RESEARCH ARTICLE

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Exploring the Efficacy and Molecular Mechanisms of Dingxuan in Alleviating Vertigo Episodes in Meniere's Disease: A Comprehensive Study

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ABSTRACT

Background: Meniere's disease (MD) poses a formidable challenge, marked by debilitating symptoms such as vertigo, hearing loss, tinnitus, and aural fullness. The traditional Chinese medicine, Dingxuan, was explored here for its potential in mitigating MD challenges. We investigated Dingxuan's efficacy in alleviating acute vertigo episodes in patients with MD and sought to understand its impact and underlying molecular mechanisms.

Methods: In a clinical trial, Dingxuan was administered to patients experiencing acute vertigo episodes. Additionally, mouse experiments were conducted to assess safety at an elevated dosage, while a guinea pig model was utilized to compare Dingxuan with betahistine. Molecular responses, modulation of water channels, and evaluation of labyrinth hydrops were performed as part of the study.

Results: In the clinical trial, Dingxuan significantly reduced the mean frequency of vertigo attacks. Impressively, even at a high dosage, Dingxuan showed no apparent liver or kidney toxicity in mouse experiments. In the guinea pig model, Dingxuan distinctively modulated water channels AQ2 and AQ5, regulated AVP-mediated signaling through cAMP, and ameliorated inner ear labyrinth hydrops.

Conclusions: Dingxuan emerges as a promising candidate for treatment of Meniere'sdisease, effectively reducing vertigo frequency. Its safety profile, even at elevated dosages, strengthens its potential for clinical applications. Further research and clinical trials are essential to validate these findings and explore broader implications for Dingxuan use with human subjects.

Keywords: Meniere's disease, Endolymphatic Hydrops, Water channel modulation, Liver and kidney toxicity, AQP2

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INTRODUCTION

Meniere's Disease (MD) is an inner ear disorder characterized by symptoms such as vertigo, hearing loss, tinnitus, and ear fullness[1-3]. The current medical interventions primarily aim to alleviate symptoms rather than address the root cause[4-6]. Despite various available treatments, there is no universally acknowledged cure for MD. The condition's underlying mechanisms are associated with endolymphatic hydrops in the inner ear[3,7-9]. Endolymphatic hydrops refers to a pathological anatomical condition where the structures surrounding the endolymphatic space undergo distension due to an increase in the volume of endolymph[10-12]. While some treatments like the Meniett device and endolymphatic sac surgery exist, their efficacy remains controversial[13]. Although Traditional Chinese Medicine (TCM) is reported by some individuals to have positive outcomes for Meniere's syndrome, scientific evidence supporting its effectiveness is limited[14,15].

Our comprehensive clinical and animal studies address this gap. Dingxuan, a traditional Chinese medicine composed of coptis, ginger pinellia, perilla, magnolia officinalis, ginger zuru, Jiaoshan gardenia, light tempeh, poria, dried ginger, and raw oysters, was the focus of this study. The primary objective was to assess through a clinical trial the effectiveness of Dingxuan in relieving acute vertigo episodes. The study also aimed to evaluate Dingxuan's safety, particularly concerning liver and kidney toxicity, even at seven times the standard dosage. Betahistine was initially approved in Europe in 1970 for addressing Ménière's disease, serving as an anti-vertigo medication[16,17]. Frequently prescribed to manage balance disorders and alleviate symptoms of vertigo, Betahistine functions as a histamine analog[18,19]. It enhances microcirculation in the inner ear by inducing vasodilation and mitigating the accumulation of fluid within the inner ear[20,21]. Additionally, a comparison between Dingxuan and betahistine in a guinea pig model explored the potential of Dingxuan in modulating the water channels AQ2 and AQ5, regulating AVP-mediated signaling via cAMP, and improving inner ear labyrinth hydrops. The promising findings suggest Dingxuan's potential in targeting water channel activity, laying the groundwork for further research and clinical trials to delve into its broader implications and efficacy in human subjects.

MATERIALS & METHODS

Plant Names

Dingxuan comprises a blend of medicinal plants, featuring key components such as Chinese goldthread, Pinellia ternata, Perilla frutescens, Officinal magnolia, bamboo shavings, gardenia, fermented soybeans, and Poria cocos. Additionally, it includes adjuvants like Zingiber officinale and raw oyster shell.

Clinical Study

The clinical efficacy of Dingxuan was substantiated through a pre-post trial, involving a significant cohort of 30 patients diagnosed with Meniere's Disease following the Clinical Practice Guideline for Meniere's Disease[22,23]. Diagnosis entailed a thorough patient history, characterized by discrete episodes of vertigo lasting 20 minutes or more, accompanied by initially low frequency sensorineural hearing loss, aural fullness, and tinnitus[24,25]. Diagnostic workup included audiometry, contrast-enhanced MRI of the internal auditory canals, and the exclusion of other conditions presenting similar symptoms, such as syphilis, autoimmune inner ear disease, perilymphatic fistula, superior semicircular canal syndrome, lyme disease, multiple sclerosis, vestibular paroxysms, and temporal bone tumors[26,27]. Additionally, a history of migraine was considered due to its high cooccurrence rate[24].

The assessment of Dingxuan's efficacy encompassed various parameters, including the frequency, severity, and duration of vertigo attacks. The Grading of Inner Ear Symptoms and Function in Vestibular Disorders (GISFaV) self-rating scale and the Dizziness Handicap Inventory (DHI) were also employed[28-30]. DHI gauges self-perceived handicapping effects related to vestibular system disease, with its final version consisting of 25 items, including 7 physical questions, 9 functional questions, and 9 emotional questions, resulting in a total score of 100 points (4 points for each item)[31,32]. Higher scores indicate more severe handicaps, with a maximum score of 100 and a minimum score of 0[30,33]. Supplementary (**Table 1**) provides comprehensive information on the DHI scales. The GISFaV self-assessment scale is primarily used to evaluate subjective symptoms related to vestibular disorders. It relies on self-reported information from individuals experiencing vestibular symptoms. Supplementary (**Table 2**) offers ample detail on the GISFaV scales.

This pre-post trial was carried out at the Otorhinolaryngology outpatient department of Wuhu Traditional Chinese Medicine Hospital in Wuhu, China, spanning the period from October 15, 2023, to January 15, 2024. The research included and managed a group of 30 patients using Dingxuan. To be eligible for a Meniere's Disease diagnosis in this study, patients had to exhibit key symptoms like distinct episodes of vertigo lasting 20 minutes or longer, coupled with initial low-frequency sensorineural hearing loss, aural fullness, and tinnitus[2,9,34]. Exclusion criteria required the exclusion of other possible causes for similar symptoms, such as vestibular migraine, labyrinthitis, perilymphatic fistula, acoustic neuroma, benign paroxysmal positional vertigoor, endolymphatic sac tumor, autoimmune inner ear disease, and certain central nervous system disorders[35-39]. The study (KY2022-005) was approved by the ethics committee of Wuhu Traditional Chinese Medicine Hospital, and written informed consent was secured from all eligible participants before enrollment.

Animal Study

All animal procedures were conducted with prior approval from the Wannan Medical College Committee on Animal

Table 1: The Dizziness Handicap Inventory (DHI).

DHI Scoring Instructions	
P1. Does looking up increase your problem?	Yes Sometimes No
E2. Because of your problem, do you feel frustrated?	Yes Sometimes No
F3. Because of your problem, do you restrict your travel for business or recreation?	Yes Sometimes No
P4. Does walking down the aisle of a supermarket increase your problems?	Yes Sometimes No
F5. Because of your problem, do you have difficulty getting into or out of bed?	Yes Sometimes No
F6. Does your problem significantly restrict your participation in social activities, such as going out to dinner, going to the movies, dancing, or going to parties?	Yes Sometimes No
F7. Because of your problem, do you have difficulty reading?	Yes Sometimes No
P8. Does performing more ambitious activities such as sports, dancing, household chores (sweeping or putting dishes away) increase your problems?	Yes Sometimes No
E9. Because of your problem, are you afraid to leave your home without having without having someone accompany you?	Yes Sometimes No
E10. Because of your problem have you been embarrassed in front of others?	Yes Sometimes No
P11. Do quick movements of your head increase your problem?	Yes Sometimes No
F12. Because of your problem, do you avoid heights?	Yes Sometimes No
P13. Does turning over in bed increase your problem?	Yes Sometimes No
F14. Because of your problem, is it difficult for you to do strenuous homework or yard work?	Yes Sometimes No
E15. Because of your problem, are you afraid people may think you are intoxicated?	Yes Sometimes No
F16. Because of your problem, is it difficult for you to go for a walk by yourself?	Yes Sometimes No
P17. Does walking down a sidewalk increase your problem?	Yes Sometimes No
E18.Because of your problem, is it difficult for you to concentrate	Yes Sometimes No
F19. Because of your problem, is it difficult for you to walk around your house in the dark?	Yes Sometimes No

E20. Because of your problem, are you afraid to stay home alone?	Yes
	Sometimes
	No
E21. Because of your problem, do you feel handicapped?	Yes
	Sometimes
	No
E22. Has the problem placed stress on your relationships with members of your family or friends?	Yes
	Sometimes
	No
E23. Because of your problem, are you depressed?	Yes
	Sometimes
	No
F24. Does your problem interfere with your job or household responsibilities?	Yes
	Sometimes
	No
P25. Does bending over increase your problem?	Yes
	Sometimes
	No

DHI Scoring Instructions

The patient is asked to answer each question as it pertains to dizziness or unsteadiness problems, specifically considering their condition during the last month. Questions are designed to incorporate functional (F), physical (P), and emotional (E) impacts on disability.

