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Postulated anti-diabetic effect of fish ear stone (Otolith) in experimental animals

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ABSTRACT

The aim of this study is to Evaluate the anti-diabetic effect of otolith (ear stone) that obtained from type of fish called "Arius Thalassinus " which has medicinal properties and traditionally used in Yemen", for treatment of diabetic mellitus type 2. Experimental animals were divided to 2 diabetic models (streptozotocin and fructose model) Composed of (9) groups , Each model contains 4 groups , 6 animals in each group , while, Group I was kept as control for both models only taken distilled water (2ml/kg) . Administration of otolith in the doses of 2g and 3g/kg of body weight in STZ model showed significant (P<0.05) reduction in blood glucose levels, cholesterol ,TG, and LDL compared to diabetic control rats, with no effect on body weight . On the other hand, administration of otolith in the doses of 2g and 3g/kg in fructose model showed significant (P<0.05) reduction in blood glucose, insulin, cholesterol, TG, LDL levels respectively as well as body weight compared to diabetic control rats. In addition, otolith 2 and3g/kg showed high reduction in cholesterol level than reference drug pioglitazone, in fructose model. Administration of otolith 3g/kg produced significant higher anti diabetic activity than otolith 2g/kg dose. In conclusion, The traditional use of otolith to treat diabetes is supported by laboratory Findings from this study.

Keywords: Otolith, Diabetes mellitus, Streptozotocin, Insulin Resistance, Fructose

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INTRODUCTION

Diabetes Mellitus (DM) is a major disease threatening the global public health that is rapidly getting worse with the highest impact in adult of working age in developing countries.[1] There is an estimated (347 million people worldwide have diabetes)**[2]** It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, which results in abnormalities in carbohydrate, lipid and protein metabolism. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels**.[3]**Oral hypoglycemic agents are used in the treatment of type 2 diabetes, among which gliclazide, a second-generation of sulfonylurea derivative, is preferred in therapy because of its selective inhibitory effect towards pancreatic K+ adenosine triphosphate (ATP) channels, antioxidant properties, low incidence of severe hypoglycemia, and hemobiological effects. **[4,5]** In addition, insulin resistance plays an important role in the development of diabetes mellitus Type II. [6]

Insulin resistance is a common pathological state in which target cells fail to respond to ordinary levels of circulating insulin resulting in disregulation in lipid homeostasis and glucose regulation [6,7].However**,** insulin sensitizers are group of agents work by enhancing glucose utilization in tissues, and so reduce insulin resistance. They activate the nuclear peroxisome proliferator-activated receptors (PPAR-Y), which alter gene expression and result in insulin-like effect. It is a target of the class of drugs known as thiazolidinediones (TZDs), for example Pioglitazone . It is used to treat type II diabetes and known to regulate lipid and carbohydrate metabolism [8]. Fish is a rich source of nutrients like polyunsaturated fatty acids , amino acids, vitamins and minerals. It plays a major role in preventing and curing coronary heart diseases , asthma, eye diseases , and nutrient deficiencies**.[9]** Yemen

has great biodiversity of marine life such as fish that uses with medicinal properties (Traditional Therapeutic) such as *Arius Thalassinus*, that's locally called "Comal". **[10]***Arius Thalassinus* contains otoliths, commonly known as "ear stones," they play an important role in sensing , balance , movement and hearing of fish . *Otoliths* are small, white structures found in the head of all fish other than sharks, rays and lampreys. **[10]**.*Otolith* is used traditionally along coastal areas especially Hodidah governorate , the people there believe that it has many health benefits and magic control of diabetes and migraine headache pain. they said that this agent control their hyperglycemia in some cases without any other supported medications .

Survey of current literature revealed that there is no previous scientific data documented for the effect of otolith in the treatment of type 2 diabetes mellitus. Therefore, the present study is undertaken to investigate the anti-diabetic activity of otolith in type 2 diabetic rats.

EXPERIMENTAL SECTION

2.1. Drugs and chemicals : Streptozotocin (STZ) was purchased from (Sigma). All other chemicals and kits were purchased from Roche Diagnostic and Merck Company.

