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ANTIOXIDANT AND HYPOGLYCEMIC EFFECT OF *OTOSTEGIA AUCHERI* METHANOLIC EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC MALE LONG-EVANS RATs

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Abstract: Present study is based on the investigation of antioxidant and antihyperglycemic effect of methanolic extract from areal parts of *Otostigea aucheri* (OA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method was used to measure the antioxidant activity of extract of the species *Otostigea aucheri*. The observed scavenging activity for the free radicals was significant and it was compared with the standard BHT inhibition method. The IC_{50} value obtained of methanolic extract was 2.23 µg/mL. The methanolic extract of OA on the blood glucose level was further studied in normal (non-diabetic), streptozotocin (STZ)-induced type I and type II diabetic male Long-Evans rats at postprandial glucose load state. The results revealed that the oral administration of methanolic extract (1.25 g/kg) of OA showed no remarkable hypoglycemic effect in normal and type I (IDDM) diabetic rats. However, the methanolic extract significantly lowered ($p < 0.005$) serum glucose level in type II diabetic (NIDDM) models when simultaneous glucose was administered. This screening for antioxidant activity interprets the pernicious effects of diabetes that have been associated with mediation through the oxidation stress. The study also suggests to introduce natural source of the potential orally active antioxidant and active antihyperglycemic phytochemicals for the future. It may also improve the impaired antioxidant defense system.

Keywords: *Otostigea aucheri* L., antioxidant activity, hypoglycemic effect, streptozotocin, diabetes

The medicinal plants contribute a lot in support of human communities all over the world (1). In addition to other uses, plants remained the main source of medicines. The traditional plant remedies have always been pragmatically practiced for various ailments like diabetes, hypertension, jaundice, cardiovascular diseases and cancer in traditional ways (2). An endocrine disorderness is the major cause of diabetes mellitus (DM) and oral hypoglycemic drugs and/or insulin is used generally for the treatment (3). As the immune system of the body diminishes, the pancreatic β cells that produce insulin for the regulation of blood glucose level, get affected, hence, develop type I (IDDM) diabetes. In type II (NIDDM) diabetes, resistance is developed against the use of insulin by cells. As a result, the need for insulin increases and pancreas gradually loses its ability to produce insulin. Although pathogenesis of both IDDM and NIDDM diabetes are varied, hyperglycemia and its related complications are common to some extent in both types (4). Cardiovascular complications are due to the increased level of lipid profile in the diabetes (5). The production of free radicals is usually increased in diabetes, hence, simultaneous decline of antioxidants defense mechanisms (6) causes oxidative stress (7). The elevation of oxidative stress consequently prop up the complications (8).

In fact, diabetes is manageable using pharmacologic products and changing life style, like controlled diet and light exercise (9, 10) along with taking effective antidiabetes phyto-medicines. Since all of the pharmacologic agents are exceptionally not without severe side effects, so the researchers are motivated to seek for remedies in traditional medicines that have milder toxicity than available allopathic drugs (11). Diabetes complications have lethal effects due to multiple defects in its pathophysiology (12). Current research trend is diverted towards traditional medicinal herbs and plants as potential alternative source to manage diabetes with its multiple pharmacologic actions (13). A large number of antidiabetic phytoconstituents were isolated and characterized from natural sources and still

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research is continuously focused to explore new antidiabetic better natural lead molecules for the benefit of humanity (14).

Although the complementary and alternative medicine consists of phytomedicine and nutraceutics as alternatives, to mainstream allopathic treatment is another better option. According to the recent estimation, up to 30% diabetics take dietary supplements as alternative medicine (15). In the third world countries hypoglycemic plants contribute a lot in controlling and management of diabetes (16). Natural products from various sources like plants, animals and microorganisms are considered to be strong candidate of pharmaceutical drugs. It has been clearly described in the ethnobotanical literature that a large number of plants species with antidiabetic potential are used worldwide (17).

In Balochistan, there is a wide arsenal of medicinal plants because of the high altitude. A huge number of medicinal plants with antidiabetic efficacy are yet to be scientifically studied and commercially formulated as modern drugs, since they have been commended for their potential hypoglycemic therapeutic in traditional medicine (18).

The genus *Otostegia* belongs to family Lamiaceae and consists of more than 31 species growing in Asia and Mediterranean region (19).

Only three species are known in Pakistan, *Otostegia limbata, Otostegia persica and Otostegia aucheri* (20). They are all biologically active and were subjected to the phytochemical isolation and characterization studies. Alcoholic extract of *O. persica* showed hypoglycemic and hypolipidemic effect in normoglycemic and streptozotocin-induced diabetic rats with significant antioxidant efficacy (21).

In recent research, indigenous plant *O. aucheri* was subjected to evaluation of the antioxidant and anti-diabetes efficacy on account of the reported literature (22).

The traditional medicinal plants should be investigated to get better understanding of their chemistry to elucidate the structure of the bioactive constituents, their efficacy, selectivity, specificity and safety. Thus, the provision of new aspects may solve the health and economical challenges in the region (23).