To each item, the following scores can be assigned:

No=0 Sometimes=2 Yes=4

Scores:

Scores greater than 10 points should be referred to balance specialists for further evaluation.

16-34 Points (mild handicap)

36-52 Points (moderate handicap)

54+ Points (severe handicap)

Table 2: Medication duration.

Clinical cases	Treatment duration (days)	Clinical cases	Treatment duration (days)
1	10	16	18
2	16	17	17
3	14	18	8
4	7	19	16
5	7	20	11
6	12	21	9
7	13	22	5
8	12	23	7
9	12	24	10
10	10	25	7
11	12	26	7
12	5	27	9
13	10	28	4
14	6	29	5
15	6	30	6

Care and aligned with ethical standards of the National Research Council Guide for the Care and Use of Laboratory Animals. Guinea pigs and mice resided in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility at Wannan Medical College, maintained at a controlled environment (21-23°C, 12-hour light-dark cycle). Guinea pigs received standard guinea pig chow, and nutritional guidelines for mice in laboratory settings were strictly followed[40]. Guinea pigs basic diet is unlimited amounts of commercial high-fiber guinea pig pellets, supplemented with smaller timothy or other low-calcium hay as well as Vitamin C each day[41].

Modeling Process

The guinea pig experiment encompasses six groups, including a control group, a Betahistine group, a model group, and a drug administration group (comprising low, medium, and high doses). The control group serves as the untreated control, while the other five groups undergo a literature-based method involving intraperitoneal

injection of desmopressin acetate to induce membranous labyrinth hydrops. An initial intraperitoneal injection of 4 μg/kg desmopressin acetate(dDAVP) (Hybio, Pharmaceutical Co., Ltd) is administered, followed by a 7-day continuous injection[42,43]. Subsequently, the dose is increased to 6 μg/kg for an additional 3 days, aiming to induce cochleosaccular hydrops, a sensitive finding in Meniere's Disease[44,45]. Ten days after modeling, the intragastric administration of betahistine and Dingxuan (low, medium, and high dosage groups) is carried out based on body weight. This administration occurs once a day for seven consecutive days. Dingxuan is orally administered three times a day, with each dose consisting of one bag (10g/bag). The guinea pig dosage for intragastric administration is determined using a conversion coefficient of 5.42, based on the comparison between humans and guinea pigs, considering an adult standard weight of 60kg[46,47]. Guinea Pig Dosage of 2.71g/kg/d is recorded as the medium dose of the Dingxuan group administered by gavage to guinea pigs. The high dose is calculated as 5.42 g/kg/d, and the low dose is 1.36g/kg/d. The Betahistine group(1.084g/kg) receives treatment according to established procedures[19,48].

Guinea Pig Vertigo Scoring Criteria

We established a standardized method to assess nystagmus in guinea pigs, defining vertigo scoring criteria as follows: Score 0: No symptoms. Indication: Absence of observable vertigo symptoms in guinea pigs. Description: The animal maintains typical behavior, movement, and posture without any signs of imbalance or abnormal eye movements (nystagmus). Score 1: Mild nystagmus. Indication: Guinea pigs exhibit mild nystagmus, characterized by involuntary rhythmic eye movements. Description: Subtle, noticeable eye movement occurs without significantly affecting overall behavior or posture. Score 2: Mild nystagmus with curling up and lying down. Indication: Guinea pigs display both mild nystagmus and additional behaviors such as curling up and lying down. Description: Alongside mild nystagmus, the guinea pig may intermittently exhibit a tendency to curl up or lie down, indicating a mild to moderate level of vertigo. Score 3: Severe nystagmus with curling up and lying down. Indication: Guinea pigs show severe nystagmus, characterized by intense, sustained involuntary eye movements. Description: The animal demonstrates pronounced vertigo symptoms, including severe nystagmus, and frequently curls up or lies down, suggesting a substantial impairment of balance and coordination.

These defined scoring criteria offer a systematic approach for evaluating the severity of vertigo symptoms in guinea pigs, encompassing both nystagmus observations and associated behaviors.

Hepatotoxicity and Renal Toxicity Assays

For toxicity assessment, Dingxuan was administered at an elevated dosage of 34.8 g/kg/d, which is 70-fold over the mouse standard dose. The primary focus was scrutinizing potential hepatotoxicity and renal toxicity. The animals were humanely euthanized following approved ethical guidelines[49,50]. The liver and kidneys were thoroughly rinsed in ice-cold physiological saline to eliminate excess blood. Subsequently, the tissue slices were fixed in formalin, embedded in paraffin, sectioned, and subjected to Hematoxylin and Eosin (H&E) staining[51,52]. Microscopic examination was conducted to identify any structural changes. The outcomes derived from histological analyses were quantified. Statistical analyses were performed to identify significant distinctions between the control and treatment groups.

Serum samples were collected and Assay kits were employed, following manufacturer instructions(BUN, AST, ALT, and CREA assay kit, Jiancheng Biotechnology Co., Ltd). A BUN assay kit (diacetyl oxime colorimetric method) and AST/ALT assay kits (microplate methods) were used to measure hepatotoxicity, while a creatinine assay kit (sarcosine oxidase method) assessed renal toxicity[53].

Hematoxylin and Eosin (H&E) Staining

Cochlear tissues were isolated, processed, and stained to assess differences in membranous labyrinth hydrops among guinea pig groups. The tissue was then refrigerated at 4°C for 48 hours. Subsequently, the specimen was transferred to a 10% EDTA decalcifying solution(Feijing Technology Co.Ltd) (pH 7.2) for about 21 days, with the solution changed daily. After decalcification, the excess softened bone was carefully removed, trimmed as needed, and rinsed with running water. The tissue was then dehydrated in a graded series of ethanol, made transparent in xylene, and embedded in paraffin. Paraffin blocks were cut in 4 μm thick slices parallel to the modiolus. The images with staining were subjected to analysis using Image J software to quantify the cross-sectional area of both the scala media and scala vestibule[54-56].

Enzyme-Linked Immunosorbent Assay (ELISA)

Following an 8-day recovery period, the guinea pigs were randomly allocated into five groups: three receiving varying doses of Dingxuan, another receiving betahistine, and a control group. The animals were subjected to a seven-day treatment regimen. Guinea pigs were administered general anesthesia through intraperitoneal injection with 10% chloral hydrate (3.5 ml/kg). Subsequently, blood samples were collected for the detection of AVP (Jianglai Technology Co. Ltd) and cAMP (Jianglai Technology Co. Ltd) activities using Enzyme-Linked Immunosorbent Assay (ELISA). For the enzyme-linked immunosorbent assay, 50 μL of the sample was incubated for 60 minutes in designated wells. AVP antibody incubation, secondary antibody addition, enzyme reaction initiation, color development, and absorbance measurement followed using a microplate reader at the appropriate wavelength[15,57,58].

Western Blot Analysis

For protein quantification, cochlear tissue from the right ear of guinea pigs was swiftly frozen at -80°C. The finely sliced tissue received 100 μl of RIPA protein lysis buffer (for

every 10 mg) and 10 μl of PMSF (for every 1 ml of RIPA lysis buffer)[59,60]. Subsequent centrifugation at 12,000 rpm for 30 minutes at 4°C yielded the protein extract supernatant[61,62]. After 12% SDS-PAGE gel electrophoresis, proteins transferred to a membrane were blocked in 5% skimmed milk powder solution. ECL luminescent solution incubation, conducted in the dark, was followed by exposure, image collection, and band gray value analysis using Image J image processing software[13,63-65].

