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Evaluation of the *in vivo* subacute toxicity of Otolith as a novel traditional hypoglycemic agent

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In coastal areas like Hodeida governorate-Yemen, otolith, or "ear stone", are used to treat diabetes. Locals believe that these formations can manage diabetes and relieve migraine pain. This study examines the *in vivo* subacute toxicity and safety of catfish otolith as a novel traditional antidiabetic agent. Twenty \ fourmale rats were evenly divided into four six-rat groups. Control group (Group 1) received 0.9% w/v sodium chloride for 21 days. The remaining three groups received oral otolith at different doses for the same time. Group 2, the low-dose group, received 5 g/day otolith per body weight. Mid-dose group 3 received 10 g/day otolith per body weight. Finally, Group 4, the high-dose group, received 15 g/day otolith per body weight. The animals' behavior and anomalies were recorded throughout the experiment. Biochemical tests like liver and kidney function and hematological tests like total blood count were done at the end of the experiment. In large doses, otoliths reduced cholesterol, LDL, and triglycerides and increased HDL. Otolith did not affect renal function or electrolyte balance at the tested doses. It affected certain hepatic enzyme functions at higher doses, but protein levels remained stable across doses, indicating neither liver injury nor considerable hepatic function loss. This study sheds light on otolith's safety, notably for renal function, electrolyte homeostasis, and liver health.

Keywords: Otolith, Safety, Subacute toxicity, Traditional antidiabetic

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Fish serves as an excellent source of essential nutrients, including amino acids, fatty acids, vitamins, and minerals. It plays a crucial role in both the prevention and treatment of various health conditions, such as asthma, heart diseases, ocular disorders, and nutritional deficiencies. To maintain optimal health, it is highly recommended to incorporate fish into one's daily dietary regimen[1]. Fish offers a wealth of nutritional benefits beyond its basic components. It is a rich source of premium protein and omega-3 fatty acids, which play vital roles in supporting cognitive function and heart health. Studies have shown that incorporating fish into one's diet regularly may lower the likelihood of developing conditions such as depression, Alzheimer's disease, and specific forms of cancer[2]. Furthermore, fish is often regarded as a more sustainable protein option in comparison to many animal products derived from land-based sources. This makes it an appealing choice for individuals who are mindful of their impact on the environment[2].

The coastal waters of Yemen boast a rich diversity of marine organisms, which are utilized in traditional medicine. One such example of this aquatic biodiversity is the fish species *Arius thalassinus*, locally known as "Comal"[3]. It contains otoliths, which are commonly referred to as "ear stones". Furthermore, otoliths play a crucial role in sensory perception, equilibrium, locomotion, and auditory function[4].

The *Arius thalassinus*, commonly known as "Comal," holds significance beyond its medicinal applications, serving as a vital gauge of marine ecosystem well-being in Yemen's coastal regions. For generations, traditional healers in Yemen have recognized the potential curative properties of otoliths, incorporating them into various treatments for conditions associated with equilibrium and auditory impairments. This remarkable convergence of folk medicine and marine science underscores the necessity of safeguarding Yemen's diverse marine

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life, not only for ecological preservation but also to maintain cultural heritage[4].

The otolith is a small, white structure present in the head of all fish except sharks, rays and lampreys[5] (Supplementary Fig. S1). There are three types of otoliths: lapillus, sagitta and asteriscus. The sagitta is considered the largest otolith[6]. Similar to the asteriscus, it plays a crucial role in sound detection and the process of hearing[7]. Furthermore, the lapillus is involved in the identification of gravitational force and sound[8].

The inner ear comprises the cochlea and vestibular organs; the cochlea facilitates hearing, whereas the vestibular organs detect head movement, crucial for spatial direction and bodily equilibrium[9]. The vestibular apparatus on either side comprises three semicircular canals and two otolith organs, namely the utricle and saccule. The otolithic receptors are located on the maculae of the utricle and saccule[10]. Consequently, the accurate creation, stabilization, and preservation of the vestibular otolith are essential for good vestibular function and the maintenance of balance[11].

Vestibular evoked myogenic potentials (VEMP) serve as objective and reliable electrophysiological assessments of otolith function[12]. The cervical VEMP (cVEMP) is a short-latency muscle response thought to originate from the saccule, while the ocular VEMP (oVEMP) is considered to originate from the utricle[13].

In coastal regions, particularly the Hodeida governorate, otolith has been traditionally utilized. Thelocal population believes it possesses numerous health advantages, including ability to manage diabetes and migraine pain. This practice has been in place for many years, with people relying on otolith for these purposes.

