benzofuranone (loliolide), and 1 sterol ( $\beta$ -sitosterol)<sup>[27]</sup>. Shu et al.<sup>[17]</sup> isolated 9 compounds using various chromatographic techniques, with ixerol B being newly identified (**Table 6**).

**Table 6.** Other chemical components in ice plant

Category	Compound	Reference
Alkaloids	Nicotinic acid	[26,27]
	3-benzyl-6-isopropylpiperazine-2, 5-dione	[26,27]
	(1S, 3S)-1-methyl-2, 3, 4, 9-tetrahydro-1H-pyrido[3, 4-b]indole-3-carboxylic acid	[17,26]
	(1R, 3S)-1-methyl-1, 2, 3, 4-tetrahydro- $\beta$ -carboline-3-carboxylic acid	[17,26]
	Cyclo(phenylalanine-valine)	[17,26]
	4-hydroxy-3-methoxybenzamide	[18]



**Table 6 (Continued)**

Category	Compound	Reference
Terpenoids	Loliolide	[26,27]
	(1R, 2S)-4-((R, E)-3-hydroxybut-1-en-1-yl)-3, 5, 5-trimethylcyclohex-3-ene-1, 2-diol	[26]
Sterols	Stigmasterol	[26]
	$\beta$ -Sitosterol	[27]
Phenylpropanoids	cis-p-Hydroxycinnamic acid	[26,27]
	Ferulic acid	[17,18,26,27]
Esters	Dibutyl phthalate	[17,27]
	Ethane-1, 2-diyl dinicotinate	[26,27]
Others	Vitamin B4 (Adenine)	[17,26,27]
	Uridine	[17,18,26,27]
	adenosine	[18]

## 4. Bioactivities of Ice Plant

Research indicates ice plant possesses antioxidant<sup>[25]</sup>, hypoglycemic<sup>[22]</sup>, antibacterial, antitumor<sup>[18]</sup>, immunoenhancing<sup>[29]</sup>, and cardiovascular protective activities, likely mediated by its polysaccharides, flavonoids, polyphenols, and other bioactive components.

### 4.1. Antioxidant Activity

Flavonoids, polysaccharides, and polyphenols contribute to antioxidant activity. Wang et al.<sup>[28]</sup> found ice plant polyphenols dose-dependently scavenged DPPH and hydroxyl radicals. At 0.10 mg/mL, scavenging rates reached 64.19% and 88.10%, respectively. Kang et al.<sup>[25]</sup> measured antioxidant activity via DPPH and hydroxyl radical scavenging, showing leaves had higher activity than stems, correlating with polyphenol content. Wang et al.<sup>[21]</sup> demonstrated ice plant flavonoid extracts (0.02-0.10 mg/mL) exhibited dose-dependent antioxidant activity against DPPH and hydroxyl radicals. Yang et al.<sup>[24]</sup> showed ice plant polysaccharides effectively scavenged hydroxyl radicals, DPPH, and nitrite ions, and exerted dose-dependent antioxidant effects in animal and vegetable oils.

### 4.2. Hypoglycemic Activity

Total flavonoids<sup>[30]</sup>, polyols like pinitol<sup>[24]</sup> and myo-inositol contribute to hypoglycemic effects. Sun et al.<sup>[22]</sup> found ice plant flavonoids inhibited  $\alpha$ -glucosidase and  $\alpha$ -amylase activity in vitro. Crude extract, purified flavonoids, and acarbose all inhibited  $\alpha$ -glucosidase more strongly than  $\alpha$ -amylase. Purified flavonoids showed inhibition comparable to acarbose and significantly higher than crude extract, suggesting potential as natural enzyme inhibitors. Kim et al.<sup>[31]</sup> reported fermented ice plant extract had stronger anti-diabetic effects (inhibiting lipid accumulation, reducing fasting blood glucose, improving glucose tolerance) than non-fermented extract in db/db mice. The mechanism involved upregulating IRS-1, PI3K, and Akt expression/phosphorylation. Zhang et al.<sup>[32]</sup> optimized D-pinitol extraction from ice plant (IPE). Optimized IPE effectively lowered fasting blood glucose, improved glucose tolerance, and protected/repaired pancreatic  $\beta$ -cells in type 2 diabetic rats, linked to improved islet function and gut microbiota modulation.

### 4.3. Antibacterial Activity

Polysaccharides, polyphenols, and flavonoids exhibit antibacterial properties. Yang et al.<sup>[24]</sup> found ice plant polysaccharides

inhibited *Escherichia coli* and *Bacillus subtilis*, with stronger activity against Gram-positive (*B. subtilis*) than Gram-negative (*E. coli*) bacteria. Wang et al.<sup>[28]</sup> demonstrated ice plant polyphenols significantly inhibited *E. coli*, with an inhibition zone diameter of 12 mm at 3 mg/mL.

#### 4.4. Other Bioactivities

Beyond antioxidant and antibacterial effects, ice plant show cognitive-enhancing<sup>[30]</sup> and immunomodulatory activities. Extracts inhibited acetylcholinesterase ( $77.72 \pm 3.30\%$ ), comparable to the positive control tacrine<sup>[33]</sup>, suggesting potential for cognitive improvement. Choi et al.<sup>[29]</sup> showed extracts enhanced IL-6 and TNF- $\alpha$  production in macrophages, upregulated iNOS gene expression, and increased NO production in IFN $\gamma$ -stimulated macrophages, indicating immunomodulatory activity. Ice plant also shows cardiovascular protective effects and inhibits tumor cell growth<sup>[34,35]</sup>.

### 5. Prospects

Ice plant possesses high nutritional value, rich in inorganic elements, amino acids, vitamins, flavonoids, and other bioactive substances, along with diverse bioactivities (antioxidant, hypoglycemic, antibacterial), giving it significant development potential. Currently, few ice plant-based products exist beyond fresh consumption. Limited research explores food applications: Yuan et al.<sup>[36]</sup> developed a fermented beverage using kale, ice plant, and purple carrot; Xiao et al.<sup>[37]</sup> optimized a jelly using ice plant and hawthorn. Taiping Town in Tianjin is actively researching saline soil cultivation and collaborating (e.g., with Tianjin Liuyefeng Co.) on medicinal food development<sup>[38]</sup>. Given its high nutritional value and significant bioactivities, ice plant is poised to become a premium raw material for low-fat, high-energy meal replacements, sports drinks, and other functional products.

### Disclosure statement

The author declares no conflict of interest.

### References

- [1] Han M, Shen W, Zhao G, 2018, Biological characteristics and key cultivation techniques of the healthy vegetable *Mesembryanthemum Crystallinum*. *Agricultural Science & Technology*, 19(05):24-28.
- [2] Agarie S, Shimoda T, Shimizu Y, 2007, Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *Journal of Experimental Botany*, 58(8): 1957-1967.
- [3] Xia J, Mattson N, Stelick A, 2022, Sensory evaluation of common ice plant (*Mesembryanthemum crystallinum* L.) in response to sodium chloride concentration in hydroponic nutrient solution. *Foods*, 11(18): 2790.
- [4] Jiao Y, 2019, Analysis and evaluation of nutritional components in *Mesembryanthemum crystallinum* L. *Food Research And Development*, 40(09): 181-185.
- [5] Rodríguez-Hernández M C, Garmendia I, 2022, Optimum growth and quality of the edible ice plant under saline conditions. *Journal of the Science of Food and Agriculture*, 102(7): 2686-2692.
- [6] Li T T, Wang L, 2024, Party building leads the characteristic road, consumption assistance helps revitalization. *Bincheng Times*, (004).
- [7] Zheng L H, Zheng H, Wang Chao, 2019, Study on Effects of Different Plant Growth Regulatorson on Seed Germination of *Mesembryanthemum crystallinum* under Salt Condition. *Chinese Wild Plant Resources*, 38(01): 33-38.
- [8] Liu H, 2019, Influences of NaCl on growth and quality of *Mesembryanthemum crystallinum*. *Jiangsu Agricultural*



- Sciences, 47(15): 184-188.
- [9] Duan R J, Wu C B, Wang J, 2019, Effect of seawater on growth and nutrient quality of *Mesembryanthemum crystallinum* L. and salt tolerance response of polyamines in leaves. *Acta Agriculturae Universitatis Jiangxiensis*, 41(05): 881-889.
  - [10] Wang Z W, Li W Y, Li W L, 2023, Constituent analysis of amino acids and inorganic elements of introduced *Mesembryanthemum Crystallinum*. *Journal of Kunming University*, 45(06): 79-82.
  - [11] Agarie S, Kawaguchi A, Kodera A, 2009, Potential of the common ice plant, *Mesembryanthemum crystallinum* as a new high-functional food as evaluated by polyol accumulation. *Plant Production Science*, 12(1): 37-46.
  - [12] Chinese Nutrition Society. *Dietary Reference Intakes for China(2023 Edition)*. Beijing: People's Medical Publishing House, 2023. 120-137+193-361.
  - [13] Farzana T, Guo Q, Rahman M S, 2023, Salinity and nitrogen source affect productivity and nutritional value of edible halophytes. *PloS one*, 18(8): e0288547.
  - [14] Zhu K, Pan C, Ji Y, 2024, Comparative metabolomic analysis of epidermal bladder cells in *Mesembryanthemum crystallinum* under salt stress. *Journal Of Tropical Biology*, 15(02): 224-231.
  - [15] Zhang S Q, 2019, *Foods for special medical purposes principles and practice*. Beijing: China Light Industry Press, 92-114.
  - [16] Liu Q, Liu J, Huang G, 2019, Study on determination of content of reduction- type vitamin cin special vegetable with molybdenum- blue colorimetry. *Farm Products Processing*, (04): 56-59.
  - [17] Shu Y, Li T T, Zeng Y B, 2020, Study on the chemical constituents of *Mesembryanthemum crystallinum* L. *Nat Prod Res Dev*, 32(10): 1704-1708.
  - [18] Oh M, Han A R, Lee J, 2024, LC-QTOF/MS-Based profiling of the phytochemicals in ice plant (*Mesembryanthemum crystallinum*) and their bioactivities. *Foods*, 13(12): 1820.
  - [19] Sun M, Feng X, Chang X, 2021, Study on extraction and antioxidant activity of total flavonoids from *Mesembryanthemum crystallinum*. *Journal of Beijing University of Agriculture*, 36(04): 116-120.
  - [20] Sun M, Qiu X, Zhou J, 2022, Optimization of extraction process, structural characterization and component analysis of total flavonoids from *Mesembryanthemum crystallinum*. *Science and Technology of Food Industry*, 43(4): 196-204.
  - [21] Wang M, Wang J, Wang Z, 2022, Study on optimization of extraction and the antioxidant activity of total flavonoids in *Mesembryanthemum crystallinum*. *Journal of Tianjin Agricultural University*, 29(01): 33-36+41.
  - [22] Sun M, Feng X, Hou X, 2021, Purification technology and in vitro hypoglycemic activity of total flavonoids macroporous resins from *Mesembryanthemum crystallinum*. *Journal of Henan Agricultural University*, 55(05): 936-944.
  - [23] Chen F, Huang G, 2018, Preparation and immunological activity of polysaccharides and their derivatives. *International Journal of Biological Macromolecules*, 112: 211-216.
  - [24] Yang Z, Wang M, Wang Z, 2023, Study on antioxidant and antibacterial activity of polysaccharide extract in *Mesembryanthemum crystallinum*, 30(01): 1-5.
  - [25] Kang Y W, Joo N M, 2023, Comparative analysis on phytochemical properties, anti-oxidative, and anti-inflammatory activities of the different organs of the common ice plant *Mesembryanthemum crystallinum* L. *Applied Sciences*, 13(4): 2527.
  - [26] Li T T, 2019, *Isolation and identification of chemical components in Mesembryanthemum crystallinum L.* Hainan University.
  - [27] Li T, Dai H, Cai C, 2020, Isolation and identification of the chemical compositions of ethyl acetate and n-butyl alcohol fractions of ethanol extract from *Mesembryanthemum crystallinum*. *Chinese Journal of Tropical Crops*, 41(06): 1234-1241.
  - [28] Wang Z, Wang J, Wang M, 2022, Study on extraction optimization of polyphenols from *Mesembryanthemum crystallinum* and its antioxidant and bacteriostatic activities. *Journal of Tianjin Agricultural University*, 29(03): 23-28.
  - [29] Choi J H, Jo S G, Jung S K, 2017, Immunomodulatory effects of ethanol extract of germinated ice plant (*Mesembryanthemum crystallinum*). *Laboratory animal research*, 33(1): 32-39.
  - [30] Calvo M M, Martín-Diana A B, Rico D, 2022, Antioxidant, antihypertensive, hypoglycaemic and nootropic activity of a polyphenolic extract from the halophyte Ice Plant (*Mesembryanthemum crystallinum*). *Foods*, 11, 1581.

- [31] Kim H L, Jung Y, Kim H I, 2023, Antidiabetic effect of fermented *Mesembryanthemum crystallinum* L. in db/db mice involves regulation of PI3K-Akt pathway. *Current Issues in Molecular Biology*, 45(8): 6415-6431.
- [32] Zhang C, Wu W, Xin X, 2019, Extract of ice plant (*Mesembryanthemum crystallinum*) ameliorates hyperglycemia and modulates the gut microbiota composition in type 2 diabetic Goto-Kakizaki rats. *Food & Function*, 10(6): 3252-3261.
- [33] Li T , Cai C, Guo Z, 2019, Inhibitory activity of acetylcholinesterase from ethanol extract of *Mesembryanthemum crystallinum*. *Chinese Journal of Tropical Agriculture*, 39(11): 104-108.
- [34] Loconsole D, Murillo-Amador B, Cristiano G, 2019, Halophyte common ice plants: A future solution to arable land salinization. *Sustainability*, 11(21): 6076.
- [35] Lin T H, Tan T W, Tsai T H, 2013, D-pinitol inhibits prostate cancer metastasis through inhibition of  $\alpha V\beta 3$  integrin by modulating fak, c-src and nf- $\kappa$ b pathways. *International Journal of Molecular Sciences*. 14, 9790–9802.
- [36] Yuan Xi, Yang Q, Wang F, 2024, Development and volatile flavor substances analysis of purple carrot compound fermented beverage. *Science and Technology of Food Industry*, 45(2): 201–209.
- [37] Xiao M, Ren H H, 2022, Optimization of processing technology for ice plant and hawthorn jelly by response surface methodology. *Journal of Zhejiang Agricultural Sciences*, 63(07): 1596-1599+1605.
- [38] Town T P, 2025, Binhai New Area. Industrial breakthrough: Signing ceremony for Taiping characteristic agricultural product medicinal food homologous project. [2025-07-22]. [https://mp.weixin.qq.com/s/S\\_CJCxrgS9UedTR6Yo7k\\_Q](https://mp.weixin.qq.com/s/S_CJCxrgS9UedTR6Yo7k_Q)

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# Rapid Determination of 16 Illegally Added Chemical Drugs in Anti-gout Chinese Patent Medicines by Ultra-high Performance Liquid Chromatography

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**Abstract:** Objective: To establish a method for the rapid determination of illegally added chemical drugs in anti-gout Chinese patent medicines. Method: Ultra-high performance liquid chromatography was used with acetonitrile-0.1% phosphoric acid (45:45) as mobile phase A and anhydrous methanol-0.1% phosphoric acid (35:65) as mobile phase B. The detection wavelength was set at 230nm, the flow rate was 1.0ml/min, the column temperature was room temperature, and the injection volume was 1μl. Results: The 16 compounds showed good linearity in the range of 1~6ng, with an average recovery rate of 98%~102% and an RSD less than 2%. Conclusion: Ultra-high performance liquid chromatography can quickly and accurately determine the chemical drugs contained in traditional Chinese medicine preparations. This method is simple, highly sensitive, reproducible, low-cost, and easy to operate, making it suitable for the detection of illegally added drugs in anti-gout Chinese patent medicines.

**Keywords:** Ultra-high Performance Liquid Chromatography; Anti-gout Chinese Patent Medicines; Illegally Added Chemical Drugs

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## 1. Introduction

Chinese herbal formula granules are granular preparations made from single or several herbs in proportion for clinical use in traditional Chinese medicine<sup>[1]</sup>. These preparations are mostly made from crushed herbal medicines, which are then processed into granules according to specific prescriptions and technological requirements. The source of these prescriptions is generally the standard herbal medicines listed in the national pharmacopoeia, and they typically consist of five parts: preparation method, technological process, prescription composition, functional indications, and usage and dosage<sup>[2]</sup>. They are mainly used to treat diseases such as bone pain, gout, gouty arthritis, and osteoporosis caused by kidney deficiency. Depending on the manufacturing process, they can be classified into two categories: Chinese patent medicines and herbal slices. Anti-gout Chinese patent medicines refer to those used in the clinical practice of traditional Chinese medicine to treat gout and other related diseases, and their components are mostly herbal slices. However, due to factors such as diverse sources and complex manufacturing processes, the illegal addition of chemical drug ingredients is often

found in anti-gout Chinese patent medicines sold in the market.

## 2. Instrumentation and Materials

### 2.1. Instrumentation

Agilent7890A ultra-high performance liquid chromatography system (Agilent Technologies, USA), Agilent ZORBAX SB-C18 column (250mm×4.6mm, 5μm), Agilent chromatography workstation; Zeiss ZBQ ultrasonic cleaner (made in Germany); ultrapure water system from Shanghai Yuan Ye Precision Instrument Co., Ltd.; Waterse2695 ultrapure water system (Waters Corporation, USA); KQ3200B electric thermostatic blast drying oven (Shanghai Yiheng Technology Co., Ltd.).

### 2.2. Reagents and Chemicals

Methanol (chromatographically pure), acetonitrile (analytically pure), potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>, Sinopharm Chemical Reagent Co., Ltd.), phosphoric acid (analytically pure), formic acid (analytically pure), p-cresol (analytically pure), ammonium acetate (analytically pure), copper sulfate (analytically pure), ferrous chloride (analytically pure), sodium citrate (analytically pure), sodium hydroxide (analytically pure), copper sulfate (analytically pure), etc. were all purchased as analytically pure reagents.

### 2.3. Samples

Six types of anti-gout Chinese patent medicines (numbered S1-S6) were purchased from the market. After eliminating non-target substances, test samples were prepared according to the relevant methods in the “Pharmacopoeia of the People’s Republic of China” (2016 edition).

**Table 1.** Experimental Sample Information

Sample ID	Chinese Patent Medicine Name (Trade Name)	Manufacturing Date	Expiry Date	Specifications
S1	Tongfengshu Capsules	Mar 20, 2023	Mar 19, 2026	0.3g×36 capsules
S2	Tongfengding Tablets	May 10, 2023	Nov 9, 2025	0.4g×24 tablets
S3	Simiao Pills	Dec 25, 2022	Dec 24, 2024	6g×10 bags
S4	Danggui Niantong Pills	Dec 25, 2022	Jan 11, 2026	5g×12 bags
S5	Shirebi Granules	Oct 30, 2023	Oct 29, 2025	3g×20 bags
S6	Qinpi Jiegu Capsules	Sep 15, 2022	Sep 14, 2024	0.35g×30 capsules

### 2.4. Detection Method

The method for detecting 16 chemical drug components employs high-performance liquid chromatography (HPLC). This approach is based on the testing conditions prescribed in the pharmacopoeia. The specific detection process involves several steps: first, determining the content of the target drug components in the sample; then, using chromatographic conditions to separate and identify the target drug components; finally, calculating the content of the target drug components in the sample by comparing with the reference standards specified in the pharmacopoeia<sup>[3]</sup>.

- (1) Sample pretreatment: Weigh 5g of the sample (according to the pharmacopoeia requirements) and place it in a 50mL volumetric flask. Add 5mL of acetonitrile, extract for 30 minutes using ultrasound, filter, and dilute to 10mL with acetonitrile. Transfer a measured amount of the test solution (take 10mL of the test solution, accurately weigh, and dilute to 5mL with methanol) to a conical flask with a stopper and dilute to 10mL with methanol.

Ultrasonically extract the above solution three times at 0, 4, 8, and 12 hours, for 20 minutes each time. Combine the three extracts in another 25mL volumetric flask and dilute to 10mL separately. Simultaneously, weigh a certain amount of the test solution, heat it at 50°C for 1 hour, cool it, add an appropriate amount of methanol to dissolve and dilute to 25mL. Accurately pipette the above solution into a 5mL volumetric flask, precisely add 10μL of the above solution, ultrasonically extract at 50°C for 30 minutes, and then filter. Process according to the above method to obtain the sample solution.

- (2) Preparation of reference solution: Take about 1g of the product, accurately weigh, place it in a conical flask with a stopper, accurately add 50mL of methanol, weigh, and treat with ultrasound (power 250W, frequency 40kHz) for 30 minutes. Cool, reweigh, make up the lost weight with methanol, and mix well. Accurately add 25mL of 5% NaOH solution, weigh, and treat with ultrasound (power 250W, frequency 40kHz) for 30 minutes. After cooling, weigh again, make up the lost weight with methanol, transfer the solution to a 10mL volumetric flask, dilute to the mark with methanol, and mix well<sup>[4]</sup>. Filter through a 0.45μm microporous membrane.
- (3) Preparation of test solution: Take about 2g of the product (containing about 1g of crude drug), accurately weigh (to 0.01g). Accurately add 20mL of 5% NaOH solution, 25mL of 50% sulfuric acid, 50mL of distilled water, and 25mL of methanol. After ultrasonic treatment (power 250W, frequency 40kHz) for 30 minutes, make up the lost weight with 20mL of 5% NaOH solution; then wash twice with 25mL of 50% sulfuric acid; wash twice again with 25mL of 50% sulfuric acid. Accurately weigh 50mL of distilled water into a 10mL volumetric flask (to 0.01g) and dilute to the mark with methanol.
- (4) Preparation of test solutions: Take about 2g (containing about 1g of crude drug), 1000mg, and 1500mg of the product, place them in three separate volumetric flasks (accurate to 0.01g), and accurately add 20mL of 5% NaOH solution, 25mL of 50% sulfuric acid, and 50mL of distilled water to each. Then rinse each of the three volumetric flasks twice with 20mL of 5% NaOH solution.

## 2.5. Chromatographic Conditions

Column: HPLC-DAD; Column temperature: Room temperature; Injection volume: 1μL; Flow rate: 1.0mL/min; Detection wavelength: 230nm; Sample volume: 20μL.

The choice of the chromatographic column depends on the characteristics of different chemical drug components. For example, polar chemical drug components such as tetrahydrofuran and benzyl alcohol require a strongly acidic chromatographic column for adequate separation. However, these components can undergo hydrolysis under alkaline conditions, reducing the actual service life of the chromatographic column. Therefore, this study selected acetonitrile-0.1% phosphoric acid (45:45) as mobile phase A and anhydrous methanol-0.1% phosphoric acid (35:65) as mobile phase B for gradient elution of tetrahydrofuran, benzyl alcohol, benzoic acid, formic acid, and methyl benzoate.

After determining the chromatographic column, this study used UPLC to verify the established chromatographic conditions. The UPLC chromatographic conditions established in this study were: LuminexC18 column (250mm×4.6mm, 5μm) as the separation column, mobile phase of acetonitrile-0.1% phosphoric acid (45:45), and a detection wavelength of 230nm.

Detection wavelengths for tetrahydrofuran, benzyl alcohol, benzoic acid, formic acid, and methyl benzoate are 230nm, while tetrahydrofuran and benzyl alcohol have detection wavelengths of 266nm and 285nm, respectively, and methyl benzoate has a detection wavelength of 270nm.

Mobile phase A: Acetonitrile-0.1% phosphoric acid (45:45); Gradient time: Inject once every 5 minutes from the start of sample injection to the end of sample delivery; Gradient flow rate: 1.0mL/min; Mobile phase A:B ratio: Water: Methanol: Disodium hydrogen phosphate = 3:4:1.



### 3. Results and Discussion

This study established an ultra-high-performance liquid chromatography method for the determination of illegally added chemical drugs in anti-gout traditional Chinese medicines and verified the reliability and stability of the method.

#### 3.1. Linearity Investigation

Under the chromatographic conditions mentioned above, an external standard method was employed, using peak area as the detection index for linear regression. The standard curve equation was calculated to be  $y = -1.4896x + 0.9713$ , with a correlation coefficient of  $r = 0.9999$ , indicating good linearity of the method.

#### 3.2. Standard Addition Recovery Test

Six samples were taken, and their total masses were precisely measured. They were then dissolved in 20 mL of acetonitrile to prepare the test solution. 5  $\mu$ L of the test solution was injected into the liquid chromatograph to measure the peak area. The peak areas in the sample solutions were determined according to the above method, and the recovery rates were calculated. To verify the accuracy and precision of the method, the sample solutions were measured. The peak times of each drug in the extraction solution are shown in **Table 2**.

**Table 2.** HPLC retention times and precision of 16 anti-gout related components (n=6)

Component Name	Retention Time (min)	RSD(%)	Component Name	Retention Time (min)	RSD(%)
Colchicine	6.25	0.8	Benzbromarone	18.73	1.2
Allopurinol	8.41	1.1	Probenecid	22.15	0.9
Allopurinol Metabolite (Oxypurinol)	9.87	1.3	Febuxostat	24.62	1.5
Xanthine	10.53	0.7	Febuxostat Metabolite (Febuxostat Acid)	26.18	1.8
Hypoxanthine	12.09	0.9	Sulfinpyrazone	28.34	1.0
Adenine	14.27	1.2	Diclofenac Sodium	30.41	1.4
Uric Acid	15.82	0.5	Indomethacin	32.75	1.6
Caffeine	17.16	0.6	Naproxen	35.03	1.3

The recovery rates of 16 chemical drugs in different batches of samples were calculated and are shown in **Table 3**. The results indicated that the recovery rates of all batches of samples were between 85% and 120%, meeting the requirements of this method<sup>[5]</sup>.

**Table 3.** Measurement results of recovery rates of 16 chemical drugs in different batches of samples (n=6)

Component Name	S1(%)	S2(%)	S3(%)	S4(%)	S5(%)	S6(%)	Average Recovery (%)	RSD (%)	Recovery Range (%)
Colchicine	98.2	102.3	96.5	101.7	97.8	99.4	99.3	2.1	96.5-102.3
Allopurinol	95.7	104.1	97.2	98.6	102.5	96.8	99.2	3.0	95.7-104.1
Oxypurinol	97.5	101.8	94.3	103.2	98.6	100.4	99.3	3.2	94.3-103.2
Xanthine	102.1	98.7	103.5	96.8	101.2	97.9	100.0	2.5	96.8-103.5
Hypoxanthine	96.4	103.6	95.8	102.3	97.1	101.5	99.4	3.3	95.8-103.6

**Table 3 (Continued)**

Component Name	S1(%)	S2(%)	S3(%)	S4(%)	S5(%)	S6(%)	Average Recovery (%)	RSD (%)	Recovery Range (%)
Adenine	98.8	101.2	97.5	99.6	103.1	96.3	99.4	2.4	96.3-103.1
Uric Acid	101.5	97.3	102.8	96.5	98.7	103.4	100.0	2.8	96.5-103.4
Caffeine	97.2	103.5	96.1	101.8	99.3	98.6	99.4	2.7	96.1-103.5
Benzbromarone	99.1	97.8	102.4	95.7	101.3	98.5	99.1	2.5	95.7-102.4
Probenecid	96.8	102.6	95.3	103.1	97.9	101.2	99.5	3.1	95.3-103.1
Febuxostat	98.5	101.7	97.2	100.4	102.8	96.3	99.5	2.6	96.3-102.8
Febuxostat Acid	97.3	103.2	96.5	101.9	98.4	99.7	99.5	2.4	96.5-103.2
Sulfinpyrazone	101.2	97.6	103.5	96.8	99.2	102.1	100.1	2.7	96.8-103.5
Diclofenac Sodium	96.5	102.3	95.8	103.6	97.4	101.8	99.6	3.2	95.8-103.6
Indomethacin	98.7	101.5	97.1	102.8	96.3	99.6	99.3	2.5	96.3-102.8
Naproxen	101.5	97.8	103.2	96.4	98.7	102.1	99.9	2.6	96.4-103.2

### 3.3. Precision Test

The content of 16 chemical drugs in six batches of anti-gout traditional Chinese medicines was determined according to the above method, including five samples with peak areas greater than or equal to 100,000. The relative standard deviation (RSD) was 1.16% (n=5), and the average recovery rate of standard addition was 99.7% (n=6).

### 3.4. Repeatability Test

The test solution (20g) and reference solution (10mL) were precisely weighed in 5mL volumetric flasks and prepared according to the above method<sup>[6]</sup>. The sample solutions were measured under the same chromatographic conditions, and the peak areas and relative standard deviations were recorded<sup>[7]</sup>.

### 3.5. Stability Test

Measurements were taken after placing the samples at room temperature (20°C), in dark conditions (10°C), and under sealed conditions (with the bottle bottom not touching water) for 30 minutes, 1 hour, 2 hours, and 4 hours<sup>[8]</sup>. The results showed no significant changes in the test solution after being stored at 4°C for 3 and 10 days, and no significant changes in peak area compared to the reference solution after being stored in dark conditions for 1 day.

### 3.6. Sample Content Determination

Three portions (one of which was a 10g test solution) were taken from each of the six anti-gout traditional Chinese medicine samples. Their total masses were precisely measured, dissolved in 20mL of acetonitrile, diluted to 1L, and set aside. 20g of each sample was precisely weighed and prepared according to the method described above. The total mass of the 20g test solution was precisely measured, dissolved in 10mL of acetonitrile, diluted to 1L, and set aside. Sample solutions were prepared and peak areas were measured according to the above method. The results showed no significant changes in peak shape when the test solutions were stored at 4°C for 3, 10, and 20 days, or when stored in dark conditions for 1, 2, 3, 4, and 6 days.

### 3.7. Standard Addition Recovery Rate Test

The recovery rates of the sample solutions were measured according to the above method, and the results showed that the

recovery rates were all between 98% and 102% (n=6).

## 4. Conclusion

As a new type of granular preparation for Chinese herbal decoction pieces, traditional Chinese medicine formula granules differ significantly from traditional Chinese herbal decoction pieces in terms of composition and formula. They are neither drugs nor decoction pieces, but a new type of traditional Chinese medicine granules<sup>[9, 10]</sup>. In this experiment, we identified “anti-gout Chinese patent medicines” from the source and established a rapid method using ultra-high-performance liquid chromatography to determine illegally added chemical drug components in such Chinese patent medicines, providing a scientific basis for the regulation of these medicines. Due to its simplicity, speed, strong specificity, and high sensitivity, this method can be used to detect illegally added chemical drug components in this type of Chinese patent medicine. Since there are many types of illegally added chemical drug components in this type of Chinese patent medicine, and they are relatively concealed, and there are currently few detection methods for illegally added chemical drug components in this type of Chinese patent medicine, the establishment of this method can provide a scientific basis for quality control of this type of Chinese patent medicine. The chromatographic conditions in this method have a significant impact on the chromatographic peaks of the samples, so it is necessary to optimize the chromatographic conditions to avoid interference from non-target components in the samples as much as possible.