2.2. Collection and Preparation of Otolith: Otolith pieces were obtained freshly from catfishes hunted from Red sea near to khokha governorate port Hodiedah –Yemen. Fresh Pieces of otolith were air dried and powdered using mechanical grinder, then, stored into suitable light resistant glass container and tightly closed. Otolith freshly prepared before use, as solution by dissolving it in hot distilled water.

2.3. Animals :
54 Male albino Wister rats (Rattusnorvegicusalbinus)aged (6 weeks± 1week) and weighing (210± 20g) were obtained from animal house (Sana'a University-Department of Biology). They were acclimatized to the laboratory conditions before starting of treatment for 1week . The animals starved overnight with water only prior to the experiment. The animals were weighted and given a specific number and mark. The study protocol has been approved by the University Ethics committee , No.(41)

2.4. Acute Toxicity Studies: Acute oral toxicity study was performed according to Organization for Economic Cooperation and Development (OECD) guidelines 423 **[12]**. After the oral administration of otolith , animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for total of 7 days.

2.5. Anti-oxidant activity:
The free radical scavenging capacity of the extract was determined using DPPH [13-14,15].

4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. 50 µl of various concentrations of otolith as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min. at 517nm using methanol as blank on UV-visible spectrometer.[16]. In addition, The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, Which is a stable free radical becomes paired off in the presence of a hydrogen donor. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present)[17]. The DPPH free radical scavenging activity was calculated using the following formula [18].

Inhibition %= Ac-As/Ac×100

Where **Ac** is the absorbance of the control and **As** is the absorbance of the sample

2.6. Study design:54 animals rats were divided to 2 diabetic models (A and B) composed of 9 groups . Each model contains 4 groups (6 animals in each group). Group I was kept as control for both models only taken distilled water (2ml/kg) .

Model A : STZ induced type II diabetes mellitus
Group II_A : Untreated group given STZ to induce type II DM .
Group III_A : Treated with 10mg/kg body weight of Gliclazide and kept as a reference group
Group IV_A: Treated with otolith 2g/kg/d .
Group V_A: Treated with otolith 3g/kg/d .

For induction of type II DM, STZ was administered to male rats i.p. after dissolving it in sodium citrate buffer at a concentration of 62.5 mg/ml by dose of(35 mg /kg)according to pilot study and a method of Netaji. *et. al.*2014[19]. Blood glucose was estimated after 2nd and7th days after the streptozotocin injection [20] [21]. Rats with consistent hyperglycemia on the 7th day (fasting blood glucose levels > 250 mg/dL) were used inthis study. Then the three treated groups were givengliclazide and otolith orally through oral gavage for 4 weeks.

Model B : (Fructose induce insulin resistance)[22]
Group II_B: Untreated group received *10% w/v* fructose solution
Group III_B:Treated with pioglitazone (3 mg/ kg)and kept as a reference group
Group IV_B:Treated with otolith 2g /kg/d.
Group V_B : Treated with otolith 3g /kg/d .

All groups except normal control, received 10% of fructose for three weeks to induce insulin resistance according to the method of Massimo Collino, *et al*, 2010.[23]. All tested drugs were given via oral gavage starting from 4th week and the duration was 6 weeks. The blood samples were collected from retro-orbital plexus and tested for the all parameters like glucose level , insulin level , lipid profile and body weight using biochemical kits .

RESULTS

3.1. Acute Toxicity Study:

from the acute toxicity studies no toxicity was found to doses of 4 up to 8g/kg and the doses selected are the low and high dose is 2g/kg and 3g/kg.

3.2. Antioxidant activity:

The antioxidant activity of otolith probably due to present trace elements like zinc , magnesium,. Copper and iron . On the other hands ,Antioxidant properties of otolith were found to be higher effect with otolith 3g/kg than 2g/kg . Based on the results obtained, the low concentration of otolith 50 and 100 (µg/ml) showed low effect as antioxidants while the concentrations 150,200,250 (µg/ml) showed highly significantly effect ($P<0.05$) as antioxidants compared with ascorbic acid . The fig.1 shows the antioxidant activities of the otolith.