People believe this agent manages their high blood sugar levels without additional supportive medications. Subacute toxicity studies are generally mandated by regulatory agencies worldwide prior to the commencement or progression of human trials for potential new drug candidates; however, the specific criteria for conducting preclinical studies vary by region. The United States, Europe, and Japan, the three main areas for pharmaceutical development, have been working together to standardize various regulatory requirements for preclinical research. These efforts have resulted in the publication of guidelines through the International Conference for Harmonization (ICH) compendium. Before the establishment of the ICH and modern general toxicology planning, it was not unusual for pharmaceutical companies to conduct Good Laboratory Practice (GLP) studies ranging from acute to chronic durations prior to beginning the clinical phase[14].

The length of repeated-dose toxicity studies typically corresponds to the intended duration, therapeutic use, and proposed dosing period of the Phase I clinical trial. In some cases, a fortnight-long study may suffice to support the Phase I clinical trial. Nevertheless, for the majority of products, a month-long study is essential to provide adequate safety data for trials involving multiple doses. Consequently, the 4-week study is conducted to bolster the toxicity information for the potential pharmaceutical product[15]. This novel research serves as a foundation for examining the subacute toxicity and safety characteristics of otolith.

Material and Methods

Collection and preparation of Otolith

Otolith pieces were obtained freshly from catfishes hunted from the Red Sea near Khokhaport, Hodeida governorate –Yemen. The dissection was performed on the top of the fish head, behind the eyes. An optimal cut removes the top of the skull, exposing the brain. By pressing the brain, the pair of otoliths from each side rise to the surface and are removed. The otoliths were then washed with distilled water and acetone before drying at room temperature. The otoliths were then ground, dissolved in water, and given to the rats orally.

Experimental animals

Male albino rats (weighing approximately 200-250 g) were obtained from the animal house of the Research Center of Sana'a University, Sana'a, Yemen. Animals were housed in a settlement room under a 12/12 h light/dark cycle at 21±2°C and had free access to water and food. All animal experiments were approved by the Research Ethical Committee of the University of Science and Technology-Yemen with reference number (EAC/UST234).

Sub-acute toxicity study

Subacute oral toxicity study of otolith were implemented according to the OECD guideline 407 for testing of chemicals according to OECD, A[16] and Haruna, Hauwa MS *et al.*[17]. Twenty-four male rats were randomly divided into four groups (n = 6).

Group 1: The control group [6 rats] received a sodium chloride solution (0.9% w/v) for 21 days Group 2: Low dose group [6 rats] received otolith 5 g/day of body weight orally/day) for 21 days. Group 3: Mid-dose group [6 rats] received otolith 10 g/day of body weight orally/day for 21 days. Group 4: High dose group [6 rats] received otolith 15 g/day of body weight orally/day for 21 days. Animals were observed for general behavior and signs of abnormalities during the experiment duration[18].

Blood sampling

After 21 days, animals were anesthetized by chloroform. Blood is collected from the venous sinus while the rat is under terminal anesthesia. The neck is gently scruffed and the eye is made to bulge. A capillary tube is inserted laterally. Blood is allowed to flow by capillary action into the capillary tube. Blood samples were collected into sterile tubes with anticoagulant (EDTA) for hematological tests and without anticoagulant tubes for biochemical tests. Blood samples (without anticoagulant) tubes were centrifuged at 5000 rpm for 15 min and serum was separated[18].

Biochemical tests such as liver and kidney function tests were performed as well as hematological tests including complete blood count and lipid profile at the end of the experiment[19,20].

Statistical analysis

Data were summarized as means ± SEM. One-way analysis of variance (ANOVA) was used to conduct the significance of association using SPSS Program version 20. Differences were considered significant at p values < 0.05.

Results

Our results discussed the subacute toxicity of otolith at different doses of 5, 10, and 15 g/day on hematological and metabolic parameters, kidney function and electrolyte levels, liver enzymes and protein levels.

Table 1 presents data on the effects of different dosages of otolith (5 g/day, 10 g/day, and 15 g/day) on various blood parameters compared with control groups. Parameters measured include Hemoglobin (Hb), Red Blood Cells (RBCs), Cholesterol, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Triglycerides (TG).

Otolith administration had a significant impact on various hematological and metabolic parameters, particularly at higher doses. Notably, elevated otolith doses led to substantial reductions in cholesterol, LDL, and triglyceride levels, whilst HDL levels increased, suggesting a beneficial effect on lipid profiles. Significant alterations in RBCs were also observed at certain dosages.