In the presented studies, methanolic extract of indigenous plant species *Otostigea aucheri* (OA) have been taken into consideration to investigate the antioxidant potential using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. The pharmacological screening of the plant extract revealed potent antioxidant activity. Further *in vivo* antidiabetic activity for the crude plant extract was also determined by streptozotocin (STZ)-induced diabetic model in rats. These results add new imminence for exploitation of source of natural antioxidant and antidiabetic drugs as the screened plant exemplified potential. Further research is awaited to explore and identify the bioactive moieties from the fractions present in the crude extract and also to determine their full spectrum of efficacy, selectivity and specificity.

EXPERIMENTAL

Plant material

The plant *Otostegia aucheri* (Lamiaceae) local name Gul Gaider was collected from Kohlu, Balochistan province, Pakistan. The species was confirmed by Prof. G. Rasool Tareen (Department of Botany, University of Balochistan, Quetta, Pakistan) and specimens (Voucher no. 335) were deposited in the herbarium of Department of Botany, University of Balochistan, Quetta, Pakistan.

Extraction

The air dried aerial parts of *O. aucheri* were finely powdered, and 10 kg sample was soaked in 80% methanol for a week. It was exhaustively extracted with methanol (3 × 10 L). The extract was evaporated under reduced pressure to yield the residue, 1.52 kg.

In vitro antioxidant assay

In this experiment, methanolic extract of *O. aucheri* and standard BHT ((butylated hydroxytoluene) were subjected to evaluation of antioxidant activity. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used for measuring free radical scavenging activity (RSA) IC_{50} (24). *O. aucheri* extract (90, 60, 30, 3.0 µg/mL) in methanol were added to 2 mL of methanolic DPPH solution (4 × 10–5 g/mL MeOH). The BHT standard was used as control. The absorption of sample was measured at 517 nm after 30 min. The percentage of inhibition of activity was calculated as follows:

$$\text{Inhibition (\%)} = 100 - (\text{Sample Absorption} - \text{Control Absorption})/\text{Blank Absorption}) \times 100$$

Analyses of at least 3 samples were carried out in triplicate.

Antidiabetic bioassay

Induction of diabetes

Male Long-Evans rats (150–210 g) were used to screen the efficacy of the crude extract. Only single dose administration of the crude extract with

simultaneous glucose was fed to normal rats, insulin dependent diabetes mellitus (IDDM) models and noninsulin dependent diabetes mellitus (NIDDM) rats.

The standard conditions of temperature and relative humidity, with a 12 h light/dark cycle were maintained in animal house. Water and commercial rat feed were provided ad *libitum*. The current experiments were carried out with a prior permission from the institutional ethical committee regarding animals used in experiments. The IDDM diabetes was induced in rats by single interperitoneal injection of freshly prepared streptozotocin (STZ) solution in citrate buffer (pH 4.5) to 12 h fasted adult rats at a dose of 65 mg/kg body weight. After 84 h of STZ administration, the serum glucose level of each rat was determined for confirmation of diabetes.

Type II diabetes (NIDDM) was produced by single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution to 48 h old pups at a dose of 90 mg/kg body weight (25). The diabetic status of the models was checked prior to experimentation by blood glucose estimation. Rats with serum glucose level above 250 mg/dL were considered as diabetic and were used in the experiments.

Plant extract administration with simultaneous glucose load

The rats were fasting for 12 h, but they had free access to water. The alcoholic extract of *O. aucheri* (1.25 g/kg body weight in 2 mL distilled water) suspension was orally administered simultaneously with glucose (2.5 g/kg body weight) by gastric tube attached with 5 mL syringe. The experimental control groups received only glucose at the same dose.

Blood sample collection

Keeping experimental rats under mild ether anesthesia, blood samples were collected by cutting the tail tip, after immersing tail in luke-warm water for 30 s for vascular dilation. The samples were collected at zero minute followed by 30 and 75 min intervals. The serum was separated by centrifugation at the rate of 5700 rpm for 5 min. The glucose level was measured immediately and rest of serum was stored at –20°C until further analyzed.

Estimation of blood glucose level

The glucose level of serum was immediately estimated by enzymatic-colorimetric method using glucose-oxidase method GOD-PAP (26). The absorbance was measured by a microplate reader (Bio-Tek, ELISA). The 10 mL of 20 mmol glucose solution was prepared in different dilutions 8, 6, 4, 2 and 1 mmol/L and distilled water was used as blank with zero mmol/L glucose. Serum sample (2.5 µL) with 5 µL of each concentration of the glucose solution were dispensed into the wells of the micro titer plate and on top of that 200 µL glucose oxidase GOD-PAP was added simultaneously by multichannel pipette, incubated for 15 min at 37°C and the absorbance was measured at 490 nm using microplate ELISA reader (Bio-Tek EL.). Each value represents the mean of duplicate measurements.

Statistical analysis

All results are tabulated as the mean ± SEM. Data analysis was done by applying unpaired Student's *t*-test and results were compared to mean values between control and treated groups. Values of p less than 0.05 and 0.005 in different experiments were considered significant.