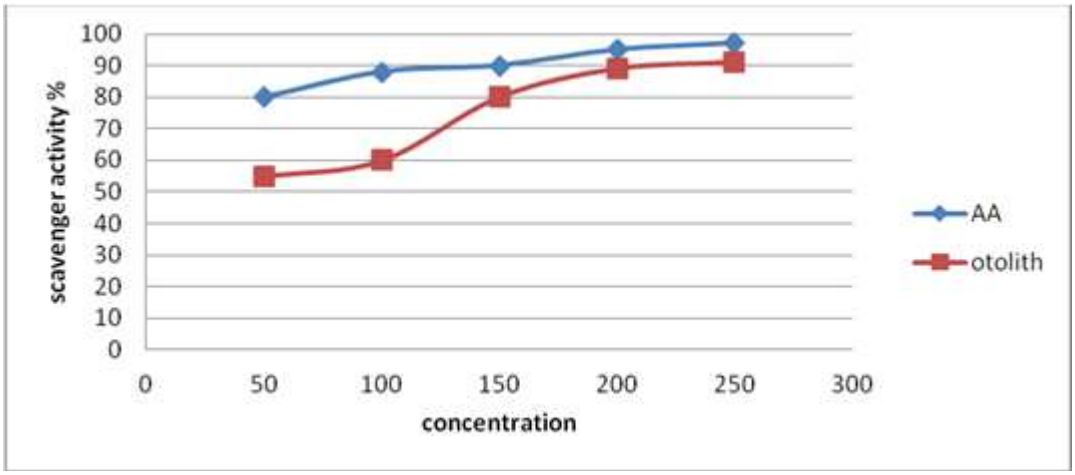


Fig1. Antioxidant s activity of otolith compared to ascorbic acid

3.3. Part A: STZ-induced type 2DM.

Animals treated with STZ significantly increased serum glucose, triglycerides , LDL and cholesterol when compared to normal control group. On the other hands, Animals treated with STZ significantly decreased in insulin level. Otolith at dose levels of 2 and 3 g/kg b.wp.o showed significant decreased ($P<0.05$) in the levels of serum glucose, triglycerides and cholesterol compared with diabetic control rats . additionally, Otolith in dose 3g/kg, also showed significantly increased in insulin level, as shown in table 1 and figure 2,3 and 4 .

Table. 1 : Effect of oral otolith administration (2 and3g/kg) average(M± SE) on lipid profile in STZ -induced type 2DM

Parameters (mg/dL)	Control (D.W. Only)	STZ (35mg/kg)	Gliclazide (10mg/kg)	Otolith (2g/kg)	Otolith (3g /kg)
Triglyceride	**88** ±1.9	**157 ±8.4***	**100 ±5.12**	**110.2 ±4.7#**	**108.25 ±5.12#**
Cholesterol	**123 ±2.76**	**172 ±2.8 ***	**143 ±2.58 #**	**152 ±3.21#**	**147 ±4.15#**
HDL	**35 ±1.6**	**33 ±1.86**	**39 ±1.73 #**	**36 ±1.92 #**	**38 ±1.4**
LDL	**79 ±1.52**	**88 ±2.28 ***	**69 ±2.19**	**83 ± 2.0**	**74.00 ± 2.3#**

Values are expressed as mean ±S.E.M. (One-way ANOVA followed by LSD test). (n=6).
**P <0.05 compared to control Group ; # P <0.05 compared to STZ Group.*