Table 1 — Impact of otolith dosing on hematological and metabolic parameters

Parameter	Otolith dose	Control mean	Otolith mean	Mean difference	Adjusted p-value	F	P-value
Hb (g /dL)						0.105	0.9562
	5 g/day	13.52	13.55	-0.03	0.9993		
	10 g/day	13.52	13.65	-0.13	0.9598		
	15 g/day	13.52	13.47	0.05	0.9976		
RBS(mg /dL)						6.622	0.0028*
	5 g/day	115	98.6	16.4	0.0424*		
	10 g/day	115	104.2	10.8	0.231		
	15 g/day	115	87.67	27.33	0.0008*		
Cholesterol (mg /dL)						26.39	<0.0001*
	5 g/day	118.2	112	6.17	0.6776		
	10 g/day	118.2	78.8	39.37	<0.0001*		
	15 g/day	118.2	69.67	48.5	<0.0001*		
LDL (mg /dL)						6.002	0.0043*
	5 g/day	54	52.4	1.6	0.9213		
	10 g/day	54	54	0	>0.9999		
	15 g/day	54	42.63	11.37	0.0049*		
HDL (mg /dL)						9.428	0.0004*
	5 g/day	28.5	23	5.5	0.0037*		
	10 g/day	28.5	28.6	-0.1	0.9997		
	15 g/day	28.5	23	5.5	0.0037*		
TG (mg /dL)						13.45	<0.0001*
	5 g/day	125.5	103	22.53	0.0171*		
	10 g/day	125.5	107	18.5	0.0545		
	15 g/day	125.5	78.8	46.7	<0.0001*	6.622	

As demonstrated in Table 2, the administration of otolith at various doses (5 g/day, 10 g/day, and 15 g/day) generally does not elicit significant alterations in the majority of kidney function and electrolyte parameters. These include serum creatinine, sodium, calcium, urea, and uric acid, with p-values exceeding 0.05. However, a notable exception is observed in potassium levels, which exhibit a significant elevation at the 15 g/day dosage ($p = 0.0335$), indicating a dose-dependent effect of otolith on potassium regulation.

The results indicate that otolith generally does not adversely affect renal function or electrolyte balance at the doses examined. However, it is advisable to closely monitor potassium concentrations when administering higher doses to prevent possible hyperkalemia-related complications.

Table 3 showed that, the Alkaline Phosphatase (ALP) levels significantly increase at all dosages of otolith (5 g/day, 10 g/day, and 15 g/day), with the most substantial increase observed at 15 g/day ($p<0.0001$). Similarly, Bilirubin levels also show a significant increase at all dosages, particularly at 15 g/day ($p<0.0001$). Glutamate Pyruvate Transaminase (GPT) levels significantly increase only at the highest dose of 15 g/day ($p=0.0324$), while Glutamate Oxaloacetate Transaminase (GOT) levels significantly increase at all dosages, with the highest increase at 15 g/day ($p<0.0001$). However, protein levels remain unaffected across all dosages, with p-values well above 0.05.

Discussion

Our results reveal a statistically significant overall effect of otolith on RBS levels, as indicated by the f-value of 6.622 and a p-value of 0.0028. This suggests that otolith supplementation has a measurable impact on blood sugar regulation, with the extent of this effect varying by dose.

At the 5 g/day dose, RBS levels decreased significantly from a control mean of 115 mg/dL to 98.6 mg/dL, with a mean difference of 16.4 mg/dL and an adjusted p-value of 0.0424. This indicates that even a low dose of otolith can effectively lower blood sugar levels. However, at the 10 g/day dose, the reduction in RBS (mean difference of 10.8 mg/dL) was not statistically significant ($p=0.231$), suggesting a potential non-linear or threshold effect in the dose-response relationship. In contrast, the 15 g/day dose showed the most pronounced reduction in RBS, with levels dropping to 87.67 mg/dL, a mean difference of 27.33 mg/dL, and a highly significant p-value of 0.0008. This demonstrates that higher doses of Otolith are particularly effective in lowering blood sugar levels.