Statistical Analysis

The results are presented as means ± Standard Error of the Mean (SEM), and statistical analyses were performed using GraphPad Prism 9 software. For Western blot and Hematoxylin and Eosin (HE) staining experiments, unpaired two-tailed Student's t-tests were employed for data analysis. Significance was considered at $P < 0.05$.

RESULTS

Effective reliefs for Meniere's symptoms reduce both vertigo frequency and intensity.

An acute episode of vertigo and other associated symptoms such as tinnitus, nausea, and vomiting manifest often frighten the patient. For this study, inclusion criteria encompassed patients experiencing a period of remission and newly diagnosed individuals over 30 years of age and under 65 years. Following 18 days of Dingxuan treatment, there was a notable decrease in the mean frequency of vertigo attacks among patients with Ménière's disease. Specifically, the frequency decreased from an onset time of 55.56 hours to 0.06 hours. Relief of symptoms with Dingxuan treatment can occur as early as 4 days and is typically achieved within a maximum period of 10 days. The difference between pre-treatment and post-treatment became significant from the end of the fourth day of treatment onward. The duration of treatment varies depending on whether the patient's vertigo symptoms resolve Supplementary (**Table 3**). 19 individuals among the 30 patients treated had GISFaV scores exclusively at 0, representing 63.3% of the overall population. This implies that, on the 18th day, the post-treatment group showed a 63.3% of patients with GISFaV scores of zero, while the pre-treatment group maintained a 0% rate Supplementary (**Table 4**). While 11 patients continue to experience symptoms such as vertigo, tinnitus, or visual vertigo, a comparison of the cumulative duration and frequency of vertigo attacks before and after treatment revealed that Dingxuan still significantly reduces Ménière syndrome's impact (**Figure 1A)**. The vertigo intensity score showed more frequent improvement in post-treatment compared to pre-treatment (**Figure 1B**). Associated symptoms such as tinnitus, aural fullness, nausea, and vomiting were also more frequently improved in post-treatment than in pre-treatment. Symptoms absent from the post-treatment records in (**Table 3**) suggest their disappearance Supplementary (**Table 1**). Similarly, six out of the seven items in the Dizziness Assessment Rating Scale showed more improvement in the post-treatment group than in the pre-treatment group. Dingxuan post-treatment significantly reduced Dizziness Handicap Inventory(DHI) scores more frequently than pre-treatment (**Figure 1C)** and Supplementary (**Table 2**).

Both Dingxuan and Betahistine alleviate vertigo, impacting inner ear fluid dynamics.

Betahistine is commonly used to treat vertigo and balance disorders, particularly associated with Ménière's disease66. Betahistine is believed to have a vasodilatory effect on the blood vessels in the inner ear, improving blood flow. Additionally, it may influence the release of histamine, acting as a histamine agonist, and affect the sensitivity of the vestibular system67. In our study, we conducted a comparative analysis involving two groups of guinea pigs, with one group receiving Betahistine and the other Dingxuan.

Table 3: The Grading of Inner Ear Symptoms and Function in Vestibular Disorders (GISFaV) self-rating scale.

	Pre-treatment				Post-treatment			
Clinical cases	**Vertigo attack intensity**	**Cumulative duration of vertigo attacks (hour)**	**Dizziness attack time per day(hour)**	**Symptom**	**Vertigo attack intensity**	**The cumulative duration of vertigo attacks after treatment (hour)**	**Dizziness attack time per day (hour)**	**Symptom**
1	moderate	5	5	Dizziness, visual rotation, vomiting, tinnitus	No vertigo attack	0	0	No vertigo attack
2	moderate	5	5	Dizziness, visual rotation, vomiting	mild	2.56	0.16	Dizziness
3	severe	24	12	Dizziness, unable to walk	mild	3.5	0.25	Dizziness
4	moderate	48	12	Dizziness, nausea and vomiting, unsteady standing	No vertigo attack	0	0	No vertigo attack
5	moderate	3	3	Dizziness, visual rotation, vomiting	No vertigo attack	0	0	No vertigo attack
6	moderate	24	12	Dizziness, blurred vision, nausea and vomiting	mild	3.96	0.33	Dizziness

7	moderate	168	12	Dizziness, visual rotation, tinnitus, vomiting	mild	1.04	0.08	Dizziness
8	moderate	72	9	Dizziness, rotating vision, inability to open eyes, tinnitus	No vertigo attack	0	0	No vertigo attack
9	moderate	240	12	Dizziness, visual rotation, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
10	severe	24	12	Dizziness, visual rotation, nausea and vomiting, tinnitus	mild	0.83	0.083	Dizziness
11	moderate	96	12	Dizziness, visual rotation, palpitation	mild	0.996	0.083	Dizziness, visual rotation,
12	moderate	96	12	Dizziness, visual rotation, vomiting	mild	0.8	0.16	Dizziness
13	moderate	24	12	Dizziness, visual rotation, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
14	moderate	2	2	Dizziness, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
15	moderate	24	8	Dizziness, blurred vision, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
16	moderate	720	12	Dizziness, visual rotation, nausea	No vertigo attack	0	0	No vertigo attack
17	moderate	12	6	Dizziness, visual rotation, tinnitus, vomiting	mild	1.411	0.083	Dizziness, tinnitus
18	moderate	120	12	Dizziness, visual rotation, tinnitus, vomiting	No vertigo attack	0	0	No vertigo attack
19	moderate	24	8	Dizziness, tinnitus	mild	2.656	0.166	Dizziness
20	moderate	8	8	Dizziness, visual rotation, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
21	moderate	8	4	Dizziness, visual rotation, vomiting	mild	0.747	0.083	Dizziness
22	moderate	7	7	Dizziness, visual rotation, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
23	moderate	72	12	Dizziness, visual rotation, nausea and vomiting	No vertigo attack	0	0	No vertigo attack
24	moderate	168	12	Dizziness, visual rotation, nausea	No vertigo attack	0	0	No vertigo attack
25	moderate	72	12	Dizziness, rotating vision, unsteady walking	mild	0.581	0.083	Dizziness
26	moderate	3	3	Dizziness, visual rotation, vomiting	No vertigo attack	0	0	No vertigo attack
27	moderate	96	12	Dizziness, visual rotation, vomiting, tinnitus	No vertigo attack	0	0	No vertigo attack
28	moderate	3	3	Dizziness, visual rotation, vomiting, hearing loss	No vertigo attack	0	0	No vertigo attack
29	moderate	3	3	Dizziness, visual rotation, vomiting	No vertigo attack	0	0	No vertigo attack
30	moderate	96	12	Dizziness, visual rotation, vomiting	No vertigo attack	0	0	No vertigo attack

Table 4: The Dizziness Handicap Inventory (DHI) score.

Clinical Case	Pre-treatment DHI Score	Post-treatment DHI Score	Clinical Case	Pre-treatment DHI Score	Post-treatment DHI Score
1	48	10	16	42	14
2	46	14	17	46	16
3	50	18	18	44	12
4	44	12	19	42	14
5	46	10	20	44	12
6	44	14	21	46	16
7	48	12	22	48	14
8	50	12	23	44	12
9	44	10	24	46	10
10	50	14	25	46	16
11	46	16	26	44	12
12	44	12	27	44	14
13	48	10	28	42	12
14	44	12	29	46	14
15	42	10	30	44	12

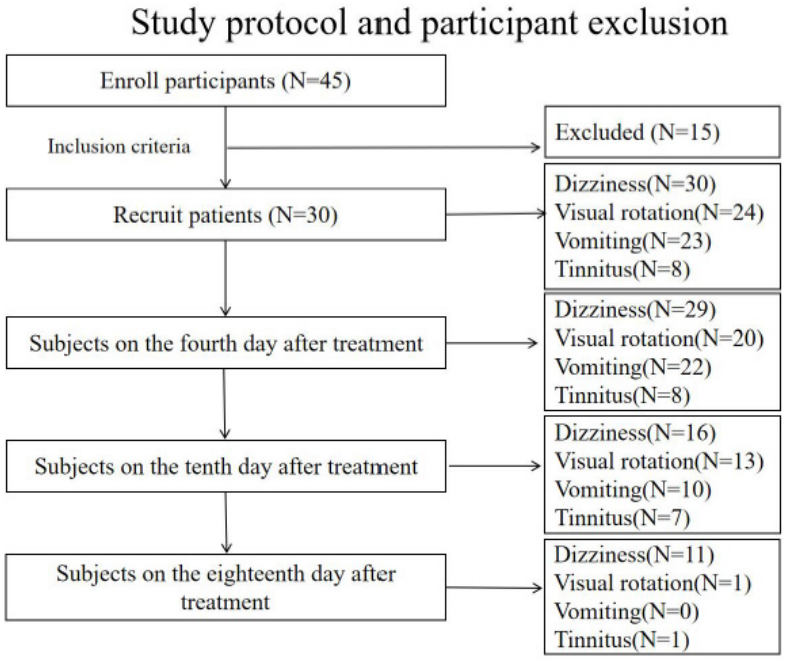

Figure 1A: Clinical trail of Dingxuan (A) Study protocol and participant exclusion.