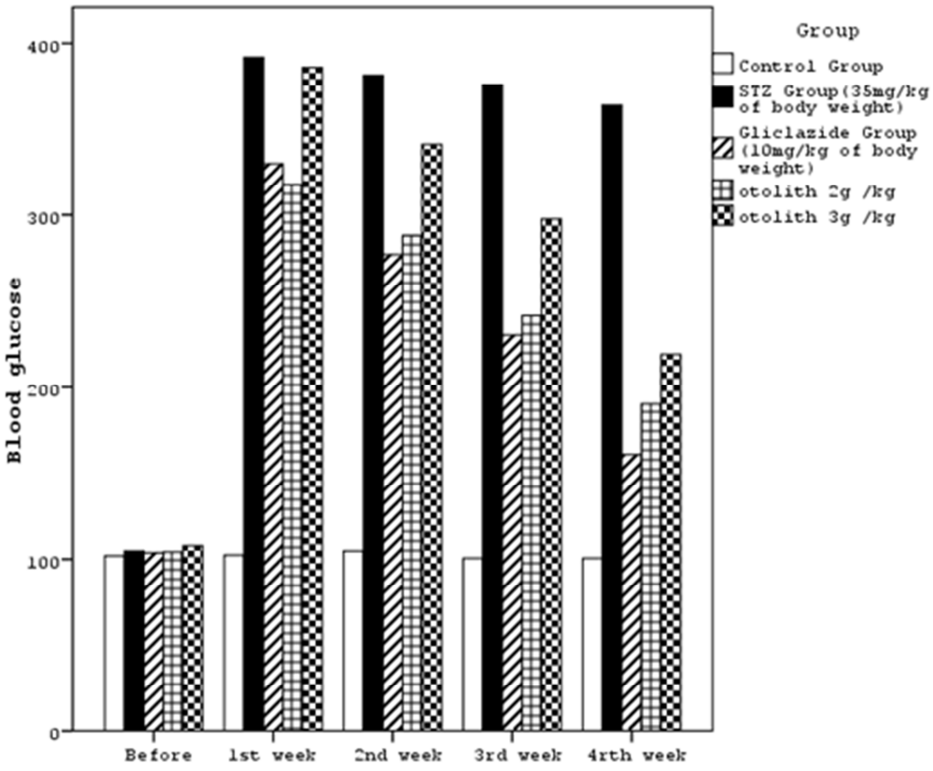
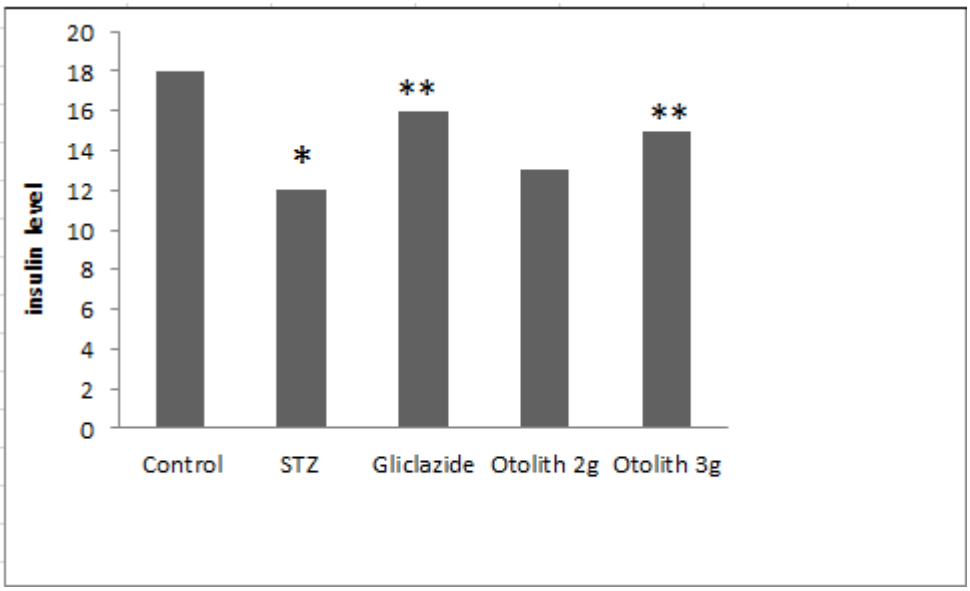
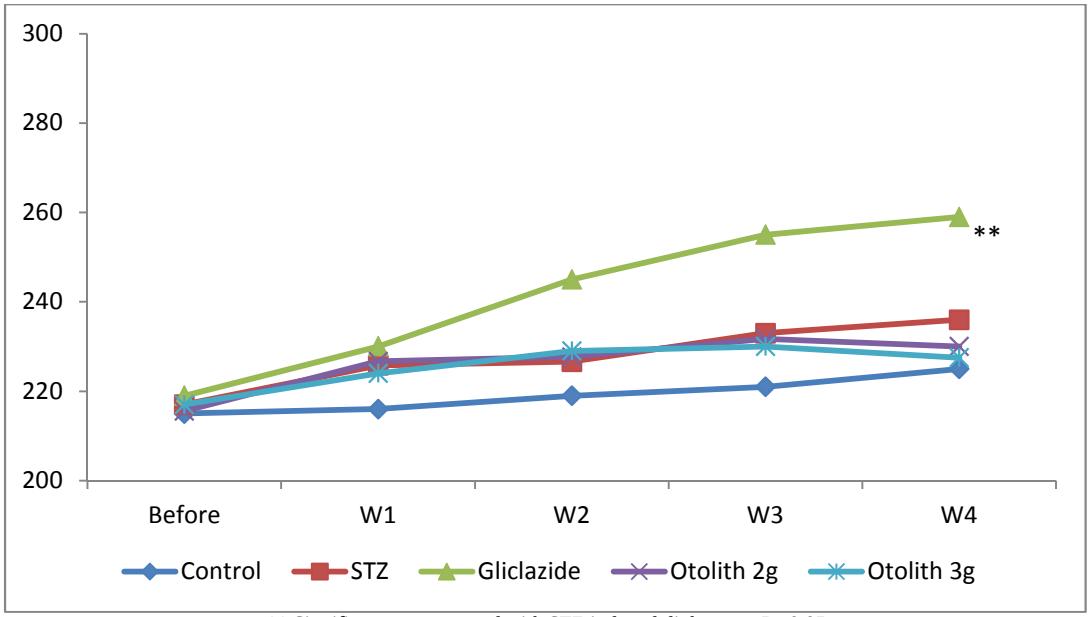


