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The Hypoglycemic Effect of Libyan Truffle "Terfezia Boudieri" in Experimentally Induced Diabetic Rats

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ABSTRACT

Recent studies have shown that many types of mushrooms may have important physiological functions in humans including antioxidant activities, regulation of blood lipid level and blood glucose level.
The present study was designed to investigate the antihyperglycemic effect of ethanolic *Terfezia boudieri* Chatin (TBC) extract on streptozotocin (STZ) induced-diabetic rats. The results showed that, rats which were fed with a concentration of 200mg/kg of TBC ethanolic extract had a significant reduction in the plasma blood glucose *(P<0.005)*. Polydipsia was stronger in the induced diabetic rats which were not fed with TBC extract than in those receiving the *T. boudieri* extract.

Keywords - *Hypoglycemic effect; Libyan truffle; Terfezia boudieri; Induced diabetic rats.*

INTRODUCTION

Truffle is a fungus, which grows wildly in desert regions depending on water rainfall. It is a collective name for the species belonging to the family Tubraceae. Four species of *Terfezia* are common around the world: *Terfezia boudieri* Chatin, *T. claveryi* Chatin, *T. leonis* Tul, and *T. metaxasi* Chatin.[1] The desert truffle tends to be 10 cm or more in diameter, sometimes up to 40 cm across weighting up to 1kg.[2] However, the only report of successful man-made desert truffle plantations to date comes from Spain.[3]

In Libya, four species of truffles (locally known as Terfase) were identified: *Terfezia boudieri* Chatin (most common), *Terfezia clavereyi* Chatin (less common), *Tirmania nivea* and *Terfezia africana*.[1] All kinds are grow in the desert area particularly in the south and west regions of Tripoli. The tuber grows underground invisible to the human eye and when becomes mature it is collected by hand and then marketed. These truffles are important as nutritive materials, usually used in cooked dishes and have long been utilized by the desert natives as substitutes for meat in their diet. Although, truffle provides large quantities of rich agreeable vegetable, it has not been given a significant attention in terms of clinical use. However, internationally it is used as a traditional medicine, folk medicine to treat some diseases of skin, eyes, and diabetes.[2,4,5]

In addition, many researches stated that truffle can be used in many purposes such as source of energy, an activation of sex hormones, and as an antibiotics against gram positive bacteria including *Bacillus subtills* and *Staphylococcus aureus*.[4-6] The only instance that truffles were reported to cause disease to human was reported in a case study, in this study a patient suffering from acute uritecaria was found infected with extensive black truffle.[4]

In fact, there is a great need for a search for an acceptable, cheap and safe blood sugar lowering oral hypoglycemic agents that would be effective in the treatment of diabetes and devoid of serious side effects of the currently used oral hypoglycemic agents.

The chemical composition of the truffle differs from one species to another, but usually it is rich in carbohydrates (60%), which probably accounts for the sweetish taste, proteins (20-27%), amino acids, macro and microelements, fatty acids (3-7.5%), crude fibre (7-13%), vitamins and appreciable amount of ascorbic acid.[3] High level of potassium and phosphate and fair amount of iron has also been reported.[3,7] Peeling of truffle before eating has been reported to considerably decrease the content of protein, fat, ash, ascorbic acid and all mineral elements especially calcium and iron.[8] Recently it is well known that some mushrooms have a hypoglycemic action that act on blood glucose through different mechanisms, some of them may inhibit endogenous glucose production by alpha-glucosidase inhibitor or interfere with gastrointestinal glucose absorption, some may have insulin like substances, and some may increase secretion of insulin from beta-cells, while others may increase beta cells in pancreas by activating regeneration of these cells.[9,10]

Nowadays, more than 1200 species of the organisms (from 725 genera belonging to 183 families) are used to treat symptoms of diabetes mellitus. The hypoglycemic property of these traditionally consumed medicines has

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been experimentally tested.[11] Half of these species have been used traditionally for controlling diabetes. Many of them can experimentally reduce blood glucose level, for example the extract of *Agaricus blazei* Murill species has significantly lowered plasma glucose level in diabetic rats.[11] However, the traditional medical practitioner believes that combination of many mushroom products will be more effective than the use of a single mushroom product.[10]

MATERIALS AND METHODS

Truffle was collected from a local folk market in spring season, and was taxonomically identified as *Terfezia boudieri* Chatin by Prof. Fathi Rateeb, Botany department, Faculty of Sciences, University of Tripoli, Libya.

Albino healthy male rats (24), with average body weight of 58-96g, and age of about 24 weeks were used throughout the study. Female rats were not used in order to minimize the biological variations. The animals were bred and maintained at the central animal house and kept in standard plastic cages having dimensions of 55×33×20 cm. Soft wood shavings were employed as bedding in the cages, and each cage contain 4 animals. Food (composed of cereals and corns) and tap water was available freely. The animals were kept at humidity of about 55 ± 5 and temperature of 20 to 30Ċ with a 12 hours dark/light cycle and over-night starved before each experiment.

