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In Vivo Antioxidants Activity of the Traditional Medicine Otolith Obtained from Catfish

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ABSTRACT

Background: Otolith, commonly known as "ear stones", Otolith is used traditionally along coastal areas especially Hodeida governorate fir treatment of diabetes mellitus, the people there believe that it has many health benefits and magic control of diabetes and migraine headache pain

Objective: To investigate the in vivo antioxidant activity of otolith obtained from catfish.

Method: Twenty male rats were randomly divided into four groups (n = 5) in each group. Otolith at various concentration (5g/d, 10g/d and 15/d) were administered for 21 days depending on the design of the experiment. Catalase (CAT) activity was measured by monitoring the decomposition of H2O2, according to the method of Johansson and Borg. In vivo superoxide radical scavenging activity was measured by reduction in riboflavin/light/nitro blue tetrazolium (NBT). Reduction of NBT is the most popular method

Results: The administration of otolith at doses of 5, 10, and 15 g/day for 21 days significantly enhanced the activities of the antioxidant enzymes SOD and catalase in the serum of rats fed with Otolith, as compared to the control group.

Conclusion: The use of Otolith at higher dosages resulted in a noticeable rise in catalase activity, indicating improved antioxidant capabilities. Similarly, superoxide dismutase activity exhibited a similar trend with increased Otolith dosages, particularly at 10/day and 15/day, which were found to be significantly different from the control group.

Keywords: Otolith, Diabetes, SOD , catalase, Antioxidants activity .

1. Introduction

Fish is a rich source of nutrients like amino acids, fatty acids, vitamins and minerals. It plays a major role in preventing and curing asthma, coronary diseases, eye diseases, and nutrient deficiencies. It is very important to include fish in our daily diet to maintain a healthy life [1] .

Yemen has great marine life biodiversity that is used for medicinal purposes in its traditional therapy. An example of this biodiversity is *Arius Thalassinus*, which is a kind of fish. It is traditionally called "Comal" (Ministry of Fisheries, Yemen, 2007). It contains otolith which is commonly known as "ear stone". Moreover, otolith plays an important role in sensing, balance, movement and hearing[2].

Otolith is a small and white structure present in the head of all fish except sharks, rays and lampreys[3]. There are three types of otolith which are lapillus, sagitta and asteriscus respectively. Sagitta is considered as the largest otolith,[4] Like Asteriscus, it plays a vital role in the detection of sound and the process of hearing. Moreover, lapillus is involved in the identify of gravitational force and sound[5].

Otolith is used traditionally along coastal areas especially Hodidah governorate. People there believe that it has many health benefits and a magic control of diabetes and migraine headache pain. They used it long years ago for that purposes. They believe that this agent controls their hyperglycemia in some cases without any other supported medications. This is the first study and also considered as a step stone in discovering the potential health benefits of the fish ear stone (otolith).

Oxidative stress is a relatively new concept, widely used in medical sciences in the past three decades. It takes an active part in the physiology of very common diseases, like diabetes, high blood pressure, preeclampsia, atherosclerosis, acute renal failure, Alzheimer's and Parkinson's. The cells, through metabolising oxygen, create reactive species of oxygen (ROS), that are potentially harmful. Under normal circumstances, the rate and amplitude of

oxidant formation is balanced by the rate of their removal. However, loss of balance between pro-oxidants and antioxidants results in oxidative stress. High levels of ROS in biological cells have a large impact on their functioning, leading to deficient cell operation, aging, or disease [6]

Free radical-induced oxidative stress is attenuated by preventive and repair mechanisms or physical and antioxidant defenses [7].

1.1 Antioxidant Classifications :

Antioxidants can be categorized as primary and secondary antioxidants based on their operating mechanism. These secondary antioxidants include enzymatic and non-enzymatic molecules that are distributed within the cytoplasm and cell organelles of the endogenous antioxidative cell defenses. These defenses also include the enzymes superoxide dismutase (intracellular Cu/Zn-SOD, Mn-SOD, and extracellular SOD), glutathione peroxidase, catalase, peroxiredoxins, and non-enzymatic antioxidants such as glutathione, thioredoxin, and uric acid[8].

