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Evaluation of Hypolipidemic Activity of Otolith Extracted from Catfish in Experimental Animals

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ABSTRACT

Background: Otolith, also referred to as "ear stone", have been traditionally utilized in coastal regions, particularly in the Hodeida governorate, for managing diabetes mellitus. The local population believes that these structures possess numerous health benefit, including a purported ability to magically regulate diabetes and alleviate migraine headache pain. **Objective:** To investigate the hypolipidemic effects of Otolith as a novel traditional neutral agent obtained from catfish. **Method**: The 24 male rats were evenly divided into four groups six-rat in each. 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 (low dose) received 5g/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. Lipid profile tests were done at the end of the experiment. **Results**: The findings indicate that Otolith markedly decreased triglycerides from 125.5 mg/dL (control) to 78.8 mg/dL (15 mg/day), cholesterol from 118.2 mg/dL to 69.67 mg/dL, and LDL from 54 mg/dL to 42.63 mg/dL, while elevating HDL from 28.5 mg/dL to 34 mg/dL at the maximum dosage (15 g/day). The dose-dependent enhancements were statistically significant $P<0.05$ for all, with the most notable effects observed at 15 g/day, suggesting Otolith's potential as a lipid-lowering agent. **Conclusion:** Otolith supplementation demonstrated a dose-dependent amelioration of lipid metabolism, marked by substantial decreases in total cholesterol, LDL, and triglyceride concentrations alongside elevated HDL levels. These beneficial effects were most pronounced at the maximum administered dosage (15 g/day), suggesting a potential therapeutic role in dyslipidemia management.

Keywords: Otolith, hypolipidemic effects, lipid profile, catfish, Yemen's coastal areas.

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INTRODUCTION

Essential nutrients such as vitamins, minerals, fatty acids, and amino acids are all abundant in fish. It is essential for the prevention and treatment of a number of illnesses, including heart disease, eye disorders, asthma, and nutritional deficiencies. It is strongly advised that people include fish in their daily diet in order to preserve optimum health [1]. The fish provides many more nutritional advantages than only its fundamental elements. Rich in both omega-3 fatty acids and quality protein, it is essential for supporting heart health and cognitive ability. Regular fish intake has been linked in studies to reduced risk of illnesses including depression, Alzheimer's disease, and particular types of cancer [2]. Moreover, fish is sometimes considered as a more sustainable source of protein than other animal goods coming from land-based sources. For those who consider their effect on the surroundings, this makes it a tempting alternative [2].

Yemen's coastal seas provide a great variety of marine life, which is used in traditional medicine. *Arius Thalassinus*, sometimes known as "Comal," is one such example of aquatic biodiversity (Yemeni Ministry of Fisheries, 2007). Otoliths, sometimes known as "ear stones," abound in it. Moreover, otoliths are absolutely important for sensory awareness, balance, mobility, and hearing [3].

A total of 31 elements have been detected in otoliths to date not including radioactive elements such as Th, Ra. The elemental composition is dominated by major elements calcium, oxygen, and carbon which make up the calcium carbonate (CaCo3). The minor elements are represented by Na, Sr, K, S, N, CL and Zn [4]. Beyond its medical uses, the *Arius Thalassinus* also known as "Comal is an important indicator of the condition of marine ecosystems in Yemen's coastal areas .

For millennia, Yemen's traditional healers have known otoliths' possible healing powers and have included them into several treatments for disorders related to equilibrium and auditory loss. This amazing intersection of traditional medicine and marine science emphasizes the need of protecting Yemen's varied marine life not only for environmental preservation but also for preservation of cultural legacy [4].

Except for sharks, ray, and lampreys, every fish has a little white substance called the otolith in the cranial area [5]. Otoliths come in three flavors: asteriscus, sagitta, and lapillus. Considered as the largest otolith is the sagitta [6]. Like the asteriscus, it is absolutely important for sound detection and hearing process. Moreover, the lapillus helps to identify gravity force and sound [7]. Study outcomes conducted in 2015, were suggested that otolith may be useful in treating type 2 Diabetes mellitus in rats with no visible signs or symptoms of toxicity indicating a high margin of safety [1].

Figure 1: Collected Otolith from catfish

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In coastal regions, particularly the Hodeida governorate, otolith has been traditionally used for their perceived health benefits. The local population believes it otolith manage diabetes and relieve migraine pain [1]. This practice has been in place for many years, with people relying on otolith for these purposes.

Hypolipidemic 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 [6]. Health data from countries around the world which records global population of 8.2 billion revealed that CVD is the leading cause of death with approximately 17.7 million deaths annually and the mortality was estimated to be 23.6 million by 2030 [8]. Hyperlipidemia plays a significant role in the onset of conditions including diabetes, liver disease, and others, as well as the decline in bone health [9, 10]. There are many other medical benefits of otoliths as people believe: that these structures possess numerous health advantages, including a purported ability to magically regulate diabetes, alleviate migraine headache pain [1], renal colic and kidney stones [11]. On the contrary, study conducted in 2020, showed that otolith has no protective effect on stone formation/nephropathy [12] as well as, otolith used for Rickets in children and trouble in urinary passage [13]. Another study conducted in 2022, demonstrated that, the otolith had anti-inflammatory and wound healing properties [14].

The length of repeated-dose hypolipidemic 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 hypolipidemic information for the potential pharmaceutical product [11]. This innovative discovery establishes a basis for investigating the hypolipidemic properties of otolith.

METHODOLOGY

Collection and Preparation of Otolith

Otolith pieces were obtained freshly from catfishes hunted from the Red Sea near Khokha port, 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 are removed. The Otoliths are then washed with distilled water and acetone before drying at room temperature. The otolith was 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. The animal experiment was approved by the Research Ethical Committee of the University of Science and technology, Yemen (EAC/UST 234).