The findings suggest a dose-dependent relationship between otolith supplementation and RBS reduction,

Table 2 — Effects of otolith treatment on kidney function and electrolyte levels

Parameter	Otolith dose	Control mean	Otolith mean	Mean diff.	Adjusted p value	F	P-value
Serum creatinine						0.2801	0.8391
(mg/dL)	5 g/day	0.65	0.60	0.05	0.9061		
	10 g/day	0.65	0.67	-0.02	0.9959		
	15 g/day	0.65	0.62	0.03	0.9691		
Potassium (K) (mmol/L)						6.888	0.0023*
	5 g/day	2.65	2.47	0.18	0.4998		
	10 g/day	2.65	2.47	0.18	0.4998		
	15 g/day	2.65	3.07	-0.42	0.0335		
Sodium (Na) (mmol/L)						1.022	0.4037
	5 g/day	119.50	123.80	-4.30	0.4176		
	10 g/day	119.50	124.20	-4.70	0.3433		
	15 g/day	119.50	122.20	-2.70	0.7572		
Calcium (Ca) (mg/dL)						1.412	0.2687
	5 g/day	8.27	8.13	0.13	0.6589		
	10 g/day	8.27	8.22	0.05	0.9688		
	15 g/day	8.27	8.00	0.27	0.1646		
Urea (mg/dL)						0.0613	0.9796
	5 g/day	19.00	19.50	-0.50	0.9857		
	10 g/day	19.00	19.60	-0.60	0.976		
	15 g/day	19.00	19.00	0.00	>0.9999		
Uric acid (mg/dL)						0.5028	0.6847
	5 g/day	2.32	2.33	-0.02	0.9989		
	10 g/day	2.32	2.18	0.13	0.6954		
	15 g/day	2.32	2.22	0.10	0.8368		

Table 3 — Otolith dose-dependent changes in liver enzymes and protein levels

Parameter	Otolith dose	Control mean	Otolith mean	Mean diff.	Adjusted p value	F	P-value
ALP (IU/L)						57.45	<0.0001*
	5 g/day	287.8	545	-257.2	0.0034*		
	10 g/day	287.8	555.7	-267.9	0.0024*		
	15 g/day	287.8	1152	-864.2	<0.0001*		
Bilirubin						22.9	<0.0001*
(mg/dL)	5 g/day	0.4	0.52	-0.12	0.3933		
	10 g/day	0.4	0.4	0	>0.9999		
	15 g/day	0.4	1	-0.6	<0.0001*		
GPT (U/L)						4.625	0.0136*
	5 g/day	59.83	60.17	-0.33	>0.9999		
	10 g/day	59.83	53.8	6.03	0.8019		
	15 g/day	59.83	81.2	-21.37	0.0324*		
GOT(U/L)						126.9	<0.0001*
	5 g/day	50.2	63.17	-12.97	<0.0001*		
	10 g/day	50.2	64.33	-14.13	<0.0001*		
	15 g/day	50.2	92.2	-42	<0.0001*		
Albumin (g/dL)						1.42	0.2663
	5 g/day	3.28	3.22	0.07	0.9433		
	10 g/day	3.28	3.5	-0.22	0.3532		
	15 g/day	3.28	3.27	0.02	0.999		
Protein (g/dL)						0.303	0.8228
	5 g/day	6.45	6.62	-0.17	0.7681		
	10 g/day	6.45	6.52	-0.07	0.9781		
	15 g/day	6.45	6.62	-0.17	0.7681		

with the 15 g/day dose being the most effective. This has important clinical implications, as it indicates that otolith could potentially be used as a therapeutic agent for managing blood sugar levels, particularly at higher doses. However, This result aligns with another study undertaken by Areqi *et al.,*[1] which revealed that otolith may be useful in treating type 2 Diabetes mellitus with no visible signs or symptoms of toxicity in rats indicating a high margin of safety. The otolith exhibited antidiabetic activity comparable to that of a standard drug (gliclazideand pioglitazone) in both models. The traditional use of otolith to treat diabetes is supported by laboratory finding from this study[1].

Our finding showed that the administration of otoliths demonstrated a dose-dependent effect on various physiological parameters, with higher doses eliciting more pronounced alterations. The most significant effect was observed in lipid profiles, with higher otolith dosages resulting in a beneficial alteration of blood lipid composition. The observed decrease in cholesterol, LDL, and triglyceride concentrations, alongside an increase in HDL levels, indicates that otoliths might be useful in addressing dyslipidemia and lowering cardiovascular risk factors. These alterations in lipid profiles could be attributed to the distinctive mineral content of otoliths, which may influence the body's lipid metabolism and transport processes.