RESULTS AND DISCUSSION

The antioxidant and antidiabetic activities of *O. aucheri* (AO*)* are presented in the tabular form. The statistical analysis of the antioxidant activity showed significant ($p < 0.05$) differences between results of extract and BHT, the results indicated that the methanolic extract has remarkable free radical scavenging activity compared to standard (Table 1).

IC_{50} of samples from *O. aucheri* with DPPH method

In male rats, it has been reported that oral single administration of extract significantly lower the

Table 1. IC_{50} of samples from *O. aucheri* with DPPH method.

Samples/Standard	DPPH/30 min (µg/mL)
Otostigea aucheri extract	2.23 ± 0.97 *
BHT (Butylated hydroxytoluene)	110.97 ± 8.25

* Values are the mean ± SD (n = 3), $p < 0.05$ considered significant

Table 2. Effect of *O. aucheri* extract on the serum glucose level (mmol/L) in normal (non diabetic), IDDM and NIDDM model rats.

Treatment groups	0 min	30 min	75 min	Δ
Normal (non-diabetic) rats				
Control (n = 12)	8.28 ± 0.22	11.94 ± 0.42	9.5 ± 0.30	4.92 ± 0.87
O. aucheri (n = 9)	7.74 ± 0.23	11.9 ± 0.51	10.0 ± 0.45	6.50 ± 0.58
IDDM model rats				
Control (n = 8)	24.3 ± 1.6	31.60 ± 1.89	27.4 ± 1.7	10.3 ± 2.9
O. aucheri (n = 8)	22.9 ± 1.32	29.9 ± 1.2	24.3 ± 1.03	8.3 ± 2.8
NIDDM model rats				
Control (n = 10)	8.8 ± 0.39	20.1 ± 1.76	15.7 ± 2.1	18.2 ± 3.2
O. aucheri (n = 7)	9.4 ± 0.50	11.3 ± 1.3*	10.9 ± 1.6	3.23 ± 2.5

Data are expressed in terms of means ± SEM; 'n' indicates the number of rats in each groups. Δ, cumulative increment over basal value, * significant compared to baseline value (0 minute)

serum glucose level and increase insulin level. Even biologically active phytochemicals are potential source of several modern drugs, which produce hypoglycemia in the diabetic animals and non-significant in normal animals (27).

The oxidative stress mediated deadly effects of diabetes, since it is associated with increased release of reactive oxygen species and impaired antioxidant immune system .Due to this lipid peroxidation, change in antioxidants enzymes, impaired glutathione metabolism and a decrease in ascorbic acid occurred mainly on account of ineffective scavenging of reactive oxygen species that play a pronounced role in diabetes mellitus complications. Hence, disturbance of antioxidant defense system in diabetes mellitus (28, 29). The alcoholic extract was further analyzed for its hypoglycemic activity.

As shown by the results, there was not remarkable change in the normal rats and IDDM model upon feeding the methanolic extract of *Otostigea aucheri* with simultaneous glucose load (Table 2). However, the oral administration of methanolic extract of *O. aucheri* significantly lowered the blood glucose level in NIDDM rats. The extract revealed to be proficient in lowering sugar levels within 30 min (*$p < 0.005$), thus indicating that the extract of *O. aucheri* might not affect insulin secretion by pancreatic cells in NIDDM rats. It may delay the development of the diabetic complications. The plant extract of *O. aucheri* may be adapting the extra-pancreatic mechanism as antidiabetes agent, which resembles the mode of action of biguanides drugs (30).

As the isolated phytoconstituents of this plant have not been studied and characterized as antidiabetic agents, therefore, the mechanism of mode of action of the extract as antidiabetic and antioxidant is focused. This study is in sequence to an ethnobotanical assessment of medicinal plant alleged for the cure of diabetes mellitus. Comparative available literature study with other ethnobotanical analysis of plants used conventionally for the management of diabetes mellitus recommends that plant genus build claim of new information on antidiabetic usefulness. According to literature, antidiabetic drugs increase the blood insulin levels of diabetic rats by improving the antioxidant status and decreased lipid peroxidation (31).

Present results support the use of *O. aucheri* as an antidiabetic agent by conventional healers in urban environment. Hence, it is necessary to open new path for developing broad-spectrum antidiabetic drugs. The administration of new plant used in the present research showed the significance of the wide variety of such ethnobotanical understanding. The executive use of *O. aucheri* results in considerable restoration of the blood glucose levels.

CONCLUSION

O. aucheri exhibited powerful antioxidant and hypoglycemic effect in type II diabetic model rats. It is known that oxidative stress is produced under diabetic conditions and *O. aucheri* extract is considered for significant hypoglycemic activity as it is also a good antioxidant. It can be utilized as natural antioxidant and a preventing agent for diseases caused by free radicals and as antidiabetic in terms of associated complications. However, further investigations must be conducted to evaluate the mode of action with antidiabetic and antioxidant effect of the plant extract and identification of phytochemicals respon-

sible for this action. These activities may not only be attributed to the presence of potential antioxidant and antidiabetic molecule of the future but also provide appropriate mechanism to explore the interconnected management of diabetes complications through natural antioxidant.

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