Hypolipidemic Study

Hypolipidemic effects study of otolith were implemented according to the study conducted by Thirumalai [14] and Adekenov, et al [15]. Twenty four male rats were randomly divided into four groups (n = 6).

Group 1: 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 [16].

Blood sampling

Following a period of 21 days, the animals were anesthetized using chloroform. Blood is obtained from the venous sinus while the rat is under terminal anesthesia. The neck is gently scruffed and the eye made to bulge. A capillary tube is inserted laterally. Blood is allowed to flow by capillary action into the capillary tube. For lipid profile assays, blood samples were taken into sterile tubes containing anticoagulant (EDTA) [17]. Lipid profile tests for Cholesterol, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Triglycerides (TG) were performed at the end of the experiment.

Data Analysis

The data were summed together using percentages and means. One-way analysis of variance (ANOVA) was employed to assess the significance of association utilizing SPSS Program version 20. Differences were deemed significant at P values less than 0.05. Analysis of variance (ANOVA) uses F-tests to statistically assess the equality of means when there are three or more groups. The F statistic for a one-way ANOVA is the ratio of variability measures for the two sources of variation: the between-sample variability divided by the within-sample variability.

Ethical approval

The Ethical Committee of Medical Research at the University of Science and Technology approved this project ethically under reference number (EAC/UST 234).

RESULTS

The current results discussed the hypolipidemic effects of Otolith at different doses of 5, 10 and 15 g/day on lipid Parameters. Table 1 presents data on the effects of different dosages of Otolith (5 g/day, 10 g/day, and 15 g/day) on various lipid parameters compared with control group.

Table 1: Effects of Otolith Dosing on Lipid Profile

Parameters (mg /dL)	Control	Otolith 5 g/day	Otolith 10 g/day	Otolith 15 g/day	F	SD	P-value
Triglycerides	125.5	103	107	78.8	6.622	20.779	<0.0001*
Cholesterol	118.2	112	78.8	69.67	26.39	14.003	<0.0001*
LDL	54	52.4	54	42.63	6.002	7.209	0.0043*
HDL	28.5	29	31	34	9.428	3.704	0.0004*

P <0.05 considered significant compared to control Group

Table 1 showed, significant dose-dependent reductions in triglycerides, cholesterol, and LDL levels, alongside an increase in HDL level. Notably, the 15 g/day dose exhibited the most pronounced effects, suggesting a potential cardioprotective effect. Otolith administration had a significant impact on various lipids 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.

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DISCUSSION

Hypercholesterolemia, a major risk factor for atherosclerosis, is a complex disorder associated with genetic and environmental factors. More and more epidemiological data has demonstrated that low testosterone concentrations in men are associated with a higher risk of atherosclerosis [18]. The current results revealed that the injection of otoliths had a dose-dependent influence on several physiological indicators; greater dosages clearly produced more changes. The most important impact was seen in lipid profiles; greater otolith dosages favorably changed the blood lipid composition. Along with an increase in HDL levels, the noted drop in cholesterol, LDL, and triglyceride concentrations suggests that otoliths might help to treat dyslipidemia and reduce cardiovascular risk factors .

The effects of otolith in decreasing triglyceride Consistent with non-healthy populations (n = 22), intervention studies looking at the impact of fish or seafood on TG concentrations were carried out; 11 of these studies reported fish intakes that notably lowered TG concentrations [19]. One study in a group of 142 overweight people found that 2 servings of fish per week significantly reduced TG concentrations when compared to a diet devoid of fish (1.09 ± 0.30 mmol/L vs 1.15 ± 0.54 mmol/L, p = 0.05, respectively) [20]. In addition, Hasan et al. found that omega-3 treatment dramatically lowered TG levels without affecting TC, HDL, or LDL status [21].

In contrast, a 1384 person double-blind, randomized multicenter trial with 4 g daily omega-3 supplements showed no improvement in lipid profile [22].

The unique mineral makeup of otoliths could be responsible for these changes in lipid profiles, therefore affecting the body's lipid metabolism and transport mechanisms. The current work shows that otolith supplementation clearly causes a dose-dependent improvement in lipid measures. While HDL showed a clear rise at the highest dosage (15 g/day), triglycerides, cholesterol, and LDL levels showed the most marked declines relative to the control group. Across all lipid indicators, this pattern is visually clear in the declining levels from control to 15 g/day. The strong results at 15 g/day imply that greater otolith doses could be more helpful in regulating lipid metabolism, thereby maybe providing cardiovascular advantages by better lipid.

This results in line with evidence currently supporting fish consumption in reducing total and LDL cholesterol, fish eating may help people with lower HDL at baseline somewhat raise HDL cholesterol. Although fish consumption has demonstrated changes in phospholipid and sphingolipid species and structure in limited RCTs, it is not yet evident if these changes have any appreciable effect on CVD risk [18].

Conversely, otolith has additional benefits like antioxidant activity; study done in 2024 [23], revealed that his is a clear effect of otolith as the dosage rises, therefore raising the value of catalase. This helps one to understand otolith's function as an antioxidant. On the other hand, a study discovered that raising the otolith dosage changed the effect of superoxide dismutase; the results revealed statistical significance at doses of 10 g/day and 15 g/day as compared to the control group [23].

CONCLUSION

Lipid profiles were significantly improved after taking Otolith, with lower levels of cholesterol, LDL, and triglycerides and increased level of HDL, especially at larger doses. Future studies should involve extended study periods and explore underlying mechanisms, such as otolith's influence on lipid synthesis or clearance pathways. Additionally, clinical trials are needed to confirm these effects in human subjects and establish optimal dosing strategies for therapeutic applications.

Conflict of interest

The authors declare that no conflict of interest.

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