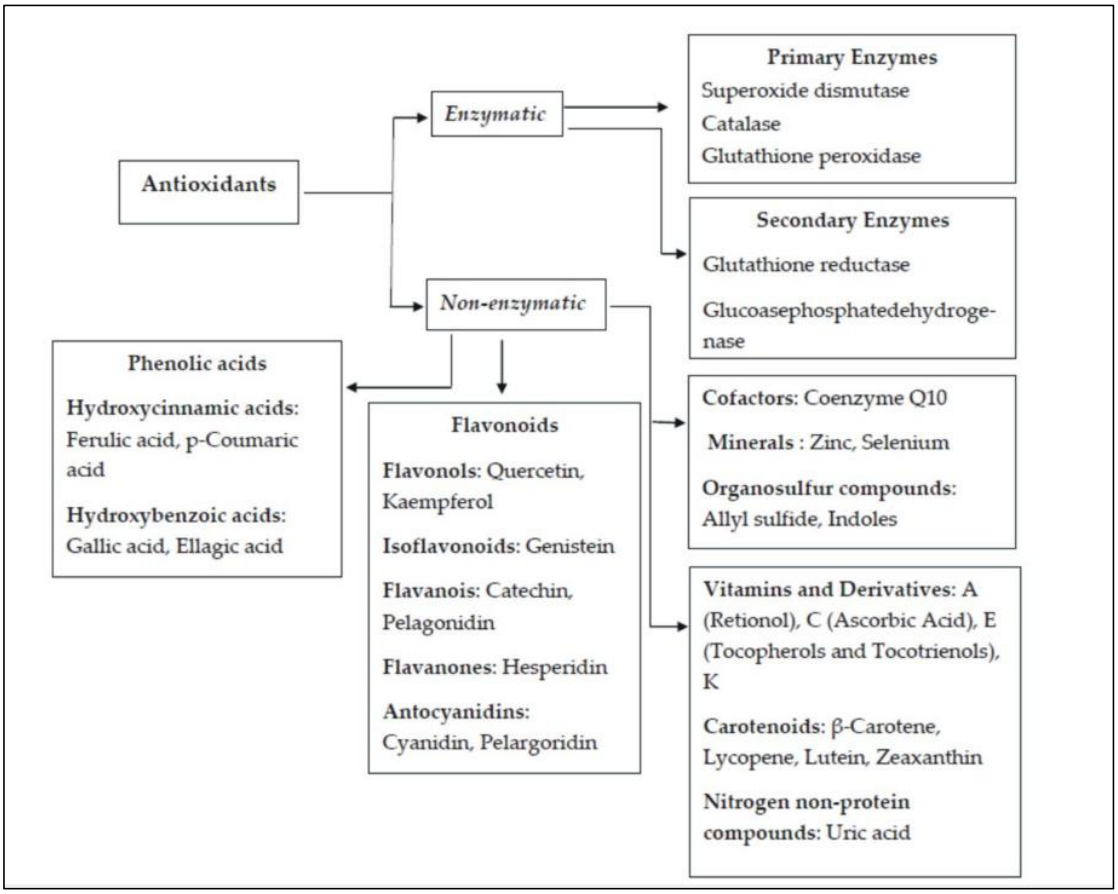

Figure 1. Application scope of antioxidants [9]

Enzymatic antioxidants are typically categorized into primary and secondary types. Primary antioxidant enzymes, for instance, SOD, several peroxidases, and catalase, initiate a series of reactions that transform ROS into more stable molecules, such as H2O and O2. Specifically, SOD converts superoxide anions into hydrogen peroxide, which serves as a substrate for catalase[10]. It has been proposed that the activation of enzymatic antioxidant defenses by ingesting exogenous antioxidants from food is more crucial than scavenging ROS in vivo.

This study aims to assess the in vivo antioxidant activity of the otolith by examining how it triggers endogenous antioxidant defenses and repair systems to conserve cellular energy in response to elevated ROS formation [11].

2. Material and Methods

2.1 Collection and Preparation of Otolith

Fresh otolith pieces were collected from catfish caught in the Red Sea near Khokha Governorate port in Hodiedah, Yemen. The otoliths were air-dried and powdered using a mechanical grinder, then transferred to a light-resistant glass container and tightly sealed. The otoliths were prepared for use as a solution by dissolving them in distilled water.

2.2 Antioxidant activity In Vivo

Twenty male rats, weighing between 120 and 170 grams and obtained from the Animal House Department of Biology at Sanaa University, will be used in the study. These animals will be housed in well-ventilated aluminum cages at room temperature and subjected to natural light/darkness cycles. The study will adhere to the recommendations of the Guide for the Care and Use of Laboratory Animals, and the University of Science and Technology Animal Ethics Committee will approve the experiment.

2.2.1 Experimental Animals.

Twenty male rats were randomly divided into four groups of five animals each. Group 1 (n=5) served as the control group and were given distilled water.

Group 2: (n=5) receive 2g/kg of otolith.

Group 3: (n=5) receive 3g/kg of otolith.,

Group 4: (n=5) receive 4g/kg of the otolith.

The animals were administered daily doses for a period of 21 days and were closely monitored for any signs of toxicity or death. Observations were conducted each day. Twenty-four hours after the final treatment, blood samples obtained through direct cardiac puncture were utilized to measure the in vivo antioxidant activity of the otolith [12].