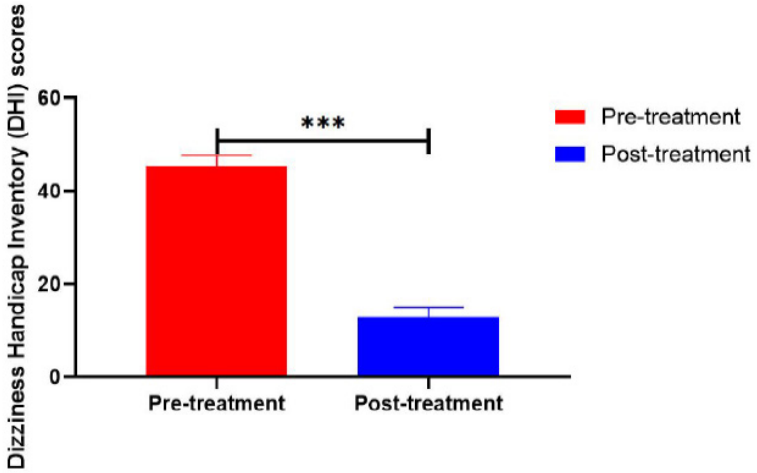

Figure 1B: Clinical trail of Dingxuan (B) Assessment of dizziness disorder scale scores in Meniere's disease patients. The Dizziness Handicap Inventory (DHI) after treatment significantly decreases compared to pre-treatment ($p < 0.05$) (n=30).

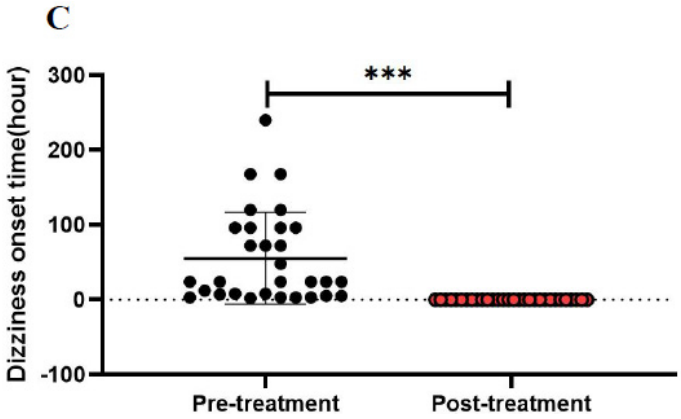

Figure 1C: Clinical trail of Dingxuan (C) Evaluation of the improvement in the frequency of vertigo attacks in Meniere's disease, showing a significant improvement ($p < 0.05$)($n=30$).

Several clinical and experimental investigations have proposed the potential involvement of Arginine Vasopressin (AVP) in the progression of endolymphatic hydrops48. Findings from studies indicate that the administration of a significant quantity of AVP can induce endolymphatic hydrops in guinea pigs68. Histological sections from these studies demonstrated the dilation of the scala media, coupled with the extension of Reissner's membrane toward the scala vestibuli. On the tenth day following the administration of arginine vasopressin to guinea pigs, we compared the vertigo index between the Dingxuan and Betahistine-treated groups. Both Dingxuan and Betahistine demonstrated the ability to improve the cochlear duct area of the inner ear in guinea pigs and reduce membranous labyrinth hydrops (**Figure 2A**). Both Dingxuan and Betahistine are postulated to influence fluid dynamics within the inner ear. The results indicated a significant improvement in the vertigo index in guinea pigs treated with both Dingxuan and Betahistine, with a statistically significant difference observed (**Figure 2B**). The Hypothalamic-Pituitary-Adrenal (HPA) axis is a complex neuroendocrine system that plays a crucial role in the body's response to stress and the regulation of various physiological processes69. Research suggests that stress-related hormonal changes and increased cortisol levels may contribute to vascular changes, inflammation, and fluid retention in various parts of the body, including the inner ear70. This therapeutic effect is attributed to the positive modulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis4. Dingxuan exhibits the ability to decrease AVP and cAMP levels, suggesting its role in regulating plasma AVP levels through the HPA axis (**Figure 2C**). Additionally, Dingxuan contributes to maintaining fluid homeostasis within the inner ear by influencing AQP2 and AQP5 to regulate the volume and pressure of endolymph (**Figure 2D**). Dingxuan reduces the activation of vasopressin receptor 2, thereby inhibiting the AVP-AQP2 system, crucial for antidiuresis and maintaining plasma osmotic stability. This leads to a benign modulation of the AVP-AQP2 system, effectively mitigating membranous hydrops.

Elevated dosage in mice reveals no adverse effects on body weight, organ indices, or liver/kidney function.

In conducting in vivo animal acute toxicity experiments in mice using Dingxuan, we administered the herbal remedy to mice at a dose 69.6 times higher than the normal dosage. Subsequently, we closely monitored the mice for potential side effects at this elevated dose. The change in body weight of mice serves as an indicator of the effects or impact of a particular intervention, treatment, or condition. A comparison of the body weight gain between the experimental group and the blank group revealed no statistically significant difference ($p>0.05$) (**Figure 3A**). The body weight of mice in both groups exhibited an increase following administration. An upward trend in body weight typically indicates growth and overall well-being in mice. The mouse organ index, which reflects the relative size of specific organs concerning the overall body weight, can also suggest various physiological conditions. We assessed the heart, lung, spleen, liver, and kidney organ indices, comparing them between the administration group and the placebo group. Monitoring these indices provides valuable information about the impact of interventions or experimental conditions on organ health and physiology in mice. No changes in organ indices suggested that there were no alterations in organ development, function, or response to treatments in administration group ($p>0.05$)(**Figure 3B**).

Monitoring for hepatotoxicity and nephrotoxicity is crucial in assessing the safety of drugs or interventions, as damage to the liver or kidneys can have serious health implications. Regular assessments of liver and kidney function, including laboratory tests and imaging studies, are typically conducted to detect and manage potential toxic effects on these organs. We evaluated the levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Urea Nitrogen (BUN), and Creatinine (CRE) in the serum of two mouse groups. No significant differences were observed between the two groups (**Figure 3C**). Moreover, the examination of mouse liver and kidney tissue sections offers valuable insights into the respective organs' conditions, revealing structural

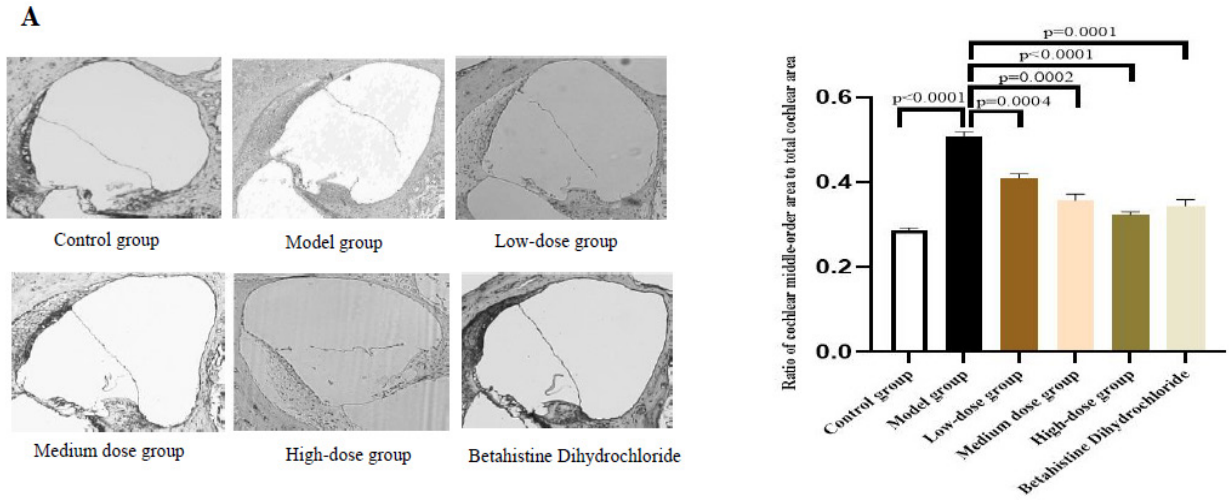

Figure 2A: Comparative Study of Dingxuan and Betahistine in the Treatment of Ménière Syndrome Induced by Membranous Hydrops in Guinea Pigs. (A) Micrographs of sectioned guinea pig cochleae show mid modiolar sections through normal and hydropic guinea pig cochlea. The endolymphatic compartment has been highlighted, revealing marked enlargement of the endolymphatic space and distension of Reissner's membrane. Various membranous structures in the ear, including those bounding the saccule, utricle, and ampullae of the semi-circular canals, may be displaced to varying degrees. The bar graph indicates that Dingxuan can reduce membranous labyrinth hydrops in guinea pigs, with the high dose exhibiting a similar effect to betahistine(n=10)