Fig2. Effect of oral otolith administration (2 and 3g/kg) onaverage(M± SE) glucose level in STZ Model



**Significant as compared with Control Group at P<0.05*
*** Significant as compared with STZ-induced diabetes at P<0.05*

Fig3. Effect of oral otolith administration (2 and 3g/kg) onaverage(M± SE) insulin level in STZ Model



*** Significant as compared with STZ-induced diabetes at P<0.05*

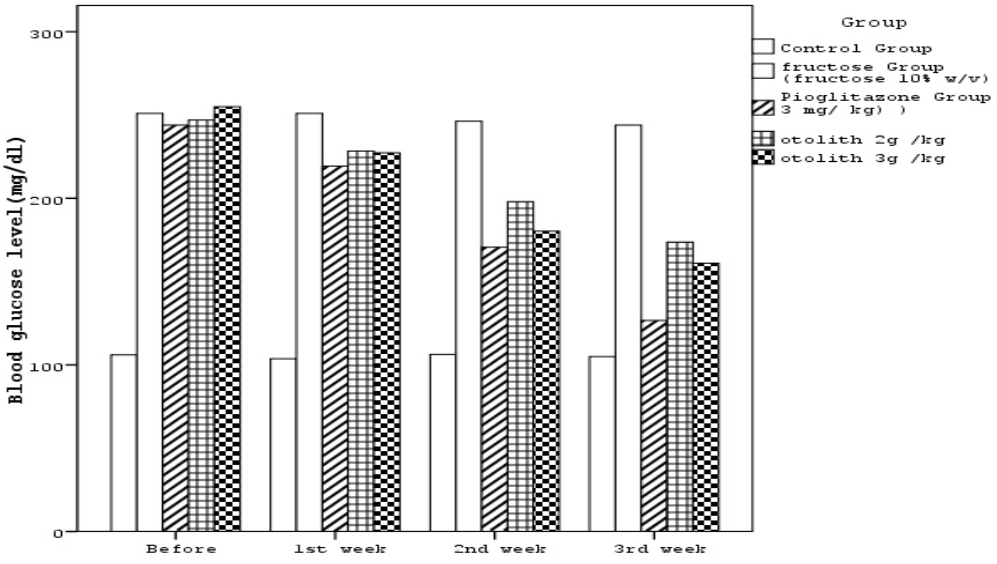
Fig4.Effect of oral otolith administration (2 and 3g/kg) on average(M± SE) body weight in STZ Model

Fructose feeding groups for 3weeks has shown significance increase in serum glucose, insulin, triglycerides, cholesterol and LDL levels when compared to normal control rats. Animals treated with otolith for 3 weeks at a dose of 2 and 3g/kg b.w showed significant (P<0.05) reduction in serum glucose, insulin, triglycerides, LDL, and cholesterol levels with higher effects with 3g/kg dose . In diabetic control group, there was a steep increase in body weight, however body weight was reverted to near normal when treated with otolith at 2 and 3g/kg b.w. Results are shown in tables 2 and figures 5&6.