In summary, ultra-high-performance liquid chromatography can quickly, easily, and sensitively detect illegally added chemical drug components in anti-gout Chinese patent medicines, providing a scientific basis for the regulation of this type of Chinese patent medicine.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Zhou C, Liu T, Li X, 2021, Determination of aristolochic acid I in Chinese patent medicines by ultra-high performance liquid chromatography tandem mass spectrometry. *China Pharmaceuticals*, 30(16): 81-84.
- [2] Chen F, Wang H, Sun J, 2025, Simultaneous determination of 10 active components in Antitumor Pill (Huoxue Yiqi Recipe) by ultra-high performance liquid chromatography. *China Pharmaceuticals*, 34(3): 81-85.
- [3] Lin R, Xia H, Sang X, 2025, Determination of nitrite in pickled vegetables by nitrosation derivatization and ultra-high performance liquid chromatography. *Journal of Food Safety and Quality*, 16(4): 279-283.
- [4] Jin Y, 2024, Determination of isoflavone content in soybeans by ultra-high performance liquid chromatography. *Modern Food*, 30(3): 186-188, 193.
- [5] Sun Y, Huang Y, Liu Jiameng, 2023, Detection of four purine compounds in 58 soybean varieties by ultra-high performance liquid chromatography. *Journal of Chinese Institute of Food Science and Technology*, 23(6): 304-313.
- [6] Mei L, Ding H, Qian Y, 2022, Determination of lycium acid in goji berries by ultra-high performance liquid chromatography. *Food and Nutrition in China*, 28(8): 29-33.
- [7] Ke J, Yang F, Jin Y, 2024, Determination of lycopene content in tomatoes by ultra-high performance liquid chromatography. *Journal of Zhejiang Agricultural Sciences*, 65(7): 1683-1686.
- [8] Lu L, He Y, Cai Y, 2025, Comparative study on the determination of lobetyolin content in roots and leaves of Guizhou *Codonopsis pilosula* by ultra-high performance liquid chromatography. *China Prescription Drug*, 23(3): 47-50.
- [9] Huang Y, 2019, Application of modern analytical methods in the determination of Chinese patent medicine content.



Consumer Guide, (14): 6.

- [10] Chen Z, Bian Y, Du S, 2023, Determination of potassium content in 32 commonly used Chinese patent medicines and its application analysis in chronic kidney disease. Herald of Medicine, 42(10): 1548-1553.

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# Systematic Review of the Efficacy and Immune Function Impact of Probiotic Preparations on Recurrent Respiratory Infections in Children

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**Abstract:** Objective: To explore the therapeutic effect of probiotic preparations on children with recurrent respiratory tract infections and their impact on their immune function. Method: 80 children with recurrent respiratory tract infections admitted to our hospital from May 2022 to May 2024 were randomly divided into a probiotic group and a control group using a random number table, with 40 cases in each group. The conventional group implemented conventional intervention measures, while the probiotic group combined probiotic preparations on the basis of conventional intervention. The treatment effects and immune function related indicators of the two groups were compared. Result: The total effective rate of the probiotic group was significantly higher than that of the conventional group, and the improvement of immune function indicators was more significant. The incidence of complications was lower than that of the conventional group ( $P < 0.05$ ). Conclusion: Probiotic preparations can effectively improve the treatment effect of children with recurrent respiratory tract infections, improve their immune function status, reduce the risk of complications, and have clinical promotion and application value.

**Keywords:** Probiotic preparations; Children; Recurrent respiratory infections; Therapeutic effect; immune function

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## 1. Introduction

Recurrent respiratory infections are a common condition among children aged 6 to 18 months, which often leads to frequent coughing, fever, and other symptoms. They not only interfere with the normal growth and development process of children, but also increase the medical burden on families. At present, conventional interventions such as anti infection and symptomatic support are commonly used in clinical practice for this type of disease. However, some children still have a high probability of recurrence after treatment, which may be related to their weak immune function<sup>[1]</sup>. Probiotic preparations have the function of regulating the balance of gut microbiota, and there is a close relationship between gut microbiota and immune function in the body. Based on this, this study compared the clinical effects of probiotic preparation intervention and conventional intervention, analyzed the impact of probiotic preparations on the efficacy and immune function of children with recurrent respiratory tract infections, and provided reference for clinical treatment work.

## 2. Data and Methods

### 2.1. General Information

Select 80 children with recurrent respiratory infections admitted to our hospital from May 2022 to May 2024 as the research subjects, and use a random number table method to divide them into two groups, with 40 cases in each group. Among the children selected for the probiotic group, there were 21 males and 19 females, with an age range of 6-18 months and an average age of  $(11.25 \pm 2.36)$  months; Among the children selected in the regular group, there were 20 males and 20 females, with an age range of 6-18 months and an average age of  $(11.58 \pm 2.41)$  months. There was no significant difference in general information between the two groups of children, indicating comparability ( $P > 0.05$ ). Inclusion criteria: (1) Meet the diagnostic criteria for recurrent respiratory tract infections, with at least 2 infections within 2 months and a healthy interval of at least 10 days between infections; (2) Children aged 6-18 months, born full-term with a birth weight of  $\geq 2500\text{g}$ ; (3) Parents of the children voluntarily participate in the study and agree to cooperate in completing the relevant research procedures. Exclusion criteria: (1) presence of underlying conditions such as immunodeficiency and congenital diseases; (2) Have used probiotic preparations or immune modulators in the past month; (3) There is an allergic reaction to the ingredients contained in probiotic preparations.

### 2.2. Method

Both groups of children received a 4-month intervention, and their parents were required to cooperate in completing the following research-related procedures: ① Accurately fill in the personal basic information and milk powder usage of the tested infants and young children; ② Participate in project-specific training to clarify the precautions during the research process; ③ Fill in the subject's diary and 8 infant growth status questionnaires as required; ④ Collect saliva and fecal samples 5 times before the start of the experiment, on day  $28 \pm 2$ , day  $56 \pm 2$ , day  $84 \pm 2$ , and day  $112 \pm 2$ , respectively; ⑤ Collect test samples from the hospital every two weeks; ⑥ One follow-up observer will record the use of the test sample through regular telephone follow-up, and conduct home visits if necessary.

#### 2.2.1. Conventional group

Adopting a routine intervention plan, the specific content includes: (1) selecting appropriate anti - infective drugs according to the severity of respiratory tract infections in children (such as using cefaclor dry suspension for bacterial infections, taking  $20\text{mg/kg/day}$  in three doses according to the child's body weight) and symptomatic treatment drugs (such as using acetaminophen suspension drops for children with fever, taking  $10\text{-}15\text{ mg/kg/time}$  according to the child's body weight); (2) Guide parents of children to carry out reasonable feeding, ensure balanced nutritional intake of children, and provide appropriate supplementation of vitamin A and D preparations for children (daily supplementation of vitamin A  $1500\text{IU}$ , vitamin D  $500\text{IU}$ ); (3) Urge children to engage in appropriate outdoor activities, enhance their physical fitness, remind parents to adjust their children's clothing in a timely manner according to weather changes, and avoid children getting cold.

#### 2.2.2. Probiotic group

On the basis of routine intervention measures, probiotic preparations (*Bacillus subtilis* bifidobacteria granules) are added. The specific dosage and administration are:  $1\text{g}$  each time, diluted with warm water below  $40^\circ\text{C}$ , taken twice a day, taken with meals, and continuously used for 4 months; If children experience symptoms of infection during this period, the use of this preparation should be temporarily suspended until the symptoms of infection have recovered.

### 2.3. Observation indicators

Compare the total effective rate of treatment, immune function indicators (serum immunoglobulin IgA, IgG, IgM, detected by immunoturbidimetry) and incidence of complications (diarrhea, rash) between two groups of children before and after intervention. Efficacy evaluation criteria: Significant efficacy is defined as the number of respiratory infections in children

within 2 months after intervention being  $\leq 1$ ; The number of respiratory infections in children within 2 months after effective intervention is 2 times; Invalid as not meeting the above criteria for effectiveness and validity; The total effective rate of treatment is equal to (number of significantly effective cases+number of effective cases) divided by the total number of cases multiplied by 100%.

## 2.4. Statistical Methods

SPSS 24.0 software was used to analyze and process the research data. t-test was used for quantitative data, and chi square test was used for count data.  $P < 0.05$  indicates statistically significant differences.

## 3. Results

### 3.1. Comparison of total effective rates between two treatment groups

The total effective rate of probiotics treatment was significantly higher than that of the conventional group ( $P < 0.05$ ), as shown in **Table 1**.

**Table 1.** Comparison of total effective rates between two treatment groups [n (%)]

Group	Significant effect	Effective	Invalid	Total effective
Regular Group(40)	12(30.00)	18(45.00)	10(25.00)	30(75.00)
Probiotic group(40)	20(50.00)	17(42.50)	3(7.50)	37(92.50)
$\chi^2$				4.501
$P$				0.034

### 3.2. Comparison of immune function indicators between two groups before and after intervention

Before intervention, there was no significant difference in various immune function indicators between the two groups of children ( $P > 0.05$ ); After intervention, the levels of serum immunoglobulin IgA, IgG, and IgM in the probiotic group were significantly higher than those in the control group, and the differences were statistically significant ( $P < 0.05$ ). The specific data are shown in **Table 2**.

**Table 2.** Comparison of immune function indicators between two groups before and after intervention ( mean  $\pm$  SD, g/L)

Group	IgA (Before intervention)	IgA (After intervention)	IgG (Before intervention)	IgG (After intervention)	IgM (Before intervention)	IgM (After intervention)
Regular Group(40)	0.32 $\pm$ 0.08	0.45 $\pm$ 0.10	5.86 $\pm$ 0.72	6.58 $\pm$ 0.81	0.51 $\pm$ 0.12	0.63 $\pm$ 0.15
Probiotic group(40)	0.33 $\pm$ 0.09	0.58 $\pm$ 0.11	5.92 $\pm$ 0.68	7.35 $\pm$ 0.78	0.52 $\pm$ 0.11	0.76 $\pm$ 0.13
$t$	0.525	5.531	0.383	4.331	0.389	4.142
$P$	0.601	0.000	0.703	0.000	0.699	0.000

### 3.3. Comparison of incidence of complications between two groups

The incidence of complications in the probiotic group was lower than that in the conventional group, and the difference was statistically significant ( $P < 0.05$ ). Among them, there were 6 cases of diarrhea and 2 cases of rash in the conventional group, and 1 case of diarrhea and 0 cases of rash in the probiotic group.

**Table 3.** Comparison of Complications

Group	Diarrhea	Rash	Overall incidence rate
Regular Group(40)	6(15.00)	2(5.00)	8(20.00)
Probiotic group(40)	1(2.50)	0(0.00)	1(2.50)
$\chi^2$			4.507
<i>P</i>			0.034

## 4. Discussions

The occurrence of recurrent respiratory infections in children is influenced by multiple factors, with age being particularly critical. Children aged 6 to 18 months have immature immune systems and weak immune function, making it difficult to effectively resist pathogen invasion, thus making them prone to recurrent respiratory infections. At the same time, the gut microbiota of children in this stage is still in the process of establishment and improvement. The balance of microbiota is easily disrupted by external factors such as feeding methods and drug use. As an important immune organ in the human body, the imbalance of microbiota will further weaken children's immune function, forming a vicious cycle of "weak immune function - microbiota imbalance - repeated infections", leading to frequent respiratory infections. The conventional intervention methods currently used in clinical practice mainly focus on symptomatic treatment and daily care during infection outbreaks, with limited improvement in children's immune function. Even if some children receive standardized treatment, they still face a high risk of infection recurrence. Therefore, it is urgent to explore more effective intervention methods.

The results of this study showed that the total effective rate of the probiotic group (92.50%) was significantly higher than that of the conventional group (75.00%). This result indicates that the combined use of probiotic preparations on the basis of conventional intervention can significantly improve the treatment effect of children with recurrent respiratory tract infections. In depth analysis of its mechanism of action shows that live bacteria in probiotic preparations can successfully colonize and proliferate on the surface of the intestinal mucosa in children's intestines, forming a stable biological barrier. This barrier can effectively prevent harmful pathogens from adhering to the intestinal mucosa, reduce the opportunity for pathogens to invade the body, and thus reduce the frequency of respiratory infections. In addition, probiotics can actively regulate the structure of gut microbiota, promote the growth and reproduction of beneficial bacteria, while inhibiting the activity of harmful bacteria, restoring the balance of gut microbiota. The balance of gut microbiota is an important foundation for maintaining normal immune function in the body, which provides strong guarantees for the improvement of treatment effectiveness. Taking the *Bacillus subtilis* dual live bacteria particles used in this study as an example, the live bacteria contained in them can decompose sugars in the intestine, produce acidic substances such as lactic acid and acetic acid, and lower the pH value in the intestine. This acidic environment is not only unfavorable for the growth of harmful bacteria, but also stimulates intestinal peristalsis, accelerates the elimination of harmful substances in the intestine, further enhances intestinal defense function, reduces the possibility of pathogens entering the bloodstream through the intestine and invading the respiratory tract, and ultimately achieves an improvement in the overall treatment efficiency<sup>[2]</sup>.

From the perspective of changes in immune function indicators, there was no significant difference in serum IgA, IgG, and IgM levels between the two groups of children before intervention. However, after intervention, all indicators in the probiotic group were significantly higher than those in the conventional group. This change fully demonstrates that probiotic preparations can effectively improve the immune function of children with recurrent respiratory tract infections. Immunoglobulin is an important immune active substance synthesized by the body's immune system. Different types of immunoglobulin play different immune defense roles: IgA mainly exists on the surface of mucous membranes such as the respiratory and digestive tracts, and is the core component of mucosal immunity. It can prevent pathogens from



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adhering to mucosal cells and neutralize toxins produced by pathogens; IgG is the highest content immunoglobulin in human serum, with multiple immune functions such as antibacterial and antiviral. It can recognize and bind to pathogens, promote phagocytosis and clearance of pathogens by phagocytic cells; IgM is the earliest synthesized immunoglobulin in the body after being stimulated by pathogens, playing a crucial role in immune defense in the early stages of infection. The core reason why probiotic preparations can increase the levels of these immunoglobulins is that they can activate the intestinal mucosal immune system, stimulate the production of more immune cells (such as B lymphocytes) in intestinal related lymphoid tissues, and B lymphocytes can further differentiate into plasma cells, which can secrete a large amount of immunoglobulins. In addition, probiotics can also regulate the secretion of immune cytokines, such as promoting the production of cytokines such as interleukin-2 and interferon -  $\gamma$ , which can enhance immune cell activity, promote B lymphocyte proliferation and differentiation, further increase the synthesis and secretion of immunoglobulin, thereby comprehensively improving children's immune function and enhancing the body's resistance to respiratory pathogens<sup>[3-4]</sup>.

In terms of the occurrence of complications, the incidence of complications in the probiotic group (2.50%) was lower than that in the conventional group (20.00%), and the difference between the two groups met the research design requirements (a difference of 15 cases). This result indicates that probiotic preparations have good safety while improving treatment efficacy. The main complications in the conventional group are diarrhea and rash, which may be related to the use of anti infective drugs: some anti infective drugs, while exerting therapeutic effects, can disrupt the balance of intestinal flora, leading to intestinal dysfunction and ultimately causing diarrhea. In addition, a small number of children may have allergic reactions to drug ingredients, leading to rash<sup>[5-6]</sup>. Probiotic preparations can regulate the gut microbiota, reduce the damage caused by anti - infective drugs to the gut microbiota, and lower the risk of diarrhea. Meanwhile, probiotics themselves have low allergenicity, and there were no cases of rash in the probiotic group in this study, further confirming their safety. However, in clinical practice, it is still necessary to pay attention to the usage norms of probiotic preparations, such as using warm water below 40 °C to avoid high temperatures damaging the activity of live bacteria and affecting the treatment effect; At the same time, it is necessary to closely observe the reactions of children after medication. If discomfort symptoms such as bloating and constipation occur, the dosage of medication should be adjusted or suspended in a timely manner to ensure the safety and effectiveness of treatment<sup>[7-8]</sup>.

In summary, the combined use of probiotic preparations in the treatment of recurrent respiratory infections in children on the basis of routine interventions can significantly improve the overall treatment efficacy, effectively improve children's immune function indicators, and have a low incidence of complications and good safety. At the same time, active cooperation from parents of children in the research process and standardized implementation of intervention plans by medical staff are important prerequisites for ensuring the effectiveness of treatment. Therefore, probiotic preparations can be promoted and applied in clinical practice as an effective intervention for treating recurrent respiratory infections in children, helping more children reduce the occurrence of respiratory infections, improve immune function status, and promote healthy growth.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Wei X, Xue N, Zhang L, Zhu L, 2024, The effect of composite probiotic preparations on gut microbiota and 5-hydroxytryptamine in children with functional constipation. *Chinese Journal of Pathogenic Biology*, 19 (08): 901-906.
- [2] Li C, Zhao H, Wu G, 2024, Observation of the therapeutic effect of probiotic preparation Bifidobacterium on children with refractory eczema. *Smart Health*, 10 (15): 103-105.



- [3] Zheng Y, Huang Z, 2024, Pay attention to the application of probiotics in children. Chinese Journal of Practical Pediatrics, 39 (01): 16-20.
- [4] Zou B, Shu S, 2024, Progress in the application of probiotics in antibiotic associated diarrhea in children. Chinese Journal of Practical Pediatrics, 39 (01): 36-42.
- [5] Huang J, Shi Z, Li Z, 2023, Observation of the therapeutic effect of probiotic preparation Bifidobacterium combined with Dinide cream in the treatment of refractory eczema in children. Chinese and Foreign Medical Journal, 42 (08): 138-142.
- [6] Li L, Zhang Y, Zhang X, 2021, Analysis of dominant bacterial genera in the gut microbiota of NEC patients and the efficacy of probiotic preparations. International Journal of Laboratory Medicine, 42 (17): 2132-2134+2140.
- [7] Ye H, Lan T, Yang W, Liu G, 2021, The efficacy of probiotics as adjuvant therapy for asthmatic bronchitis and their impact on the immune function of pediatric patients. Chinese Journal of Medical Sciences, 11 (20): 205-208.
- [8] Cheng H, Liu X, Tian C, Zhao L, 2021, Rationality analysis of probiotic preparations in two children's hospitals in Beijing for the treatment of inflammatory bowel disease in children. Chinese Hospital Drug Evaluation and Analysis, 21 (09): 1105-1108.

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# The Advantages of Shenrongbian Pills Combined with PDE5 Inhibitors in the Treatment of Impotence

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**Abstract:** Objective: To investigate the clinical advantages of Shenrongbian Pills combined with PDE5 inhibitors in the treatment of impotence. Methods: A total of 160 patients with impotence were randomly divided into a combination therapy group and a monotherapy group, with 80 patients in each group. The combination therapy group was treated with Shenrongbian Pills combined with PDE5 inhibitors, while the monotherapy group was treated with PDE5 inhibitors alone. The clinical efficacy, symptom improvement, onset time, and incidence of adverse reactions were compared between the two groups. Results: The total effective rate in the combination therapy group was significantly higher than that in the monotherapy group ( $P < 0.05$ ). The combination therapy group showed superior improvement in impotence symptoms and symptoms related to kidney yang deficiency compared to the monotherapy group ( $P < 0.05$ ). There was no significant difference in the initial onset time between the two groups ( $P > 0.05$ ), but the sustained onset time in the combination therapy group was significantly longer than that in the monotherapy group ( $P < 0.05$ ). The incidence of adverse reactions did not differ significantly between the two groups ( $P > 0.05$ ). Conclusion: The combination of Shenrongbian Pills and PDE5 inhibitors demonstrates significant advantages in the treatment of impotence, as it not only provides rapid onset of action but also offers root-cause conditioning, potent nourishment, and addresses both symptoms and underlying causes, with good safety.

**Keywords:** Shenrongbian Pills; PDE5 inhibitors; impotence; combination therapy; clinical advantages

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## 1. Introduction

Impotence, also known as erectile dysfunction (ED), is a common sexual dysfunction disorder in males. Its incidence rate gradually increases with age, seriously affecting patients' quality of life and family harmony <sup>[1]</sup>. Currently, there are

numerous clinical treatment methods for impotence, among which PDE5 inhibitors (phosphodiesterase type 5 inhibitors, PDE5) are commonly used Western medications known for their rapid onset. Traditional Chinese medicine (TCM) also boasts a long history and unique advantages in treating impotence, with Shenrongbian Pills being a classic representative. The pathogenesis of impotence is complex, involving multiple systems such as the nervous, endocrine, and vascular systems. From a modern medical perspective, it is primarily related to dysfunction in the relaxation of penile corpus cavernosum smooth muscle, abnormal penile hemodynamics, hormonal imbalances, and psychological factors <sup>[2]</sup>. From the perspective of TCM, impotence is mostly associated with kidney-yang deficiency. Kidney-yang deficiency is an important TCM syndrome characterized by the decline of yang qi in the kidneys, leading to a weakened warming function. Kidney-yang deficiency can result in sexual dysfunction, manifesting as impotence and premature ejaculation, along with other symptoms such as soreness or cold pain in the lower back and knees, aversion to cold, morning diarrhea, and frequent nocturia <sup>[3]</sup>. PDE5 inhibitors are currently the first-line treatment for impotence. Their mechanism of action involves selectively inhibiting type 5 phosphodiesterase (PDE5) in the penile corpus cavernosum, reducing the degradation of cyclic guanosine monophosphate (cGMP), thereby promoting the relaxation of penile corpus cavernosum smooth muscle, increasing penile blood flow, and achieving penile erection <sup>[4]</sup>. PDE5 inhibitors are known for their rapid onset, typically taking effect within 30 minutes to 1 hour after administration, quickly improving patients' erectile function and enhancing sexual quality of life. Shenrongbian Pills originated from "Shenrong Decoction," as recorded in "Treatise on Differentiation and Treatment of Warm Diseases" (1798) by Wu Jutong, a renowned medical expert in the Qing Dynasty. After centuries of clinical practice and inheritance, the formula of Shenrongbian Pills has been continuously optimized and refined, becoming a classic prescription for treating sexual dysfunction caused by kidney-yang deficiency <sup>[5]</sup>. The formula of Shenrongbian Pills contains four types of animal penis medicinal materials: donkey penis, ox penis, dog penis, and mink penis. Traditional Chinese medicine (TCM) holds that animal penises possess the effects of nourishing the kidney and strengthening yang, as well as replenishing essence and marrow. Adhering to the theory of "like cures like," they can effectively replenish kidney yang qi and alleviate various symptoms caused by kidney yang deficiency <sup>[6]</sup>. The formula of Shenrongbian Pills emphasizes the balance of yin and yang. It not only incorporates various warm and yang-tonifying medicinal herbs but also combines them with cold and yin-nourishing herbs (two yin herbs and two cold herbs). The two yin herbs are Goji berries and *Rehmannia glutinosa* (prepared), while the two cold herbs are *Asparagus cochinchinensis* and *Lycium barbarum* cortex. This combination imparts a warm yet non-drying characteristic to the entire formula, ensuring that while it warms and tonifies kidney yang qi, it does not cause adverse reactions such as internal heat <sup>[7]</sup>. Shenrongbian Pills can improve sexual dysfunction (impotence, premature ejaculation) caused by kidney yang deficiency, as well as other symptoms such as soreness or cold pain in the lower back and knees, aversion to cold, morning diarrhea, and frequent nocturia. Its mechanism of action involves nourishing kidney yang qi, regulating the body's endocrine and immune systems, fundamentally improving the patient's constitution, and achieving both symptomatic relief and root cause treatment <sup>[8]</sup>. This study aims to explore the advantages of combining Shenrongbian Pills with PDE5 inhibitors in the treatment of impotence, providing a reference for clinical treatment. The findings are reported as follows.

## 2. Materials and Methods

### 2.1. General Information

A total of 160 patients with impotence who were treated at our hospital from January 2023 to January 2024 were selected as the study subjects. Inclusion criteria: meeting the TCM and Western medicine diagnostic criteria for impotence; TCM differentiation as kidney yang deficiency syndrome; no recent use of any medications for impotence or medications that could affect treatment outcomes; age between 25 and 60 years; informed consent obtained from patients and their families, with signed informed consent forms. Exclusion criteria: severe diseases of vital organs such as the heart, liver, and kidneys; mental illness; allergy to the medications used in this study; recent use of other medications for impotence. The 160 patients were randomly divided into a combination therapy group and a monotherapy group, with 80 patients in

each group. In the combination therapy group, the age range was 25-58 years, with an average age of  $(42.52 \pm 6.84)$  years; the disease duration ranged from 6 months to 5 years, with an average of  $(2.31 \pm 1.14)$  years. In the monotherapy group, the patients were aged between 26 and 60 years, with an average age of  $(43.21 \pm 7.18)$  years; their disease duration ranged from 8 months to 5 years, with an average of  $(2.52 \pm 1.24)$  years. There was no statistically significant difference ( $P > 0.05$ ) in general information such as age and disease duration between the two groups, indicating comparability.

## 2.2. Methods

**Monotherapy Group:** Patients were treated with a PDE5 inhibitor (sildenafil), administered orally at a dose of 50 mg, taken 1 hour before sexual activity.

**Combination Therapy Group:** On the basis of the treatment in the monotherapy group, patients additionally received Shenrongbian Pills (comprising deer antler, dog penis (parched), mink penis (parched), donkey penis (parched), morinda officinalis, ox penis (parched), dodder seed (roasted), actinolite (calcined), prepared aconite root, sparrow, epimedium (processed), cynomorium songaricum, eucommia bark (charred), dark plum salt, semen allii tuberosi, clove, sulfur (processed), psoralea fruit (salt-fried), hippocampus (processed), wolfberry fruit, prepared rehmannia root, cortex lycii radices, asparagus root, stone sparrow (calcined), amomum fruit, licorice root, cyathula root, cassia bark, and red ginseng), manufactured by Dalian Meiluo Traditional Chinese Medicine Co., Ltd., with approval number Z21020982. The pills were taken orally at a dose of 10 pills each time, twice daily (morning and evening). It was recommended to take the pills 1-2 hours before meals with light salt water or plain water. For patients with chronic conditions, a 15-day continuous treatment course was recommended, followed by a 2-day break, before resuming treatment for a second course. The recommended treatment duration was 3-6 months.

Both groups of patients received continuous treatment for 3 months. During the treatment period, they were advised to avoid using other medications for erectile dysfunction and to maintain regular sexual activity.

## 2.3. Observation Indicators

### 2.3.1. Clinical Efficacy

Efficacy was evaluated based on the International Index of Erectile Function (IIEF-5) scoring criteria. Cure: An IIEF-5 score  $\geq 22$  points, with normal erectile function enabling satisfactory sexual activity. Markedly effective: An increase in IIEF-5 score of  $\geq 10$  points from the pre-treatment score, but  $< 22$  points, with significant improvement in erectile function allowing completion of sexual activity. Effective: An increase in IIEF-5 score of  $\geq 5$  points from the pre-treatment score, but  $< 10$  points, with some improvement in erectile function enabling completion of sexual activity with difficulty. Ineffective: An increase in IIEF-5 score of  $< 5$  points from the pre-treatment score, with no significant improvement in erectile function, preventing completion of sexual activity. The overall effective rate = (Number of cured cases + Number of markedly effective cases + Number of effective cases) / Total number of cases  $\times 100\%$ .

### 2.3.2. Symptom Improvement

The improvement of kidney-yang deficiency-related symptoms (such as soreness or cold pain in the lower back and knees, aversion to cold, morning diarrhea, frequent nocturia, clear and excessive vaginal discharge, etc.) in both groups before and after treatment was observed and recorded. Symptom scoring was used for evaluation, with each symptom categorized into four levels: none, mild, moderate, and severe, assigned 0, 1, 2, and 3 points respectively. The total score was the sum of scores for each symptom, with higher scores indicating more severe symptoms.

### 2.3.3. Onset Time

The initial time (from medication intake to the first effective erection enabling completion of sexual activity) and the duration of effectiveness (from the first effective erection to the time when erectile function could no longer meet sexual demands) after each medication intake were recorded for both groups.

### 2.3.4. Incidence of Adverse Reactions

Adverse reactions experienced by both groups during treatment, such as headache, facial flushing, dizziness, and nausea, were observed and recorded.

## 2.4. Statistical Methods

Data analysis was conducted using SPSS 22.0 statistical software. Continuous data are presented as mean  $\pm$  standard deviation ( $\bar{x} \pm \bar{s}$ ), and comparisons between groups were made using the t-test. Categorical data are expressed as rates (%), and the  $\chi^2$  test was employed. A P-value of less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Comparison of Clinical Efficacy Between Two Groups of Patients

The total effective rate in the combination therapy group was 92.50%, significantly higher than the 77.50% observed in the monotherapy group, with a statistically significant difference ( $P < 0.05$ ), as shown in **Table 1**.

**Table 1.** Comparison of Clinical Efficacy Between Two Groups of Patients (Cases, %)

Group	Cured	Markedly Effective	Effective	Ineffective	Total Effective Rate
Combination Therapy Group (n=80)	35 (43.75)	28 (35.00)	11 (13.75)	6 (7.50)	74 (92.50)
Monotherapy Group (n=80)	20 (25.00)	26 (32.50)	16 (20.00)	18 (22.50)	62 (77.50)
$\chi^2$					7.059
P-value					<0.05

### 3.2. Comparison of Symptom Scores Before and After Treatment Between Two Groups of Patients

Before treatment, there was no statistically significant difference in symptom scores between the two groups ( $P > 0.05$ ). After treatment, symptom scores in both groups significantly decreased ( $P < 0.05$ ), with the combination therapy group showing significantly lower scores than the monotherapy group, a statistically significant difference ( $P < 0.05$ ). See **Table 2**.

**Table 2.** Comparison of Symptom Scores Before and After Treatment Between Two Groups of Patients (mean  $\pm$  standard deviation, points)

Group	n	Before Treatment	After Treatment
Combination Therapy Group	80	12.51 $\pm$ 3.22	4.22 $\pm$ 1.51
Monotherapy Group	80	12.82 $\pm$ 3.54	7.62 $\pm$ 2.11
t-value		0.580	11.721
p-value		>0.05	<0.05

### 3.3. Comparison of Onset Time Between Two Groups of Patients

There was no statistically significant difference in the initial onset time between the two groups ( $P > 0.05$ ); however, the duration of the effect in the combination therapy group was significantly longer than that in the monotherapy group, with a statistically significant difference ( $P < 0.05$ ). See **Table 3**.



**Table 3.** Comparison of Onset Time Between Two Groups of Patients (mean  $\pm$  standard deviation, minutes)

Group	n	InitialOnsetTime(min)	DurationofEffect(min)
Combination Therapy	80	25.21 $\pm$ 10.54	60.54 $\pm$ 30.23
Monotherapy	80	27.14 $\pm$ 11.22	40.32 $\pm$ 25.61
t-value		1.121	4.565
p-value		>0.05	<0.05

### 3.4. Comparison of the Incidence of Adverse Reactions Between the Two Groups

In the combination therapy group, there were 2 cases of headache, 1 case of facial flushing, and 1 case of dizziness, resulting in an adverse reaction rate of 5.00%. In the monotherapy group, there were 3 cases of headache, 2 cases of facial flushing, 1 case of dizziness, and 1 case of nausea, leading to an adverse reaction rate of 8.75%. There was no statistically significant difference in the incidence of adverse reactions between the two groups ( $P > 0.05$ ).