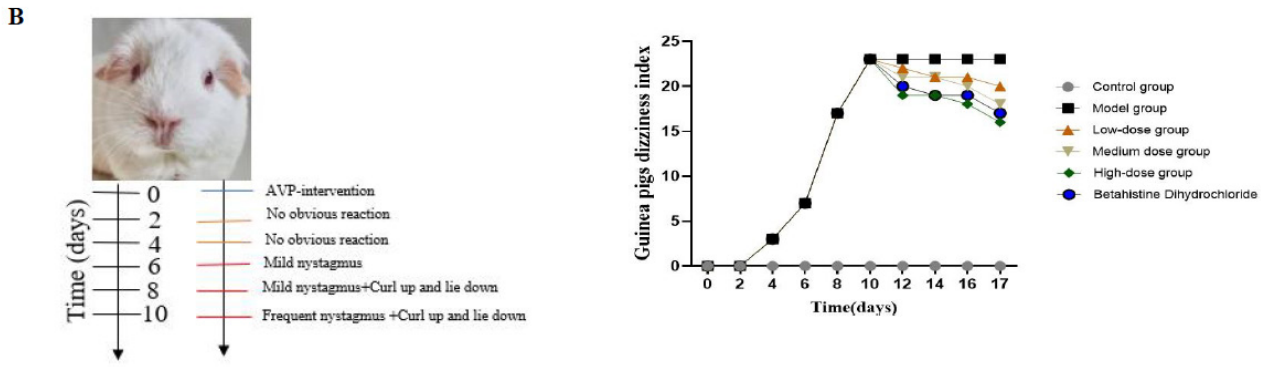

Figure 2B: Comparative Study of Dingxuan and Betahistine in the Treatment of Ménière Syndrome Induced by Membranous Hydrops in Guinea Pigs. (B) Mean dizziness curves illustrate the maximal performance of guinea pigs on the rotating beam. Results are expressed in dizziness scores as a function of posttreatment time in days (on the abscissa)(n=10).

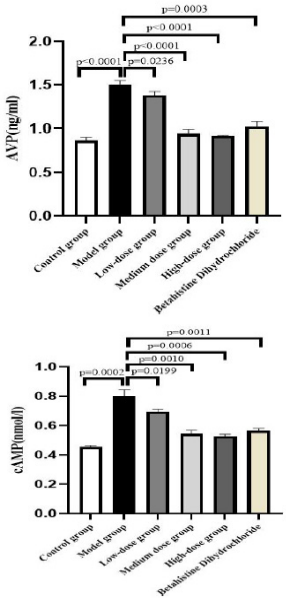

Figure 2C: Comparative Study of Dingxuan and Betahistine in the Treatment of Ménière Syndrome Induced by Membranous Hydrops in Guinea Pigs. (C) Dingxuan demonstrates the ability to decrease AVP and cAMP levels, suggesting its role in regulating plasma AVP levels through the HPA axis(n=10).

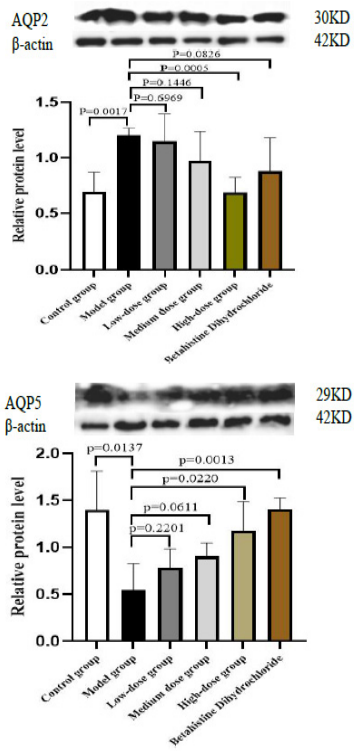

Figure 2D: Comparative Study of Dingxuan and Betahistine in the Treatment of Ménière Syndrome Induced by Membranous Hydrops in Guinea Pigs. (D) Dingxuan contributes to maintaining fluid homeostasis within the inner ear by influencing AQP2 and AQP5 to regulate the volume and pressure of endolymph. Compared with betahistine, Dingxuan rescues fluid homeostasis(n=10).

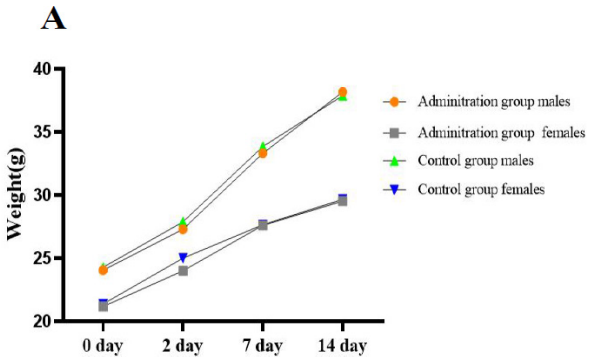

Figure 3A: Study of safety and tolerance of Dingxuan (A) Body Weight Analysis, The body mass index of mice, with an equal distribution of male and female subjects in each group (n=10), revealed no significant changes in body weight between the Dingxuan and control groups.

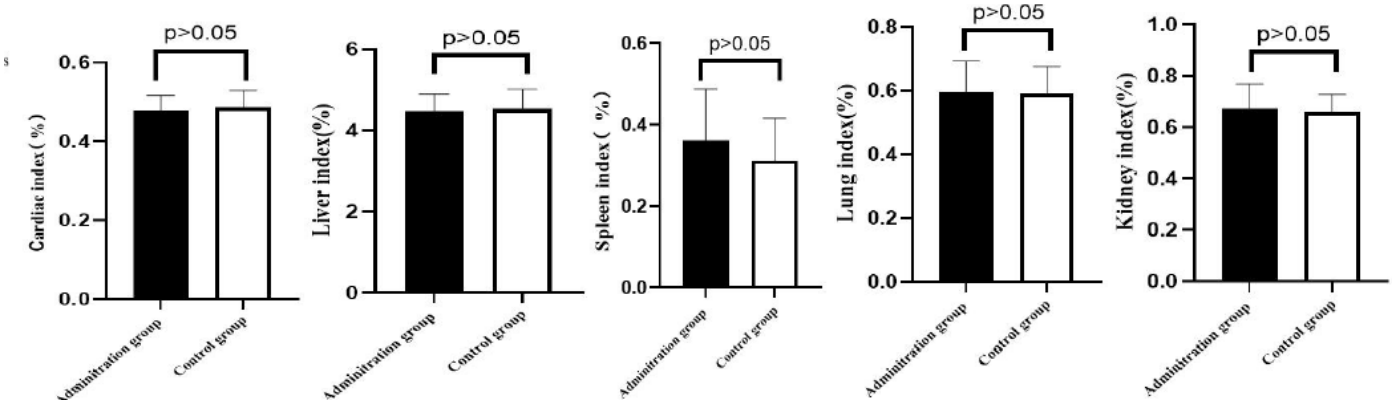

Figure 3B: Study of safety and tolerance of Dingxuan (B) Organ Mass Index, Evaluation of the main organ mass index, including the heart, liver, lungs, spleen, and kidneys, showed no remarkable differences between the Dingxuan and control groups. This comprehensive assessment considered variations in body weight (n=10).