2.3. Analytical Methods :

2.3.1: Serum Preparation.

Blood will be collected for serum preparation through direct heart puncture using a 21 G needle attached to a 5 mL syringe, after administering mild chloroform anesthesia to the rats.

The serum will be prepared using a standard method, as outlined by Yesufu et al.[13]. Following a 30-minute period for blood clotting, the sample will be centrifuged at 2500 rpm for 15 minutes to isolate the serum.

The activities of Superoxide Dismutase (SOD) and Catalase were measured using commercially available kits from Sigma, an American company, following the manufacturer's instructions.

2.3.2. Estimation of Catalase Activity.

The modified method described by Atawodi[14]will be used to evaluate the catalase activity in serum. To begin, 10 μL of serum will be added to a test tube containing 2.80mL of 50mM potassium phosphate buffer (pH 7.0). Next, 0.1mL of fresh 30mM hydrogen peroxide will be added to initiate the reaction. The decomposition rate of hydrogen peroxide will be measured at 240nm on a spectrophotometer for 5 minutes. The molar extinction coefficient of 0.041mM−1 cm−1 will be used to calculate the catalase activity.

2.3.3 Estimation of superoxide dismutase (SOD).

The ability of Superoxide anion radical-scavenging was assessed by a non-enzymatic method, which was adapted slightly (Kuda, Hishi, & Maekawa, 2006) based on the original method by Nishikimi, Rao, & Yagi (1972). To conduct the test, a 0.025 ml sample solution was mixed with 0.1 ml of 25 mM phosphate buffer (pH 7.2), 2 mM NADH (0.025 ml), and 0.5 mM NBT (0.025 ml). The absorbance at 560 nm was measured as a baseline value. Following a 10-minute incubation at room temperature with 0.025 ml of 0.03 mM PMS, the absorbance was measured again[15].

2.3.4 Ethical consideration:

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).

3. Results and Discussion:

3.1. Antioxidant activity of otolith on Superoxide level

In this study, we examined the ability of Otolith extracts to scavenge or prevent the formation of superoxide anion free radicals. As shown in Table 1 and Fig. 2, the scavenging activity of the otolith extract increased with increasing concentration. The highest scavenging activity of the otolith extract was 118.6 at a concentration of 15g/day, followed by 70.8 at a concentration of 10g/day, and 37.8 at a concentration of 5g/day.

Table 1: In vivo antioxidant effects of Otolith in rats (mean ± SEM).

Groups / Parameters	Control (D.W.only)	Otolith 5mg/day	Otolith 10mg/day	Otolith 15mg/day
Catalase (μmol/mg protein)	2.0118 ±	37.8 ±1.9*	70.833±4.1*	6.8 *118.6 ±
Superoxide (unit/mL)	±0.812.1	±1.1 14.5	1.3*19 ±	1.1* ±21.3