The truffles were immediately washed with copious tap water to remove foreign matters, spread on a mat to drain the water off, cut into thin slices and then kept for ten days away from direct sun light in a well ventilated room. The slices were flipped on daily basis to prevent spoilage. The dried slices were then powdered by a household grinder prior to extraction procedures. The fine powder was extracted by maceration technique which was conducted at room temperature by mixing the ground truffle with ethanol (1:5). The mixture was left for 3 days with occasional shaking. The procedure was repeated 3 times with fresh solvent. The extract was filtered and rotary evaporator was used to get rid of ethanol. Finally, the dried extract was 235 gm.

STZ (Sigma) was dissolved in 3ml of 0.9% normal saline then 20 rats out of 24 were intraperitoneally injected with 50 mg/kg of STZ for 3 consecutive days to induce type 1 diabetes mellitus. Animals with a fasting blood glucose range above 200 mg/dl were considered diabetic. After one week of acclimatization in standard conditions at animal house, all rats were divided into six groups. Two sets of experiments were performed with four animals in each treatment group. Daily for four weeks, except one group, each group of animals was orally instilled of different concentrations (200- 400- 600 mg/dl) of ethanol *T. boudieri* Chatin extract (ETBCE) in a volume of 5ml distilled water contained 5% gum acacia by using an I.G.T.

Blood samples from all groups were collected in days 15, 21, 28 from the first injection and placed in both heparinised tubes for CBC, and in non-heparinised tubes for blood biochemical examination. Blood glucose levels were also estimated in days of 0, 4, 8,12, 16, 20, 24 and 28 by using an electronic glucometer (ACCU-CHEK).

Body weight of all rats was recorded by using a sensor balance throughout the whole experimental period.

Group 1: Animals were orally supplied with 200 mg/kg.b.wt of ETBCE.

Group 2: Animals were orally supplied with 400 mg/kg.b.wt of ETBCE.

Group 3: Animals were orally received 600 mg/kg.b.wt of ETBCE.

Group 4: Animals were orally supplied with both 5mg/kg GLIB and 200mg/kg of ETBCE.

Group 5 (diabetic control): Animal were I.P injected with 50 mg/kg.b.wt of STZ and no extract was given.

Group 6 (normal control): Animals were injected with 3 ml/kg.b.wt of 0.9% normal saline only.

RESULTS

In STZ-induced diabetic rats showed a significant increase in blood glucose level (Figure 1).

It has been observed that at dose of 200 mg/kg b.w the blood glucose level was significantly decreased in diabetic rats starting from zero day (3 days after the first day of ETBCE treatment) until the last day of treatment compared with the control ($P < 0.05$) (Figure 2), however, rats treated with 400 mg/kg b.w of truffle extract exhibited a mild hypoglycemic effect compared with the control one ($P < 0.05$) (Figure 3). Interestingly, when the truffle extract was administered at concentration of 600 mg/kg b.w, it showed a less significant decrease in blood glucose level ($P < 0.05$) (Figure 4). It seems that the strong hypoglycemic effect of truffle extract was achieved when the animal treated with concentration of 200 mg/kg b.w of the extract. Moreover, table 1 showed that all diabetic rats have a decrease in their weights compared with the

Table 1: Changes in blood glucose level and weight amongst different groups of animals.

Groups	Blood glucose level (gm/dl) ±MSE		Weight(gm) ±MSE	
	First day record	Last day record	First day record	Last day record
1	374 ± 28.21	173.7 ± 39.27	86 ± 5.37	142.31 ± 2.2
2	197.3 ± 58.39	357.3 ± 2.51	92.33 ± 2.04	126.94 ± 3.51
3	278.3 ± 104.74	319 ± 128.96	91 ± 5.49	127.15 ± 13.31
4	492.7 ± 26.85	504 ± 36.66	82.33 ± 6.25	69.41 ± 0.63
5	106.3 ± 18.50	120 ± 7.81	87 ± 5.09	145.66 ± 2.25

normal one, however, diabetic rats treated with ETBCE showed a significant reduction in their weights toward the normal weight.

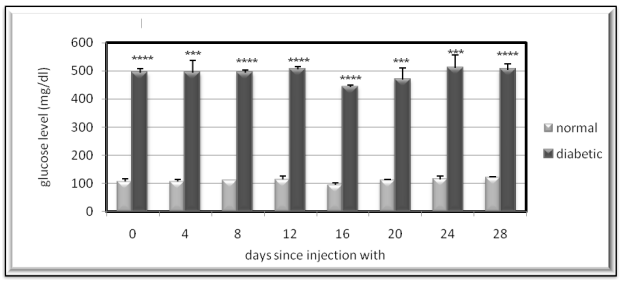

Figure 1: Effect of STZ on blood glucose level
Rats were injected with STZ to induce type 1DM.

Blood sample was taken every 4 days and fasting glucose level was measured. Results show a significant increase in total blood glucose since the first measurement. Data are from 4 rats per time point and show the mean ± sem. Normal rats do not show any significant change in total blood glucose.

The results of student's paired *t* test, comparing means and standard errors of experimental to normal, are presented as stars over each experimental point indicating the *P*-values. * *P<0.05,* ** *P< 0.02,* *** *P<0.01,* **** *P< 0.001*. Points without stars are not significantly different from their normal *(P>0.05)*.