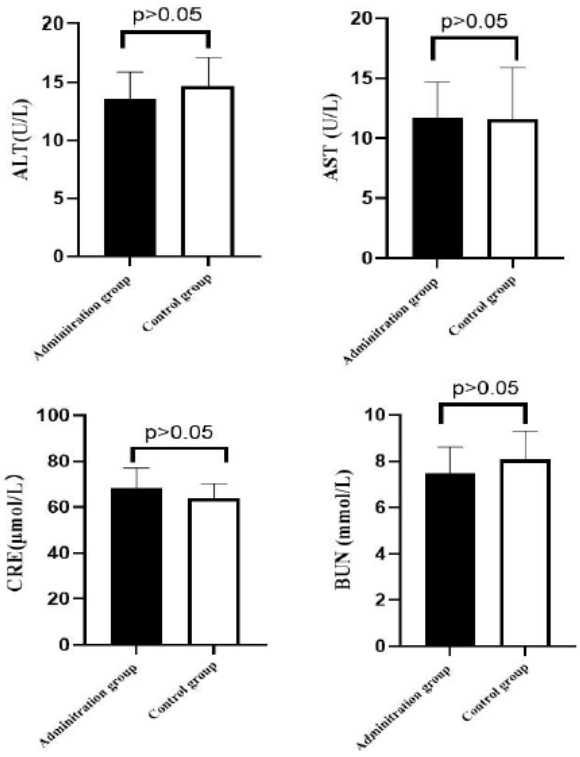

Figure 3C: Study of safety and tolerance of Dingxuan (C) Serum Biochemical Analysis, Analysis of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen (BUN), and creatinine (CRE) displayed no significant differences between the two mouse groups(n=10).

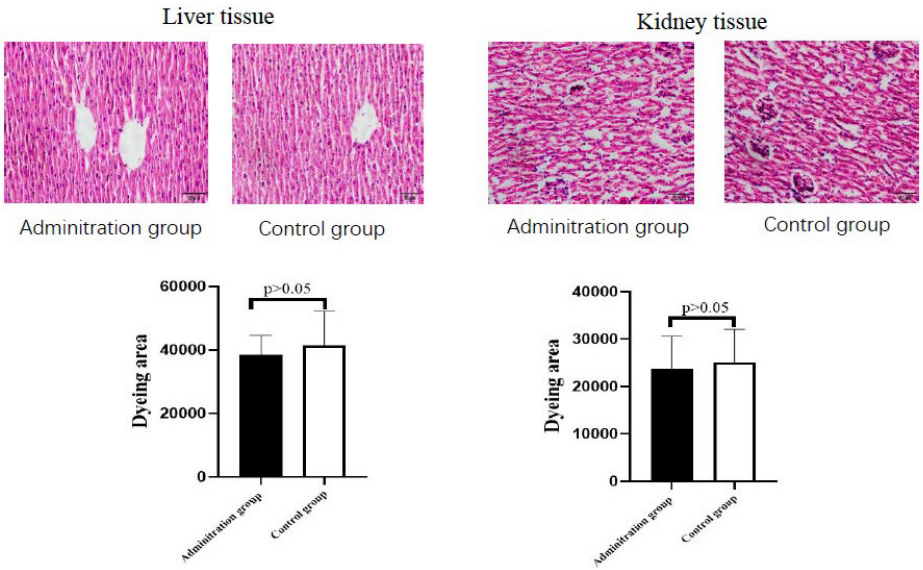

Figure 3D: Study of safety and tolerance of Dingxuan (D) Tissue Section Examination, Microscopic examination of liver and kidney tissue sections provided valuable insights into the structural and functional characteristics of these organs(n=10). The assessment aimed to identify potential abnormalities and evaluate organ responses to Dingxuan treatments or conditions.

and functional characteristics, identifying potential abnormalities, and assessing responses to treatments or conditions. The findings indicated that Dingxuan had no adverse effects on mouse liver and kidney (**Figure 3D**).

DISCUSSION

Meniere's Disease (MD) is a complex inner ear disorder marked by troubling symptoms, including vertigo, hearing loss, tinnitus, and aural fullness[2]. Since its original diagnosis in the 1860s, the precise mechanisms underlying MD development remain elusive[9]. Researchers have identified Endolymphatic Hydrops (ELH), an abnormal accumulation of endolymph fluid in the inner ear's membranous labyrinth, as a prominent histopathological feature[5]. Current treatments primarily target management of MD symptoms rather than addressing the root cause, lacking definitive cures. Meniere's syndrome complexity presents clinical challenges, and existing

medications have inherent pitfalls. Traditional Chinese herbal medicines, known for their intricate formulations, can restore body homeostasis and address specific symptoms[71,72]. The synergistic effects of multiple herbs, particularly those with anti-inflammatory properties[52,73], are considered beneficial in reducing inner ear inflammation linked to Meniere's syndrome.

In this study, patients meeting inclusion criteria, including those in remission and newly diagnosed individuals aged 30 to 65 years, exhibited significant improvements after 18 days of Dingxuan treatment. The mean frequency of vertigo attacks markedly decreased, indicating the potential efficacy of the treatment. Betahistine, an anti-vertigo medication, is widely prescribed for balance disorders and vertigo relief, and gained initial registration in Europe in 1970 for Meniere's disease[17]. Our study explored the effects of Betahistine and Dingxuan, an herbal prescription widely used for Meniere's disease management. AVP, a hormone regulating water reabsorption in the kidneys, could contribute to abnormal fluid retention in elevated levels, potentially causing Meniere's disease symptoms[74,75]. cAMP, a second messenger in cellular processes, influences protein expression[62,76,77]. AQP2, vital for water reabsorption, and AQP5, regulating water movement across cell membranes, play roles in osmotic balance[78-81]. In comparison to betahistine, Dingxuan showed promising outcomes in a guinea pig model. Dingxuan modulated water channels AQ2 and AQ5, regulated AVP-mediated signaling through cAMP, and alleviated inner ear labyrinth hydrops. Importantly, no signs of liver and kidney toxicity were observed even at seventy times the standard dosage. This Chinese patent medicine holds potential clinical applications in targeting water channel activity, introducing a novel avenue for Meniere's syndrome treatment. Further research and clinical trials are necessary to explore its broader implications and efficacy in human subjects.

CONCLUSION

While grappling with the complexities of Meniere's disease management, this study illuminates the strides taken in comprehending its pathophysiology and advancing tailored treatments. Dingxuan emerged as a promising contender, showcasing efficacy in modulating water channels and regulating AVP-mediated signaling. Its capacity to ameliorate inner ear hydrops is noteworthy, accompanied by an absence of discernible liver or kidney toxicity even at elevated dosages. The prospects for Dingxuan as a Chinese patent medicine targeting water channel activity in clinical applications appear auspicious.

Declaration of competing interest

No conflicts of interest exist; the research adheres to ethical standards and transparently reports funding sources and affiliations.

REFERENCES

1. Basura GJ, Colandrea M, Walsh SA, Kuch AA, Monjur TM. Plain language summary: méniere's disease. Otolaryngol Head Neck Surg. 2020;162(4):435-45.
2. Mohseni-Dargah M, Falahati Z, Pastras C, Khajeh K, Mukherjee P, Razmjou A, et al. Meniere's disease: Pathogenesis, treatments, and emerging approaches for an idiopathic bioenvironmental disorder. Environ Res J. 2023:116972.
3. Webster KE, Galbraith K, Lee A, Harrington-Benton NA, Judd O, Kaski D, et al. Intratympanic gentamicin for Ménière's disease. Cochrane Database Syst Rev. 2023(2).
4. Basura GJ, Adams ME, Monfared A, Schwartz SR, Antonelli PJ, Burkard R, et al. Clinical practice guideline: Ménière's disease. Otolaryngol Head Neck Surg. 2020;162:S1-55.
5. Morita N, Kariya S, Farajzadeh Deroee A, Cureoglu S, Nomiya S, Nomiya R, et al. Membranous labyrinth volumes in normal ears and Meniere disease: a three-dimensional reconstruction study. Laryngoscope. 2009;119(11):2216-20.
6. Webster KE, Galbraith K, Harrington-Benton NA, Judd O, Kaski D, Maarsingh OR, et al. Systemic pharmacological interventions for Ménière's disease. Cochrane Database Syst Rev. 2023(2).
7. Hoskin JL. Ménière's disease: new guidelines, subtypes, imaging, and more. Curr Opin Neurol. 2022;35(1):90-7.
8. George B, Harrington-Benton NA, Judd O, Kaski D, Maarsingh OR, MacKeith S, et al. Surgical interventions for Ménière's disease. Cochrane Database Syst Rev. 2023(2).
9. Strupp M, Dlugaiczyk J, Ertl-Wagner BB, Rujescu D, Westhofen M, Dieterich M. Vestibular disorders: diagnosis, new classification and treatment. Dtsch Arztebl Int. 2020;117(17):300.
10. Bae CH, Na HG, Choi YS. Current diagnosis and treatment of vestibular neuritis: a narrative review. J Yeungnam Med Sci. 2022;39(2):81.
11. Belyantseva IA, Boger ET, Friedman TB. Myosin XVa localizes to the tips of inner ear sensory cell stereocilia and is essential for staircase formation of the hair bundle. Proc Natl Acad Sci. 2003;100(24):13958-63.
12. Webster KE, Lee A, Galbraith K, Harrington-Benton NA, Judd O, Kaski D, et al. Intratympanic corticosteroids for Ménière's disease. Cochrane Database Syst Rev. 2023(2).
13. Zhang YX, Zhang XT, Li HJ, Zhou TF, Zhou AC, Zhong ZL, et al. Antidepressant-like effects of helicid on a chronic unpredictable mild stress-induced depression rat model: Inhibiting the IKK/IκBα/NF-κB pathway through NCALD to reduce inflammation. Int Immunopharmacol. 2021;93:107165.
14. Chen JY, Guo ZQ, Wang J, Liu D, Tian E, Guo JQ, et al. Vestibular migraine or Meniere's disease: a diagnostic dilemma. J Neurol. 2023;270(4):1955-68.
15. Li Y, Dai M, Wang L, Wang G. Polysaccharides and glycosides from Aralia echinocaulis protect rats from arthritis by modulating the gut microbiota composition. J Ethnopharmacol. 2021;269:113749.
16. Murdin L, Hussain K, Schilder AG. Betahistine for symptoms of vertigo. Cochrane Database Syst Rev. 2016(6).
17. Van Esch B, van der Zaag-Loonen H, Bruintjes T, van Benthem PP. Betahistine in Meniere's disease or syndrome: a systematic review. Audiol Neurootol. 2022;27(1):1-33.