In addition to the effects on lipid profiles, significant alterations in RBCs parameters were observed at specific dosages. The changes in red blood cell characteristics might influence oxygen-carrying capacity and overall blood health. These results suggest that otoliths may effectively improve various health markers, positioning them as a potential therapeutic intervention. The varied effects of otolith administration on both metabolic and hematological factors highlight its potential as a novel therapeutic strategy.

The results of our study provide substantive insights into the safety profile of otolith with regard to renal function and electrolyte balance. At the doses examined, otolith exhibited a generally favorable safety profile, with no significant adverse effects on kidney function or electrolyte homeostasis.

This finding is significant for individuals who may need to take otolith for extended periods. It suggests the medication can be administered over the long term without posing significant risks to kidney function or disturbing the body's crucial electrolyte balance. The finding that higher doses may influence potassium levels requires serious attention in medical settings. Clinicians should establish consistent monitoring

procedures for individuals receiving increased amounts of otolith, particularly emphasizing the measurement of potassium in the blood.

The beneficial effects of otoliths were also confirmed in animal studies, where reductions in blood sugar, triglycerides, cholesterol, and LDL levels were observed. This supports otolith's potential for broader applications in metabolic health. Another study evaluating otolith's cytotoxicity, anti-inflammatory properties, and wound healing potential demonstrated that otoliths might also have applications in biomedical fields[12].

However, the study also highlights the importance of vigilant monitoring, particularly when higher doses of otolith are prescribed. This proactive approach may facilitate the detection and prevention of potential hyperkalemia, a condition that, if unaddressed, could result in severe cardiac complications. Through maintaining vigilant monitoring of electrolyte balance, particularly potassium, clinicians can optimize the safety and efficacy of otolith treatment whilst minimizing the risk of adverse events associated with electrolyte imbalances.

Our results suggest that the otolith has a dose-dependent impact on liver enzyme activity, indicating potential hepatotoxic effects at higher dosages, while protein levels remain stable. The observed alterations in enzyme levels suggest that otolith may influence hepatic function, particularly at elevated dosages. The consistency of protein concentrations across various dose levels indicates that otolith's impact on the liver may be specific to certain metabolic pathways rather than inducing generalized hepatic dysfunction.

While otolith demonstrates the capacity to alter the function of specific hepatic enzymes, particularly at elevated dosages, it does not appear to induce generalized hepatic damage. The consistency of protein levels across varying doses suggests that the overall hepatic function is not significantly compromised by otolith administration.

These results warrant further investigation into the long-term effects of Otolith consumption on liver function, particularly at higher dosages. Future studies could explore the mechanisms underlying the observed increases in liver enzyme activity and determine if these changes are reversible upon discontinuation of otolith supplementation. Additionally, monitoring other markers of liver health and function could provide a more comprehensive understanding of otolith's impact on hepatic processes.

Conclusion

The study found that the investigated doses of otolith (5 g/day, 10 g/day, and 15 g/day) were well-tolerated and did not show any adverse effects on renal function or electrolyte balance. This is an important finding, as it suggests that otolith supplementation is safe for the kidneys and does not disrupt the body's essential electrolyte levels, even at higher doses. Additionally, while higher doses of otolith were observed to alter the activity of certain hepatic enzymes, these changes did not translate into widespread liver damage or significant impairment of overall liver function. This conclusion is supported by the consistent levels of proteins across all dosage groups, indicating that liver function remained stable throughout the study.

One of the most notable outcomes of otolith administration was its positive impact on lipid profiles. The study documented significant improvements in key lipid markers, including reductions in total cholesterol, LDL (low-density lipoprotein), and triglycerides. These changes were particularly pronounced at higher dosages, suggesting a dose-dependent relationship. Furthermore, otolith supplementation was associated with an increase in HDL (high-density lipoprotein) levels, which is often referred to as "good cholesterol" due to its role in protecting cardiovascular health. The improvements in lipid profiles, especially at higher doses, highlight the potential of otolith as a therapeutic agent for managing dyslipidemia and reducing the risk of cardiovascular diseases. Overall, these findings underscore the safety and efficacy of otolith in improving metabolic health without compromising renal or hepatic function.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24(6)(2025)536-542_SupplData.pdf

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

AAA, NMEM and DAI conceived the study. AH conducted investigations and data analysis. AMMS prepared the formulation and analysis. AAA drafted the manuscript.

Ethics Approval

Ethical approval for this study was granted by the Ethical Committee of Medical Research at the University of Science and Technology, with reference number (EAC/UST234).

Data Availability

The authors confirm that the data supporting the findings of this study are presented within the article and/or its supplementary materials, and will be made available upon reasonable request.

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