## 4. Discussion

As a classic prescription with a century-long heritage, Shenrongbianwan Pills are characterized by their “four-penis tonic, shape-replenishing shape” properties, effectively replenishing kidney yang and alleviating various symptoms caused by kidney yang deficiency. Additionally, its “warm but not dry” formula ensures that patients do not experience adverse reactions such as excessive internal heat during administration, enhancing patient compliance with the medication. Moreover, Shenrongbianwan Pills address both the symptoms and root causes, not only improving sexual dysfunction but also alleviating other symptoms of kidney yang deficiency, such as soreness or cold pain in the lower back and knees, and cold intolerance, thereby further enhancing patients’ quality of life <sup>[9]</sup>.

The results of this study indicate that the total effective rate of treating impotence with Shenrongbianwan Pills combined with PDE5 inhibitors is significantly higher than that of using PDE5 inhibitors alone. This outcome fully underscores the significant advantage of combination therapy in enhancing clinical efficacy. From the pathological mechanism of impotence, its onset involves issues such as smooth muscle relaxation dysfunction of the penile corpus cavernosum and hemodynamic abnormalities, as recognized by modern medicine, as well as being closely related to kidney yang deficiency in traditional Chinese medicine theory. Although monotherapy with PDE5 inhibitors can rapidly promote penile erection by inhibiting the activity of the PDE5 enzyme and increasing cGMP concentration, this effect primarily intervenes in a specific aspect of the erection process and fails to fundamentally address the underlying issues causing impotence.

The treatment regimen combining Shenrongbian Pills and PDE5 inhibitors achieves multi-target intervention in the pathological mechanisms of impotence. PDE5 inhibitors take effect rapidly, swiftly improving patients’ erectile function, meeting their immediate needs, alleviating psychological stress, and enhancing treatment confidence. Shenrongbian Pills, from the perspective of traditional Chinese medicine (TCM) syndrome differentiation and treatment, target the core pathogenesis of kidney yang deficiency. By nourishing the kidney yang and regulating the body’s overall functions, they work in synergy with PDE5 inhibitors. This combination not only rapidly improves symptoms but also gradually repairs damaged bodily functions, thereby enhancing the overall therapeutic effect.

The combined medication group also demonstrated significant advantages in improving symptoms associated with kidney yang deficiency. While the single-drug group could improve erectile function to some extent, its effectiveness in alleviating symptoms of kidney yang deficiency, such as soreness and coldness in the waist and knees, fear of cold, and morning diarrhea, was limited. In contrast, the various herbs in Shenrongbian Pills possess clear kidney-tonifying and yang-strengthening, as well as warming yang and dispersing cold properties, effectively targeting and improving these symptoms. This indicates that the combined medication approach not only focuses on the primary symptom of impotence



but also emphasizes the improvement of the patient's overall health, reflecting the TCM principles of "holistic concept" and "treatment based on syndrome differentiation."

In terms of onset time, there was no significant difference in the initial onset time between the two groups, which aligns with the rapid onset characteristic of PDE5 inhibitors, indicating that the combined medication did not affect the rapid onset effect of PDE5 inhibitors. However, the sustained onset time of the combined medication group was significantly longer than that of the single-drug group, a result of great clinical significance. The duration of action of PDE5 inhibitors is relatively short, often limited by factors such as drug metabolism rate. The addition of Shenrongbian Pills may extend the duration of erectile function by regulating the endocrine system, improving blood supply and tissue structure of the corpus cavernosum. This prolonged sustained onset time not only enhances patient satisfaction with sexual life but also reduces medication frequency and psychological burden, further improving their quality of life<sup>[10]</sup>.

In conclusion, the treatment of impotence with Shenrongbian Pills combined with PDE5 inhibitors offers significant clinical advantages, and the combined medication regimen is relatively safe, making it worthy of clinical promotion and application.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Yan B, Zhang J, Gao Q, 2019, Research progress on the mechanisms of sexual dysfunction caused by chronic prostatitis/chronic pelvic pain syndrome. *Chinese Journal of Andrology*, 33(2): 69-72.
- [2] Wang K, 2023, Research progress on the pathological mechanisms of chronic prostatitis complicated with erectile dysfunction. *Chinese Journal of Andrology*, 37(3): 110-114.
- [3] Zhang S, 2023, Investigation on lifestyle-related risk factors and distribution of TCM syndrome elements in young male patients with erectile dysfunction. Beijing: China Academy of Chinese Medical Sciences.
- [4] He H, Yang M, Zhu J, 2023, Clinical application progress of phosphodiesterase type 5 inhibitors. *International Journal of Urology and Nephrology*, 43(3): 545-548.
- [5] Lin Y, 2006, Application of the method of "Xin Gan Hua Yang" in "Treatise on Febrile Diseases". *Fujian Journal of Traditional Chinese Medicine*, 37(6): 59-60.
- [6] Liu B, Hu B, Zhao H, 2025, A brief analysis of the research progress on the treatment of impotence with traditional Chinese medicine from the perspectives of theory, treatment principles, formulas, and herbs. *Guangming Journal of Chinese Medicine*, 40(2): 407-411.
- [7] He F, 2021, Observation on the effect of Shenrongbianwan combined with dapoxetine in the treatment of premature ejaculation. *World Latest Medicine Information*, 21(101): 253-254.
- [8] He F, 2021, Analysis of the effect of Shenrongbianwan combined with PDE5 inhibitors in the treatment of impotence. *World Latest Medicine Information*, 21(83): 461-462.
- [9] Li Y, 2021, Application effect of Shenrongbianwan in kidney deficiency. *World Latest Medicine Information*, 21(98): 541-542.
- [10] Liu Y, Chen B, Gao X, 2021, Research progress on the potential therapeutic effects of type 5 phosphodiesterase inhibitors on chronic complications of diabetes. *Modern Practical Medicine*, 33(4): 558-560.

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# Research on the Interaction Mechanism of Molecular Signaling Pathways in Tumor Occurrence and Development in the Junction Area of Liver, Gallbladder, Pancreas and Spleen

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**Abstract:** Tumors in the junction area of the liver, gallbladder, pancreas and spleen are complex and highly fatal malignant tumors. Due to their involvement in multiple organs such as the liver, gallbladder, pancreas and spleen, they have become a tumor-prone area. In recent years, advancements in molecular biology have promoted in-depth research on the occurrence and development of tumors in this region, especially the interactions of molecular signaling pathways. The occurrence and progression of tumors are the result of the combined action of multiple signaling pathways. This article reviews the main molecular signaling pathways and their interaction mechanisms in tumors at the junction of the liver, gallbladder, pancreas and spleen, explores their roles in the tumor microenvironment, drug resistance and metastasis, and looks forward to future clinical applications.

**Keywords:** Tumor in the junction area of liver, gallbladder, pancreas and spleen; Molecular signaling pathway; Tumorigenesis; Interaction mechanism; Tumor microenvironment

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## 1. Introduction

Tumors in the junction area of the liver, gallbladder, pancreas and spleen refer to malignant tumors that occur in the junction area of the liver, gallbladder, pancreas and spleen. These tumors have a high degree of malignancy due to their complex anatomical structure and multiple tissue components. With the development of modern molecular biology, the occurrence and development of tumors are no longer merely studied from the traditional anatomical and pathological perspectives, but are more focused on exploring the pathogenesis of tumors at the molecular level. Studies have shown that the occurrence of tumors in the junction area of the liver, gallbladder, pancreas and spleen is caused by the abnormal activation or inhibition of multiple molecular signaling pathways. These signaling pathways jointly promote the occurrence and progression of tumors through complex interactions. This article will explore the molecular signaling pathways and their interaction mechanisms of tumors in the junction area of the liver, gallbladder, pancreas and spleen, with the aim of providing new ideas and directions for the early diagnosis and treatment of tumors.

## **2. Characteristics and Distribution of Tumors in the Junction Area of the Liver, Gallbladder, Pancreas and Spleen**

### **2.1. Anatomical features and clinical manifestations**

The junction area of the liver, gallbladder, pancreas and spleen includes the liver, gallbladder, pancreas and spleen. These organs are closely adjacent anatomically and play a closely coordinated role in physiological functions. The liver, as the center of metabolism, plays a crucial role in tumorigenesis. The gallbladder stores and secretes bile and is one of the main sources of gallbladder cancer. The pancreas has important digestive and endocrine functions. The incidence of pancreatic cancer is increasing year by year, and the prognosis is poor. The spleen, as an immune organ, although tumors are relatively rare, the occurrence of tumors in it may affect the tumor development in the entire borderline region<sup>[1]</sup>. The clinical symptoms of tumors in the junction area of the liver, gallbladder, pancreas and spleen are usually manifested as abdominal pain, jaundice, weight loss, etc. However, these symptoms are relatively non-specific, making early diagnosis difficult and increasing the challenges of clinical treatment.

### **2.2. Tumor type**

Tumor types in the junction area of the liver, gallbladder, pancreas and spleen include liver cancer, pancreatic cancer, gallbladder cancer and spleen tumors, etc. Liver cancer and pancreatic cancer are the most common tumor types in this region, and they are mostly discovered at an advanced stage, usually featuring strong malignant characteristics. Liver cancer is usually closely related to chronic liver diseases such as hepatitis and liver cirrhosis. The symptoms of pancreatic cancer are often not obvious, which leads to patients being diagnosed at an advanced stage by the time they are diagnosed. Although gallbladder cancer is relatively rare, it often occurs in the context of cholecystitis and gallstones, and the condition progresses rapidly<sup>[2]</sup>. Spleen tumors are generally benign, but in a few cases, they may also transform into malignant tumors. Overall, tumors in the junction area of the liver, gallbladder, pancreas and spleen mostly show malignant progression, and the early symptoms are not obvious, significantly increasing the difficulty of early intervention and the challenge of treatment.

### **2.3. Diagnosis and treatment of tumors**

The early diagnosis of tumors in the junction area of the liver, gallbladder, pancreas and spleen faces huge challenges, mainly because the symptoms are often similar to those of other common diseases and lack specificity. Currently, diagnosis mainly relies on imaging examinations such as CT, MRI, and ultrasound, as well as histopathological examinations. These methods can help confirm the presence of tumors and classify them. With the advancement of molecular biology, genetic testing and molecular marker screening techniques have gradually been applied to the early diagnosis of tumors, especially showing promising prospects in the early detection of liver cancer and pancreatic cancer. In terms of treatment, tumors in the junction area of the liver, gallbladder, pancreas and spleen usually require a comprehensive treatment strategy, including surgical resection, radiotherapy, chemotherapy, targeted therapy, etc. In recent years, immune checkpoint inhibitors have achieved certain clinical results in the treatment of liver cancer and pancreatic cancer, showing a relatively positive prospect. However, the early diagnosis of tumors, personalized treatment and precision medicine remain the key research directions at present. In the future, it is necessary to continuously explore more efficient and precise treatment methods and early screening approaches<sup>[3]</sup>.

## **3. The molecular mechanism of tumor occurrence and development in the junction area of the liver, gallbladder, pancreas and spleen**

### **3.1. The main molecular signaling pathways**

The occurrence of tumors in the junction area of the liver, gallbladder, pancreas and spleen is closely related to multiple signaling pathways, among which the most crucial ones include the Wnt/ $\beta$ -catenin pathway, PI3K/Akt/mTOR pathway, MAPK/ERK pathway and Notch pathway, etc. These signaling pathways play a significant role in the proliferation,

survival, migration and drug resistance formation of tumor cells.

Abnormal activation of the Wnt/ $\beta$ -catenin signaling pathway is commonly seen in the occurrence of liver cancer, promoting tumor proliferation and metastasis through nuclear translocation of  $\beta$ -catenin. The PI3K/Akt/mTOR pathway is common in pancreatic cancer and gallbladder cancer, which can promote cell survival and inhibit cell apoptosis. The MAPK/ERK pathway is activated in various tumor types, promoting cell proliferation and metastasis. The Notch signaling pathway also plays a significant role in the differentiation, proliferation and drug resistance of tumors.

### **3.2. The role in the tumor microenvironment**

The tumor microenvironment plays a crucial role in the occurrence and development of tumors. Tumor cells interact with surrounding stromal cells, immune cells, vascular endothelial cells, etc., to form the microenvironment of the tumor. Studies have shown that in the microenvironment of tumors at the junction of the liver, gallbladder, pancreas and spleen, factors such as inflammatory response, hypoxia and immune escape all promote the occurrence and metastasis of tumors<sup>[4]</sup>. Tumor-related inflammatory responses not only promote tumor growth but also enhance the drug resistance of tumor cells to external treatments. The hypoxic state in the tumor microenvironment also activates multiple signaling pathways, further enhancing the invasiveness and metastasis ability of the tumor. The immune escape mechanism helps tumor cells evade clearance by the host immune system by altering the expression of surface antigens on tumor cells or by recruiting immunosuppressive cells. In addition, angiogenesis in tumors is also an important component of the tumor microenvironment, which supports the rapid growth and metastasis of tumors by providing nutrients and oxygen.

### **3.3. The mechanism of interaction among molecular signaling pathways**

During the occurrence of tumors in the junction area of the liver, gallbladder, pancreas and spleen, multiple molecular signaling pathways do not act independently but interweave and interact with each other. Taking the PI3K/Akt/mTOR pathway and the Wnt/ $\beta$ -catenin pathway as examples, the activation of the PI3K/Akt/mTOR pathway can not only promote cell survival and proliferation, but also further activate the Wnt/ $\beta$ -catenin pathway by up-regulating the stability of  $\beta$ -catenin, thereby promoting tumor growth and metastasis. In addition, the activation of the MAPK/ERK pathway can also interact with the Notch signaling pathway through feedback mechanisms, enhancing the proliferation and invasiveness of tumor cells. The MAPK/ERK pathway can activate Notch receptors, thereby promoting drug resistance and proliferation of tumor cells, forming a vicious cycle<sup>[5]</sup>. The interaction of these signaling pathways enables tumor cells to evade normal growth control and cell cycle checkpoints, thereby promoting tumor progression and metastasis.

## **4. The role of molecular signaling pathways in tumor metastasis and drug resistance**

### **4.1. The molecular mechanism of tumor metastasis**

Tumor metastasis is one of the important factors leading to tumor-induced death, especially in tumors at the junction of the liver, gallbladder, pancreas and spleen. The metastasis of tumor cells is usually accompanied by abnormal activation of multiple signaling pathways. Studies have shown that the Wnt/ $\beta$ -catenin pathway, TGF- $\beta$  pathway and PI3K/Akt pathway play a crucial role in the process of tumor metastasis. These pathways promote the invasion and metastasis of tumor cells by regulating their adhesion, motility and the degradation of extracellular matrix. The Wnt/ $\beta$ -catenin pathway promotes the metastasis of tumor cells from the primary site to distant sites by altering cell adhesion and motility<sup>[6]</sup>. TGF- $\beta$  signaling promotes metastasis by regulating the epithelial-mesenchymal transition (EMT) process and enhancing the invasiveness of tumor cells. The PI3K/Akt pathway helps tumor cells degrade the matrix and cross it by enhancing the survival ability of tumor cells and promoting the secretion of matrix metalloproteinases, thereby facilitating the metastasis process.

### **4.2. The formation of tumor drug resistance**

Tumor drug resistance is an important cause of chemotherapy failure and poor prognosis, especially in tumors at the



junction of the liver, gallbladder, pancreas and spleen. Drug resistance is usually closely related to changes in multiple molecular signaling pathways. For instance, the activation of the PI3K/Akt/mTOR pathway can enhance the survival ability of tumor cells and prevent apoptosis induced by chemotherapy drugs. The activation of the MAPK/ERK pathway promotes cell proliferation and slows down the killing effect of chemotherapy drugs, thereby making tumor cells resistant to the drugs. In addition, tumor cells further enhance their tolerance to drugs through mechanisms such as upregulating drug resistance genes and altering the ways drugs are taken up and excreted. Through in-depth research on drug resistance mechanisms, it is expected to discover new therapeutic targets to overcome these drug resistances.

### **4.3. Drug resistance treatment strategy**

Targeted therapy and immunotherapy have provided new treatment directions for the drug resistance of tumors in the junction area of the liver, gallbladder, pancreas and spleen. Certain progress has been made in the development of drugs that target and inhibit signaling pathways such as the PI3K/Akt/mTOR pathway and the Wnt/ $\beta$ -catenin pathway. Targeting and inhibiting these pathways can effectively slow down the proliferation of tumor cells and enhance their sensitivity to chemotherapy drugs. In addition, the application of immune checkpoint inhibitors has achieved positive results in clinical treatment<sup>[7]</sup>. They can eliminate the immune escape of tumors, enhance the immune response of the body, and thereby overcome the drug resistance of tumors. In the future, combined treatment strategies may become an important means to deal with tumor drug resistance, especially in the face of tumor drug resistance mechanisms involving multiple signaling pathways.

## **5. Future research directions and clinical applications**

### **5.1. The discovery of early diagnostic markers**

With the continuous advancement of molecular biology techniques, the discovery of early diagnostic markers has become an important direction in the research of tumors at the junction of the liver, gallbladder, pancreas and spleen. By conducting in-depth analysis of the changes in tumor-related molecular signaling pathways and integrating high-throughput genomics, proteomics and metabolomics research, scientists are expected to screen out tumor markers with high specificity and sensitivity. These markers can help detect tumors at an early stage, especially in the early stage of tumors at the junction of the liver, gallbladder, pancreas and spleen, where the symptoms are not obvious and are easily overlooked. The discovery of early diagnostic markers will significantly enhance the early screening ability for tumors, thereby improving the prognosis of patients, enhancing treatment outcomes, and providing more precise diagnostic tools for clinical practice<sup>[8]</sup>.

### **5.2. The development of targeted therapy**

Targeted therapy, as one of the important means for treating tumors in the junction area of the liver, gallbladder, pancreas and spleen, will continue to play a key role in the future. By precisely targeting the key molecular signaling pathways in tumor cells, targeted therapy can selectively inhibit the proliferation and growth of tumor cells, avoid damage to normal cells, thereby enhancing the therapeutic effect and reducing side effects<sup>[9]</sup>. Future research will focus on developing new targeted drugs, especially those for difficult-to-treat types such as liver cancer and pancreatic cancer. Exploring the interactions among different signaling pathways and combining them with individualized treatment will provide more effective therapeutic strategies for the precise treatment of tumors in the junction area of the liver, gallbladder, pancreas and spleen, and promote the development of targeted therapy.

### **5.3. The application of immunotherapy**

Immunotherapy, as a breakthrough in the field of cancer treatment in recent years, has broad application prospects in tumors at the junction of the liver, gallbladder, pancreas and spleen. Tumor cells evade the recognition and attack of the immune system through multiple mechanisms<sup>[10]</sup>. Therefore, research on the immune escape mechanisms of tumors is

of vital importance. By developing new immunotherapy strategies, such as immune checkpoint inhibitors, CAR-T cell therapy, and tumor vaccines, the immune response of the body can be effectively enhanced, thereby changing the treatment pattern of tumors in the junction area of the liver, gallbladder, pancreas and spleen. Future research will focus on enhancing the effectiveness of immunotherapy, overcoming the side effects and drug resistance issues related to immunotherapy, providing more targeted and personalized treatment plans for clinical practice, and further improving the treatment outcome.

## 6. Conclusion

The occurrence and development of tumors in the junction area of the liver, gallbladder, pancreas and spleen is a complex process involving the interaction of multiple molecular signaling pathways. In-depth research on the interaction mechanisms of these signaling pathways can help us better understand the occurrence and development of tumors, providing new theoretical basis and clinical strategies for the early diagnosis and treatment of tumors. In the future, with the continuous advancement of molecular biology techniques, the diagnosis and treatment of tumors in the junction area of the liver, gallbladder, pancreas and spleen will enter a new stage.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Yin P, Yang J, 2024, The application progress and current situation of membrane Anatomy in Hepatobiliary, Pancreatic and splenic surgery. *Chinese and Foreign Medical Sciences*, 43(36):195-198.
- [2] Song H Y, Ding R F, Ji Q F, 2024, Retrospective Analysis of the Impact of Standardized Surgical Nursing Cooperation on Patients converting from robot to open Surgery in Hepatobiliary, Pancreatic and splenic Surgery. *Shanghai Nursing Association. Compilation of Papers from the 6th Shanghai International Nursing Conference (Part 2). Shanghai Changhai Hospital*, 343.
- [3] Yin H, 2024, Hepatobiliary, Pancreatic and Splenic Surgery Team of the First Affiliated Hospital of Naval Medical University (Shanghai Changhai Hospital) Changhai has a four-step battle against the “King of Cancer”. *Doctor’s Journal*, (A07).
- [4] Guo H, 2024, Practice and Prospect of Big Data Technology in Postoperative Care of Patients in Hepatobiliary Surgery: A Review of “Nursing in Hepatobiliary and Pancreatic Surgery”. *Contemporary Chemical Engineering*, 53(07):1765.
- [5] Ma H, 2024, The Application Value of Magnetic Nursing Management Based on the Current Situation - Background - Assessment - Recommendation Communication Model Implemented by Nursing Staff in Hepatobiliary Surgery. *Primary Medicine Forum*, 28(20):1-3+15.
- [6] Li J W, Zhao K F, Wu G, 2024, Clinical application progress of single-port laparoscopic surgery in the treatment of liver, gallbladder, pancreas and spleen diseases. *Journal of Laparoscopic Surgery*, 29(07):538-540+545.
- [7] Liu H, Wang Y Y, Qian Y B, 2024, Analysis of the Effect of Clinical Pharmacists’ Participation in Nutritional Assessment and Intervention in the Treatment of Patients with Hepatobiliary and Pancreatic Malignancies. *Chinese Journal of Hospital Pharmacy*, 44(17):2038-2043.
- [8] Mo J H, Wei Q, Pang Q X, 2024, Application of 3D Model Reconstruction Technology Combined with Mind Mapping in Clinical Teaching of Hepatobiliary and Pancreatic Surgery Nursing. *Minimally invasive Medicine*, 19(03):335-339.
- [9] Feng D, Ge L, 2024, Analysis of the Application Effect of Case Teaching in Hepatobiliary Surgery Nursing Teaching. *Yulin*



Medical Association. Proceedings of the Fifth National Medical Research Forum (Part II). Department of Hepatobiliary, Pancreatic and Spleen Surgery, Affiliated Hospital of Inner Mongolia Medical University, B District, 123-128.

- [10] Zhao H P, 2020, “Grassland Talent” Innovation Team - Liver, Gallbladder, Pancreas and Spleen Tumor and Minimally Invasive Surgery Innovation Team. Inner Mongolia Medical University Affiliated Hospital, Inner Mongolia Autonomous Region.

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# Efficacy and Safety Study of Qirui Weishu Capsule in Treating Chronic Gastritis with Abdominal Pain Symptoms

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**Abstract:** Objective: To evaluate the efficacy and safety of Qirui Weishu Capsule as monotherapy for chronic gastritis with abdominal pain, providing evidence-based support for clinical application. Methods: A prospective real-world study (January to November 2024) enrolled 60 patients with chronic gastritis and abdominal pain (NRS  $\geq 4$ ). Participants received Qirui Weishu Capsule (4 capsules/dose, twice daily before meals). Efficacy was assessed using NRS pain scores, the Gastrointestinal Quality of Life Index (GIQLI), and the Gastrointestinal Symptom Rating Scale (GSRS). Results: Treatment significantly improved gastrointestinal quality of life (GIQLI) and reduced symptom severity (GSRS). Pain/discomfort (NRS) showed sustained relief within 2–4 weeks, with an average pain-relief onset time of  $6.67 \pm 1.53$  days. The pain improvement efficacy rate reached 96% ( $P < 0.05$ ). Safety: No adverse reactions (e.g., hepatic/kidney impairment, coagulation abnormalities) were observed. Conclusion: Qirui Weishu Capsule rapidly alleviates abdominal pain (within 2–4 weeks), significantly enhances quality of life, and demonstrates favorable safety, making it a preferred treatment for chronic gastritis with abdominal pain.

**Keywords:** Chronic gastritis; Abdominal pain; Qirui Weishu Capsule

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## 1. Introduction

There are 120 million patients with gastrointestinal diseases in China, with a chronic gastritis incidence rate of 30% and an abdominal pain incidence rate of 60% to 80%<sup>[1]</sup>. The pain is mainly located in the upper abdomen. The mechanism of chronic gastritis with abdominal pain is complex and may be accompanied by symptoms such as nausea, vomiting, and loss of appetite. The emergence of these symptoms not only affects patients' daily lives but may also lead to increased psychological burden, further affecting patients' treatment compliance and quality of life<sup>[2]</sup>. Studies have shown that abdominal pain in patients with chronic gastritis is closely related to factors such as the degree of inflammation of the gastric mucosa, the presence of *Helicobacter pylori* infection, and lifestyle. The severity of abdominal pain is also significantly associated with the patient's psychological state<sup>[3]</sup>.

In the treatment of chronic gastritis, medication is the primary intervention. Qirui Weishu Capsule mainly consists of *Panax notoginseng*, dried alum, calcined flowerstone, and wine-processed rhubarb. Among them, *Panax notoginseng* can "stop bleeding without leaving stagnation, and resolve stagnation without injuring the healthy qi," while rhubarb can "dredge the fu organs, purge heat, detoxify, and promote blood circulation," and has a broad-spectrum antibacterial effect.

When used together, alum and flowerstone can converge eroded gastric mucosa and promote gastric mucosa repair<sup>[4]</sup>. They can promote blood circulation to remove blood stagnation, dry dampness, and relieve pain. They are mainly used for mild to moderate chronic non-atrophic gastritis with erosion and dampness-heat stagnation syndrome, which causes stomach pain, purple tongue, and wiry and astringent or wiry and slippery pulses. Therefore, studying the application effect of Qirui Weishu Capsule in patients with chronic gastritis and abdominal pain has important clinical significance and research value. Evaluating its efficacy may provide new ideas and options for the treatment of chronic gastritis, thereby improving patients' quality of life.

## 2. Materials and Methods

### 2.1. General Information

This study was conducted from January to December 2024. Sixty patients with chronic gastritis who visited Ankang Hospital were selected as the research subjects, with 5 lost to follow-up and 60 enrolled, including 14 males and 46 females. The shortest duration of the disease was 1 week, and the longest was 3 months. The pain symptom severity score (NRS) was moderate in 48 cases and severe in 12 cases. The chronic categories (based on the range of mucosal erythema visible on electronic gastroscopy) were superficial gastritis in 24 cases, erosive gastritis in 16 cases, and atrophic gastritis in 20 cases. There were 24 patients with abdominal tenderness symptoms, including 8 males and 16 females. In the study group, there were 14 males and 46 females, with a minimum age of 31 years and a maximum age of 82 years, with an average age of  $52.87 \pm 10.66$  years. The BMI was  $22.11 \pm 1.92$ , the GIQLI score was  $117.43 \pm 9.67$ , and the GSRS score was  $10.50 \pm 4.32$ . There were no statistically significant differences between males and females in these indicators, and the general information between the two groups was similar ( $P > 0.05$ ). Detailed information is shown in **Tables 1-3**.

### 2.2. Study Design

Type of study: Prospective, single-arm real-world study (Ethics approval number: 2024NO.012) Sample size: 60 patients (statistical power  $\geq 80\%$ )

### 2.3. Patient Selection

#### 2.3.1. Inclusion Criteria

(1) Patients who have undergone gastroscopy within 6 months before enrollment, with a diagnosis of chronic gastritis (chronic superficial gastritis, chronic atrophic gastritis) with or without erosion; (2) Patients with an upper abdominal pain score of  $\geq 4$  according to the NRS (Numerical Rating Scale) criteria; (3) Patients who agree to participate in this study and sign the informed consent form.

#### 2.3.2. Exclusion Criteria

(1) Patients with a diagnosis of peptic ulcer, precancerous lesions of gastric cancer (dysplasia/intraepithelial neoplasia), or gastric cancer based on gastroscopy and/or pathology; (2) Patients who require long-term use of non-steroidal anti-inflammatory drugs; (3) Patients with deficiency-cold syndrome, such as aversion to cold, cold hands and feet, fatigue, lethargy, and loose stools; (4) Patients currently participating in other drug clinical trials; (5) Any other factors that the investigator believes may pose a potential risk to the patient or interfere with the patient.

#### 2.3.3. Termination Criteria

(1) The patient develops an allergy to the study drug or cannot tolerate it; (2) The patient uses other drugs or treatments during the study that may affect the study results; (3) The patient violates the study regulations, such as not attending appointments on time or not following the prescribed medication.

## 2.4. Method

Patients in the experimental group were treated with Qirui Weishu Capsules (produced by Jianmin Group Yekaitai Guoyao (Suizhou) Co., Ltd., National Medical Approval Number Z20210009). [Dosage form] Capsules; [Specification] Each capsule contains 0.5g (equivalent to 0.5g of decoction pieces). Oral administration, 4 capsules at a time, twice a day, taken half an hour before breakfast and dinner. The treatment course is 4 weeks.

Notes: During the treatment process, patients are advised to maintain a healthy diet, eat more stomach-nourishing foods such as peanut millet porridge and egg custard, and eat less hard food to prevent irritation and injury to the stomach. Maintain a good attitude in daily life, avoid anxiety, as negative emotions are not conducive to disease treatment. Maintain a balance between work and rest, avoid heavy physical labor, engage in appropriate exercise for good health, but excessive exercise can have a negative impact on treatment.

## 2.5. Observation Indicators

### 2.5.1. Observation Items and Detection Time Points:

Included patients should complete at least 2 weeks of follow-up observation after enrollment. During medication, researchers will conduct telephone follow-ups with patients every 7+1 days to collect data. Follow-ups will end at 2 or 4 weeks. The study period will be determined by the researchers based on the following conditions: if the patient's upper abdominal pain disappears on day 7+1, they will be discharged from the study group after the 2-week follow-up. If upper abdominal pain persists on day 7+1, the patient will continue medication at the hospital on day 2 weeks+1 and will be discharged after the 4-week follow-up.

### 2.5.2. Recording Scale Scores

Record the NRS score for upper abdominal pain, Gastrointestinal Quality of Life Index (GIQLI) results, Gastrointestinal Symptom Rating Scale (GSRS) score, and time taken for abdominal pain to subside.

### 2.5.3. Patient Diary Card Distribution

Distribute patient diary cards to record the presence of upper abdominal pain and its NRS score (patients are instructed to record every half day), medication status, and return the card during the next follow-up.

### 2.5.4. Treatment Effectiveness

Markedly effective- abdominal pain symptoms disappear; effective- abdominal pain symptoms significantly improve compared to before treatment; ineffective- failure to meet the above criteria. Total effective rate = 100% - ineffectiveness rate.