18. Sayin I, Koç RH, Temirbekov D, Gunes S, Cirak M, Yazici ZM. Betahistine add-on therapy for treatment of subjects with posterior benign paroxysmal positional vertigo: a randomized controlled trial. Braz J Otorhinolaryngol. 2022;88:421-6.

19. Zamergrad MV, Kunelskaya NL, Guseva AL, Amelin AV, Lilenko SV, Samartcev IN, et al. Betahistine in vestibular disorders: current concepts and perspectives. Vestn Otorinolaringol. 2021;86(2):73-81.

20. Adrion C, Fischer CS, Wagner J, Gürkov R, Mansmann U, Strupp M. Efficacy and safety of betahistine treatment in patients with Meniere's disease: primary results of a long term, multicentre, double blind, randomised, placebo controlled, dose defining trial (BEMED trial). BMJ. 2016;352.

21. Holmes S, Lalwani AK, Mankekar G. Is Betahistine Effective in the Treatment of Meniere's Disease. Laryngoscope. 2021;131(12):2639-40.

22. Quatre R, Attyé A, Karkas A, Job A, Dumas G, Schmerber S. Relationship between audio-vestibular functional tests and inner ear MRI in Meniere's disease. Ear Hear. 2019;40(1):168-76.

23. Shi S, Guo P, Li W, Wang W. Clinical features and endolymphatic hydrops in patients with MRI evidence of hydrops. Ann Otol Rhinol Laryngol. 2019;128(4):286-92.

24. Jian H, Wang S, Li X, Zhao H, Liu S, Lyu Y, et al. Effect of Late-Stage Meniere's Disease and Vestibular Functional Impairment on Hippocampal Atrophy. Laryngoscope. 2024;134(1):410-8.

25. Xiao H, Guo X, Cai H, Lin J, Lin C, Fang Z, et al. Magnetic resonance imaging of endolymphatic hydrops in Ménière's disease: A comparison of the diagnostic value of multiple scoring methods. Front Neurol. 2022;13:967323.

26. Angeli RD, Piccirillo E, Di Trapani G, Sequino G, Taibah A, Sanna M. Enlarged translabyrinthine approach with transapical extension in the management of giant vestibular schwannomas: personal experience and review of literature. Otol Neurotol. 2011;32(1):125-31.

27. Zhou F, Shi S, Wang D, Guo P, Wang W. MR imaging and clinical characteristics of Lermoyez syndrome. Acta Otolaryngol. 2020;140(7):528-32.

28. Koppelaar-van Eijsden HM, Schermer TR, Bruintjes TD. Measurement properties of the dizziness handicap inventory: a systematic review. Otol Neurotol. 2022;43(3):e282-97.

29. Mutlu B, Serbetcioglu B. Discussion of the dizziness handicap inventory. J Vestib Res. 2013;23(6):271-7.

30. Szostek-Rogula S, Zamysłowska-Szmytke E. Validation of the Polish version of the Dizziness Handicap Inventory. Med PR. 2019;70.

31. Kamo T, Ogihara H, Tanaka R, Kato T, Tsunoda R, Fushiki H. Relationship between physical activity and dizziness handicap inventory in patients with dizziness–A multivariate analysis. Auris Nasus Larynx. 2022;49(1):46-52.

32. McDonnell MN, Hillier SL. Vestibular rehabilitation for unilateral peripheral vestibular dysfunction. Cochrane Database Syst Rev. 2015(1).

33. Thomeer H, Bonnard D, Franco-Vidal V, Porez F, Darrouzet P, Liguoro D, et al. Prognostic factors of balance quality after transpetrosal vestibular schwannoma microsurgery: an instrumentally and DHI-based prospective cohort study of 48 patients. Otol Neurotol. 2015;36(5):886-91.

34. Bai P, Zhu R, Wang P, Jiang F, Zhen J, Yao Y, et al. The efficacy and safety of fingolimod plus standardized treatment versus standardized treatment alone for acute ischemic stroke: A systematic review and meta-analysis. Pharmacol Res Perspe. 2022;10(3):e00972.

35. Knox GW, McPherson A. Menière's disease: differential diagnosis and treatment. American family physician. 1997;55(4):1185-1194.

36. Kumagami H, Loewenheim H, Beitz E, Wild K, Schwartz H, Yamashita K, et al. The effect of anti-diuretic hormone on the endolymphatic sac of the inner ear. Pflugers Arch. 1998;436:970-5.

37. Le CH, Truong AQ, Diaz RC. Novel techniques for the diagnosis of Meniere's disease. Curr Opin Otolaryngol Head Neck Surg. 2013;21(5):492-6.

38. Minor LB, Schessel DA, Carey JP. Meniere's disease. Curr Opin Neurol. 2004;17(1):9-16.

39. Phillips JS, Murdin L, Rea P, Sutton L. Clinical subtyping of Ménière's disease. Otolaryngol Head Neck Surg. 2018;159(3):407-9.

40. Pan M, Zhao F, Xie B, Wu H, Zhang S, Ye C, et al. Dietary □-3 polyunsaturated fatty acids are protective for myopia. Proc Natl Acad Sci. 2021;118(43):e2104689118.

41. Lin X, Lei Y, Pan M, Hu C, Xie B, Wu W, et al. Augmentation of scleral glycolysis promotes myopia through histone lactylation. Cell Metab. 2024;36(3):511-525.

42. Jiang LY, He JJ, Chen XX, Sun XJ, Wang XZ, Zhong S, et al. Arginine vasopressin-aquaporin-2 pathway-mediated dehydration effects of electroacupuncture in guinea pig model of AVP-induced eendolymphatic hydrops. Chin J Integr Med. 2019;25:763-9.

43. Knapstad MK, Goplen FK, Nordahl SH, Berge JE. The Dizziness Handicap Inventory and sickness absence: a cross-sectional study. Disabil Rehabil. 2023;45(2):286-90.

44. Liyuan J, Jiaojun H, Xixi C, Huade C. Effect of electroacupuncture on arginine vasopressin-induced endolymphatic hydrops. J Tradit Chin Med. 2019;39(2).

45. Sajjadi H, Paparella MM. Meniere's disease. Lancet. 2008;372(9636):406-14.

46. Guo LL, Gao RY, Wang LH, Lin SJ, Fang BH, Zhao YD. *In Vivo* pharmacokinetic/pharmacodynamic (PK/PD) profiles of Tulathromycin in an experimental intraperitoneal Haemophilus parasuis infection model in Neutropenic Guinea Pigs. Front Vet Sci. 2021;8:715887.

47. Ma M, Wei N, Yang J, Ding T, Song A, Chen L, et al. Schisandrin B promotes senescence of activated hepatic stellate cell via NCOA4-mediated ferritinophagy. Pharm Biol. 2023;61(1):621-9.