* Significant as compared with Control Group at P<0.05

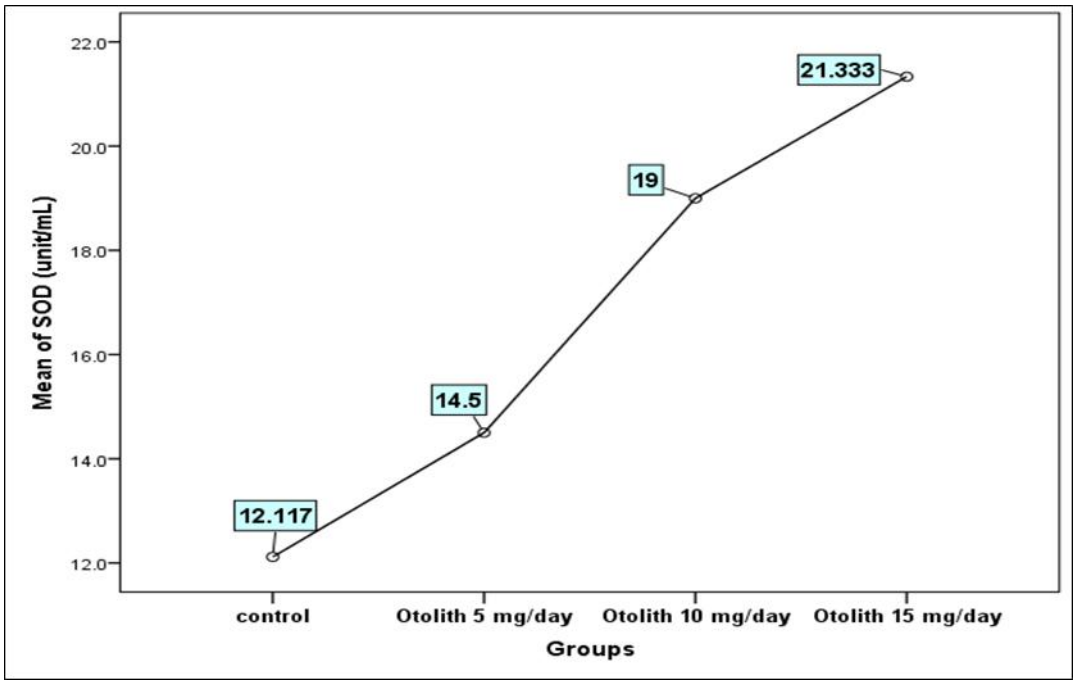

Figure 2: Effects of Otolith on Superoxide activity

Superoxide dismutase (SOD) is a vital antioxidant enzyme that is derived from living organisms. It serves as the first line of defense against active oxygen by scavenging it, and plays a crucial role in the body's antioxidant system.

Superoxide dismutase (SOD) is a widely distributed enzyme found in a variety of organisms, including animals, plants, and microorganisms. During normal metabolism and under various environmental stresses, SOD produces both living oxygen and free radicals. The accumulation of active oxygen and free radicals can lead to cellular damage by disrupting the structure and function of cells.

SOD is a critical component in the defense mechanism of cells against oxidative damage. This is because it possesses the unique ability to specifically eliminate harmful free radicals from the body, thereby mitigating the cellular damage caused by oxidative stress.

3.1. Effects of otolith on catalase activity

Our findings indicated that the administration of otolith at doses of 10 and 15 g/day resulted in a significant impact on catalase activity. We examined the effects of the otolith extract on catalase levels at different concentrations, and it was observed that the highest increase in catalase level was 21.3 units/mL at an otolith concentration of 15 g/day.

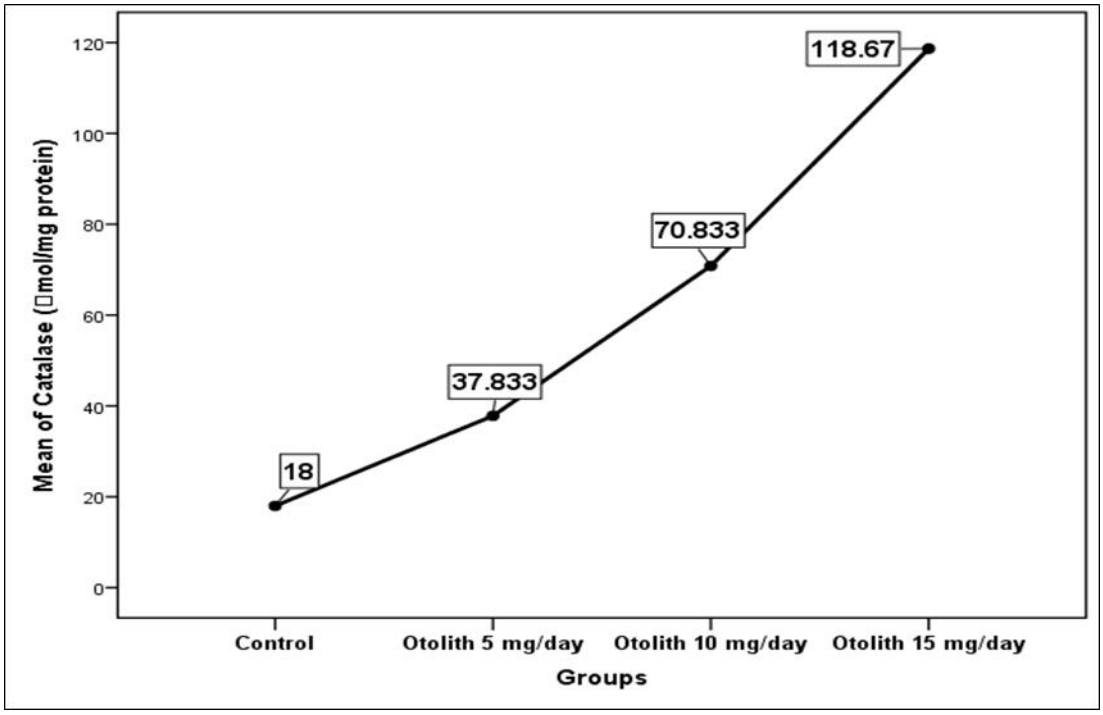

Figure 3: Effects of otolith on catalase activity

4. Conclusion:

The administration of Otolith at increased dosages led to a marked enhancement in catalase activity, which suggests an improvement in antioxidant capabilities. Furthermore, the activity of superoxide dismutase displayed a parallel trend with the rise in Otolith dosages, particularly at 10g/day and 15g/day, which were found to be statistically significant compared to the control group.

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