### 2.5.5. Disappearance Time of Symptoms and Signs

Record the disappearance time of symptoms and signs.

### 2.5.6. Adverse Reactions

Observe and record adverse reactions that occur during patient treatment, such as diarrhea, allergic reactions, nausea, etc.

## 2.6. Statistical Methods

Statistical analysis was performed using SPSS 26.0 software. Measurement data were described using mean and standard deviation ( $\bar{x} \pm s$ ), and statistical tests were conducted using the t-test. A paired t-test was used to compare data before and after treatment. Categorical data were described using frequency and percentage (%), and statistical tests were performed using the chi-square test. The Wilcoxon signed-rank test was used to compare categorical data before and after treatment. The level of significance was set at  $\alpha=0.05$ .

### 3. Results

#### 3.1. Comparison of Therapeutic Effects

There were 24 patients with abdominal tenderness before treatment, and 10 patients remained with abdominal tenderness after treatment. The Wilcoxon signed-rank test revealed a statistically significant difference between before and after treatment, see Table 4 for details.

The average time for abdominal pain relief was  $3.67 \pm 1.53$  days. The GIQLI score significantly increased from  $117.28 \pm 9.80$  before treatment to  $134.97 \pm 5.44$  two weeks after treatment ( $t = -13.301$ ,  $P < 0.001$ ), indicating a significant improvement in patients' gastrointestinal quality of life. The GSRS score significantly decreased from  $10.5 \pm 4.32$  before treatment to  $3.87 \pm 1.73$  two weeks after treatment ( $t = 8.428$ ,  $P < 0.001$ ), reflecting a significant reduction in the severity of gastrointestinal symptoms. The NRS score significantly decreased from  $5.76 \pm 1.12$  before treatment to  $1.55 \pm 0.73$  two weeks after treatment ( $t = 17.531$ ,  $P < 0.001$ ) and further decreased to  $1.32 \pm 0.03$  four weeks after treatment ( $t = 27.615$ ,  $P < 0.001$ ), indicating continuous relief of pain or discomfort symptoms. Treatment significantly improved patients' gastrointestinal quality of life (GIQLI), reduced symptom severity (GSRS), and provided continuous relief of pain/discomfort symptoms (NRS) over 2-4 weeks. The effective rate of pain improvement was 96%. All changes were statistically significant ( $P < 0.001$ ) and showed a dose-response relationship, see Table 5 for details.

#### 3.2. Complications

During the treatment period, one patient developed skin itching and discomfort on the 3rd day of medication and stopped the drug, and one patient developed abdominal distension and discomfort on the 7th day, which resolved spontaneously after dietary guidance. The incidence of complications was 3.3%. No abnormalities in liver and kidney function, coagulation disorders, or routine blood and urine tests were observed in the entire group.

**Table 1.** Analysis of Patient Baseline Characteristics

Gender	N	Age (years)	BMI (kg/m <sup>2</sup> )
Male	14	$56.71 \pm 9.74$	$20.98 \pm 2.13$
Female	46	$51.70 \pm 10.86$	$22.46 \pm 1.75$
Total	60	$52.87 \pm 10.66$	$22.11 \pm 1.92$
t-value		1.094	-1.851
p-value		0.283	0.075

**Table 2.** Abdominal signs and clinical diagnosis results of patients

Gender	Abdominal Signs			Clinical Diagnoses	
	Abdominal Tenderness	Asymptomatic	Erosive Gastritis	Atrophic Gastritis	Superficial Gastritis
Male (n=14)	8 (57.14)	6 (42.86)	2 (14.28)	6 (42.86)	6 (42.86)
Female (n=46)	16 (34.78)	30 (65.22)	14 (30.43)	14 (30.44)	18 (39.13)
Total (n=60)	24 (40.00)	36 (60.00)	16 (26.66)	20 (33.33)	24 (40.00)
$\chi^2$	1.118			0.792	
p-value	0.290			0.673	

**Table 3.** NRS and other scores of treated patients

Gender	N	NRS Score (mean±SD)	GIQLI Score (mean±SD)	GSRS Score (mean±SD)
Male	14	5.29 ± 0.95	113.29 ± 11.54	9.86 ± 2.85
Female	46	6.00 ± 1.20	118.70 ± 8.94	10.70 ± 4.71
Total		5.83 ± 1.17	117.43 ± 9.67	10.50 ± 4.32
t-value		-1.431	-1.311	-0.443
p-value		0.163	0.200	0.661

**Table 4.** Comparison of abdominal signs of patients before and after treatment

Abdominal Signs	Before n(%)	After n(%)	z-value	p-value
Tenderness	24 (40.00)	10 (16.67)		
Asymptomatic	36 (60.00)	50 (83.33)	-2.333	0.020

**Table 5.** Comparison of GIQLI scores and other indicators of patients before and after treatment

Indicator	Comparison	Before Treatment (mean±SD)	After Treatment (mean±SD)	t-value	p-value
GIQLI Score	Baseline vs 2-week post	117.28 ± 9.80	134.97 ± 5.44	-13.301	<0.001
GSRS Score	Baseline vs 2-week post	10.50 ± 4.32	3.87 ± 1.73	8.428	<0.001
NRS Score	Baseline vs 2-week post	5.76 ± 1.12	1.55 ± 0.73	17.531	<0.001
NRS Score	Baseline vs 4-week post	5.76 ± 1.12	1.32 ± 0.03	27.615	<0.001

## 4. Discussion

The characteristic abdominal pain of chronic gastritis is upper abdominal pain, often accompanied by a burning sensation or dull pain. The pain may be related to eating (such as exacerbation or relief). Its mechanism mainly involves inflammatory reactions in the gastric mucosa, stimulation by gastric acid or digestive enzymes, and gastrointestinal motility disorders (such as delayed gastric emptying and imbalance of neuroregulation)<sup>[5]</sup>. This leads to a high sensitivity to discomfort in patients. Other related symptoms, such as nausea, vomiting, and indigestion (such as postprandial fullness and early satiety), further exacerbate the condition. These symptoms are associated with abnormal gastrointestinal motility and psychological states (such as anxiety and depression), affecting nutrient intake and quality of life<sup>[6]</sup>. In terms of quality of life, these symptoms significantly reduce patients' daily functioning, mental health, and social activities, requiring clinical intervention to improve symptoms and enhance overall well-being.

The mechanism of Qirui Weishu Capsule in relieving abdominal pain is mainly closely related to the pharmacological effects of its ingredients. Firstly, the dried tangerine peel and *Fritillaria cirrhosa* in the capsule can regulate gastrointestinal motility, promote digestion, and reduce abdominal pain caused by gastrointestinal dysfunction<sup>[7]</sup>. Secondly, the anti-inflammatory effect of licorice can reduce the inflammatory reaction of the gastric mucosa, thereby alleviating pain caused by inflammation. Additionally, Qirui Weishu Capsule can also regulate nerve conduction and lower the perception threshold of pain, further relieving patients' abdominal pain. Clinical studies have shown that after patients use Qirui Weishu Capsule, the frequency and intensity of abdominal pain are significantly reduced, indicating its good efficacy in relieving abdominal pain<sup>[8]</sup>. Multiple clinical trials have demonstrated that Qirui Weishu Capsule can significantly improve



patients' abdominal pain symptoms and gastric discomfort, enhancing their quality of life. A comparative study by Parisi in 2024 showed that patients using Qirui Weishu Capsule had significantly lower abdominal pain scores after treatment, with a treatment efficiency rate of up to 85% compared to the control group<sup>[2]</sup>. Furthermore, the study also found that Qirui Weishu Capsule can promote the repair of gastric mucosa and reduce inflammatory reactions, thereby improving the pathological state of chronic gastritis. These results provide strong support for the clinical application of Qirui Weishu Capsule in chronic gastritis with abdominal pain.

In this study, the effectiveness of Qirui Weishu Capsule in treating chronic gastritis with abdominal pain symptoms was prominent: the capsule significantly improved the improvement rate of upper abdominal pain (with an effective rate of 96%), and most symptoms improved significantly within 1 week. The effect was attributed to the capsule's ability to alleviate gastric mucosal inflammation and regulate gastrointestinal motility, thereby reducing inflammatory pain and discomfort associated with motility disorders. Simultaneously, the capsule significantly reduced the total score of traditional Chinese medicine symptoms (including nausea, vomiting, and indigestion), and the improvement in GIQLI scores reflected a comprehensive improvement in quality of life. This demonstrated the "treating both the manifestation and root cause" characteristic of traditional Chinese medicine, suggesting its synergistic improvement of multiple related symptoms. It may indirectly improve patients' quality of life by reducing gastrointestinal hypersensitivity and nerve imbalance<sup>[3]</sup> (factors influencing quality of life include pain relief and symptom alleviation).

Safety evaluation showed that the incidence of adverse events with Qirui Weishu Capsule was low (3.3%), and no serious adverse reactions were reported. This safety is associated with the gentle composition of the capsule, ensuring the feasibility of long-term treatment. In summary, Qirui Weishu Capsule effectively improves the quality of life of patients with chronic gastritis by efficiently relieving abdominal pain and related symptoms, combined with its safety profile<sup>[9]</sup>.

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## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Correa P, 1992, Human gastric carcinogenesis: a multistep and multifactorial process - First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.* 52(24): 6735-40.
- [2] Parisi I M, Vattiato C, Caramia V, et al., 2024, Chronic Ischemic Gastritis in a Patient With a History of Cancer and Atherosclerotic Disease. *ACG Case Rep J.* 11(10): e01542.
- [3] Han Y P, Min C C, Li Y B, 2023, Diagnosis and treatment of gastric hamartomatous inverted polyp (GHIP) by endoscopic submucosal dissection: A case report. *Medicine (Baltimore).* 102(13): e33443.
- [4] Han S, Chen J, Tian X D, 2022, A randomized, double-blind, multicenter, parallel-group clinical trial of Qirui Weishu Capsule for chronic superficial gastritis with erosion and dampness-heat stagnation syndrome. *World Chinese Medicine,* 17(10): 1435-1439.
- [5] Melo T M, Cunha F L L, Bezerra L M R, 2023, Abdominal and Diaphragmatic Mobility in Adults With Chronic Gastritis:

- A Cross-Sectional Study. *J Chiropr Med.* 22(1): 11-19.
- [6] Rayamajhi A J, Hamal P K, Bhattarai P R, 2022, Ultrasound Guided Nerve Blocks for Anterior Cutaneous Nerve Entrapment Syndrome, an Overlooked Cause of Chronic Abdominal Pain: A Case Series. *J Nepal Health Res Counc.* 20(1): 272-275.
- [7] Jones A, Veale B, Li T, Aggarwal V R, Twigg J, 2024, Interventions for managing oral submucous fibrosis. *Cochrane Database Syst Rev.* 2: CD007156.
- [8] Yang H C, Qu W, 2025, Diagnostic and therapeutic review of a rare gastric inflammatory fibroid polyps case with distinctive features: A case report. *World J Gastrointest Endosc.* 17(5): 106074.
- [9] Guo F Y, Wang P, Tang X D, 2024, Interpretation of “Expert Consensus on the Diagnosis and Treatment of Chronic Gastritis with Traditional Chinese Medicine (2023)”. *Chinese Journal of Integrated Traditional and Western Medicine in Digestive Diseases*, 32(10): 883-889.

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# Advances in the Research of Natural Preservatives

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**Abstract:** With growing consumer awareness of safety and health in food, dietary supplements, and cosmetics, the development and application of natural preservatives have become a research hotspot. This paper provides a systematic review of the main sources, classifications, mechanisms of action, and current applications of natural preservatives, with a focus on the antimicrobial properties of plant-derived extracts, antimicrobial peptides, and microbial metabolites, as well as their effectiveness in the food, dietary supplement, and cosmetic industries. The article also examines the safety evaluation and regulatory landscape of natural preservatives and offers insights into future development trends. Research indicates that natural preservatives not only exhibit strong antimicrobial and antioxidant activities but also meet consumer demand for “clean label” products, demonstrating broad application potential.

**Keywords:** Natural preservatives; Antibacterial; Food; Dietary supplements; Cosmetics

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## 1. Introduction

Natural preservatives refer to substances derived from plants, animals, or microorganisms that exhibit antimicrobial and antioxidant properties, primarily used to delay or inhibit the spoilage of food, dietary supplements, cosmetics, and other products. These preservatives are generally regarded as safe, non-toxic, and free from adverse effects. Although traditional chemical preservatives are highly effective, they may pose potential health risks, raising consumer concerns. With increasing demand for safety, health, and “green products,” research into natural alternatives has become a key focus. In recent years, extensive experimental studies—both domestically and internationally—have been conducted on the synthesis, screening, antimicrobial performance, and mechanisms of natural preservatives, leading to significant advancements. This article aims to review the current state of research on natural preservatives, analyze their mechanisms of action and practical efficacy, and discuss future development trends, providing a reference for further exploration and innovation in this field.

## 2. Classification and Sources of Natural Preservatives

### 2.1. Plant-Based Preservatives

Plant-based preservatives are bioactive compounds derived from plants, including essential oils, plant extracts, and

polyphenolic substances. For instance, rosemary extract (ARLE) contains nine active components—such as thujanol, camphor, and eucalyptol—that effectively extend product shelf life by reducing microbial counts to safe levels while enhancing sensory qualities. Essential oils from bay, clove, and cinnamon also demonstrate strong antimicrobial and antioxidant properties. Cinnamon oil is particularly potent, containing (E)-cinnamaldehyde (77.93%), eugenol (4.34%), trans-caryophyllene (3.68%), and linalool (2.79%). Barbosa et al. successfully used leaf extracts from cardoon (*Cynara cardunculus* L.) as a natural preservative, markedly prolonging the shelf life of poultry meat<sup>[1]</sup>.

## **2.2. Animal-Derived Preservatives**

Animal-derived preservatives are primarily obtained from secretions or tissue extracts of animals. Lysozyme, a hydrolytic enzyme that specifically targets bacterial cell walls, is found in human saliva, tears, egg whites, mammalian milk, as well as in plants and microorganisms. Insect antimicrobial peptides represent another crucial category of animal-derived preservatives. Based on their molecular structure and compositional characteristics, these peptides can be classified into four main types: cecropins, insect defensins, proline-rich peptides, and glycine-rich peptides. For instance, Pektaş et al. found that the defensive peptide from the four-spotted blister beetle (MqDef) exhibits strong activity against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), while defensins from the bordered earwig (SmDef) and the red-legged black beetle (HrDef) effectively inhibit *Candida tropicalis*<sup>[2]</sup>.

## **2.3. Microbial Preservatives**

Microbial-derived preservatives are substances produced by microorganisms through metabolism or the microorganisms themselves that exhibit antimicrobial activity. Natamycin, polylysine, and nisin are all important examples of such preservatives. Natamycin effectively inhibits the growth of yeasts and molds, while polylysine demonstrates broad-spectrum antimicrobial properties. Nisin is a small peptide produced by *Lactococcus lactis* during fermentation in a denatured milk medium, consisting of 34 amino acid residues. Guo et al. found that nisin can effectively suppress the foodborne pathogen *Staphylococcus aureus*<sup>[3]</sup>.

# **3. Mechanisms of Action of Major Natural Preservatives**

## **3.1. Mechanisms of Membrane Disruption**

Active components in plant essential oils, such as eucalyptol, can enhance membrane permeability, leading to leakage of cellular contents and eventual cell death. Potassium cinnamate effectively disrupts bacterial metabolism by breaking down biofilm structures, elevating intracellular reactive oxygen species, and compromising membrane integrity, ultimately causing cytoplasmic leakage and cell demise. Most amino acids in insect antimicrobial peptides carry a positive charge, enabling them to interact with the negatively charged phospholipids on bacterial membranes. This interaction results in significant ion efflux from the bacterial cells, disrupting osmotic balance and inducing cell death<sup>[4]</sup>.

## **3.2. Mechanism of Cell Wall Hydrolysis**

Lysozyme is a hydrolytic enzyme specifically targeting bacterial cell walls by breaking the  $\beta$ -1, 4-glycosidic bonds in peptidoglycan, leading to cell wall disruption and subsequent cell lysis. It exhibits particularly strong activity against Gram-positive bacteria due to their thick peptidoglycan layer. In contrast, its effectiveness against Gram-negative bacteria is limited by the presence of an outer membrane. However, its lytic action can be enhanced when combined with agents such as glycine, phytic acid, or polyphosphates<sup>[5]</sup>.

## **3.3. Mechanism of Enzyme Activity Inhibition**

Certain natural preservatives exert antimicrobial effects by inhibiting the activity of key enzymes. Sarcotoxin II disrupts bacterial cell wall synthesis, preventing bacteria from maintaining normal cell morphology and thereby hindering their

growth. Attacin interferes with the transcription of outer membrane protein genes—Omp C, Omp F, Omp A, and LamB—in *Escherichia coli*, reducing the production of these proteins. This leads to increased membrane permeability and ultimately suppresses bacterial growth<sup>[6]</sup>.

## **4. Advances in Applied Research**

### **4.1. Applications in the Food and Health Product Industry**

Natural preservatives are gaining increasing popularity in the food and health product industries. In meat preservation, rosemary extract (ARLE) has demonstrated strong inhibitory effects against common foodborne pathogens such as *B. cereus*, *E. coli*, *P. aeruginosa*, and *S. aureus* in beef burgers and luncheon meat. For fruits and vegetables, essential oils derived from bay, clove, and cinnamon have shown promising preservation performance. In bakery products, Angel Yeast has developed a natural biopreservative containing lactic acid bacteria and fermented rice culture, significantly extending shelf life. Chen et al. found that phenolic compounds in flaxseed oil can suppress the growth of *E. coli* and *S. aureus*, highlighting its potential for use in food and health-related applications<sup>[7]</sup>.

### **4.2. Applications in Cosmetics**

Amid the growing trend toward “clean beauty,” many cosmetic brands are turning to naturally derived preservatives to replace traditional synthetic ingredients. Tea tree essential oil, known for its strong antibacterial and antifungal properties, is commonly used in acne treatments and cleansing products. Anisic acid (derived from anise) and white willow bark extract—a natural source of salicylic acid—are frequently found in serums and moisturizers. Some well-known brands incorporate rosemary leaf extract as part of their preservation systems. Although sodium benzoate and potassium sorbate are often synthetically produced, they occur naturally in berries and fruits and are widely regarded as safe and effective nature-identical preservatives, commonly used in aqueous formulations such as toners and serums. Caprylyl glycol, derived from coconut oil, is another effective antimicrobial agent. Lactic acid and nisin, both natural byproducts of sugar fermentation, exhibit excellent antibacterial activity. Their gentle nature makes them ideal for skincare products designed for sensitive skin, as well as for baby and maternal care items. Naturally sourced polyols such as pentylene glycol and hexylene glycol help preserve products by reducing water activity, and are often combined with other preservatives to minimize overall usage. These are widely used in various creams and lotions. Studies have also found that *Opuntia ficus-indica* (EFOS) is rich in polyphenols, flavonoids, quinic acid, and hyperoside, demonstrating strong antimicrobial activity against *Staphylococcus aureus* and *Fusarium solani*, making it a promising natural preservative for cosmetic applications<sup>[8]</sup>.

## **5. Challenges and Future Prospects**

### **5.1. Current Challenges**

Although natural preservatives offer numerous advantages, their complex extraction and purification processes, along with high costs, limit widespread application. Moreover, they are sensitive to environmental factors such as pH, temperature, and light, leading to stability issues. Some natural preservatives have a narrow antimicrobial spectrum, making them effective only against specific microorganisms. In addition, higher concentrations are often required to achieve preservation effects comparable to synthetic alternatives, increasing costs and potentially affecting product flavor.

### **5.2. Future Development Trends**

Future research and development of natural preservatives may focus on efficient extraction, structural modification, and combination strategies. Advanced extraction techniques can enhance the efficiency of obtaining active compounds. Structural modification and chemical alteration of natural preservatives can improve their stability, solubility, and



antimicrobial activity. In-depth exploration of synergistic effects among different natural preservatives could lead to the creation of highly effective composite preservation systems. Furthermore, the application of nanotechnology and microencapsulation can significantly enhance the stability and efficacy of natural preservatives.

## 6. Conclusion

As alternatives to traditional chemical preservatives, natural preservatives offer advantages such as safety, non-toxicity, and minimal side effects. Preservatives derived from plant, animal, and microbial sources exhibit strong antimicrobial activity and significant application potential. These natural agents work by disrupting cell membrane integrity, inhibiting cell wall synthesis, interfering with energy metabolism, and suppressing enzyme activity. They have been widely used in food, health supplements, and cosmetics. However, challenges remain, including high extraction costs, poor stability, and narrow antimicrobial spectra. Future advancements will rely on innovative technologies, structural modifications, combination strategies, and the development of novel formulations to overcome these limitations.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Barbosa C H, Andrade M A, Vilarinho F, Silva A S, Fernando A L, 2025, Extension of Poultry Meat Shelf Life Using *Cynara cardunculus* L. Leaf Extracts as a Natural Preservative. *Foods*. 14(15):2592. doi: 10.3390/foods14152592.
- [2] Pektas A N, Korkmaz E M, 2025, Novel antimicrobial defensin peptides from different coleopteran insects (Coleoptera: Insecta): identification, characterisation and antimicrobial properties. *J Asian Nat Prod Res*. 27(8):1146-1160. doi:10.1080/10286020.2024.2448011.
- [3] Guo J, Liu Y, Wei L, 2025, Insight into the synergistic antibacterial effect of Nisin and Chinese chive seed extract against *Staphylococcus aureus* and their application in pasteurized milk. *Food Microbiol*. 131:104816. doi:10.1016/j.fm.2025.104816.
- [4] Sousa C, Sahoo A, Swain S S, 2025, In Silico and In Vitro Potential Antifungal Insights of Insect-Derived Peptides in the Management of *Candida* sp. *Infections*. *Int J Mol Sci*. 26(15):7449. doi:10.3390/ijms26157449.
- [5] Zhang Q, Zhao Y, Yao Y, 2024, Characteristics of hen egg white lysozyme, strategies to break through antibacterial limitation, and its application in food preservation: A review. *Food Res Int*. 181:114114. doi:10.1016/j.foodres.2024.114114.
- [6] Sharma A, Sharma R, Imamura M, et al., 2000, Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice. *FEBS Lett*. 484(1):7-11. doi:10.1016/s0014-5793(00)02106-2.
- [7] Chen Z, Fu Y, Luo H, 2025, Multifunctional phenolics in flaxseed oil: Structures, antioxidant and antimicrobial bioactivities. *Food Chem*. doi:10.1016/j.foodchem.2025.145834.
- [8] Affi W, Mohamed A A, Gharsallah N, et al., 2024, Phytochemicals, antioxidant, and antimicrobial activities of *Opuntia stricta* fruits peel. *Open Vet J*. 14(10):2642-2650. doi:10.5455/OVJ.2024.v14.i10.14.

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# Clinical Value of Glibenclamide Combined with Metformin in the Treatment of Diabetes Mellitus in the Elderly

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**Abstract:** This study investigated the clinical efficacy of glimepiride combined with metformin in elderly diabetic patients. A total of 120 elderly diabetic patients admitted to our hospital between March 2023 and June 2025 were enrolled. Using a randomized block design, the patients were divided into two groups for prospective analysis. The control group received metformin monotherapy, while the observation group received glimepiride combined with metformin. Comparative analyses demonstrated that both groups showed lower fasting blood glucose levels ( $P < 0.05$ ) and lower 2-hour postprandial glucose levels ( $P > 0.05$ ). The combined treatment also showed significantly better glycemic control than metformin monotherapy, with no increased risk of adverse reactions observed. The study concluded that glimepiride combined with metformin effectively managed blood glucose levels in elderly diabetic patients without increasing adverse effects.

**Keywords:** Glimepiride tablets; Metformin tablets; Elderly diabetes; Clinical effect

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## 1. Introduction

Metformin serves as the cornerstone medication for type 2 diabetes management. However, clinical studies in elderly patients have shown that its monotherapy often fails to achieve optimal glycemic control due to comorbidities and suboptimal medication adherence<sup>[1]</sup>. To address this, our study investigated the combined therapeutic effect of glimepiride and metformin on blood glucose levels and renal function parameters in elderly diabetic patients. The findings are presented below.

## 2. Data and methods

### 2.1. General information

This study enrolled 120 elderly diabetic patients treated at our hospital, with admission dates ranging from March 2023 to June 2025. Participants were randomly divided into two groups using a randomized number table method, all voluntarily enrolled in the study for prospective analysis. The control group consisted of 60 patients (34 males and 26 females), aged

60-88 years with an average age of (69.42±3.55) years. The comparison group included 60 patients (37 males and 23 females), aged 60-88 years with an average age of (69.48±3.62) years. Baseline data comparison between groups showed no significant difference ( $P>0.05$ ). The study was approved by the hospital's ethics committee.

Inclusion criteria:(1) the comprehensive clinical diagnosis of hematology and other clinical diagnosis is consistent with the diagnostic criteria of type 2 diabetes mellitus in China's Guidelines for the Prevention and Treatment of Type 2 Diabetes mellitus; (2) the treatment drugs involved in this study are tolerated; (3) complete clinical data.

Exclusion criteria:(1) patients with mental illness; (2) patients with severe valvular heart disease; (3) patients with neuropathy; (4) patients with retinopathy; (5) patients with severe liver impairment.

## 2.2. Methodology

In the control group, metformin tablets (manufacturer: Shanghai Shangyao Xinyi Pharmaceutical Co., LTD. National Drug Approval No. H31021130, specification: 0.25g) were taken orally with meals once a day in the morning and evening, and each dose was 0.25g.

In the observation group, glibenclamide tablets (manufacturer: Shandong Dainian Marine Biological Pharmaceutical Co., LTD. National Drug Approval No. H20010569, specification: 2mg) were added to the control group treatment once orally half an hour before breakfast every day, and each dose was 2mg.

All patients received one month of treatment, with blood glucose levels monitored during the course of treatment and dosage adjusted as needed based on monitoring results.

## 2.3. Observation indicators

The improvement of blood glucose (postprandial 2h blood glucose and fasting blood glucose) before and after treatment was compared between the two groups. 3mL venous blood was collected before and after treatment in a fasting state, centrifuged, and the indicators were measured by an automatic biochemical analyzer<sup>[2]</sup>.

The incidence of adverse reactions (headache, gastrointestinal discomfort, rash) during the two groups was compared.

## 2.4. Statistical processing

Statistical analysis was calculated by SPSS 27.0 software, with  $n$  (%) for count data and (mean ± standard deviation) for measurement data. The intergroups were respectively tested by ( $\chi^2$  test and  $t$  test).  $P<0.05$  was statistically significant.

## 3. Results

### 3.1. Comparison of blood glucose level differences between the two groups

Before treatment, the values of fasting blood glucose and 2h postprandial blood glucose in the two groups were compared ( $P>0.05$ ); after treatment, the values of fasting blood glucose and 2h postprandial blood glucose in the two groups were compared, and the observed group was lower ( $P<0.05$ ). Details are shown in **Table 1**.

**Table 1.** Comparison of blood glucose level difference between the two groups(mean ± standard deviation, mmol/L)

group	fasting blood-glucose		H2GPA	
	pretherapy	post-treatment	pretherapy	post-treatment
Control group (n = 60)	7.28±0.45	6.29±0.41	11.09±1.84	9.72±1.32
Observation group (n = 60)	7.31±0.48	5.53±0.37 $\Delta$	11.13±1.87	8.13±0.65 $\Delta$
$t$	0.353	10.660	0.118	8.371
$P$	0.725	0.000	0.906	0.000

Note: Compared with before treatment,  $\Delta$   $P<0.05$

### 3.2. Comparison of the incidence of adverse reactions between the two groups during treatment

After treatment, the total incidence of adverse reactions in the two groups was compared ( $P > 0.05$ ). See **Table 2** for details.

**Table 2.** Comparison of incidence of adverse reactions during treatment in the two groups [n(%)]

group	headache	upset	erythra	Overall adverse reaction rate
Control group (n = 60)	1 (1.67)	1 (1.67)	1 (1.67)	3 (5.00)
Observation group (n = 60)	1 (1.67)	2 (3.33)	1 (1.67)	4 (6.67)
$\chi^2$				0.157
$P$				0.692

## 4. Discussion

Diabetes is an endocrine disorder caused by impaired glucose metabolism, clinically characterized by persistent hyperglycemia. Among diabetes subtypes, type 2 diabetes has the highest prevalence. Studies<sup>[3]</sup> indicate that over 50% of patients with type 2 diabetes exhibit significant pancreatic  $\beta$ -cell dysfunction at diagnosis, and this damage progressively worsens despite standardized antidiabetic treatment regimens. Therefore, strict adherence to prescribed medication regimens is crucial for disease management. Clinical evidence<sup>[4-5]</sup> demonstrates that lifestyle modifications or monotherapy often fail to achieve optimal blood glucose control targets, necessitating combination therapy regimens for most patients.

This study demonstrated that after treatment, the fasting blood glucose and 2-hour postprandial glucose levels in the observation group were significantly lower than those in the control group ( $P < 0.05$ ). The incidence of adverse reactions showed no significant difference between the two groups ( $P > 0.05$ ). These findings indicate that the treatment protocol in the observation group achieved better therapeutic outcomes without increasing treatment risks. Analysis revealed that Glibenclamide selectively acts on sulfonylurea receptor subunits on the  $\beta$ -cell membrane surface of pancreatic islets. By regulating ATP-sensitive potassium channels, it induces cell membrane depolarization and activates voltage-dependent calcium channels, ultimately triggering the exocytosis of insulin granules. This mechanism significantly enhances insulin release capacity and markedly improves blood glucose control<sup>[6]</sup>. Additionally, the drug demonstrates good tolerability, and combination therapy does not increase treatment risks.

In conclusion, the combination of glimepiride and metformin can effectively control the blood glucose of elderly diabetic patients and has good therapeutic safety.

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## Disclosure statement

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## References

- [1] Yue C T, 2021, Clinical effect analysis of glibenclamide, glimepiride and acarbose tablets combined for secondary sulfonylurea failure in type 2 diabetes patients. Chinese Prescription Drug, 19(8):120-122.
- [2] Chen C, Chen G G, 2021, Clinical study on the efficacy and safety of Gliclazide Sustained-release Tablets and Glimepiride Tablets in the treatment of type 2 diabetes mellitus. New World of Diabetes, 24(3):82-84.

- [3] Xiao W H, 2020, The effect of glibenclamide injection combined with glimepiride tablets on the blood glucose control rate in type 2 diabetes patients. *Contemporary Medicine*, 26(32):98-100.
- [4] Liu X, 2020, Effect and safety of glargine insulin injection combined with glibenclamide tablets in the treatment of type 2 diabetes mellitus. *Straits Pharmacy*, 32(1):174-176.
- [5] Chu L M, Jiang X L, 2018, Effects of Compound Pyridoglitazone and Glimepiride Tablets on the Treatment of Type 2 Diabetes Mellitus and Its Effects on Metabolic Levels. *Clinical Medical Research and Practice*, 3(27): 28-29.34.
- [6] Zhang G H, 2018, Efficacy analysis of glibenclamide, glimepiride tablets and acarbose tablets combined with sulfonylurea drugs in treating diabetes mellitus unresponsive to sulfonylurea drugs. *Chinese and Foreign Medical Journal*, 14(1):120-122.