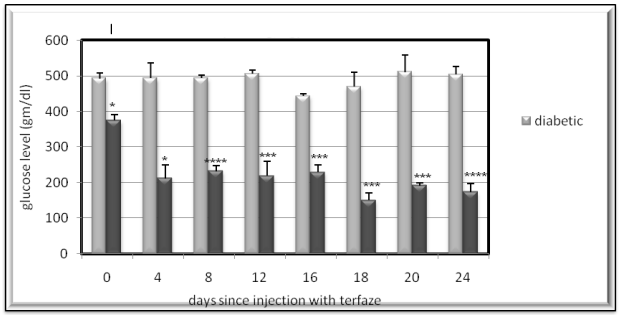

Figure 2: Represents a comparison of results between control and experimental rats show a significant decrease in total blood glucose since the first treatment (4 days after injection with 200mg/kg of ETBCE).

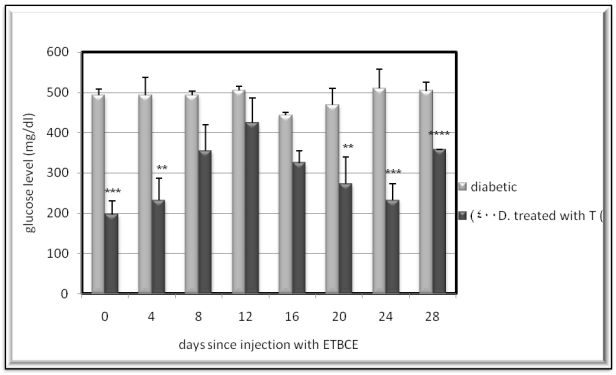

Figure 3: Represents a comparison of results between control and experimental rats, show a significant decrease in total blood glucose since the first measurement (4 days after injection with 400mg/kg of ETBCE).

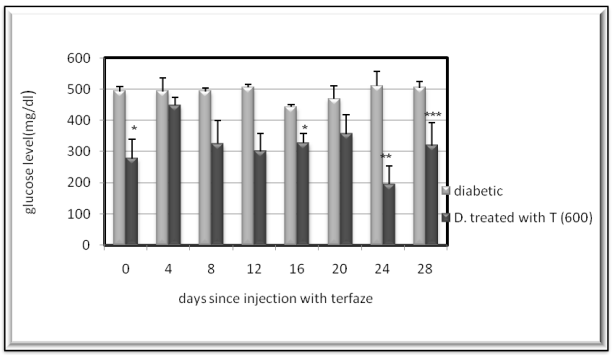

Figure 4: Represents a comparison of results between control and experimental rats, show a significant decrease in total blood glucose since the first measurement (4 days after injection with 600mg/kg of ETBCE).

DISCUSSION

Although various types of oral hypoglycemic agents are currently available along with insulin for treating diabetes mellitus, there is a growing interest in herbal remedies due to the side-effects associated with the existing therapeutic hypoglycemic agents. For instance, chemically, certain kinds of TBC contain natural products of antioxidants such as vitamins, enzymes, flavonoids, terpenenes and alkaloids. These products have been documented as hypoglycemic agents which act as protective agents to maintaine pancreatic β-cells from damage and can facilitate insulin secretion due to facilitation of calcium into the β-cells.

The hypothesis in this study stated that oral administration of ETBCE may be used as a protective agent against raising blood glucose. Oral administration of 200 mg/kg body weight of ETBCE was found to be more potent to reduce blood glucose level in STZ-induced diabetic rats than the other two doses of the extract (400 and 600 mg/kg body weight). We suggested that, the aqueous extract of the edible TBC possibly stimulates the action of pancreatic β-cell mechanism to facilitate the synthesis and/or the secretion of insulin hormone. The extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. This hypothesis is in an agreement with earlier observations by several researchers who reported that certain mushroom such as ganoderma lucidum can stimulate insulin secretion from isolated islets β-cells and prevent type 2 DM.[13,14]

No side effect was observed when the animals treated with ETBCE and this is in an agreement with Ahamed et. al. (1981)[1] and others who discovered that certain kind of truffles have strong effect on the blood glucose as hypoglycemic agent with no evidence for toxic effect.

According to this, the truffle extract may be a putatively useful agent against elevation of blood glucose and this leads to envisage that such extract could reduce the consumption of anti-diabetic drugs thus increase the chance of the pancreas to educate itself in the fight against raising of glucose level. Hence more use of truffle

substances and less anti-diabetic drugs might be beneficial to prevent development of diabetes if the hypothesis holds true.

Continued work on truffle is recommended and should focus on the other sides that were not covered in the current study, healthy volunteers as well as patients should be considered.

CONCLUSION

The results of the current study showed that the oral administration of an aqueous extract of truffle protects against lethal diabetes. This was demonstrated by the lowering of blood glucose level and the higher survival rate of the treated animals. Because truffle may have no side effects, the observed action was most probably occurs *via* the pancreatic beta cells to release insulin hormone. We thought that in the near future, such truffle may have prophylactic and therapeutic effects against high blood glucose as "alternatives anti-diabetic drugs".

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