Parameters (Mg/dL)	Control (D.W. only)	fructose Group %10	Pioglitazone (3 mg/ kg)	Otolith (2g/kg)	Otolith (3g /kg t)
Triglyceride	**88 ±1.9**	**203 ±1.9***	**181 ±3.7 #**	**197 ±4**	**186 ±3.4 #**
Cholesterol	**123 ±2.8**	**242 ±4.1 ***	**192 ±3.5#**	**175 ±3.7 #**	**159 ±2.7 #**
HDL	**35 ±1.6**	**33 ±1.3**	**42 ±1.7 #**	**38 ±1.4#**	**41 ±1 #**
LDL	**79 ±1.5**	**167 ± 7.3***	**98 ±3.5 #**	**112 ±3.5 #**	**102 ±2.4 #**

.P <0.05 compared to control Group ; # P <0.05 compared to fructose Group.Values are expressed as mean ±S.E.M. (one-way ANOVA followed by LSD test). (n=6)

Fig5.Effect of oral otolith administration (2 and 3g/kg) on average(M± SE) glucose level in fructose model.



**Significant as compared with Control Group at P<0.05*
*** Significant as compared with Fructose-induced diabetes at P<0.05*

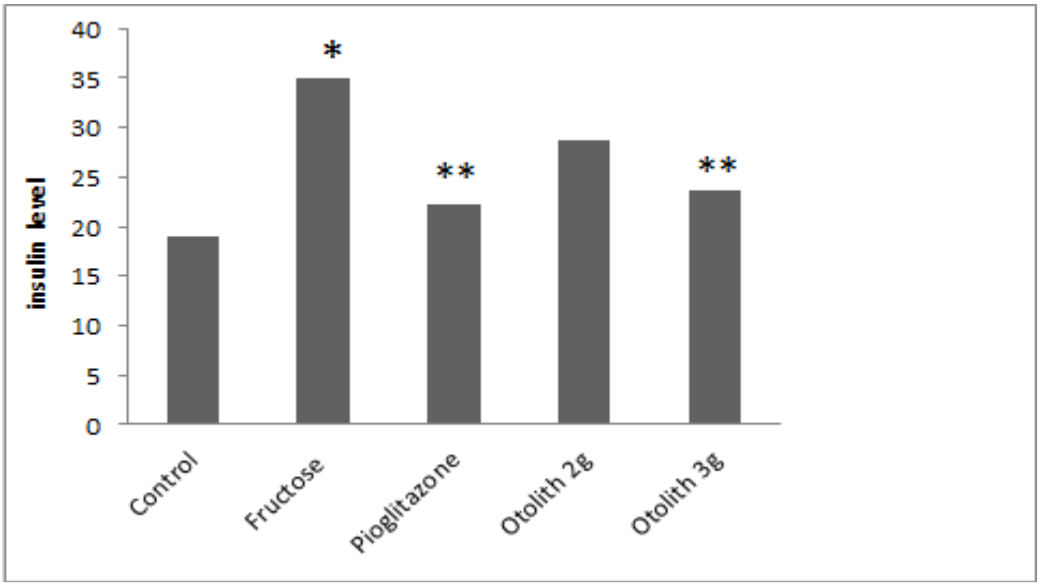
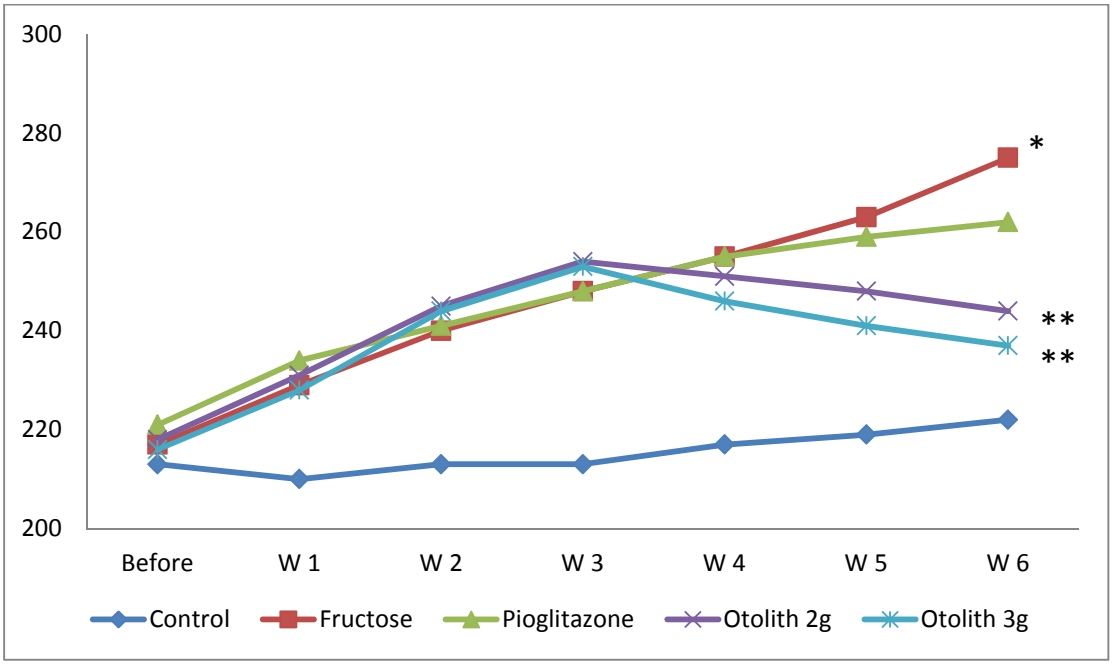