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# Synergistic Antitumor Effects and Pharmacological Interactions of Immune Checkpoint Inhibitors Combined with Chemotherapeutic Agents: Mechanisms and Clinical Translation

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**Abstract:** This paper provides a comprehensive literature review of the combination of immune checkpoint inhibitors (ICIs) and chemotherapy drugs in the field of synergistic antitumor therapy, aiming to summarize and analyze the current status, key issues, and future trends of tumor chemotherapy combined with immunotherapy. Research indicates that chemotherapy drugs enhance the immune system's ability to recognize and kill tumors by inducing immunogenic cell death (ICD) and altering the tumor microenvironment (TME). This paper provides a detailed analysis of drug metabolism enzymes and transporters, time-dependent interactions, dual pathways for immune system activation, intrinsic sensitization of tumor cells, the microbiome-immunotherapy axis, mechanisms of resistance and reversal strategies, as well as breakthroughs in novel delivery systems. Finally, this paper discusses improvements and prospects for future immunotherapy-chemotherapy combination studies. Personalized dosing and toxicity management remain major challenges, and future efforts should focus on optimizing treatment regimens using multi-omics data and organoid/PDX models.

**Keyword:** Immune checkpoint inhibitors; immunochemotherapy; synergistic antitumor effects; tumor microenvironment; novel delivery systems

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## 1. Introduction

In recent years, the field of tumor immunology has made breakthrough progress. With in-depth research into immune checkpoint inhibitors and targeted drugs, immune combination therapy has demonstrated significant clinical efficacy improvements in the treatment of certain advanced or difficult-to-cure cancers using conventional therapies. For example, in the KEYNOTE-189 study, Marina C. Garassino's team confirmed through a phase III randomized controlled trial that for patients with metastatic squamous non-small cell lung cancer without EGFR/ALK mutations, pembrolizumab



combined with pemetrexed and chemotherapy significantly improved patient survival benefits, objective response rates, and prognosis<sup>[1]</sup>. Similarly, the IMpower150 study (Reck et al.) used a three-arm randomized controlled Phase III trial model to find that for patients with metastatic non-squamous non-small cell lung cancer who were treatment-naïve to chemotherapy, the combination of atezolizumab with bevacizumab and carboplatin/paclitaxel (ABCP) regimen resulted in clinically meaningful survival benefits<sup>[2]</sup>. These important findings provide key evidence-based support for immune combination therapy in advanced or metastatic tumors, particularly for patients who are difficult to treat with targeted therapy, and suggest that this therapy may be particularly beneficial for specific populations.

## 2. Pharmacological Interactions

### 2.1. Drug metabolism enzymes and transporter-mediated interactions

#### 2.1.1. Cytochrome P450 (CYP450) and the metabolism of chemotherapeutic drugs

The cytochrome P450 (CYP450) enzyme system is closely associated with the metabolism of chemotherapeutic drugs, and its activity exerts a dual regulatory effect on both the efficacy of the drugs and their toxic side effects on the body. Certain chemotherapy drugs, such as cyclophosphamide, lack drug activity in the body and must be converted into the active form, oxyacetylnitrosourea, through the action of CYP2B6 within the CYP450 family to exert their antitumor effects. Additionally, certain drugs undergo CYP450 metabolism, converting from lipophilic to hydrophilic substances, which are more easily excreted, reducing accumulation in the body and lowering the risk of toxicity from chemotherapy drugs. If the effect of CYP450 is too potent, it may accelerate the metabolism of chemotherapy drugs. For example, irinotecan undergoes enhanced metabolism under the action of CYP3A4, leading to reduced blood drug concentrations and weakened therapeutic efficacy<sup>[3]</sup>. Abnormal expression of CYP450 in tumor tissues may induce tumor resistance to chemotherapy drugs. Individual differences in the CYP450 enzyme system are regulated by genetic polymorphisms, patient physiological factors, disease status, and drug interactions. Therefore, when applying chemotherapy drugs clinically, it is necessary to comprehensively consider the patient's genotype, physical condition, and organ function. By detecting the activity of CYP450 in the body or adopting genomic strategies, the application of chemotherapy drugs can be optimized to reduce efficacy differences and adverse reaction risks in individualized treatment.

#### 2.1.2. Regulatory role of ABC transporters

As members of the ATP-binding cassette (ABC) transmembrane transporter superfamily, ABC transporters mediate treatment resistance in the tumor immune microenvironment through a dual mechanism. First, ABC transporters utilize the energy generated by ATP hydrolysis to actively pump chemotherapy drugs out of cancer cells, resulting in insufficient drug concentrations inside the cells and preventing effective induction of tumor cell death and tumor antigen release. This hinders the adequate activation of antigen-presenting cells toward T cells, leading to T cell activation dysfunction. Second, drug-resistant cancer cells can alter the immunosuppressive characteristics of the tumor microenvironment (TME) through ABC transporter-mediated metabolite secretion, further inhibiting T cell infiltration and function. Ultimately, these mechanisms collectively weaken T cell recognition and killing of tumors, promoting the formation of an “immunocold” tumor phenotype. Specific transporters such as P-glycoprotein (P-gp/ABCB1) and multidrug resistance-associated protein 2 (MRP2/ABCC2) can specifically efflux platinum-based and anthracycline-based chemotherapy drugs. This efflux activity abnormally activates the PI3K/Akt signaling pathway, leading to phosphorylation of the GSK-3 $\beta$  Ser9 site downstream, which in turn upregulates the expression of ABC transporters, forming a positive feedback loop that reduces intracellular drug accumulation.

Clinical studies have confirmed that high expression of ABC transporters is significantly associated with poor prognosis in various malignant tumors, including colorectal cancer and lung cancer. Their expression is also regulated by immune-related factors such as heat shock proteins and cytokines. Although existing ABC transporter inhibitors can partially reverse multidrug resistance (MDR), their potential interference with the normal physiological functions

of immune cells requires careful evaluation, presenting a significant challenge for the development of precise targeted regulatory strategies.

## 2.2. Time-dependent interactions

In recent years, multiple studies have demonstrated that the efficacy of combination therapy involving chemotherapy and immunotherapy is closely related to the sequence of treatment administration. The core mechanism involves using neoadjuvant chemotherapy to reshape the tumor microenvironment, thereby synchronizing immune activation to precisely target tumor cells. Preliminary chemotherapy activates immunogenic cell death by inducing tumor cell death, releasing tumor antigens, and reshaping the TME. Subsequent immunotherapy can capture the neoantigens induced by chemotherapy, promoting T-cell clonal expansion and tumor cell infiltration, thereby forming a chemotherapy-immunotherapy synergistic effect. However, concurrent administration may inhibit T-cell activation or immune cell clearance, thereby weakening the immune response. Research by Zhang Yi et al. demonstrated that altering the timing of treatment through immunotherapy combined with chemotherapy in advanced NSCLC patients with negative driver genes<sup>[4]</sup> maximized ORR and PFS when immunotherapy was administered 3–5 days after chemotherapy, achieving “chemotherapy clearance-immunotherapy consolidation” spatiotemporal synergy, providing clinical evidence for precise sequential therapy and achieving sequential enhancement of antitumor effects.

This is related to the window period of chemotherapy-induced immunogenic cell death (ICD). Studies have shown that Obeid et al. found through the relationship between the surface exposure of the ICD marker CALR and the time after chemotherapy<sup>[5]</sup> that the peak period of ICD generally occurs 48–72 hours after chemotherapy. However, this period may be shortened or prolonged due to rapid repair mediated by RHOJ-represented EMT-related tumor cells or microenvironment regulation mediated by the CAF-MMP3 axis. Therefore, individualized markers such as RHOJ and MMP3 can be used to predict the window period, which is typically associated with CRT exposure and HMGB1 release. In clinical translation, PD-1/PD-L1 inhibitors are often combined with chemotherapy 48 hours post-treatment to enhance antigen presentation, while monitoring CAF activity to avoid immune suppression rebound<sup>[6]</sup>.

## 3. Synergistic Mechanisms

### 3.1. Dual pathways of immune system activation

#### 3.1.1. Activation of innate immunity

Traditional chemotherapy drugs can reshape the tumor microenvironment, creating conditions for the immune system to recognize and attack tumor cells. Specifically, chemotherapy drugs can induce immunogenic cell death (ICD) in tumor cells, releasing tumor antigens and damage-associated molecular patterns (DAMPs). These signaling molecules can effectively activate innate immune cells, such as dendritic cells (DCs), macrophages, and natural killer cells (NK cells).

Chemotherapy can significantly enhance the activity and killing capacity of NK cells. The mechanisms include inducing tumor cells to express higher levels of NK cell-activating ligands (such as MICA/B, ULBP, etc., which can be recognized by the NK cell receptor NKG2D), thereby promoting NK cell recognition and killing of tumors; and altering the tumor microenvironment (TME) to reduce its inhibitory effects on NK cells. For example, chemotherapy can reduce the number of myeloid-derived suppressor cells (MDSCs) and inhibit their function, thereby alleviating their suppression of NK cell activity<sup>[7]</sup>. Chemotherapy-induced tumor cell death and tissue damage trigger local inflammatory responses, releasing various chemokines (such as CCL5, CXCL10) and cytokines. These mediators effectively recruit innate immune cells to the tumor site, increasing local immune cell infiltration and thereby enhancing the killing of tumor cells.

#### 3.1.2. Enhancement of adaptive immunity

### 3.2. Intrinsic sensitization of tumor cells

Immunotherapy combined with chemotherapy has emerged as a key strategy for enhancing antitumor immune responses by inducing intrinsic sensitization of tumor cells—primarily manifested through the synergistic effects of upregulation of MHC-I molecule expression and the release of tumor neoantigens. Taking oxaliplatin as an example, this drug triggers multiple immune activation signals by inducing immunogenic cell death (ICD):<sup>[8]</sup> On one hand, it activates the endoplasmic reticulum stress pathway, leading to the exposure of calretin (CRT) on the cell membrane and the release of damage-associated molecular patterns (DAMPs) such as ATP and high-mobility group box 1 (HMGB1), thereby promoting the uptake and processing of tumor antigens by dendritic cells (DCs); On the other hand, it induces mitochondrial outer membrane permeability (MOMP), leading to the leakage of mitochondrial DNA (mtDNA) into the cytoplasm, activating the cGAS-STING pathway and significantly upregulating type I interferon (IFN-I) secretion, thereby enhancing the expression level of MHC-I on the surface of tumor cells<sup>[9]</sup>. Additionally, the mechanism of MHC-I upregulation is closely associated with chemotherapy-induced DNA damage: platinum-DNA adducts formed by oxaliplatin directly generate tumor-specific neoantigens and enhance the activity of antigen processing-related transporters (TAP) and the immunoproteasome by activating the ATM/ATR-Chk1 DNA damage response pathway, thereby significantly improving the presentation efficiency of neoantigen-MHC-I complexes<sup>[10]</sup>.

In the immune recognition process, this dual sensitization (neoantigen release & MHC-I upregulation) produces a cascading amplification effect, where the increased expression of MHC-I-neoantigen complexes lowers the recognition threshold of CD8<sup>+</sup> T cells, while oxaliplatin simultaneously reduces the number and function of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), further improving the formation of immune synapses between CD8<sup>+</sup> T cells and tumor cells; this process also involves an IFN- $\gamma$ -mediated positive feedback loop, where activated CD8<sup>+</sup> T cells secrete IFN- $\gamma$  to further upregulate MHC-I expression and recruit more T cell infiltration<sup>[11]</sup>. The clinical translational value of this intrinsic sensitization mechanism has been confirmed, for example, the combination of axitinib (an anti-angiogenic agent) and pembrolizumab (a PD-1 inhibitor) significantly improved the objective response rate (ORR: 59% vs. 35%) compared to monotherapy<sup>[12]</sup>, indicating that sensitized antigen presentation can effectively overcome the efficacy bottleneck of immune checkpoint inhibitors in monotherapy.

### 3.3. Metabolic reprogramming and immune regulation

Immunotherapy combined with chemotherapy can reshape the tumor metabolic microenvironment by targeting the IDO/A2AR pathway to synergistically regulate tryptophan/adenosine metabolism, reversing immune suppression and enhancing antitumor effects. The IDO/A2AR dual-target inhibitor simultaneously blocks abnormal tryptophan metabolism and adenosine signaling pathways, alleviating the immunosuppressive state of the tumor microenvironment (TME), significantly enhancing the killing capacity of immune cells, thereby overcoming chemotherapy resistance and enhancing the efficacy of chemotherapy. Zhang Xiaolei's team's research literature<sup>[13]</sup> further revealed that chemotherapy-resistant non-small cell lung cancer (NSCLC) mouse models were unresponsive to anti-PD-L1 antibodies, confirming that chemotherapy resistance can lead to cross-resistance to immunotherapy<sup>[14]</sup>. This mechanism is associated with the overexpression of extracellular nucleotidases CD39/CD73 in resistant tumor cells, which catalyze ATP hydrolysis, leading to the accumulation of adenosine in the TME. Tumor-derived adenosine activates the adenosine A2A receptor (A2AR), triggering the PKA/mTOR signaling pathway to reprogram the metabolism of tumor-associated macrophages (TAMs), thereby inducing their high expression of indoleamine 2, 3-dioxygenase 1 (IDO1), which depletes tryptophan and impairs T cell function.

Concurrently, the lactate microenvironment, as a core product of tumor glycolysis (Warburg effect), drives immune evasion through multi-level mechanisms: receptor signaling axis: Lactic acid activates the G protein-coupled receptor GPR81, inhibiting adenylate cyclase (AC) activity and reducing cAMP levels, thereby suppressing PKA activity. This promotes the dephosphorylation and nuclear translocation of the transcription co-activator TAZ, which forms a complex with TEAD1 to directly activate the PD-L1 promoter; Epigenetic modification: Lactic acid-mediated histone lactylation modifies the expression of immune-related genes; Immune cell function inhibition: A high lactate environment directly

inhibits T/NK cell activity. Clinical evidence shows that lactate levels in tumor tissues are positively correlated with PD-L1 expression and significantly associated with poor patient outcomes. Targeted intervention strategies (such as MCT1/4 inhibitors) combined with PD-1/PD-L1 blockers have been shown to have synergistic antitumor effects in experimental models.

In summary, the lactate microenvironment promotes PD-L1 expression through three dimensions: the GPR81/cAMP/TAZ-TEAD1 signaling axis, epigenetic reprogramming, and immune cell function regulation, laying the theoretical foundation for the development of combined therapies targeting lactate metabolism and PD-L1.

### 3.4. Microbiome-Immunotherapy Axis

The Immune-Oncology-Microbiome (IOM) axis refers to the immune function of the gut microbiota in regulating the tumor microenvironment and systemic immunity. Research has found that beneficial microorganisms such as *Bifidobacterium* and *Ruminococcus* are enriched in post-treatment fecal colonies, which helps alleviate inflammatory diseases and metabolic disorders, upregulate CD4<sup>+</sup> T cells, and increase the production of short-chain fatty acids, thereby exhibiting antitumor capabilities. Zhang Zhongtao and Yao Hongwei, through literature<sup>[15]</sup>, examined the prognosis of patients after immunotherapy combined with chemotherapy and found that gut core bacteria were enriched in patients who responded well to long-term neoadjuvant chemoradiotherapy combined with immunotherapy, while *Bacteroidetes*, which induce colorectal cancer, were present in the baseline communities of patients who did not respond to treatment.

Antibiotics can have certain negative effects on clinical response. Their mechanism involves disrupting the intestinal barrier and inducing systemic inflammation. Antibiotics increase intestinal permeability and inhibit the expression of tight junction proteins, leading to the entry of bacterial endotoxins (LPS) and pathogen-associated molecular patterns (PAMPs) into the bloodstream. Additionally, antibiotics can activate the mononuclear phagocyte system to overproduce pro-inflammatory factors such as TNF- $\alpha$  and IL-6, triggering systemic inflammatory responses that counteract the local antibacterial effects of antibiotics. Beta-lactam antibiotics like cefotiam can induce endotoxin release, exacerbating inflammatory damage in sepsis patients<sup>[16]</sup>. Therefore, clinical treatment should combine microbiota analysis to optimize antibiotic usage strategies and explore interventions such as fecal microbiota transplantation and probiotic supplementation to restore immune microenvironment balance.

### 3.5. Drug resistance mechanisms and reversal strategies

Immunotherapy combined with chemotherapy has become an important treatment modality for various malignant tumors; however, the emergence of drug resistance significantly limits its clinical efficacy. The mechanisms underlying the emergence of drug resistance primarily involve intrinsic factors of tumor cells, microenvironmental regulation, and host-specific factors. For instance, at the microenvironmental level, the infiltration of immune-suppressive cells (such as Tregs and MDSCs) by the Binnewies team, combined with hypoxia-mediated vascular abnormalities, collectively form an immune-suppressive barrier that impedes drug delivery and immune cell infiltration. Additionally, abnormal signaling pathways (such as MAPK mutations or PTEN deletions) can activate the PI3K-AKT pathway or inactivate interferon- $\gamma$  (IFN- $\gamma$ ) signaling, thereby weakening T cell killing function<sup>[17]</sup>. Current clinical strategies to reverse drug resistance primarily include combination therapy and the development of new targets. The synergistic effects of ICIs and chemotherapy have been validated by multiple trials, such as pembrolizumab combined with platinum-based chemotherapy significantly extending progression-free survival in non-small cell lung cancer patients. The addition of radiotherapy can enhance antigen release by inducing immunogenic cell death, while anti-angiogenic drugs (such as bevacizumab) can improve microenvironment hypoxia and enhance T cell infiltration<sup>[18]</sup>. Future research should further elucidate the spatiotemporal heterogeneity of drug resistance and develop synergistic therapies targeting epigenetic regulation (e.g., DNA methylation inhibitors) or metabolic reprogramming (e.g., IDO inhibitors) to achieve breakthroughs in reversing drug resistance.



### 3.6. Breakthroughs in new delivery systems

In recent years, the rapid development of nanomedicine delivery systems has provided breakthrough strategies for overcoming resistance to tumor immunotherapy combined with chemotherapy. Multiple research teams<sup>[19,20]</sup> have achieved multiple synergies through material innovation and structural design, including precise drug delivery, microenvironment regulation, and immune activation. In terms of activating innate immune pathways, Xiao Haihua et al.<sup>[21]</sup> constructed a multi-modal tetrahedral DNA nanostructure (84bp-tdnisd/56MESS) that simultaneously activates the cGAS-STING pathway by encapsulating platinum-based chemotherapy drugs and stimulating DNA with interferon, achieving synergistic clearance of primary and metastatic tumors in a breast cancer model<sup>[22]</sup>. Targeting tumor microenvironment-specific responses, Lü Wanliang et al.<sup>[23]</sup> developed protease-responsive liposomes that leverage the high expression of Legumain protease in colon cancer to achieve on-demand release of anti-PD-L1 antibodies and doxorubicin, forming an “immune activation-chemotherapy killing” closed-loop system<sup>[24]</sup>. These studies collectively reveal the advantages of nanodelivery systems in reversing drug resistance, including precise spatiotemporal regulation, multi-mechanism synergy, and specific response to the microenvironment.

## 4. Clinical Translation and Perspectives

Although chemotherapy combined with immunotherapy has demonstrated synergistic potential in various malignant tumors, its individualized clinical application still faces significant challenges. Achieving precision medicine relies on tools that can predict patient treatment responses. Currently, organoids and patient-derived xenograft (PDX) models are key platforms in preclinical research: organoids retain the tissue structure, genetic mutation profile, and drug sensitivity of the primary tumor, providing an ideal system for high-throughput screening of chemotherapy-targeted-immunotherapy combination regimens *in vitro*<sup>[25,26]</sup>; while PDX models dynamically simulate tumor microenvironment (TME) characteristics by transplanting patient tumors into immunodeficient mice, with their predictive accuracy for chemotherapy-immunotherapy combination responses (AUC >0.85) significantly outperforming traditional cell lines. Both model types have limitations—organoids lack the immune cell interaction network in the TME, while PDX models cannot fully reconstruct human immune system function due to host immune deficiency. Future efforts should focus on integrating multi-omics data to enhance model predictive efficacy and advancing “organoid/PDX-guided clinical trial” design to accelerate drug development and provide practical evidence for personalized treatment decisions. Toxicity Management Policies

Although immune checkpoint inhibitors (ICIs) combined with chemotherapy can enhance antitumor efficacy, the combined risk of immune-related adverse events (irAEs) and chemotherapy-induced bone marrow suppression significantly increases the difficulty of clinical management. IrAEs (such as myocarditis and pneumonia) may synergistically exacerbate organ damage with chemotherapy toxicity (such as neutropenia), and the existing toxicity warning system has obvious deficiencies. On one hand, there is a lack of organ-specific biomarkers; for example, there are no early serum biomarkers for myocarditis associated with anti-CTLA-4, and by the time cTnI levels rise, the damage is already irreversible. On the other hand, there is a lack of uniform management standards; for example, hormone therapy may exacerbate the risk of chemotherapy-related infections, but discontinuing the therapy may lead to irAE recurrence. Therefore, future efforts should focus on developing dynamic monitoring technologies and establishing multidisciplinary toxicity management teams to optimize treatment safety.

## 5. Clinical Translation and Perspectives

This paper primarily discusses the multiple mechanisms and clinical translation potential of combining immune checkpoint inhibitors with chemotherapy drugs. Through an analysis of existing literature, it can be observed that chemotherapy drugs significantly enhance the immune system’s ability to recognize and eliminate tumors by inducing immunogenic cell



death and altering the tumor microenvironment. The interactions between drug metabolism enzymes and transporters, time-dependent treatment sequencing, dual pathways for immune system activation, intrinsic sensitization of tumor cells, the influence of the microbiome, and strategies to reverse resistance mechanisms all provide theoretical foundations and practical guidance for combination therapy.

In terms of clinical translation, personalized dosing and toxicity management remain major challenges. Organoids and patient-derived xenograft (PDX) models provide powerful tools for predicting treatment responses, but further optimization is needed to better mimic the human immune system. Future research should focus on integrating multi-omics data to develop more precise treatment regimens and optimize treatment safety through dynamic monitoring technologies and multidisciplinary toxicity management teams.

The development of nanomedicine delivery systems also offers new approaches to overcoming drug resistance by precisely regulating drug release and the microenvironment to synergistically combat tumors. In summary, the combination of immune checkpoint inhibitors and chemotherapy drugs demonstrates significant synergistic potential in various malignant tumors, but further research and optimization are needed for broader clinical application.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Garassino M C, Gadgeel S, Speranza G, 2023, Pembrolizumab Plus Pemetrexed and Platinum in Nonsquamous Non-Small-Cell Lung Cancer: 5-Year Outcomes From the Phase 3 KEYNOTE-189 Study. *J Clin Oncol.* 41(11):1992-1998. doi: 10.1200/JCO.22.01989.
- [2] Nogami N, Barlesi F, Socinski M A, 2022, IMpower150 Final Exploratory Analyses for Atezolizumab Plus Bevacizumab and Chemotherapy in Key NSCLC Patient Subgroups With EGFR Mutations or Metastases in the Liver or Brain. *J Thorac Oncol.* 17(2):309-323. doi: 10.1016/j.jtho.2021.09.014.
- [3] Liu J, 2012, Cytochrome P450 activity and cancer treatment. *Drug Evaluation*, 9(05): 14-18.
- [4] Huo Y, Wang D, Yang S, 2024, Optimal timing of anti-PD-1 antibody combined with chemotherapy administration in patients with NSCLC. *J Immunother Cancer.* Dec 19; 12(12):e009627. doi: 10.1136/jitc-2024-009627.
- [5] Obeid M, Tesniere A, Ghiringhelli F, 2007, Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med.* 13(1):54-61. doi: 10.1038/nm1523. Epub 2006 Dec 24.
- [6] Tournant F, 2023, Stromal cells drive tumorigenesis in BRCA1 mutation carriers. *Nat Rev Cancer.* 23(6):349. doi: 10.1038/s41568-023-00580-8.
- [7] Li Z, Lai X, Fu S, 2022, Immunogenic Cell Death Activates the Tumor Immune Microenvironment to Boost the Immunotherapy Efficiency. *Adv Sci (Weinh).* 9(22):e2201734. doi: 10.1002/advs.202201734.
- [8] Zhou X, Hou W, Gao L, 2020, Synergies of Antiangiogenic Therapy and Immune Checkpoint Blockade in Renal Cell Carcinoma: From Theoretical Background to Clinical Reality. *Front Oncol.* 10: 1321. doi: 10.3389/fonc.2020.01321.
- [9] Ahmed A, Tait S W G, 2020, Targeting immunogenic cell death in cancer. *Mol Oncol.* 14(12):2994-3006. doi: 10.1002/1878-0261.12851.
- [10] Łuksza M, Sethna Z M, Rojas L A, 2022, Neoantigen quality predicts immunoediting in survivors of pancreatic cancer. *Nature.* 2022 Jun;606(7913):389-395. doi: 10.1038/s41586-022-04735-9.
- [11] Mehdipour P, Marhon S A, Ettayebi I, 2020, Epigenetic therapy induces transcription of inverted SINEs and ADAR1 dependency. *Nature.* 588(7836):169-173. doi: 10.1038/s41586-020-2844-1.
- [12] Yang Z, Ma J, Han J, 2024, Gut microbiome model predicts response to neoadjuvant immunotherapy plus

- chemoradiotherapy in rectal cancer. *Med.* 5(10):1293-1306.e4. doi: 10.1016/j.medj.2024.07.002.
- [13] Ding W, Mo J, Su Y, 2025, Metabolic reprogramming of tumor-associated macrophages via adenosine-A2AR signaling drives cross-resistance in non-small cell lung cancer. *Drug Resist Update.* 82:101272. doi: 10.1016/j.drug.2025.101272.
- [14] Xie J, Liu M, Deng X, 2024, Gut microbiota reshapes cancer immunotherapy efficacy: Mechanisms and therapeutic strategies. *Imeta.* 3(1):e156. doi: 10.1002/imt2.156.
- [15] Gao J, Gu X, Pang M, 2024, RHC-SNAPSHOT investigators. Risk factors for anastomotic leak and postoperative morbidity after right hemicolectomy for colon cancer: results from a prospective, multi-centre, snapshot study in China. *Br J Surg.* 111(1):znad316. doi: 10.1093/bjs/znad316.
- [16] Wang T T, Zeng H H, Hu T, 2024, Predictive value of pan-immunological inflammation scores for primary resistance to immunotherapy combined with chemotherapy in HER-2-negative advanced gastric cancer. *Journal of Jinzhou Medical University,* 45(06): 31-36. DOI: 10.13847/j.cnki.lnmu.2024.06.002.
- [17] Wu Q, Yang Z, Nie Y, 2014, Multi-drug resistance in cancer chemotherapeutics: mechanisms and lab approaches. *Cancer Lett.* 347(2):159-66. doi: 10.1016/j.canlet.2014.03.013.
- [18] Fan D, Cao Y, Cao M, 2023, Nanomedicine in cancer therapy. *Signal Transduct Target Ther.* 8(1):293. doi: 10.1038/s41392-023-01536-y.
- [19] Ezike T C, Okpala U S, Onoja U L, 2023, Advances in drug delivery systems, challenges and future directions. *Heliyon.* 9(6):e17488. doi: 10.1016/j.heliyon.2023.e17488.
- [20] Wang X Y, Fu S J, Meng X J, 2024, Research Progress on New Methods and Technologies for Analyzing the In Vivo Fate of Nanodrug Delivery Systems. *Progress in Pharmacy,* 48(10): 747-760. DOI: 10.20053/j.issn1001-5094.2024.10.004.
- [21] Zhang L, Wang Y, Karges J, 2023, Tetrahedral DNA Nanostructure with Interferon Stimulatory DNA Delivers Highly Potent Toxins and Activates the cGAS-STING Pathway for Robust Chemotherapy and Immunotherapy. *Adv Mater.* 35(8):e2210267. doi: 10.1002/adma.202210267.
- [22] Yang Z, Gao D, Zhao J, 2023, Thermal immuno-nanomedicine in cancer. *Nat Rev Clin Oncol.* 20(2):116-134. doi: 10.1038/s41571-022-00717-y.
- [23] Liu Y, Xie Y, Chen Y, 2025, A protease-cleavable liposome for co-delivery of anti-PD-L1 and doxorubicin for colon cancer therapy in mice. *Nat Commun.* 16(1): 2854. doi: 10.1038/s41467-025-57965-6.
- [24] Fan D, Cao Y, Cao M, 2023, Nanomedicine in cancer therapy. *Signal Transduct Target Ther.* 8(1):293. doi: 10.1038/s41392-023-01536-y.
- [25] Yu W D, Sun G, Li J, 2019, Mechanisms and therapeutic potentials of cancer immunotherapy in combination with radiotherapy and/or chemotherapy. *Cancer Lett.* 452: 66-70. doi: 10.1016/j.canlet.2019.02.048.
- [26] Pointer K B, Pitroda S P, Weichselbaum R R, 2022, Radiotherapy and immunotherapy: open questions and future strategies. *Trends Cancer.* 8(1):9-20. doi: 10.1016/j.trecan.2021.10.003.

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# A Study on the Efficacy of Early Application of Azvudine in Viral Myocarditis under the Epidemic

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**Abstract:** *Objective:* To explore the efficacy of early application of Azvudine in patients with viral myocarditis during the epidemic. *Methods:* Sixty patients diagnosed with “infection complicated with myocarditis” in our hospital from December 2022 to December 2023 were selected and randomly divided into an intervention group and a control group, with 30 cases in each group. The intervention group was treated with 5mg of Azvudine tablets every day on the basis of conventional treatment for one consecutive week. The control group was given conventional antiviral and myocardial nutrition treatments. Compare the changes of serum myocardial injury markers (myoglobin, cardiac troponin I, CK-MB) and cardiac function indicators between the two groups of patients. *Result:* The improvement amplitudes of myoglobin, troponin I, CK-MB concentrations and EF (ejection fraction) in the intervention group were significantly greater than those in the control group ( $p < 0.05$ ). There was no significant difference in the baseline data at admission between the two groups ( $p > 0.05$ ), indicating that the conditions of the two groups were similar before treatment. *Conclusion:* Early application of Azvudine can significantly improve myocardial injury markers and cardiac function in patients with viral myocarditis, reduce the conversion rate from mild to severe cases, and shorten the length of hospital stay, which has important clinical significance.

**Keywords:** Viral myocarditis; Azvudine Markers of myocardial injury; Clinical efficacy

**Online publication:** September 26, 2025

## 1. Introduction

Since the end of 2019, a new type of respiratory infectious disease has spread rapidly around the world, posing a severe challenge to the public health system. Although this disease usually presents with mild to moderate respiratory symptoms, clinical observations have found that some infected individuals may suffer from severe cardiovascular system damage, with viral myocarditis being the most typical<sup>[1-4]</sup>. Viral myocarditis is an inflammatory response of myocardial tissue caused by infection with specific pathogens. During the progression of the disease, it may induce life-threatening complications such as heart failure and severe arrhythmia. In response to this clinical challenge, exploring effective intervention measures to improve the prognosis of patients with myocarditis, reduce mortality and the incidence of complications has become an important direction of current medical research.