48. Pei H, Sutton AK, Burnett KH, Fuller PM, Olson DP. AVP neurons in the paraventricular nucleus of the hypothalamus regulate feeding. Mol Metab. 2014;3(2):209-15.

49. Borresen SW, Klose M, Glintborg D, Watt T, Andersen MS, Feldt-Rasmussen U. Approach to the patient with glucocorticoid induced adrenal insufficiency. J Clin Endocrinol Metab. 2022;107(7):2065-76.

50. Sun X, Zheng Y, Tian Y, Xu Q, Liu S, Li H, et al. Astragalus polysaccharide alleviates alcoholic-induced hepatic fibrosis by inhibiting polymerase I and transcript release factor and the TLR4/JNK/NF-κB/MyD88 pathway. J Ethnopharmacol. 2023;314:116662.

51. Chen Y, Guo M, Qu D, Liu Y, Guo J, Chen Y. Furin-responsive triterpenine-based liposomal complex enhances anticervical cancer therapy through size modulation. J Drug Deliv. 2020;27(1):1608-24.

52. Ding M, Tang Z, Liu W, Shao T, Yuan P, Chen K, et al. Burdock fructooligosaccharide attenuates high glucose-induced apoptosis and oxidative stress injury in renal tubular epithelial cells. Front pharmacol. 2021;12:784187.

53. Sun S, Wang Y, Du Y, Sun Q, He L, Zhu E, et al. Oxidative stress-mediated hepatotoxicity in rats induced by ethanol extracts of different parts of Chloranthus serratus. Pharm Biol. 2020;58(1):1286-98.

54. Deng Z, Gao S, An Y, Huang Y, Liu H, Zhu W, et al. Effects of earthworm extract on the lipid profile and fatty liver induced by a high-fat diet in guinea pigs. Ann Transl Med. 2021;9(4).

55. Regal JF, Fraser DG, Weeks CE, Greenberg NA. Dietary Phytoestrogens Have Anti-Inflammatory Activity in a Guinea Pig Model of Asthma (44504). Exp Biol Med. 2000;223(4):372-8.

56. Zhang R, Liu Y, Zhong W, Hu Z, Wu C, Ma M, et al. SIK2 improving mitochondrial autophagy restriction induced by cerebral ischemia-reperfusion in rats. Front Pharmacol. 2022;13:683898.

57. Zhang J, Zhou W, Chen Y, Wang Y, Guo Z, Hu W, et al. Small molecules targeting Pin1 as potent anticancer drugs. Front Pharmacol. 2023;14:1073037.

58. Zhang S, Zhu P, Yuan J, Cheng K, Xu Q, Chen W, et al. Non-alcoholic fatty liver disease combined with rheumatoid arthritis exacerbates liver fibrosis by stimulating co-localization of PTRF and TLR4 in rats. Front Pharmacol. 2023;14:1149665.

59. Chen J, Cao D, Jiang S, Liu X, Pan W, Cui H, et al. Triterpenoid saponins from Ilex pubescens promote blood circulation in blood stasis syndrome by regulating sphingolipid metabolism and the PI3K/AKT/eNOS signaling pathway. Phytomedicine. 2022;104:154242.

60. Ding D, Shen X, Yu L, Zheng Y, Liu Y, Wang W, et al. Timosaponin BII inhibits TGF-β mediated epithelial-mesenchymal transition through Smad-dependent pathway during pulmonary fibrosis. Phytother Res. 2023;37(7):2787-99.

61. Chen Y, Wang S, Hu Q, Zhou L. Self-emulsifying System Co-loaded with Paclitaxel and Coix Seed Oil Deeply Penetrated to Enhance Efficacy in Cervical Cancer. Curr Drug Deliv. 2023;20(7):919-26.

62. Song A, Ding T, Wei N, Yang J, Ma M, Zheng S, et al. Schisandrin B induces HepG2 cells pyroptosis by activating NK cells mediated anti-tumor immunity. Toxicol Appl Pharmacol. 2023;472:116574.

63. Radakovich LB, Marolf AJ, Culver LA, Santangelo KS. Calorie restriction with regular chow, but not a high-fat diet, delays onset of spontaneous osteoarthritis in the Hartley guinea pig model. Arthritis Res Ther. 2019;21:1-4.

64. Tveden-Nyborg P, Birck MM, Ipsen DH, Thiessen T, de Bie Feldmann L, Lindblad MM, et al. Diet-induced dyslipidemia leads to nonalcoholic fatty liver disease and oxidative stress in guinea pigs. Am J Transl Res. 2016;168:146-60.

65. Zhu F, Yuan C, Zhang X, Wang Z, Wang Q, Wang H. A-kinase anchoring protein 5-ancored calcineurin regulates the remodeling of H9c2 cardiomyocytes exposed to hypoxia and reoxygenation. Biomed Pharmacother. 2022;155:113689.

66. Kahn L, Hautefort C, Guichard JP, Toupet M, Jourdaine C, Vitaux H, et al. Relationship between video head impulse test, ocular and cervical vestibular evoked myogenic potentials, and compartmental magnetic resonance imaging classification in meniere's disease. Laryngoscope. 2020;130(7):E444-52.

67. Mu D, Ma C, Cheng J, Zou Y, Qiu L, Cheng X. Copeptin in fluid disorders and stress. Clin Chim Acta. 2022;529:46-60.

68. Takeda K, Meyer-Lehnert HA, Kim JK, Schrier RW. AVP-induced Ca fluxes and contraction of rat glomerular mesangial cells. Am J Physiol Renal Physiol. 1988;255(1):F142-50.

69. Douglass AM, Resch JM, Madara JC, Kucukdereli H, Yizhar O, Grama A, et al. Neural basis for fasting activation of the hypothalamic–pituitary–adrenal axis. Nature. 2023;620(7972):154-62.

70. Ma Y, Liu T, Li X, Kong A, Xiao R, Xie R, et al. Estrogen receptor β deficiency impairs gut microbiota: a possible mechanism of IBD-induced anxiety-like behavior. Microbiome. 2022;10(1):160.

71. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178.

72. Zang L, Xu H, Huang C, Wang C, Wang R, Chen Y, et al. A link between chemical structure and biological activity in triterpenoids. Recent Pat Anticancer Drug Discov. 2022;17(2):145-61.

73. Sun S, Li S, Du Y, Wu C, Zhang M, Li J, et al. Anti-inflammatory effects of the root, stem and leaf extracts of *Chloranthus serratus* on adjuvant-induced arthritis in rats. Pharm Biol. 2020;58(1):528-37.

74. Hu YH, Han J, Wang L, Shi C, Li Y, Olatunji OJ, et al. α-Mangostin alleviated inflammation in rats with adjuvant-induced arthritis by disrupting adipocytes-mediated metabolism-immune feedback. Front Pharmacol. 2021;12:692806.

75. Jiang TT, Ji CF, Cheng XP, Gu SF, Wang R, Li Y, et al. α-Mangostin alleviated HIF-1α-mediated angiogenesis in rats with adjuvant-induced arthritis by suppressing aerobic glycolysis. Front Pharmacol. 2021;12:785586.

76. Wang DD, Li Y, Wu YJ, Wu YL, Han J, Olatunji OJ, et al. Xanthones from *Securidaca inappendiculata* antagonized the antirheumatic effects of methotrexate *in vivo* by promoting its secretion into urine. Expert Opin Drug Metab Toxicol. 2021;17(2):241-50.

77. Zhou Y, Liu L, Xiang R, Bu X, Qin G, Dai J, et al. Arctigenin mitigates insulin resistance by modulating the IRS2/GLUT4 pathway via TLR4 in type 2 diabetes mellitus mice. Int Immunopharmacol. 2023;114:109529.

78. Ando F. Activation of AQP2 water channels by protein kinase A: therapeutic strategies for congenital nephrogenic diabetes insipidus. Clin Nephrol. 2021 Oct;25:1051-6.

79. Deng S, Chen X, Lei Q, Lu W. AQP2 promotes astrocyte activation by modulating the TLR4/NFκB-p65 pathway following intracerebral hemorrhage. Front immunol. 2022;13:847360.

80. Gao C, Zhang L, Chen E, Zhang W. Aqp2+ progenitor cells maintain and repair distal renal segments. Clin J Am Soc Nephrol. 2022;33(7):1357-76.

81. Zhou Y, Xiang R, Qin G, Ji B, Yang S, Wang G, et al. Xanthones from Securidaca inappendiculata Hassk. attenuate collagen-induced arthritis in rats by inhibiting the nicotinamide phosphoribosyltransferase/glycolysis pathway and macrophage polarization. Int Immunopharmacol. 2022;111:109137.