Fig6.Effect of oral otolith administration (2 and 3g/kg) on average(M± SE) insulin level in fructose model



**Significant as compared with Control Group at P<0.05*
*** Significant as compared with Fructose-induced diabetes at P<0.05*

Fig7.Effect of oral otolith administration (2 and 3g/kg) on average(M± SE) body weight in fructose model

DISCUSSION

Streptozotocin induces diabetes by free radical generation, which causes a massive reduction of insulin secreting beta cells of the islets of Langerhans, resulting in a decrease in endogenous insulin release [24]. In present study, administration of 35mg/kg of STZ showed significant increase in glucose, triglyceride, cholesterol and LDL levels. While, showed decrease in the insulin level and body weight. , Otolith contains some essential components for example calcium, zinc, magnesium, copper and iron which probably play important roles as anti-hyperglycemic and antioxidant activity. However, administration of otolith in the doses of 2 and 3g/kg/d showed significant decrease ($P<0.05$) in blood glucose level , This effect probably due to present of Ca^{+2} ,which stimulates insulin releasing by opening voltage-dependent calcium channels in the β cell resulting in calmodulin activation, which in turn leads to exocytosis of insulin containing secretory granules (Hoich et al., 1986). [25] Additionally, another hypothesis for this effect , otolith shows poor absorption through the gut wall suggested that it may exert its antihyperglycemic effect in the intestinal tract before absorption and concluded that its effect is probably due to its ability to inhibit α- glycosidase and decrease glucose transport through the intestinal epithelium.