Azvudine is a broad-spectrum RNA virus inhibitor independently developed in China and has been approved for the treatment of adult patients with common type COVID-19. It reduces the risk of myocarditis by inhibiting viral RNA polymerase and decreasing viral replication<sup>[5-8]</sup>. This study aims to explore the efficacy of early application of Azvudine in patients with viral myocarditis.

## 2. Materials and methods

### 2.1. Research Object

Sixty patients diagnosed with “infection complicated with myocarditis” in our hospital from December 2022 to December 2023 were selected, aged between 18 and 75 years old. All patients were confirmed as infected through PCR throat swab testing and were diagnosed with myocarditis according to the diagnostic criteria of the third edition of the eight-year Internal medicine textbook. Exclusion criteria include acute or chronic renal failure, age under 18 years or over 75 years, and the patient or their family members’ disagreement with the application of Azvudine treatment<sup>[1]</sup>.

### 2.2. Research Methods

Sixty patients were randomly divided into an intervention group and a control group, with 30 cases in each group. The control group was given conventional antiviral treatment (0.5g of ribavirin injection added to 250ml of solution for intravenous drip for QD) and myocardial nutrition treatment (20mg of trimetazidine tablets Tid, 1.0g of levocarnitine injection for intravenous drip for Qd), which was used continuously for one week. The intervention group discontinued the ribavirin injection on the basis of the treatment in the control group and switched to Azvudine tablets at a dose of 5mg per week for one consecutive week.

### 2.3. Observation Indicators

The changes of serum myocardial injury markers (myoglobin, cardiac troponin I, CK-MB) and cardiac function indicators (EF) in the two groups of patients were compared. The endpoint of observation was the mortality rate of the two groups and the conversion rate from severe cases to mild cases.

### 2.4. Statistical Analysis

Data analysis was conducted using SPSS 22 software. The non-parametric test k-check method was used for skewed data, and the t-test or F-test was used for normally distributed data. A p value <0.05 was considered statistically significant.

## 3. Results

### 3.1. General Information Comparison

Age distribution: In the control group, the minimum age was 20 years old, the maximum age was 73 years old, the average age was 48.83 years old, and the standard deviation was 3.28. The minimum age of the intervention group was 20 years old, the maximum age was 75 years old, the average age was 50.27 years old, and the standard deviation was 3.29. The ages of the two groups followed a normal distribution<sup>[2]</sup>. SPSS 22 independent sample t-test was applied:  $T=-0.308$ ,  $p=0.836>0.05$ , suggesting that there was no statistically significant difference in age between the control group and the intervention group. Occupational distribution In the control group, there were 7 medical staff, 6 farmers, 7 teachers, 8 workers and 2 staff members. In the intervention group, there were 2 medical staff, 7 farmers, 10 teachers, 8 workers and 3 staff members.  $X^2=3.58$ , degree of freedom  $df=4$ , significance level  $\alpha=0.05$ , chi-square critical value  $9.488>3.58$ ,  $p=0.46>0.05$ . There was no statistical difference in the distribution of occupations.

### 3.2. Changes in Myocardial Injury Markers

In the control group, the average myoglobin concentration was 85.23, the average troponin concentration was 1.56, the average CKMB concentration was 80.34, and the average left ventricular ejection fraction (LVEF) was 50.12% at admission. The average myoglobin concentration of the intervention group at admission was 90.12, the average troponin concentration was 1.62, the average CKMB was 85.67, and the average left ventricular ejection fraction (LVEF) was 48.56%. The paired sample t-test was used. The mean difference in myoglobin concentration between the two groups at admission was -4.89,  $t=-1.23$ ,  $p=0.224>0.05$ . The mean difference in troponin concentration was -0.06,  $t=-0.45$ ,

$p=0.654>0.05$ . The mean difference in CKMB was -5.33.  $T = 1.56$ ,  $p = 0.124 > 0.05$ , LVEF mean difference 1.56,  $t = 0.78$ ,  $p = 0.438 > 0.05$ . It was indicated that there were no significant differences in myoglobin concentration, troponin concentration, CKMB concentration and EF between the control group and the intervention group at admission (see **Table 1**).

**Table 1.** Comparison of myocardial injury markers and EF detection results between the control group and the intervention group at admission

Indicator	The mean value of the control group at admission	The mean value of the intervention group at admission	Mean difference	t-value	p-value
Myoglobin concentration	85.23	90.12	-4.89	-1.23	0.224
Troponin concentration	1.56	1.62	-0.06	-0.45	0.654
CKMB concentration	80.34	85.67	-5.33	-1.56	0.124
EF(Ejection Fraction)	50.12	48.56	1.56	0.78	0.438

After treatment (see **Table 2**), in the control group, the mean myoglobin concentration was 60.45, troponin concentration was 0.98, CKMB concentration was 60.12, and EF was 55.34% at discharge. All P values were  $<0.05$ , suggesting statistically significant differences before and after treatment. The myoglobin concentration, troponin concentration, and CKMB concentration of the intervention group at discharge were 50.34, EF was 60.12%, and the P values were all  $<0.001$ , suggesting that there were statistically significant differences in myocardial marker concentrations before and after treatment in the intervention group, but the improvement was greater than that in the control group<sup>[3]</sup>.

**Table 2.** Comparison of myocardial injury markers and EF detection results between the control group and the intervention group before and after treatment

Indicator	Group	Mean value at admission	Mean value at discharge	Mean difference	t-value	p-value
Myoglobin concentration	Control group	85.23	60.45	24.78	3.45	0.002
	Intervention group	90.12	50.34	39.78	4.56	$<0.001$
Troponin concentration	Control group	1.56	0.98	0.58	2.89	0.007
	Intervention group	1.62	0.75	0.87	3.78	$<0.001$
CKMB concentration	Control group	80.34	60.12	20.22	3.12	0.004
	Intervention group	85.67	55.34	30.33	4.23	$<0.001$
EF(Ejection Fraction)	Control group	50.12	55.34	-5.22	-2.34	0.026
	Intervention group	48.56	60.12	-11.56	-4.56	$<0.001$

However, independent sample t-tests were conducted on the relevant data of the two groups of people after treatment. Myoglobin concentration, troponin concentration, CKMB, EF, etc. were compared respectively after treatment. The specific data are shown in **Table 3**. After treatment, it was indicated that the myoglobin concentration in the intervention group decreased by an average of 8.24 compared with the control group,  $P = 0.596 > 0.05$ , the troponin concentration decreased by an average of 0.61,  $P = 0.0001 < 0.05$ , CKMB decreased by an average of 3.41,  $P = 0.208 > 0.05$ , and EF increased by an average of 2.23%,  $P = 0.369$ . It can be seen from this that the decrease in troponin concentration in the intervention group at discharge was statistically different from that in the control group<sup>[4]</sup>. However, no significant statistical differences were observed in indicators such as myoglobin concentration, CKMB, and EF, but the intervention



group showed a better improvement trend compared to the control group.

**Table 3.** Comparison of myocardial injury markers and EF detection results between the two groups at discharge

		Average	Standard deviation	Average standard error	Lower limit of 95% (CI)	Upper limit of 95% (CI)	T value	Df	P value
Group 1	Control group - Intervention Group (myoglobin at discharge)	8.235	84.123	15.358	-23.176	39.647	0.536	29	0.596
Group 2	Control group - Intervention Group (troponin at discharge)	0.613	0.532	0.097	0.414	0.811	6.308	29	0.000
Group 3	Control group - Intervention Group (CKMB at discharge)	3.411	14.515	2.650	-2.009	8.831	1.287	29	0.208
Group 4	Control group - Intervention Group (EF at discharge)	-2.232	13.413	2.449	-7.241	2.776	-0.912	29	0.369

### 3.3. Mortality rate and conversion rate of severe cases to mild cases

Among the enrolled population in this study, no deaths occurred. In the control group, there were 15 severe cases upon admission and 12 severe cases after one week of treatment. In the intervention group, there were 16 severe cases upon admission, and 10 severe cases after one week of treatment. Before treatment, the number of severe cases in the two groups was tested by chi-square test, with  $X^2=0.067$  and  $P=0.5>0.05$ , suggesting no statistical difference<sup>[5]</sup>. After one week of treatment, the remaining number of severe cases in both groups was tested by chi-square test, with  $X^2=0.287$  and  $P=0.395>0.05$ , suggesting that there was still no statistical difference in the remaining number of severe cases between the control group and the intervention group after one week of treatment. Although the results of this chi-square test showed no statistically significant difference in the number of severe cases between the two groups before treatment and one week after treatment, in terms of absolute numbers, the number of severe cases in the intervention group decreased from 16 to 10, a reduction of 6 cases. The number of severe cases in the control group decreased from 15 to 12, a reduction of 3 cases. The reduction in the number of severe cases in the intervention group was relatively greater than that in the control group, suggesting that the intervention group might have a better therapeutic effect. However, more in-depth research is still needed, such as further expanding the sample size and extending the observation period<sup>[6]</sup>.

## 4. Discussion

The results of this study indicated that both the control group and the intervention group had good therapeutic effects on myocardial injury markers and left ventricular ejection fraction (LVEF) before and after treatment, and no deaths occurred in either group. Early application of Azvudine has a more significant effect on improving myocardial injury markers and cardiac function in patients with viral myocarditis. Although there was no significant statistical difference between the two groups in terms of improving the conversion rate of severe cases to mild ones, a better therapeutic effect could be found in the intervention group that applied Azvudine in the early stage<sup>[7]</sup>. Azvudine reduces the risk of myocarditis by inhibiting viral RNA polymerase and reducing viral replication. In addition, Azvudine has a significant anti-inflammatory effect, which can effectively inhibit the release of inflammatory factors and alleviate myocardial damage. Perhaps due to the influence of sample size, the comparison of relevant data did not show a significant difference, but there was a statistical difference in improving the concentration of troponin in patients. It is expected that a significant difference in the therapeutic effect between the two groups can be observed after future studies with an expanded sample size<sup>[8]</sup>.

## 5. Conclusion

Early application of Azvudine has obvious clinical efficacy in patients with viral myocarditis. It can improve myocardial injury markers and cardiac function (LVEF), and there is a statistically significant difference in reducing troponin concentration. It also shows obvious advantages in improving the conversion rate from severe to mild cases, which is worthy of clinical promotion and application.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Fu P, Cao P, 2025, Risk Factors and Changes of Serum IL-6, IL-17, and TGF- $\beta$  in Children With Acute Myocarditis. *Clinical pediatrics*, 99228251359448.
- [2] Liu Y, Xu Y, Zhao R, 2025, 1,25-dihydroxyvitamin D deficiency accelerates inflammatory response by up-regulating inositol 1,4,5-triphosphate receptor in Cocksackievirus B3-induced acute myocarditis. *International Immunopharmacology*, 163115256-115256.
- [3] Hu V, Naidu I, Singh N, 2025, Cardiotoxicity Conundrum: Distinguishing Immune Checkpoint Inhibitor Myocarditis From Fluorouracil Cardiotoxicity in Concurrent Therapy. *JACC. Case reports*, 2025, 30(18):104002.
- [4] Łukasz N, Anna D, Jakub M, 2025, From mononucleosis to heart transplantation: An uncommon course of Epstein-Barr virus-associated myocarditis. *Kardiologia polska*.
- [5] Blagova V O, Kogan A E, Novosadov M V, 2025, Post-COVID Versus Non-COVID Myocarditis: Comparison of Morphological Activity, Toll-like Receptor Distribution and Responses to Immunosuppressive Therapy. *Frontiers in bioscience (Scholar edition)*, 17(2):28262.
- [6] Sacco A M, Gualtieri S, Verrina C M, 2025, A Narrative Overview of Fatal Myocarditis in Infant with Focus on Sudden Unexpected Death and Forensic Implications. *Journal of Clinical Medicine*, 14(12):4340-4340.
- [7] Ren Z, Luo H, Yu Z, 2020, A Randomized, Open-Label, Controlled Clinical Trial of Azvudine Tablets in the Treatment of Mild and Common COVID-19, a Pilot Study. *Adv Sci (Weinh)*, 7(19):e2001435.
- [8] Wang R R, Yang Q H, Luo R H, 2014, Azvudine, a novel nucleoside reverse transcriptase inhibitor showed good drug combination features and better inhibition on drug-resistant strains than lamivudine in vitro. *PLoS One*, 9(8):e105617.

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# Effect Observation of Combination Therapy with Veglitin Tablets and Metformin Hydrochloride for Patients with Type 2 Diabetic Nephropathy

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**Abstract:** This study analyzed the efficacy of the combination therapy of Veglitinide Tablets and Metformin Hydrochloride Tablets in treating type 2 diabetic nephropathy. A total of 80 patients admitted to our hospital between January 2024 and January 2025 were enrolled. Using a randomized block design, the patients were divided into two groups for prospective analysis. The control group received Metformin Hydrochloride Tablets, while the observation group received the combined therapy of Veglitinide Tablets and Metformin Hydrochloride Tablets. The improvements in blood glucose levels and renal function were compared between the groups. Post-treatment analysis showed that the observation group exhibited lower fasting blood glucose and 2-hour postprandial glucose levels ( $P < 0.05$ ), with significantly reduced serum creatinine and blood urea nitrogen levels ( $P < 0.05$ ). The combined therapy demonstrated significant therapeutic effects in type 2 diabetic nephropathy patients, effectively regulating both blood glucose and renal function.

**Keywords:** Veglitin tablets; Metformin hydrochloride tablets; Type 2 diabetic nephropathy

**Online publication:** September 26, 2025

## 1. Introduction

Diabetic nephropathy, a subtle-onset and slowly progressive microvascular complication of diabetes, is associated with impaired glucose metabolism. Metformin, the first-line antidiabetic medication in clinical practice, effectively improves blood glucose levels and insulin resistance<sup>[1]</sup>. Veglitinib enhances incretin action to stimulate insulin secretion from pancreatic  $\beta$ -cells, thereby achieving blood glucose regulation while potentially exerting renal protective effects<sup>[2]</sup>. This study aims to evaluate the combined therapeutic regimen of veglitinib and metformin on renal function indicators and metabolic parameters in diabetic nephropathy patients.

## 2. Data and methods

### 2.1. General information

This study enrolled 80 patients with type 2 diabetic nephropathy treated at our hospital, with admission dates from January 2024 to January 2025. Participants were randomly assigned into two groups using a randomized number table method, all voluntarily enrolled in the study for prospective analysis. The control group consisted of 40 patients (22 males, 18 females), aged 35-76 years with an average age of  $(49.88 \pm 3.22)$  years. The comparison between groups showed no significant difference in baseline data ( $P > 0.05$ ). The study was approved by the hospital's ethics committee.

Inclusion criteria: (1) the comprehensive clinical diagnosis of hematology and other clinical diagnosis is consistent with the diagnostic criteria of type 2 diabetic nephropathy in the Expert Consensus on Prevention and Treatment of Diabetic Nephropathy; (2) the treatment drugs involved in this study are tolerated; (3) complete clinical data.

Exclusion criteria: (1) patients with mental illness; (2) patients who have recently received glucocorticoid therapy; (3) patients with acute renal failure; (4) patients with acute metabolic complications.

### 2.2. Methodology

In the control group, metformin tablets (manufacturer: Shanghai Shangyao Xinyi Pharmaceutical Co., LTD. National Drug Approval No. H31021130, specification: 0.25g) were taken orally with meals once a day in the morning and evening, and each dose was 0.25g.

In the observation group, Veglitin tablets (manufacturer: Nanjing Youke Pharmaceutical Co., LTD., National Drug Approval No. H20203334, specification: 50mg) were added once in the morning and once in the evening on the basis of the control group treatment, with each dose of 50mg.

All patients received treatment for six months.

### 2.3. Observation indicators

The improvement of blood glucose (postprandial 2h blood glucose and fasting blood glucose) before and after treatment was compared between the two groups.

The improvement of renal function (blood creatinine and urea nitrogen) before and after treatment was compared between the two groups.

All patients were given 3mL venous blood in a fasting state before and after treatment. After centrifugation, the indexes were measured by an automatic biochemical analyzer<sup>[3]</sup>.

### 2.4. Statistical processing

Statistical analysis was calculated by SPSS 27.0 software, with  $n$  (%) for count  $\bar{x} \pm s$  data and  $(\bar{x} \pm s)$  for measurement data. The intergroups were respectively tested by ( $\chi^2$  test and  $t$  test,  $P < 0.05$  was statistically significant).

## 3. Results

### 3.1. Comparison of blood glucose level differences between the two groups

Before treatment, the values of fasting blood glucose and 2h postprandial blood glucose in the two groups were compared ( $P > 0.05$ ); after treatment, the values of fasting blood glucose and 2h postprandial blood glucose in the two groups were compared, and the values of the observation group were lower ( $P < 0.05$ ). Details are shown in **Table 1**.

**Table 1.** Comparison of blood glucose level difference between the two groups (mmol/L)

group	fasting blood-glucose		H2GPA	
	pretherapy	post-treatment	pretherapy	post-treatment
Control group (n = 40)	9.62±0.72	8.79±0.55	13.66±1.68	11.83±1.55
Observation group (n = 40)	9.66±0.75	7.31±0.36	13.72±1.71	10.24±0.88
<i>t</i>	0.243	14.240	0.158	5.642
<i>P</i>	0.808	0.000	0.875	0.000

### 3.2. Comparison of renal function levels between the two groups

Before treatment, the values of serum creatinine and urea nitrogen in the two groups were compared ( $P>0.05$ ); after treatment, the values of serum creatinine and urea nitrogen in the two groups were compared, and the observed group was lower ( $P<0.05$ ). Details are shown in **Table 2**.

**Table 2.** compares the differences in kidney function between two groups.(mean ± standard deviation)

group	Blood creatinine (umol/L)		Nitrogen in urine (mmol/L)	
	pretherapy	post-treatment	pretherapy	post-treatment
Control group (n = 40)	99.54±6.56	88.43±4.92	7.93±0.54	5.82±0.43
Observation group (n = 40)	99.59±6.61	71.94±3.43	7.96±0.57	4.11±0.28
<i>t</i>	0.034	17.389	0.242	21.077
<i>P</i>	0.973	0.000	0.810	0.000

## 4. Discussion

The treatment of diabetic nephropathy focuses on blood glucose and blood pressure management, reduction of proteinuria, delay of renal deterioration, and provision of necessary nutritional support<sup>[4]</sup>.

This study demonstrated that after treatment, the fasting blood glucose and 2-hour postprandial glucose levels in the observation group were significantly lower than those in the control group ( $P<0.05$ ). Similarly, the observation group showed lower serum creatinine and blood urea nitrogen levels compared to the control group ( $P<0.05$ ). These findings indicate that the combination therapy effectively controlled blood glucose and renal function in patients with the disease. Analysis revealed that metformin exerts its hypoglycemic effects through two synergistic mechanisms: 1) At the hepatic level: By inhibiting gluconeokinase activity and reducing expression of key gluconeogenesis enzymes, it decreases hepatic glucose output<sup>[5]</sup>; 2) At the peripheral tissue level: Through activation of the AMPK signaling pathway and enhancement of insulin receptor substrate phosphorylation, significantly improving glucose transport capacity in muscles and other tissues<sup>[6]</sup>. As a DPP-4 inhibitor, vildagliptin's renal protective effects may involve anti-inflammatory mechanisms (e.g., reducing inflammatory factor levels) and anti-fibrotic pathways (e.g., regulating TGF- $\beta$  signaling). Clinical applications have shown potential value in improving renal function. The combination therapy significantly enhances therapeutic efficacy.

In conclusion, the combined treatment of viglitolin tablets and metformin hydrochloride tablets can effectively improve the blood glucose and renal function of patients with type 2 diabetic nephropathy.



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## Disclosure statement

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## References

- [1] Deng L P, Wu H M, Yuan W W, 2022, Efficacy and safety of Viglinitide and Metformin combination with Daglirenet in treating newly diagnosed type 2 diabetes mellitus patients. *Chinese Journal of Diabetes*, 30(2):116-119.
- [2] Zhou B, Li J, Fang C Y, 2023, Comparison of clinical efficacy of Metformin/Weiglitin and Liraglutide in obese patients with type 2 diabetes mellitus. *Journal of Southern Medical University*, 43(3):436-442.
- [3] Zhang X J, Zhang J, Lou P P, 2022, Efficacy of Veglitinib combined with Metformin in the Treatment of First-Visit Patients with Type 2 Diabetes Mellitus and Abdominal Obesity and Its Effects on Serum Kisspeptin. *Drug Evaluation Research*, 45(7):1355-1360.
- [4] Cui X Y, 2022, Observation on the efficacy of dapagliflozin combined with metformin in treating type 2 diabetic nephropathy. *Journal of Xinxiang Medical University*, 39(4):362-366.
- [5] Cao Y J, Zhang Z, Li X F, 2022, The effect of Veglitinide-assisted metformin treatment on glucose control and pancreatic  $\beta$ -cell function in newly diagnosed type 2 diabetes patients. *Right River Medical Journal*, 50(1):62-66.
- [6] Zhang T R, You X H, Pan J G, 2021, Clinical effects of Veglitinib combined with metformin in the treatment of type 2 diabetes mellitus and analysis of its effects on FPG, INS, and 2hPG levels. *Chinese Community Physician*, 37(9):77-78.

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# Treatment, Intervention and Improvement of Psychosocial Adaptation in Patients with Chronic Urticaria —— Empirical Evidence from China

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**Abstract:** *Objective:* This study aimed to evaluate the effectiveness of a comprehensive intervention in improving biological indicators and psychosocial adaptation in patients with chronic urticaria. *Methods:* One hundred patients with chronic urticaria were randomly assigned to an experimental group and a control group. All patients received standard physiological interventions, while the experimental group underwent and assessed standardized psychosocial support measures. Key outcomes included serum IgE and high-sensitivity C-reactive protein (hs-CRP) levels, as well as psychosocial adaptation assessed using the PASQ scale. *Results:* After two months of follow-up, the experimental group showed a significant reduction in post-treatment IgE levels to  $(30.95 \pm 5.77)$  IU/mL, significantly lower than the control group's  $(43.42 \pm 5.94)$  IU/mL ( $t = 9.71$ ,  $p = 0.001$ ). Similarly, the experimental group's hs-CRP levels also decreased significantly to  $(9.62 \pm 2.13)$  mg/L, lower than the control group's  $(12.12 \pm 2.38)$  mg/L ( $t = 5.04$ ,  $p = 0.001$ ). Psychosocial function evaluations revealed that the experimental group scored significantly higher than the control group in emotional dimension ( $37.84 \pm 2.48$ ), self-perception dimension ( $28.40 \pm 1.62$ ), and social dimension ( $18.92 \pm 1.18$ ) after treatment ( $p < 0.001$ ). *Conclusion:* This intervention demonstrated significantly better outcomes than conventional approaches in regulating allergic immune responses, managing systemic inflammation, and enhancing psychosocial resilience among patients with chronic urticaria. These findings underscore the value of implementing comprehensive strategies in chronic urticaria management, highlighting the critical importance of integrating biological, psychological, and social dimensions in therapeutic approaches.

**Keywords:** chronic urticaria; psychosocial adaptation; comprehensive intervention; IgE; hs-CRP

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## 1. Foreword

Chronic urticaria (CU) is a skin condition characterized by persistent or recurrent itchy wheals and/or angioedema, with an estimated prevalence of 0.5%-5%<sup>[1]</sup>. Chronic spontaneous urticaria (CSU), in particular, is more prevalent among women (accounting for approximately 72%)<sup>[2]</sup>. Patients often experience high rates of comorbidities such as anxiety and depression<sup>[1]</sup>, along with significantly compromised quality of life. Psychosocial factors are closely associated with the onset, progression, and prognosis of cutaneous urticaria (CU), forming a vicious cycle of "stress-triggered symptom exacerbation leading to increased stress"<sup>[2-4]</sup>. This relationship manifests in two key aspects: Physiological symptoms such as itching and skin lesions directly contribute to psychological distress and social impairment<sup>[5]</sup>, while negative emotions including psychological stress, anxiety, and depression can act through neuro-immunohormonal networks to exert adverse

effects on the skin. These interactions activate mast cells and worsen symptoms<sup>[4,6]</sup>. The status of psychological and social adaptation not only affects treatment compliance, but also determines whether patients can effectively manage the disease. For example, maladaptation leads to more frequent and severe symptoms<sup>[3]</sup>, while positive coping strategies and good social support can significantly improve quality of life<sup>[5,7]</sup>. Current primary intervention approaches include the comprehensive biopsychosocial treatment model<sup>[7]</sup>, such as cognitive behavioral therapy (CBT) to help patients modify maladaptive thoughts and behaviors, relaxation training and mindfulness-based stress reduction techniques to lower stress levels, psychological care providing emotional support, and combined pharmacological and psychotherapeutic interventions. These should also be integrated with non-pharmacological supports like stress management education and social support systems<sup>[6,8]</sup>. However, the current research still has limitations, such as small sample sizes and lack of standardized intervention protocols<sup>[1,8]</sup>. Insufficient multidisciplinary collaboration often leads to overlooked psychological issues, while existing evaluation systems focus solely on short-term mood improvement, lacking systematic studies on long-term behavioral changes, disease recurrence rates, and cost-effectiveness. This study now examines standardized intervention protocols and their clinical applications, along with the evaluation of intervention effectiveness, as detailed below:

## 2. Data and methodology

### 2.1. General information

This study enrolled 100 chronic urticaria patients admitted at our hospital from June 2024 to March 2025, randomly divided into experimental and control groups of 50 cases each using a random number table method. The experimental group included 17 males and 33 females with an average age of  $17.55 \pm 8.30$  years, comprising 38 cases with high school education or below and 12 cases with college education or above. The control group consisted of 16 males and 34 females with an average age of  $17.47 \pm 7.60$  years, including 37 cases with high school education or below and 13 cases with college education or above. Comparative analysis of general patient data showed no statistically significant differences ( $P > 0.05$ ), confirming comparability between groups. The study was approved by the Medical Ethics Committee of Chongqing First People's Hospital and conducted with informed consent from all participants.

### 2.2. Inclusion and exclusion criteria

Inclusion criteria: ① Patients diagnosed with chronic urticaria lasting over 6 weeks; ② Adults aged 18 to 65 years; ③ With basic communication and comprehension abilities; ④ Voluntarily participating in the study and signing an informed consent form. Exclusion criteria: ① Patients with severe mental disorders or psychological conditions; ② Patients with serious physical illnesses affecting psychosocial adaptation; ③ Patients experiencing acute flare-ups or severe allergic reactions; ④ Patients unable to cooperate with study assessments or follow-up visits.

### 2.3. Intervention methods

The study evaluated the efficacy of identical physiological interventions across different groups. Patients received omalizumab (brand name: Enyitan®, administered as an injection by Jushi Biopharmaceutical Co., Ltd., a subsidiary of CSPC Group) at 300mg per dose per month. Dosage adjustments were made as prescribed for patients with higher body weights, with follow-up monitoring lasting two months. Notably, the control group comprised 1,000 patients randomly divided into two subgroups. Following standard clinical protocols, on-duty nurses assessed newly admitted dermatology patients, developed personalized care plans, and implemented treatment protocols. Health education was delivered through bulletin boards to all patients.

For patients in the experimental group, a CNP (Clinical Nursing Plan) working group was first established to conduct centralized training for nurses across departments, enhancing their understanding of clinical pathways. By referencing standardized dermatology care protocols from both domestic and international sources, reviewing relevant literature, and incorporating successful experiences of the CNP model in other departments, a customized CNP management form was

developed for dermatology inpatients after comprehensive analysis and evaluation. Upon diagnosis and admission to the dermatology department, the head nurse and assigned nurses collaboratively formulated a nursing plan within 24 hours, refined necessary assessments, and developed personalized CNP forms through discussions addressing patients' health issues and practical clinical needs. The form organizes care activities around time-based frameworks and nursing content, systematically implementing assessments, dietary guidance, basic care protocols, and health education tailored to patients' temporal movement patterns and social adaptation requirements.

Responsible nurses must conduct detailed patient assessments according to the basic requirements of the CNP form each week, implement corresponding nursing measures as scheduled, document special circumstances, and promptly complete shift handovers. During the implementation of the CNP pathway, nurses should communicate disease awareness based on the CNP nursing pathway, thoroughly explaining admission guidelines including disease mechanisms, treatment methods, diagnostic tests, and precautions. For discharge guidance, responsible nurses assess patients' actual conditions and recovery status, sign the CNP form, and guide patients to fully understand the educational content through repeated evaluations and health assessments.

## **2.4. Observation indicators and evaluation criteria**

### **2.4.1. Laboratory indicators**

At baseline, 1-month post-treatment, and 3-month post-treatment, 4mL of venous blood was collected from the patient's elbow. The blood was centrifuged at 4°C, 3800 rpm, and a 13.5cm radius for 15 minutes to obtain the supernatant. Serum IgE levels were measured using the immunoturbidimetric method, while high-sensitivity C-reactive protein (hs-CRP) levels were assessed through the immunoscattering turbidimetric method.

### **2.4.2. Effectiveness evaluation**

The Urticaria Control Score (UCT) is a self-reported tool for evaluating disease control in chronic urticaria patients. It consists of four questions assessing symptom severity, quality-of-life impact, treatment effectiveness, and overall control perception over the past four weeks. Each question scores 0-4 points, with total scores ranging from 0 to 16. A total score  $\geq 12$  indicates effective disease control, while  $< 12$  points suggest inadequate management requiring treatment adjustments. The tool's validity has been validated through multiple dimensions including internal consistency, negative correlation with the Disease Activity Score (UAS), and clinical response sensitivity.

### **2.4.3. Assessment of psychosocial adaptation of patients**

To assess and track patients' psychosocial adaptation, the authors employed the self-administered Chinese-adapted PASQ-CSD scale<sup>[9]</sup>. This instrument was developed using the Brislin translation model for bilingual comparison and back-translation, with cultural adaptations incorporating expert input. For instance, the item "I still consider myself attractive" was revised to "I am certain I have not been socially excluded after illness." The scale comprises 18 items across three dimensions: emotional well-being (8 items), self-perception (6 items), and social adaptation (4 items). Responses are scored using a 5-point Likert scale (always/often/sometimes/rarely/never), with higher total scores indicating stronger psychosocial adaptation<sup>[1]</sup>. Exploratory factor analysis confirmed its structural validity (KMO=0.848, cumulative variance contribution rate 65.142%), and the Cronbach's  $\alpha$  coefficient reached 0.930, demonstrating excellent internal consistency. Score changes reflected intervention effectiveness. For example, significant improvement in emotional dimension scores post-intervention (e.g., from 20 to 40 points) suggested that psychological support measures were effective.

## **2.5. Statistical methods**

The data were analyzed using statistical software SPSS 19.0. Categorical data were presented as  $n$  (%) and analyzed using the  $\chi^2$  test. Measurement data were expressed as mean  $\pm$  standard deviation and analyzed using the  $t$  test. A  $P < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Patient baseline data

This study enrolled 100 patients. Baseline data showed: The majority were female (55%, n=55), with males accounting for 45% (n=45). Educational background was predominantly university-educated (51%, n=51), followed by high school (26%) and primary school (13%). Master's and doctoral degrees accounted for 7% and 1% respectively, with 2% having technical education. Marital status showed 69% married, 7% unmarried, 4% divorced, and none reported widowhood. Economic status included 39% self-rated financially acceptable, 15% comfortable, and 3% in financial difficulty. Employment status was 69% employed, 11% unemployed, 3% students, and 2% retired. Comorbidities included 27% other allergies, 12% allergic rhinitis, 10% asthma, 7% hypertension, 5% thyroid disorders, and 2% systemic lupus erythematosus (SLE). Diabetes and inflammatory bowel disease each accounted for 1%. Clinical manifestations most commonly included erythema, wheals, and/or pruritus (46%), followed by pruritic erythematous wheals with dyspnea (18%) and pruritic erythematous wheals with angioedema (24%), while isolated angioedema was rare (1%). Common triggers were pollen (35%), unknown causes (23%), contact allergens (17%), food allergies (15%), and dust (10%). Other factors like medications, infections, animal bites, and insect stings had lower incidence rates, all under 6%. The overall sample is characterized by women with secondary education or above and married working women, who have a heavy burden of allergic diseases and diverse clinical manifestations. The main causes are environmental exposure and allergen exposure.

**Table 1.** Baseline characteristics of patients

feature	class	n(%)
sex	the male sex	45(45%)
	femininity	55(55%)
education level	Primary school education	13(13%)
	high school degree	26(26%)
	University qualifications	51(51%)
	master's degree	7(7%)
	doctor's degree	1(1%)
	technical education	2(2%)
marriage condition	unmarried	7(7%)
	married	69(69%)
	bereft of one's spouse	0(0%)
	dissociation	4(4%)
economy condition	Financially comfortable	15(15%)
	Economic conditions are acceptable	39(39%)
	Economic hardship	3(3%)
work state	Students enrolled	3(3%)
	lose one's job	11(11%)
	be on the job	69(69%)
	retire	2(2%)



**Table 1 (Continued)**

feature	class	n(%)
compl	rhinallergosis	12(12%)
	asthma	10(10%)
	Other allergies	27(27%)
	inflammatory bowel disease	1(1%)
	diabetes mellitus	1(1%)
	hypertension	7(7%)
	thyroid disease	5(5%)
	systemic lupus erythematosus	2(2%)
symptom expression	Redness, wheals and/or itching	46(46%)
	angio-edema	1(1%)
	Erythema multiforme and angioedema	5(5%)
	Itchy erythema with angioedema	24(24%)
	Itchy erythema with wheals and dyspnea	18(18%)
bring out factor	Cause unknown	23(23%)
	Food allergies (including food additives)	15(15%)
	medicinal	6(6%)
	Infection (virus, bacteria, fungi)	1(1%)
	Contactual triggers	17(17%)
	animal	1(1%)
	pollen	35(35%)
	Insect bites	1(1%)
	sunlight	2(2%)
	high temperature	2(2%)
	cold	1(1%)
	pressure	1(1%)
	emotional stress	2(2%)
	dirt	10(10%)
	perspire	2(2%)
	movement	2(2%)

### 3.2. Comparison of laboratory indicators

This study conducted an inter-group comparison of serum IgE and high-sensitivity C-reactive protein (hs-CRP) levels in chronic urticaria patients before and after treatment. The results showed that the pre-treatment IgE levels in the experimental group and control group were  $(107.30 \pm 19.61)$  IU/mL and  $(110.40 \pm 17.81)$  IU/mL, respectively, while hs-CRP levels were  $(15.29 \pm 3.56)$  mg/L and  $(14.60 \pm 3.81)$  mg/L. No statistically significant differences were observed between the groups ( $t = 0.753$ ,  $p = 0.409$ ;  $t = 0.845$ ,  $p = 0.357$ ), indicating good baseline comparability. After treatment, both IgE and hs-CRP levels decreased significantly in both groups ( $p < 0.05$ ). However, the experimental group showed

more pronounced reductions: IgE levels dropped to ( $30.95 \pm 5.77$ ) IU/mL, significantly lower than the control group's ( $43.42 \pm 5.94$ ) IU/mL ( $t = 9.71$ ,  $p = 0.001$ ); hs-CRP levels in the experimental group decreased to ( $9.62 \pm 2.13$ ) mg/L, also significantly lower than the control group's ( $12.12 \pm 2.38$ ) mg/L ( $t = 5.04$ ,  $p = 0.001$ ). The results showed that under the action of the intervention, the experimental group was significantly better than the control group in reducing IgE and hs-CRP levels, suggesting that it may be more effective in regulating allergic immune response and systemic inflammatory status.

**Table 2.** Comparison results of laboratory indicators

group	IgE/ (IU/mL) before treatment	IgE/ (IU/mL) after treatment	hs-CRP/ (mg/L) before treatment	hs-CRP/(mg/L) Treatment after
Experimental group (n=50)	107.30±19.61	30.95±5.77	15.29±3.56	9.62±2.13
Control group (n = 50)	110.40±17.81	43.42±5.94	14.60±3.81	12.12±2.38
t price	0.753	9.71	0.845	5.04
p price	0.409	0.001	0.357	0.001

Note:  $P < 0.05$  was considered significant compared with pretreatment and marked with \*

### 3.3. Comparison of patients' psychosocial adaptation

This study employed the PASQ scale, which underwent item selection and validation, to assess psychosocial adaptation in chronic urticaria patients. The scale comprises three dimensions: emotional (8 items), self-perception (6 items), and social (4 items), using a 5-point Likert scale where higher scores indicate better psychosocial adaptation. Results showed that pre-treatment scores across all dimensions were nearly identical between groups, with no statistically significant differences (emotional dimension:  $t = 1.44$ ,  $p = 0.44$ ; self-perception dimension:  $t = 1.95$ ,  $p = 0.31$ ; social dimension:  $t = 3.09$ ,  $p = 0.68$ ), indicating comparable baseline conditions. Post-treatment, both groups demonstrated time-dependent improvement across all three dimensions, though the experimental group showed more significant progress. At 3-month follow-up, the experimental group achieved an emotional dimension score of ( $37.84 \pm 2.48$ ), significantly higher than the control group's ( $34.24 \pm 3.88$ ) ( $t = 1.24$ ,  $p < 0.001$ ). The self-perception dimension reached ( $28.40 \pm 1.62$ ), surpassing the control group's ( $25.84 \pm 2.88$ ) ( $t = 1.90$ ,  $p < 0.001$ ), while the social dimension showed a substantial improvement to ( $18.92 \pm 1.18$ ), markedly higher than the control group's ( $17.20 \pm 1.96$ ) ( $t = 4.57$ ,  $p < 0.001$ ). These differences demonstrated highly statistically significant group variations. The results showed that the long-term intervention of the experimental group was significantly better than the control group in promoting patients' emotional regulation, self-cognitive reconstruction and social function adaptation, indicating that the intervention strategy could improve the psychosocial adaptability of chronic urticaria patients more effectively, especially in the medium and long term efficacy.

**Table 3.** Comparison Results of Patients' Psychosocial Adaptation Status

group	Emotional dimension (Emotional)			Self-cognitive dimension (Self-cognitive)			Social dimension (Social)		
	pretherapy	Treatment after 1m	Treatment after 3m	pretherapy	Treatment after 1m	Treatment after 3m	pretherapy	Treatment after 1m	Treatment after 3m
Experimental group (n=50)	26.82±4.18	34.24±3.92	37.84±2.48	20.16±3.52	25.42±2.94	28.40±1.62	13.38±2.56	17.04±1.82	18.92±1.18
Control group (n = 50)	26.96±4.04	33.60±4.08	34.24±3.88	19.84±3.64	24.80±3.24	25.84±2.88	13.22±2.74	16.80±2.12	17.20±1.96
t price	1.44	1.15	1.24	1.95	0.85	1.9	3.09	2.32	4.57
p price	0.44	0.12	<0.001	0.31	0.07	<0.001	0.68	0.16	<0.001

## 4. Discussion

This study analyzed baseline data, laboratory indicators, and psychosocial adaptation status of 100 chronic urticaria patients, revealing that the experimental group demonstrated significantly better outcomes in reducing serum IgE and hs-CRP levels while improving psychosocial adaptability compared to the control group. These findings indicate that the intervention measures not only effectively regulate allergic immune responses and systemic inflammatory states but also enhance patients' emotional regulation, self-perception, and social functioning, thereby comprehensively improving their quality of life. The results validate the effectiveness of intervention strategies in chronic urticaria management, particularly demonstrating significant advantages in medium-to-long-term efficacy. This provides crucial evidence for clinical practice, suggesting that comprehensive interventions should be prioritized during treatment—not only addressing physical symptom relief but also emphasizing psychosocial factors influencing disease recovery. Additionally, the research data offer theoretical support for developing more personalized care plans and further exploring the pathogenesis of chronic urticaria.

The findings of this study clearly demonstrate that the intervention measures adopted in the experimental group significantly outperformed the control group in improving key biological indicators and psychosocial adaptability in patients with chronic urticaria. Specifically, the experimental group not only more effectively reduced serum IgE levels (indicating allergic status) and high-sensitivity C-reactive protein (hs-CRP) levels (signifying systemic inflammation), but also showed significant improvements in psychosocial adaptation (PASQ) scores across emotional, self-perception, and social functioning dimensions. When contextualized within existing research frameworks, these findings reveal both consistency with prior studies and unique complementary value and innovative contributions.

First, the results of this study are consistent with previous literature. For example, Bakay et al. and Holgersen et al. reported that serum IgE and hs-CRP levels were elevated in patients with chronic urticaria, which was positively correlated with disease activity<sup>[10,11]</sup>. However, the reduction in IgE and high-sensitivity C-reactive protein (hs-CRP) levels post-treatment in the experimental group (down to 30.95 IU/mL and 9.62 mg/L respectively) outperformed most conventional interventions such as antihistamines or glucocorticoids<sup>[12]</sup>, which may be attributed to the unique characteristics of the therapeutic approach. For instance, while omalizumab has demonstrated significant IgE reduction, its efficacy in improving hs-CRP remains controversial<sup>[13]</sup>. The intervention in this study likely achieved simultaneous regulation of both immune and inflammatory pathways. In terms of psychological and social adaptation, the PASQ scale used in this study showed that the experimental group had significant improvement in emotional, self-cognition and social dimensions (e.g., emotional dimension reached 37.84 points), which was consistent with the conclusion of Goksin et al. that chronic urticaria patients generally have psychological distress<sup>[2]</sup>, but the improvement rate was better than conventional psychological support therapy<sup>[14]</sup>. The reasons for the differences may include: the sample of this study was mainly middle-aged, married working women (55%), and the comorbidities were mostly allergic diseases (e.g., allergic rhinitis 12%), while the previous studies had a high heterogeneity of samples<sup>[15,16]</sup>; the intervention measures may integrate the multi-dimensional effects of biopsychosocial factors rather than a single drug or psychotherapy. The innovation of this study lies in the simultaneous verification of the synergistic improvement effect of intervention on biological indicators (IgE, hs-CRP) and psychosocial adaptation, which provides new evidence for the integrated treatment of chronic urticaria and makes up for the limitation of insufficient psychosocial dimension intervention in existing guidelines<sup>[17]</sup>.

A notable feature of this study lies in its psychosocial function assessment using the PASQ scale. While previous research predominantly employed the Dermatological Quality of Life Index (DLQI) or Chronic Urticaria Quality of Life Questionnaire (CU-QoL) to evaluate disease-related quality impacts<sup>[9]</sup>, the PASQ scale adopted here provides a more nuanced perspective through its three-dimensional framework—emotional well-being, self-perception, and social functioning—to examine patients' psychological recovery processes. Although no specific psychometric validation studies for PASQ in chronic urticaria were identified in the literature, our study demonstrates that experimental interventions deliver deeper psychological benefits beyond symptom relief, offering new assessment tools and research directions. The superior efficacy observed in the experimental group likely stems from adopting a multi-target, comprehensive treatment

strategy, such as integrated Chinese-Western therapies or holistic care models that simultaneously regulate immune responses, suppress inflammatory pathways, and incorporate psychological support.

## 5. Conclusions

The significance of this study extends beyond confirming an effective intervention. More importantly, through a three-dimensional “biopsychosocial” assessment framework that simultaneously evaluates IgE (allergy), hs-CRP (inflammation), and PASQ (psychosocial adaptation), it establishes a new benchmark for comprehensive and in-depth evaluation of chronic urticaria treatment outcomes. This groundbreaking approach underscores the critical importance of holistic patient management in clinical practice, emphasizing the need to simultaneously monitor physiological indicators and psychological well-being.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Konstantinou G N, 2019, Psychiatric comorbidity in chronic urticaria patients: a systematic review and meta-analysis// *Clinical and Translational Allergy*. 9: Article 52.
- [2] Ulusoy Ş, Şahin R, 2022, Depression, anxiety, stress and life satisfaction in patients with chronic spontaneous urticaria. *Annals of Medical Research*, 29(12): 1342–1348.
- [3] Donnelly J, Ridge K, O'Donovan R, et al., 2023, Psychosocial factors and chronic spontaneous urticaria: a systematic review//*BMC Psycholog*. 11: Article 291.
- [4] Śmierciak N, 2023, Unraveling the complex nexus of chronic spontaneous urticaria: Immunological, infectious and psychosomatic dimensions. *Psychology & Psychological Research International Journal*, 8(3): 1–10.
- [5] Zhang X, Xu H, Feng L, et al., 2021, Exploring psychosocial adaptation among people with chronic skin disease: A grounded theory study. *Nursing Open*, 8(5): 2673–2685.
- [6] Tomaszewska K, Słodka A, Tarkowski B, et al., 2023, Neuro-immuno-psychological aspects of chronic urticaria. *Journal of Clinical Medicine*, 12(4): Article 1391.
- [7] Jerković H, Bešlić I, Česić D, et al., 2021, The psychosocial burden of urticaria. *Rad Hrvatske Akademije Znanosti i Umjetnosti. Medicinske Znanosti*, 56–57, 98–103.
- [8] Grattan C, Zuberbier T, Maurer M. Other Interventions for Chronic Urticaria//*Urticaria and Angioedema*. 2021: 177-206.
- [9] Li S, Chen A, 2025, Validation of the Reliability and Validity of PSAQCSD, a Psychosocial Adaptation Assessment for Patients with Chronic Urticaria. *Dermatological Health*, 3(2): 1-10.
- [10] Karstarli B O S, Demir B, Cicek D, et al., 2023, In chronic spontaneous urticaria, IgE and C-reactive protein are linked to distinct microRNAs and interleukin-31//*Clinical and Translational Allergy*. 13(2): Article e12215.
- [11] Ghazanfar M N, Sørensen J A, Zhang D, et al., 2023, Occurrence and risk factors of mental disorders in patients with chronic urticaria//*World Allergy Organization Journal*. 16(1): Article 100742.
- [12] Chen J, 2019, Changes in Immune-inflammatory Related Indicators and Plasma Dimer Levels in Chronic Urticaria and Correlation Analysis. *China Journal of Modern Drug Application*, 13(19): 52-54.
- [13] Magen E, Chikovani T, Waitman D A, et al., 2019, Factors related to omalizumab resistance in chronic spontaneous urticaria. *Allergy and Asthma Proceedings*, 40(4): 273–278.
- [14] Liu K, Zhang S Y, Wang R, et al., 2024, Research Advances in Psychosomatic Intervention for Chronic Urticaria. *Journal of*

the PLA Medical University, 45(09):996-999+1005.

- [15] Tawil S, Irani C, Kfoury R, et al., 2023, Association of Chronic Urticaria with Psychological Distress: A Multicentre Cross-sectional Study[Z]//Acta Dermato-Venereologica.103: Article adv00882.
- [16] Gyawalee M, Paudel V, 2023, Socio-demographic and clinical characteristics of chronic urticaria among patients attending Dermatology Clinic in a Tertiary Care Hospital. Our Dermatology Online, 14(2): 240–248.
- [17] Zuberbier T, Abdul L A H, Abuzakouk M, et al., 2022, The international EAACI/GA<sup>2</sup>LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy, 77(3): 734–766.

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# The Efficacy of Ultrasound-guided Drug Delivery of Diclofenac Diethylamine Emulsion Combined with Rehabilitation Training in the Treatment of Elbow Ossifying Myositis

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**Abstract:** *Objective:* To explore the clinical efficacy of ultrasound-guided drug delivery of diclofenac diethylamine emulsion combined with rehabilitation training in the treatment of elbow ossifying myositis (EOM), and to provide evidence-based support for clinical treatment. *Methods:* 86 patients with elbow ossifying myositis admitted to the orthopedics department of our hospital from January 2022 to December 2023 were selected as the study subjects. They were divided into a control group (43 cases) and an observation group (43 cases) using a random number table method. The control group received rehabilitation training only, while the observation group received ultrasound-guided drug delivery of diclofenac diethylamine emulsion in addition to the rehabilitation training. Both groups were treated continuously for 8 weeks. The range of motion (ROM), visual analogue scale (VAS) for pain, and Mayo Elbow Performance Score (MEPS) were compared between the two groups before and after treatment, and adverse reactions were recorded. *Results:* Before treatment, there were no statistically significant differences in elbow ROM (flexion, extension, pronation, and supination angles), VAS score, and MEPS score between the two groups ( $P>0.05$ ). After 8 weeks of treatment, the observation group had significantly better elbow flexion angle ( $115.23\pm10.56^\circ$ ), extension angle ( $-5.12\pm2.34^\circ$ ), pronation angle ( $78.45\pm8.12^\circ$ ), and supination angle ( $75.36\pm7.89^\circ$ ) compared to the control group's ( $92.67\pm9.87^\circ$ ), ( $-12.34\pm3.12^\circ$ ), ( $65.78\pm7.56^\circ$ ), and ( $62.13\pm6.98^\circ$ ), respectively ( $P<0.05$ ). The VAS score of the observation group ( $1.23\pm0.56$ ) was significantly lower than that of the control group ( $3.56\pm0.89$ ) ( $P<0.05$ ). The excellent and good rate of the observation group was significantly higher than that of the control group ( $P<0.05$ ). There was no statistically significant difference in the incidence of adverse reactions between the observation group (4.65%) and the control group (6.98%) ( $P>0.05$ ). *Conclusion:* Ultrasound-guided drug delivery of diclofenac diethylamine emulsion combined with rehabilitation training can effectively improve joint mobility, reduce pain, and enhance elbow function in patients with elbow ossifying myositis. This treatment approach is safe and worthy of clinical promotion and application.

**Keywords:** elbow osteomyelitis; ultrasonic drug delivery; diclofenac diethylamine emulsion; rehabilitation training; range of joint motion; pain score

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## 1. Introduction

Elbow osteomyelitis is a common chronic progressive disease in orthopedic clinics, mostly caused by abnormal ossification of local muscles, tendons, and fascial tissues after elbow trauma, surgery, or inflammation. The main symptoms include elbow pain and limited range of motion. In severe cases, it can lead to joint stiffness and dysfunction, greatly affecting patients' daily life and work quality<sup>[1]</sup>. Currently, there are various clinical treatments for elbow osteomyelitis, including drug therapy, physical therapy, rehabilitation training, and surgical treatment. Among them, rehabilitation training is a basic treatment method that can delay the progression of ossification and improve joint function through joint range of motion training and muscle strength training. However, when applied alone, it has a slower onset and poor pain relief effect<sup>[2]</sup>. As a non-steroidal anti-inflammatory drug, diclofenac diethylamine emulsion has anti-inflammatory and analgesic effects, which can effectively relieve local inflammatory reactions and pain symptoms. However, under the traditional external application method, the drug penetration depth is limited, and the bioavailability is low<sup>[3]</sup>. Ultrasonic drug delivery technology is a new drug delivery method that utilizes the mechanical vibration and thermal effects of ultrasonic waves to break the skin stratum corneum barrier, promote the directional penetration of drug molecules, increase the drug concentration in local tissues, and enhance the efficacy<sup>[4]</sup>. Based on this, this study combined ultrasonic drug delivery of diclofenac diethylamine emulsion with rehabilitation training for patients with elbow osteomyelitis to explore its clinical efficacy and safety. The report is as follows.

## 2. Materials and Methods

### 2.1. General Information

Eighty-six patients with elbow ossifying myositis admitted to the orthopedic department of our hospital from January 2022 to December 2023 were selected as the study subjects. Inclusion criteria were: (1) meeting the diagnostic criteria for elbow ossifying myositis in "Orthopedics"<sup>[5]</sup>, confirmed by X-ray, CT, or MRI examination to have ossification foci around the elbow joint; (2) significant elbow pain with a VAS score  $\geq 4$ ; (3) limited elbow joint mobility, with a flexion angle  $< 100^\circ$  or extension limitation  $> 10^\circ$ ; (4) disease duration of 3 months to 1 year; (5) informed consent from the patient and their family members, with signed informed consent forms. Exclusion criteria were: (1) severe cardiac, liver, kidney, or other organ dysfunction; (2) allergy to diclofenac diethylamine emulsion or ultrasound therapy; (3) damaged, infected, or open wounds on the skin of the elbow joint; (4) unhealed fractures, dislocations, or other joint diseases; (5) pregnant or breastfeeding women; (6) patients with mental illness unable to cooperate with treatment and follow-up. The patients were divided into a control group and an observation group using a random number table method, with 43 cases in each group. In the control group, there were 25 males and 18 females, aged between 22 and 58 years old, with an average age of  $(38.56 \pm 8.23)$  years. The disease duration ranged from 3 to 12 months, with an average of  $(6.89 \pm 2.15)$  months. Injury types included 20 cases of elbow fracture surgery, 12 cases of elbow dislocation, and 11 cases of soft tissue injury. In the observation group, there were 24 males and 19 females, aged between 23 and 59 years old, with an average age of  $(39.12 \pm 8.56)$  years. The disease duration ranged from 3 to 11 months, with an average of  $(6.78 \pm 2.03)$  months. Injury types included 19 cases of elbow fracture surgery, 13 cases of elbow dislocation, and 11 cases of soft tissue injury. There were no statistically significant differences in general information such as gender, age, disease duration, and injury type between the two groups ( $P > 0.05$ ), indicating comparability. This study was approved by the Medical Ethics Committee of our hospital.

### 2.2. Treatment Methods

#### 2.2.1. Control Group: Rehabilitation Training Only

A personalized rehabilitation training program will be developed by a professional rehabilitation therapist, consisting of 8 consecutive weeks of treatment, with 5 training sessions per week, each lasting 40-60 minutes. The specific contents are as follows:

- (1) Joint Range of Motion Training: ① Passive Movement Training: The therapist assists the patient in performing

slow passive movements of elbow flexion, extension, pronation, and supination. Each movement is maintained for 10-15 seconds and repeated 10-15 times, depending on the patient's tolerance and absence of significant pain.

② Active-Assisted Movement Training: With the assistance of the therapist or elastic bands, the patient actively performs elbow movements, gradually increasing the range of motion. Each training session lasts 20 minutes. ③ Stretching Training: Static stretching is used to stretch the muscles around the elbow (such as the biceps, triceps, and forearm flexors). Each area is stretched for 15-20 seconds and repeated 5-8 times.

- (2) Muscle Strength Training: ① Isometric Contraction Training: The patient sits or lies down, with the elbow joint maintained in a neutral position or at a specific angle, performing isometric muscle contractions. Each contraction lasts 10-15 seconds, followed by a 10-second rest, and repeated 10-15 times. The contraction intensity is gradually increased. ② Isotonic Contraction Training: Depending on the patient's muscle strength, 1-2 kg dumbbells or elastic bands are used for isotonic contraction training of elbow flexion, extension, pronation, and supination. Each movement is repeated 10-15 times in 3 sets. The load is adjusted weekly based on the patient's tolerance.
- (3) Physical Factor Therapy: Low-frequency pulsed electrotherapy (Beijing Hengkang Kailin Medical Instrument Co., Ltd., Neuromuscular Rehabilitation Instrument Model NMR-1) is used. Electrode pads are placed on the painful areas of the elbow, with a frequency of 50 Hz. The current intensity is adjusted until the patient feels a slight soreness and numbness. Each treatment lasts 20 minutes, 5 times a week.

### 2.2.2. Observation Group: Rehabilitation Training Combined with Ultrasonic Drug Delivery of Diclofenac Diethylamine Emulsion

The rehabilitation training program for the observation group was the same as that for the control group, but with the addition of ultrasonic drug delivery treatment. The specific operations were as follows:

- (1) Instrumentation and Medication: An ultrasonic drug delivery device (DS-UCMF2B, Nanjing Dingshi Ultrasonic Electro-conductive Targeted Drug Delivery System) with a probe frequency of 1MHz was used. The medication selected was diclofenac diethylamine emulsion (produced by Sino-American Tianjin SmithKline & French Laboratories Limited, National Medical Approval Number HJ20181225, specification: 20g/tube).
- (2) Operation Method: The patient was positioned in a comfortable posture, exposing the treatment site of the elbow joint. After cleaning the skin, the diclofenac diethylamine emulsion was evenly applied to the skin around the painful and ossified areas of the elbow joint, with a thickness of about 1-2mm and an area covering the size of the probe (approximately 5cm×5cm). After applying coupling agent to the ultrasonic probe, it was placed closely on the drug-applied area. The instrument parameters were adjusted as follows: output power of 0.8-1.2W/cm<sup>2</sup>, continuous wave mode, treatment duration of 30 minutes per session, 5 sessions per week, synchronized with rehabilitation training, and continuous treatment for 8 weeks.

### 2.3. Observation Indicators

- (1) Elbow Range of Motion (ROM): Before treatment and after 8 weeks of treatment, a goniometer was used to measure the flexion, extension, pronation, and supination angles of the patient's elbow joint. Flexion angle: the maximum angle of elbow flexion from the extended position; Extension angle: full extension of the elbow joint was considered as 0°, and limited extension was recorded as a negative value; Pronation and supination angles: with the elbow joint close to the body side and the forearm in a neutral position as 0°, pronation was the angle of palm rotation downward, and supination was the angle of palm rotation upward.
- (2) Pain Score: The Visual Analog Scale (VAS) was used to evaluate the patient's pain level, with a scoring range of 0-10. A score of 0 indicated no pain, while a score of 10 indicated severe pain. Patients marked their scores on the scale based on their own pain perception, and measurements were taken before treatment and after 8 weeks of treatment.
- (3) Elbow Joint Function Score: The Mayo Elbow Performance Score (MEPS) was used to evaluate patients' elbow

joint function, including pain (40 points), range of motion (20 points), muscle strength (20 points), and daily activities (20 points), with a total score ranging from 0-100. A higher score indicates better elbow joint function, where 90-100 is excellent, 75-89 is good, 60-74 is average, and <60 is poor. Evaluations were conducted before treatment and 8 weeks after treatment, and the excellence rate (excellent + good) was calculated.

- (4) Adverse Reactions: Adverse reactions such as skin redness and swelling, itching, rash, and local tingling that occurred in both groups during treatment were recorded, and the incidence of adverse reactions was calculated.

## 2.4. Statistical Methods

Data analysis was performed using SPSS 26.0 statistical software. Measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Paired t-tests were used for comparisons before and after treatment within the group, and independent sample t-tests were used for comparisons between groups. Count data were expressed as rates (%), and comparisons were made using the  $\chi^2$  test. A P-value  $< 0.05$  was considered statistically significant, and all data were rounded to two decimal places.

## 3. Results

### 3.1. Comparison of Elbow ROM Before and After Treatment in Both Groups

Before treatment, there was no statistically significant difference in elbow flexion, extension, pronation, and supination angles between the two groups ( $P > 0.05$ ). After 8 weeks of treatment, the above ROM indicators in both groups were significantly improved compared to before treatment ( $P < 0.05$ ), and the improvement in the observation group was significantly better than that in the control group, with a statistically significant difference ( $P < 0.05$ ). See **Table 1** for details.

**Table 1.** Comparison of Elbow ROM Before and After Treatment in Both Groups (mean  $\pm$  standard deviation,  $^{\circ}$ )

Indicator	Group	Before Treatment	After Treatment	t-value	P-value
Flexion Angle	Control (n=43)	75.34 $\pm$ 9.12	92.67 $\pm$ 9.87	8.560	<0.05
	Observation (n=43)	76.12 $\pm$ 8.98	115.23 $\pm$ 10.56	15.890	<0.05
	t-value	0.403	10.823	-	-
	P-value	>0.05	<0.05	-	-
Extension Angle	Control (n=43)	-18.56 $\pm$ 3.89	-12.34 $\pm$ 3.12	8.230	<0.05
	Observation (n=43)	-17.98 $\pm$ 3.67	-5.12 $\pm$ 2.34	18.760	<0.05
	t-value	0.723	12.772	-	-
	P-value	>0.05	<0.05	-	-
Pronation Angle	Control (n=43)	52.34 $\pm$ 7.23	65.78 $\pm$ 7.56	8.900	<0.05
	Observation (n=43)	53.12 $\pm$ 7.01	78.45 $\pm$ 8.12	14.560	<0.05
	t-value	0.513	7.855	-	-
	P-value	>0.05	<0.05	-	-
Supination Angle	Control (n=43)	49.87 $\pm$ 6.89	62.13 $\pm$ 6.98	7.890	<0.05
	Observation (n=43)	50.23 $\pm$ 6.78	75.36 $\pm$ 7.89	13.240	<0.05
	t-value	0.247	8.627	-	-
	P-value	>0.05	<0.05	-	-



### 3.2. Comparison of VAS scores before and after treatment between the two groups

Before treatment, the VAS score of the control group was ( $6.89 \pm 1.23$ ) points, and that of the observation group was ( $6.78 \pm 1.15$ ) points. There was no statistically significant difference between the two groups ( $t=0.428$ ,  $P>0.05$ ). After 8 weeks of treatment, the VAS score of the control group was ( $3.56 \pm 0.89$ ) points, and that of the observation group was ( $1.23 \pm 0.56$ ) points. Both groups showed significant reductions compared to before treatment (control group  $t=14.383$ , observation group  $t=28.453$ ,  $P<0.05$ ), and the observation group had significantly lower scores than the control group ( $t=14.530$ ,  $P<0.05$ ).

### 3.3. Comparison of MEPS scores before and after treatment between the two groups

After 8 weeks of treatment, the excellent and good rate of MEPS in the control group was 65.12% (28/43), including 8 excellent cases, 20 good cases, 10 average cases, and 5 poor cases. The excellent and good rate in the observation group was 90.70% (39/43), including 20 excellent cases, 19 good cases, 3 average cases, and 1 poor case. The excellent and good rate in the observation group was significantly higher than that in the control group ( $P<0.05$ ). See **Table 2** for details.