In this study, STZ induced diabetes produced marked loss in body weight in diabetic group compared to treated groups. in addition, Diabetes is usually associated with weight loss; this is probably due to the body switches to burning fatty acids due to insulin shortage, and converts glycogen stores in liver and muscles to glucose in the diabetic state by gluconeogenesis process [26] **.**Insulin resistance precedes the development of type-2- diabetes, obesity, atherosclerosis and other associated cardiovascular diseases.[27] High fructose consumption leads to obesity and metabolic abnormalities as observed in insulin resistance syndrome. Fructose as such doesn't stimulate insulin secretion from pancreatic-β-cells, leptinan adipose derived hormone production is regulated by insulin in response to meals, consumption of foods and beverages containing fructose reduces circulating leptin concentration leading to insulin resistance [27-28].In the current work, The rats fed with high fructose showed a weight gain, hyperinsulinemia, hyperlipidemia, and hyperglycemia, This result was compatible with the study of Sharon et al. 2002 and Neeharika V et al. 2012.[27] [29]. Indeed, The use of 10% w/v fructose in drinking water for a period of 21 days showed a significant increase of glucose, insulin, triglycerides, and cholesterol levels.

In this work, oral administration of otolith to diabetic rats showed a significant reduction in the level of glucose associated with significant decrease in the level of insulin as compared to untreated diabetic rats. This finding probably due to enhances peripheral and hepatic sensitivity of insulin or because otolith maybe activate the _ nuclear peroxisome proliferator-activated receptors (PPAR-Ɣ), lead to decrease insulin resistant, like pioglitazone effect.

Moreover, Zinc is one of the essential trace element present in otolith , is a component of many enzymes, and plays an important role in the maintenance of several tissue functions,[30] including the synthesis, storage and release of insulin.[31]. Moreover , Zinc has been found to enhance the effectiveness of insulin in vitro, and it has been postulated that zinc deficiency may aggravate the insulin resistance in non- insulin dependent diabetes mellitus (NIDDM)[32] . The development of glucose intolerance in rats after dietary zinc deprivation, together with the occurrence of zinc deficiency in diabetes mellitus, suggest a role for zinc deficiency in the pathogenesis of diabetes mellitus[33]. It has been suggested that zinc repletion could improve insulin sensitivity in patients with NIDDM[34].

There are numerous studies in which dietary fructose has been shown to induce hyperlipidemia in rodents [35, 36,37].These studies reported that rats fed a high-fructose diet had sustained elevations in levels of lipid profile (triglyceride ,cholesterol , and LDL) moreover, The main cause of the increase in lipid profile is the increased free fatty-acid (FFA) release from insulin-resistant fat cells.[38,39,40]. Hepatic metabolism of fructose favors *de novo* lipogenesis, and this may be linked with both hyperlipidemia and increased body fat stores [41].

From the results obtained in the present study, otolith administration in fructose model showed significant ($P<0.05$) reduction in levels of triglyceride ,cholesterol , and LDL. This finding maybe due to inhibit release of free fatty-acid from fat cell. In addition, dietary fructose metabolism leads to high concentration of FFA in liver, which in turn enhances hepatic gluconeogenesis [42]. Thus plasma glucose levels increase by the increased dietary fructose. Glucose produced as a result of fructose metabolism stimulates insulin release but the fructose induced insulin resistance prevents the insulin from effectively metabolizing glucose, resulting in hyperglycemia [43]. Insulin resistance also leads to compensatory hyperinsulinemia , and this explains the increase level of glucose and insulin at the same time, which showed in the results of current study. However, administration of otolith at doses of 2 and 3 g/kg b.w prevented the development of hyperglycemia, hyperinsulinemia and hypertriglyceridemia, as there were the outcomes of this study.

In this work, fructose feeding group only, showed significantly increase in body weight through 21 days, diabetic groups treated with otolith 2 and 3g/kg/day showed significantly reduction in body weight, So, otolith play important role in improvement of insulin resistance associated with weight gain. On the other hands , otolith showed low or no efficacy in reduction of body weight in STZ induced type 2 D.M .

Otolith might have improved insulin sensitivity in peripheral tissues, as this was evident from the results showing decreased glucose and insulin production. Thus the above results indicate that otolith has preventive effect on fructose induced insulin resistance.

CONCLUSION

The present study outcomes were suggested 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 anti-hyperglycemic activity comparable to that of a standard drugs (Gliclazide and pioglitazone) in both models. The traditional use of otolith to treat diabetes is supported by laboratory finding from this study.

Further studies using more technical methods to elucidate the constituent (s) of otolith responsible for these benefits and biological activity are required in order to approve and expand these findings.

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