**Table 2.** Comparison of MEPS score grades after treatment between the two groups (n, %)

Group	n	Excellent	Good	Fair	Poor	Excellent-Good Rate
Control	43	8 (18.60)	20 (46.51)	10 (23.26)	5 (11.63)	28 (65.12)
Observation	43	20 (46.51)	19 (44.19)	3 (6.98)	1 (2.33)	39 (90.70)
$\chi^2$ value						8.174
P value						<0.05

### 3.4. Comparison of adverse reactions between the two groups

During the treatment period, there were 2 cases of skin itching and 1 case of local tingling in the control group, with an adverse reaction rate of 6.98% (3/43). In the observation group, there were 1 case of skin redness and swelling and 1 case of itching, with an adverse reaction rate of 4.65% (2/43). There was no statistically significant difference in the incidence of adverse reactions between the two groups ( $\chi^2=0.000$ ,  $P>0.05$ ). All adverse reactions were relieved after suspending treatment for 1-2 days or applying calamine lotion locally, without affecting subsequent treatment.

## 4. Discussion

The pathogenesis of elbow ossifying myositis is not fully understood, but it is currently believed to be related to local bleeding, inflammatory response, abnormal proliferation and differentiation of fibroblasts, and abnormal expression of bone morphogenetic protein (BMP) after elbow injury [6]. After injury, local tissue hypoxia and ischemia can activate inflammatory cells to release inflammatory factors (such as IL-1 and TNF- $\alpha$ ), promoting the transformation of fibroblasts into osteoblasts, which leads to ectopic ossification, compressing surrounding nerves, blood vessels, and soft tissues, causing pain and limited joint movement [7]. Therefore, the key to clinical treatment lies in reducing local inflammatory response, inhibiting the progression of ossification, and improving joint range of motion and function. Rehabilitation training is a basic treatment for elbow ossifying myositis. Joint range of motion training can break the adhesion of surrounding tissues and delay the further maturation of ossification foci; muscle strength training can enhance the muscle strength around the elbow joint, improve joint stability, and reduce joint load; low-frequency pulsed electrotherapy can promote local blood circulation through electrical stimulation, relieve muscle spasms, and reduce pain [8]. In this study, the control group received simple rehabilitation training, and the elbow joint ROM, VAS score, and MEPS score were all improved compared with those before treatment, confirming the effectiveness of rehabilitation



training, but there is still room for improvement in the overall efficacy.

The main component of diclofenac diethylamine emulsion is diclofenac diethylamine, which can exert anti-inflammatory and analgesic effects by inhibiting the activity of cyclooxygenase (COX-1, COX-2) and reducing prostaglandin synthesis. However, when applied externally in the traditional way, the drug needs to pass through the skin stratum corneum barrier, and the penetration efficiency is low, making it difficult for the local drug concentration to meet the treatment demand, thus affecting the efficacy. Ultrasonic drug delivery technology utilizes the “cavitation effect”, “mechanical vibration effect”, and “thermal effect” of ultrasonic waves to destroy the lipid structure of the stratum corneum, forming temporary pores, and simultaneously promoting local tissue blood circulation, accelerating drug molecule diffusion and penetration, increasing local tissue drug concentration, and enhancing anti-inflammatory and analgesic effects. In addition, ultrasonic waves can directly act on the surrounding tissues of ossification foci, softening fibrous tissues, relieving tissue adhesion, and creating favorable conditions for rehabilitation training to improve joint range of motion.

The results of this study showed that the observation group had significantly better elbow ROM (flexion, extension, pronation, and supination angles) than the control group after treatment. The VAS score was significantly lower in the observation group, while the MEPS score and excellent rate were significantly higher than those in the control group ( $P < 0.05$ ). This suggests that the combined therapy has more pronounced advantages in improving joint range of motion, reducing pain, and enhancing elbow function. The reasons for this can be analyzed as follows: On one hand, rehabilitation training can improve joint range of motion and enhance muscle strength through active and passive activities; on the other hand, ultrasound-guided drug delivery can increase the local drug concentration of diclofenac diethylamine emulsion, more effectively inhibiting inflammatory responses and reducing pain, thus providing support for the smooth implementation of rehabilitation training. These two approaches form a synergistic effect, further enhancing the treatment effect.

In terms of safety, there was no statistically significant difference in the incidence of adverse reactions between the two groups ( $P > 0.05$ ), and the adverse reactions were minor and resolved after symptomatic treatment. This indicates that ultrasound-guided drug delivery of diclofenac diethylamine emulsion has high safety. This may be related to the precise control of drug penetration depth achieved by ultrasound-guided drug delivery, which reduces systemic drug absorption and lowers the risk of adverse reactions such as gastrointestinal and cardiovascular events that may be caused by oral nonsteroidal anti-inflammatory drugs.

This study has certain limitations: the sample size is small, and it is a single-center study, so the results may be biased; the follow-up time is short, and the long-term efficacy of combined therapy and the progression of ossification foci have not been observed. Future studies need to expand the sample size, conduct multi-center, long-term follow-up studies, further verify the efficacy and safety of combined therapy, and explore the effects of different ultrasound parameters and drug dosages on the efficacy, providing more evidence for the optimization of clinical treatment plans.

In summary, the combination of ultrasonic drug delivery of diclofenac diethylamine emulsion and rehabilitation training for the treatment of elbow ossifying myositis can effectively improve patients' elbow range of motion, reduce pain symptoms, enhance elbow function, and has few adverse reactions and high safety. It is an efficient and safe treatment plan worthy of clinical promotion and application in orthopedics.

## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Wang J, 2024, Meta-analysis of integrated traditional Chinese and western medicine treatment for traumatic ossifying

myositis of the elbow joint. Shanxi Medical University.

- [2] Qin J B, Zhuang X P, Zhang L H, 2021, Observation on the efficacy of ultrasound-guided drug delivery of diclofenac diethylamine emulsion combined with rehabilitation training in the treatment of ossifying myositis of the elbow joint. *Massage and Rehabilitation Medicine*, 12(12): 45-47.
- [3] Wang B, Sun J P, Wu Yongtao, et al., 2021, Analysis of clinical treatment strategies for post-traumatic ossifying myositis of the elbow joint in children. *Chinese Journal of Bone and Joint*, 10(03): 175-180.
- [4] Hou C J, Li J, Zhang Z J, 2020, Observation on the preventive effect of external application of Huoxue Ruanjian Powder on ossifying myositis after elbow joint injury. *Traditional Chinese Medicine and Technology*, 27(06): 900-902.
- [5] Shao Y G, Zhu Z K, Shen Z W, et al., 2020, Treatment of 34 cases of ossifying myositis after elbow joint injury surgery with Xugu Huoxue Decoction. *Zhejiang Journal of Traditional Chinese Medicine*, 55(02): 108-109.
- [6] Wang J, 2019, Treatment of 39 cases of ossifying myositis of the elbow with traditional Chinese medicine combined with small needle knife. *Journal of Practical Traditional Chinese Medicine*, 35(03): 290-291.
- [7] Liang G G, 2018, Observation on the efficacy of oral administration of Ruanjian Decoction combined with traditional Chinese medicine scalding therapy in the treatment of ossifying myositis of the elbow joint in adults. *Clinical Research of Traditional Chinese Medicine*, 10(27): 65-67.
- [8] Luo L, Zeng W B, 2018, Clinical observation on the treatment of early traumatic ossifying myositis of the elbow joint with milli-fire needle combined with manipulation. *Chinese Ethnic Medicine*, 27(06): 94-95+98.

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# A Study on the Long-Term Efficacy and Safety of Budesonide-Formoterol Versus Montelukast in the Treatment of Seasonal Asthma

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**Abstract:** *Objective:* To compare the long-term efficacy and safety of budesonide-formoterol and montelukast in patients with seasonal asthma. *Methods:* A total of 270 outpatient asthma patients with seasonal exacerbation characteristics were selected from our hospital from March 2022 to September 2025 and randomly divided into three groups (A, B, and C), with 90 patients in each group. All patients received asthma health education. Patients in Group A inhaled budesonide-formoterol inhalation powder, patients in Group B took oral montelukast sodium tablets, and patients in Group C did not use any asthma control medications but only used salbutamol aerosol for symptomatic treatment during acute exacerbations. All patients were followed up for more than 2 years, and ACT scores, lung function indicators, and safety were compared. *Results:* (1) The ACT scores of Groups A and B were higher than those before treatment at 3, 6, 12, and 30 months of treatment ( $P < 0.05$ ), and continued to increase with the prolongation of treatment time, while there was no significant change in Group C ( $P > 0.05$ ). When comparing between groups, the ACT scores of Groups A and B were higher than those of Group C at 3, 6, 12, and 30 months of treatment ( $P < 0.05$ ), and Group A was significantly higher than Group B at 6, 12, and 30 months ( $P < 0.05$ ). (2) The FEV1 and FEV1/FVC of Groups A and B were higher than those before treatment at 3, 6, 12, and 30 months of treatment ( $P < 0.05$ ), while there was no significant change in Group C ( $P > 0.05$ ). When comparing between groups, the FEV1/FVC of Groups A and B were higher than those of Group C at 3, 6, 12, and 30 months of treatment ( $P < 0.05$ ), and Group A was significantly higher than Group B at 6, 12, and 30 months ( $P < 0.05$ ). (3) During the treatment period, there was no significant difference in the incidence of adverse reactions between Group A and Group B ( $P > 0.05$ ). During the follow-up period, Group C experienced a total of 15 cases of asthma exacerbation complicated by other conditions, including 14 cases of pulmonary infection and 1 case of respiratory failure. All patients improved after symptomatic treatment, and no deaths occurred due to complications. *Conclusion:* Implementing preventive treatment for patients with seasonal asthma is of utmost importance. Both budesonide-formoterol and montelukast therapies can effectively alleviate patients' symptoms and exhibit comparable safety profiles. However, budesonide-formoterol demonstrates superior performance in long-term symptom control and lung function improvement. Clinical decision-making should comprehensively consider the specific conditions and treatment responses of patients to develop suitable individualized treatment plans.

**Keywords:** Seasonal asthma; Budesonide; Formoterol; Montelukast sodium; ACT score; Lung function; Safety

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## 1. Introduction

Seasonal asthma is a common subtype of bronchial asthma, closely associated with climatic changes or seasonal allergens such as pollen and mold spores. It predominantly affects children, the elderly, and individuals with allergic predispositions. The typical symptoms of this condition include mucosal edema and increased secretions triggered by airway hyperresponsiveness, accompanied by recurrent episodes of wheezing, shortness of breath, chest tightness, and coughing. Patients generally experience worsening symptoms at night and in the early morning, which, in severe cases, can induce acute respiratory insufficiency and significantly diminish their quality of life <sup>[1]</sup>. Epidemiological surveys indicate an increasing annual prevalence of asthma globally, making it a significant public health concern <sup>[2]</sup>.

According to the “2025 GINA Global Strategy for Asthma Management and Prevention,” the key treatment strategies for asthma involve controlling airway inflammation and alleviating bronchial spasm <sup>[3]</sup>. Currently, commonly used clinical medications include inhaled corticosteroids (ICS), long-acting beta-agonists (LABA), and leukotriene receptor antagonists (LTRA), among others. Budesonide-formoterol dry powder inhaler is widely used in the long-term management of bronchial asthma. It is a combination formulation of ICS/LABA, in which budesonide can inhibit airway inflammation, reduce the aggregation of inflammatory cells and the release of inflammatory mediators, and decrease airway hyperresponsiveness <sup>[4]</sup>; formoterol, on the other hand, can activate the  $\beta_2$  receptors on the airway smooth muscle cell membrane, relax the bronchial smooth muscle, and thereby alleviate airway spasms <sup>[5]</sup>. Montelukast sodium, as an LTRA, reduces allergen-induced airway constriction and inflammation by inhibiting cysteinyl leukotriene receptors <sup>[6]</sup>. Although these two drugs have been widely used in the clinical treatment of asthma, research data on the comparison of their efficacy and safety in the long-term management of seasonal asthma remain relatively limited. This study aims to provide a more sufficient theoretical basis for clinical rational drug use by comparing the long-term efficacy and safety of budesonide-formoterol and montelukast in patients with seasonal asthma.

## 2. Materials and Methods

### 2.1. General Information

A total of 270 outpatient asthma patients with seasonal onset characteristics were selected from our hospital from March 2022 to September 2025 and randomly divided into three groups (A, B, and C), with 90 patients in each group. Among them, there were 119 males and 151 females, aged between 17 and 56 years, with a disease duration of 5-11 years, a body mass index (BMI) of 20-28 kg/m<sup>2</sup>, and an Asthma Control Test (ACT) score of 16-24 points. The general information of the three groups was comparable, with no statistically significant differences ( $P > 0.05$ ) (see **Table 1**).

**Table 1.** Comparison of General Information Among the Three Groups

Indicator	Group A	Group B	Group C	F/ $\chi^2$ value	P-value
Gender (n, Male/Female)	38/52	40/50	41/49	0.210	0.900
Age (Mean $\pm$ SD, years)	35.79 $\pm$ 5.01	36.33 $\pm$ 5.24	36.61 $\pm$ 4.98	0.606	0.546
Disease Duration (Mean $\pm$ SD, years)	6.02 $\pm$ 1.17	5.94 $\pm$ 1.33	5.81 $\pm$ 1.41	0.592	0.554
BMI (Mean $\pm$ SD, kg/m <sup>2</sup> )	23.24 $\pm$ 1.81	23.06 $\pm$ 1.79	23.19 $\pm$ 1.84	0.236	0.790
ACT Score (Mean $\pm$ SD, points)	19.32 $\pm$ 2.76	20.01 $\pm$ 2.09	19.94 $\pm$ 2.31	2.248	0.108

### 2.2. Inclusion and Exclusion Criteria

Inclusion criteria: (1) Patients primarily presented with recurrent symptoms such as wheezing, dyspnea, chest tightness, or coughing, and during episodes, scattered or diffuse wheezing sounds predominantly in the expiratory phase could be auscultated in both lungs, with prolonged expiration; (2) The symptoms of acute exacerbation improved after

treatment with antiasthmatic drugs; (3) Outpatient visits ruled out wheezing, shortness of breath, chest tightness, or coughing caused by other diseases; (4) At least one of the following three criteria is positive: a positive bronchial provocation test or exercise test; a positive bronchodilation test; a diurnal PEF variation rate greater than 20%. After meeting the diagnostic criteria for bronchial asthma, patients still need to meet the inclusion criteria, including seasonal exacerbations occurring from April to May and August to September each year; (5) No acute exacerbations of bronchial asthma in the past three months and no maintenance treatment with any medications.

Exclusion criteria: (1) Recent (within the past month) onset of upper respiratory tract infections or other diseases; (2) Concomitant diagnosis of other respiratory system diseases; (3) Current smoking, alcohol consumption, or consumption of beverages containing caffeine; (4) Allergy to the medications used in this study; (5) Breastfeeding and pregnant women; (6) Inability to cooperate and complete this study or withdrawal midway.

### 2.3. Methods

All three groups of patients received asthma health education, including avoidance of allergens, proper use of inhalation devices, and identification and pre-treatment of acute exacerbations. On this basis:

Group A: Inhaled budesonide/formoterol fumarate inhalation powder (AstraZeneca AB, National Medical Products Administration Approval Number: H20140458, specification: 60 inhalations per device, each inhalation containing 160 µg budesonide and 4.5 µg formoterol fumarate), 1 inhalation per dose, twice daily, with rinsing of the mouth with water after inhalation; Group B: Oral montelukast sodium tablets (Hangzhou MSD Pharmaceutical Co., Ltd., National Medical Products Administration Approval Number: J20130047, specification: 10 mg), 10 mg once nightly; Group C: No asthma control medications were used, and only salbutamol aerosol was used for symptomatic treatment during acute exacerbations. The start date for preventive medication was from March 1st to April 1st and August 1st to September 1st each year.

All three groups of patients were treated and followed up for 30 months. During this period, a WeChat group for patients was established, and follow-up was conducted once every four weeks through on-site visits, video calls, and phone calls. During the follow-up period, patients conducted regular peak expiratory flow rate measurements at home and filled out asthma diaries based on their actual conditions. Regular medication administration and dynamic monitoring continued until the occurrence of an acute exacerbation. For patients without acute exacerbations, preventive medication was administered until September 31, 2025, after which their diaries were collected.

### 2.4. Observation Indicators

- (1) Comparison of ACT Scores Among the Three Groups: ACT scores were assessed at 3 months, 6 months, 12 months, and 30 months of treatment. The total score for this test is 25 points, with 25 points indicating complete control, 20-24 points indicating good control, and <20 points indicating inadequate control.
- (2) Comparison of Pulmonary Function Indicators Among the Three Groups: Pulmonary function indicators, including Forced Expiratory Volume in One Second (FEV1) and the ratio of FEV1 to Forced Vital Capacity (FEV1/FVC), were measured using a spirometer at 3 months, 6 months, 12 months, and 30 months of treatment. Prior to testing, patients were required to sit quietly for 15 minutes. Each measurement was repeated three times, and the best value was recorded.
- (3) Safety Analysis: Adverse drug reactions occurring during preventive treatment in Groups A and B were recorded, including throat discomfort, gastrointestinal reactions, headaches, allergic reactions, palpitations, etc. The incidence of complications during the study period in Group C patients, such as pulmonary infections and respiratory failure, was also recorded.

### 2.5. Statistical Methods

Data were processed using SPSS 26.0 statistical software. Continuous variables were expressed as “Mean±SD” and



analyzed using the t-test. Categorical variables were expressed as (n,%) and analyzed using the chi-square ( $X^2$ ) test. A p-value of  $<0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Comparison of ACT Scores Among the Three Groups

After 3 months, 6 months, 12 months, and 30 months of treatment, the ACT scores of Groups A and B were higher than those before treatment ( $P < 0.05$ ), and they continued to increase with the prolongation of treatment duration. In contrast, Group C showed no significant change ( $P > 0.05$ ). In inter-group comparisons, the ACT scores of Groups A and B were higher than those of Group C at 3 months, 6 months, 12 months, and 30 months of treatment ( $P < 0.05$ ). Furthermore, at 6 months, 12 months, and 30 months, Group A had significantly higher scores than Group B ( $P < 0.05$ ). See **Table 2**.

**Table 2.** Comparison of ACT Scores Before and After Intervention Among the Three Groups (Mean $\pm$ SD, points)

Time Point	Group A	Group B	Group C	F-value	P-value
Before Treatment	19.32 $\pm$ 2.76	20.01 $\pm$ 2.09	19.94 $\pm$ 2.31	2.248	0.108
3 Months	20.51 $\pm$ 1.94	20.82 $\pm$ 2.04	19.87 $\pm$ 1.61	6.027	0.003
6 Months	23.51 $\pm$ 2.01	21.30 $\pm$ 2.11	19.90 $\pm$ 2.41	62.546	$<0.001$
12 Months	24.21 $\pm$ 1.54	22.15 $\pm$ 1.75	20.10 $\pm$ 2.22	110.030	$<0.001$
30 Months	24.75 $\pm$ 1.01	23.14 $\pm$ 1.20	20.52 $\pm$ 2.07	182.464	$<0.001$
F-value (Time)	137.964	37.564	1.417	/	/
P-value (Time)	$<0.001$	$<0.001$	0.227	/	/

#### 3.2. Comparison of Pulmonary Function Indicators Among the Three Groups

After 3 months, 6 months, 12 months, and 30 months of treatment, the FEV1 and FEV1/FVC values of Groups A and B were higher than those before treatment ( $P < 0.05$ ), while Group C showed no significant change ( $P > 0.05$ ). In inter-group comparisons, the FEV1/FVC values of Groups A and B were higher than those of Group C at 3 months, 6 months, 12 months, and 30 months of treatment ( $P < 0.05$ ). Additionally, at 6 months, 12 months, and 30 months, Group A had significantly higher values than Group B ( $P < 0.05$ ). See **Table 3**.

**Table 3.** Comparison of Pulmonary Function Indicators Before and After Intervention Among the Three Groups (Mean $\pm$ SD)

Indicator	Time Point	Group A	Group B	Group C	F-value	P-value
FEV <sub>1</sub> (L)	Before Treatment	1.64 $\pm$ 0.23	1.59 $\pm$ 0.35	1.62 $\pm$ 0.33	0.602	0.549
	3 Months	1.92 $\pm$ 0.42	1.86 $\pm$ 0.51	1.60 $\pm$ 0.21	16.250	$<0.001$
	6 Months	2.21 $\pm$ 0.54	2.04 $\pm$ 0.41	1.63 $\pm$ 0.32	42.702	$<0.001$
	12 Months	2.82 $\pm$ 0.42	2.56 $\pm$ 0.33	1.63 $\pm$ 0.30	219.568	$<0.001$
	30 Months	2.94 $\pm$ 0.23	2.66 $\pm$ 0.47	1.62 $\pm$ 0.24	344.575	$<0.001$
F-value (Time)	/	190.137	107.129	0.167	/	/
P-value (Time)	/	$<0.001$	$<0.001$	0.955	/	/

**Table 3 (Continued)**

Indicator	Time Point	Group A	Group B	Group C	F-value	P-value
FEV <sub>1</sub> /FVC (%)	Before Treatment	66.25 ± 4.23	65.98 ± 4.46	65.53 ± 4.51	0.614	0.542
	3 Months	72.85 ± 5.61	71.82 ± 4.91	65.94 ± 4.02	45.529	<0.001
	6 Months	78.55 ± 4.67	76.15 ± 5.99	65.79 ± 5.06	149.063	<0.001
	12 Months	83.52 ± 8.31	82.61 ± 6.57	64.89 ± 3.97	232.733	<0.001
	30 Months	84.50 ± 6.51	78.14 ± 7.30	64.12 ± 5.25	238.216	<0.001
F-value (Time)	/	144.072	105.940	2.403	/	/
P-value (Time)	/	<0.001	<0.001	0.050	/	/

### 3.3. Safety Analysis Adverse Reactions in Groups A and B

There was no significant difference in the incidence of adverse reactions between the two groups during treatment ( $P > 0.05$ ). See **Table 4**.

**Table 4.** Incidence of Adverse Reactions in Groups A and B (n,%)

Adverse Reaction	Group A	Group B	$\chi^2$ Value	P-value
Throat Discomfort (n)	2	1	/	/
Gastrointestinal Reaction (n)	1	2	/	/
Headache (n)	1	2	/	/
Allergic Reaction (n)	0	0	/	/
Palpitations (n)	0	0	/	/
Total Incidence	4 (4.44%)	5 (5.56%)	0.117	0.732

Complications in Group C: During the follow-up period, Group C had a total of 15 cases of complications due to acute asthma exacerbation, including 14 cases of pulmonary infection and 1 case of respiratory failure. All patients improved after symptomatic treatment, and no patients died from complications.

## 4. Discussion

Seasonal asthma is a type of asthma associated with allergens, which can lead to asthma symptoms and respiratory inflammation. It is a common clinical asthma phenotype, and its long-term management requires a balance between “inflammation control” and “exacerbation prevention.” This means alleviating bronchial spasms in patients by suppressing airway hyperresponsiveness and reducing the release of inflammatory factors, thereby controlling the progression of the disease<sup>[7-8]</sup>. Currently, ICS, LABA, and LTRA are commonly used in clinical treatment<sup>[9-11]</sup>. Therefore, this study compared the efficacy and safety of budesonide-formoterol and montelukast sodium through a 30-month follow-up.

The ACT score is an effective tool for monitoring and evaluating asthma conditions during treatment, and its changes directly reflect the effectiveness of the treatment plan. Before treatment in this study, there were no significant differences in ACT scores among the three groups ( $P > 0.05$ ). However, after prophylactic treatment in Groups A and B, the corresponding scores increased significantly, and continued to rise with the extension of follow-up time ( $P < 0.05$ ). This indicates that both budesonide-formoterol and montelukast sodium can effectively control disease symptoms in patients

with seasonal asthma, while relying solely on salbutamol treatment cannot achieve long-term disease control. Further inter-group comparisons showed that the scores in Group A were significantly higher than those in Group B at 6, 12, and 30 months of treatment ( $P<0.05$ ). The reason is that budesonide-formoterol, as a combination preparation of ICS and LABA, can rapidly dilate the bronchi while suppressing airway inflammation, providing a dual therapeutic effect. Montelukast sodium, on the other hand, is an LTRA that can only treat the disease by antagonizing the inflammatory pathway mediated by leukotrienes, and its effect in long-term asthma management is relatively limited<sup>[12-13]</sup>.

Improvements in asthma control levels are often accompanied by improvements in patients' lung function. FEV1 and FEV1/FVC are commonly used indicators for lung function assessment, which can evaluate the patient's patency and degree of airflow limitation. Three months after treatment, the FEV1 and FEV1/FVC in Groups A and B began to be significantly higher than before treatment ( $P<0.05$ ), and the scores in Group A were significantly higher than those in Group B at 6, 12, and 30 months of treatment ( $P<0.05$ ), while Group C remained at a consistently low level. Both types of drugs can alleviate inflammatory responses and spasms, thereby protecting lung function. However, the synergistic effect of budesonide-formoterol not only relieves immediate airflow limitation but also achieves long-term improvement by delaying airway remodeling. In contrast, montelukast sodium has a weaker intervention effect on remodeling<sup>[14-15]</sup>, which is a significant reason why its long-term improvement in lung function is inferior to that of the former.

In terms of safety, the results showed that the incidence of adverse reactions was low in both Group A and Group B, with mild symptoms such as throat discomfort and gastrointestinal reactions being predominant. No inflammatory adverse reactions were observed, indicating that both drugs have favorable therapeutic effects in seasonal asthma. In Group C, 15 cases of complications were reported, including 14 cases of pulmonary infection and 1 case of respiratory failure. This further demonstrates that relying solely on symptomatic treatment without using control medications increases the risk of complications during acute asthma exacerbations, highlighting the necessity of standardized preventive treatment.

In summary, preventive treatment is crucial for patients with seasonal asthma. Both budesonide-formoterol and montelukast can effectively improve patient symptoms, with comparable safety profiles. However, budesonide-formoterol demonstrates superior performance in long-term symptom control and lung function improvement. Clinical decision-making should consider the patient's specific condition and treatment response to develop an appropriate individualized treatment plan. Nevertheless, this study has certain limitations, such as a relatively small sample size, which may affect a comprehensive assessment of the long-term safety of the drugs. Additionally, although the use of symptomatic treatment during acute exacerbations in the blank control group meets ethical requirements, it may have a certain impact on the evaluation of therapeutic efficacy. Subsequent studies will further optimize the research protocol to provide more meaningful preventive treatment strategies for asthma.

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## Disclosure statement

The author declares no conflict of interest.

## References

- [1] Creticos PS, Gunaydin FE, Nolte H, et al., 2024, Allergen Immunotherapy: The Evidence Supporting the Efficacy and Safety of Subcutaneous Immunotherapy and Sublingual Forms of Immunotherapy for Allergic Rhinitis/Conjunctivitis and Asthma. *Journal of Allergy and Clinical Immunology: In Practice*, 12(6): 1415-1427.

- [2] Douglass JA, Lodge C, Chan S, et al., 2022, Thunderstorm asthma in seasonal allergic rhinitis: The TAISAR study. *Journal of Allergy and Clinical Immunology*, 149(5): 1607-1616.
- [3] Genis C, Tas D, Yılmaz D, et al., 2025, Unraveling the effects of smoking in asthmatic adolescents: clinical outcomes, spirometry findings, and risk analysis. *European Journal of Pediatrics*, 184(8): 481.
- [4] Sobieraj DM, Weeda ER, Nguyen E, et al., 2018, Association of Inhaled Corticosteroids and Long-Acting  $\beta$ -Agonists as Controller and Quick Relief Therapy With Exacerbations and Symptom Control in Persistent Asthma: A Systematic Review and Meta-analysis. *JAMA*, 319(14): 1485-1496.
- [5] Laitano R, Calzetta L, Martino M, et al., 2024, Asthma management with triple ICS/LABA/LAMA combination to reduce the risk of exacerbation: an umbrella review compliant with the PRIOR statement. *Expert Opinion on Pharmacotherapy*, 25(8): 1071-1081.
- [6] Rayner DG, Liu M, Chu AWL, et al., 2024, Leukotriene receptor antagonists as adjunctive therapy to antihistamines for urticaria: A systematic review and meta-analysis of randomized clinical trials. *Journal of Allergy and Clinical Immunology*, 154(4): 996-1007.
- [7] Tian R, Yang Y, Liu L, et al., 2022, Seasonal distribution of inhaled allergens in allergic asthma patients with or without allergic rhinitis. *Chinese Medical Journal (English Edition)*, 135(15): 1867-1869.
- [8] Papadopoulos NG, Miligkos M, Xepapadaki P. A, 2022, Current Perspective on Allergic Asthma: From Mechanisms to Management. *Handbook of Experimental Pharmacology*, 268: 69-93.
- [9] Miller RL, Grayson MH, Strothman K., 2021, Advances in Asthma: New Insights into Asthma's Natural History, Risk Factors, Underlying Mechanisms, and Clinical Management. *Journal of Allergy and Clinical Immunology*, 148(6): 1430-1441.
- [10] Tiotiu A, Steiropoulos P, Novakova S, et al., 2025, Airway Remodeling in Asthma: Mechanisms, Diagnosis, Treatment, and Future Directions. *Archivos de Bronconeumologia*, 61(1): 31-40.
- [11] Zhou X, Zhang P, Tan H, et al., 2023, Progress in the Diagnosis and Treatment of Difficult-to-Treat Asthma in Children. *Therapeutic Advances in Respiratory Disease*, 17: 17534666231213637.
- [12] Reddel HK, Bacharier LB, Bateman ED, et al., 2022, Global Initiative for Asthma Strategy 2021: Executive Summary and Rationale for Key Changes . *American Journal of Respiratory and Critical Care Medicine*, 205(1): 17-35.
- [13] Rodriguez-Martinez CE, Sossa-Briceño MP, Castro-Rodriguez JA., 2018, Cost-Effectiveness of Pharmacological Treatments for Asthma: A Systematic Review. *Pharmacoeconomics*, 36(10): 1165-1200.
- [14] Kaplan A, FitzGerald JM, Buhl R, et al., 2020, Comparison of LAMA with LABA and LTRA as Add-On Therapies in Primary Care Asthma Management. *NPJ Primary Care Respiratory Medicine*, 30(1): 50.
- [15] Cividini S, Sinha I, Donegan S, et al., 2023, Optimal Step-Up Treatments for Children with Uncontrolled Asthma: A Systematic Review and Network Meta-Analysis of Individual Participant Data. *European Respiratory Journal*, 62(6): 2301011.
- [16] Kaplan A, FitzGerald JM, Buhl R, et al., 2020, Comparison of LAMA with LABA and LTRA as Add-On Therapies in Primary Care Asthma Management. *NPJ Primary Care Respiratory Medicine*, 30(1): 50.
- [17] Cividini S, Sinha I, Donegan S, et al., 2023, Optimal Step-Up Treatments for Children with Uncontrolled Asthma: A Systematic Review and Network Meta-Analysis of Individual Participant Data. *European Respiratory Journal*, 62(6): 2301011.

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