Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.074

RESEARCH ARTICLE

Anti-Hyperglycemic Efficacy of *Derris ovalifolia* in Alloxan-Induced Diabetic Wister Rats

Muhammad Naeem Bari[1], Uzma Saleem[1], Muhammad Naeem Faisal[2*], Malik Hassan Mehmood[1], Muhammad Asif[1], Wafa Majeed[2], Haseeb Anwar[3], Humaira Muzaffar[2], Aisha Mahmood[2] and Ahmed Abdelsadik[4]

[1]Department of Pharmacy, Government College University, Faisalabad, Pakistan; [2]Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan; [3]Department of Physiology, Government College University, Faisalabad, Pakistan; [4]Department of Zoology, Aswan University, Aswan, Egypt
*Corresponding author: m.naeem.faisal@uaf.edu.pk

ARTICLE HISTORY (19-152)

Received:	April 08, 2019
Revised:	April 27, 2019
Accepted:	May 08, 2019
Published online:	May 21, 2019

Key words:
Amylin
Anti-hyperglycemic effect
Derris ovalifolia
Glucokinase
Leptin
Methanolic extract
Serum insulin

ABSTRACT

Diabetes has been characterized by abnormal glucose homeostasis arising from various pathogenic mechanisms involving β-cells dysfunction that results in hyperglycemia. Despite latest advancement for the treatment of diabetes, numerous challenges are still come across regarding use of available drugs and their complications. Current study was designed to assess the anti-hyperglycemic effects of *Derris ovalifolia* in diabetic rats. For this purpose, methanolic extract of *D. ovalifolia* was administered in graded doses (400 & 600 mg/kg body weight) to alloxan-induced diabetic rat model for a period of 4 weeks. Qualitative, quantitative phytochemical analysis, proximate composition and DPPH assay of methanolic extract of *D. ovalifolia* were performed. Blood samples were collected for monitoring glucose level, serum insulin level and other biochemical parameters. The data were subjected to statistical analysis by applying ANOVA and DMR. Significant anti-hyperglycemic effect was observed by reducing serum glucose and increasing serum insulin level in comparison to positive diabetic group. Treatment with methanolic extract of *D. ovalifolia* also led to improvement in serum amylin, glucokinase (GCK) and leptin.

To Cite This Article: Bari MN, Saleem U, Faisal MN, Mehmood MH, Asif M, Majeed W, Anwar H, Muzaffar H, Mahmood A and Abdelsadik A, 2020. Anti-hyperglycemic efficacy of *Derris ovalifolia* in alloxan-induced diabetic wister rats. Pak Vet J, 40(1): 108-112. http://dx.doi.org/10.29261/pakvetj/2019.074

INTRODUCTION

Diabetes has been characterized as an assortment of endocrine disorders (dysfunctioning of glucose homeostasis) that arises from various pathogenic mechanisms all that results in hyperglycemia. In type 2 diabetes (T2D) there is progressive β-cells dysfunction and requires long term therapeutic management (Wang *et al*., 2017). The secretion of insulin from β-islets cells become inadequate or absent with or without concurrent decrease in responsiveness of insulin sensitive peripheral tissues. Consequently, the secretion of insulin is increased through expansion of β-cells however, not sufficient to compensate insulin resistance in peripheral tissues. T2D is related with high mortality and morbidity in humans (Wang *et al*., 2017). The rate of development of disease is determined by various factors including abnormal insulin secretion, insulin resistance, decrease insulin mediated glucose uptake and its utilization (Shori *et al*., 2015). The detrimental effects of hyperglycemia result in damage to DNA along with signaling alteration due to oxidative stress in the endothelial cells of susceptible tissues. (Gordon *et al*., 2018). It is a complex disorder in which, body fails to convert food into energy. Hence, glucose is not utilized in the cells, and concentration of glucose in the blood increases. Resultantly it leads to development of metabolic abnormalities and acute symptoms that incorporates functional and structural alterations in the organs (Shori *et al*., 2015).

Despite latest advancement in the treatment of diabetes numerous challenges still come across regarding various complications associated with presently available drugs including hypoglycemia, cardiovascular risks, increase risk of morbidity (Akinmoladun *et al*., 2014) and GIT disturbances (Shori *et al*., 2015). This has led the scientists to search for new bioactive compounds with higher margins of safety among the best treatment option i.e. herbal drugs derived from plants being more reliable and used since ages (Hassan *et al*., 2010; Bhadoriya *et al*., 2018) and also various active compounds have been characterized and isolated for the purpose (Berraaouan *et al*., 2015). In developing countries, about 90% of the residents in rural areas are exclusively dependent on use of traditional medicines for ailment of various diseases including both humans and animals (Hassan *et al*., 2010; Abbas *et al*., 2017a, 2017b, 2018, 2019; Idris *et al*., 2017; Khater *et al*., 2018; Fayyaz *et al*., 2019).

Keeping in view the importance of medicinal plants the present study was designed to investigate the anti-hyperglycemic effects of one of undiscovered plant *Derris ovalifolia* (Wight & Arn) Benth., evidenced by biochemical parameters, along with antioxidant activity analysis in diabetic Wistar rats. The plant belongs to Fabaceae family with a synonym Pongamia *ovalifolia* (Balachandran and Gastmans, 1997), in English it is called Moulmein *rosewood*, locally it is known as Vilayti shisham (urdu) and Shiva in (Pashto). Different chemical constituents have previously been isolated from the plants, i.e flavonoids, flavones, flavonones and are used in folkloric medicines for treatment of diabetes, infections, fever and pain (Rahman *et al*., 2015).

MATERIALS AND METHODS

Collection of plant: Stem and leaf parts of plant *Derris ovalifolia* were used in the study. The plant was collected from the local area of City Faisalabad during the month August 2018 and was duly authenticated by a Taxonomist (Dr. Mansoor Hameed) Department of Botany, University of Agriculture Faisalabad. For future reference, a specimen voucher was assigned and preserved in herbarium vide No. 66-2-2018.

Preparation of extract: The leaves and stem parts 1.8 kg in weight were shade dried and grinded to fine powder. Methanol 8 liter in volume was added into the powder with occasional shaking for three days. Filtration was carried by using muslin sieve later on with Wattman filter paper number 4 for fine filtration. The ultimate filtrate was let to dry by using rotary evaporator operated at 35-40 rpm with temperature 40°C.

Proximate composition determination: Various physicochemical properties including dry matter, crude proteins, crude fat, fiber contents and total ash of dried powdered plant were studied by adopting AOAC (19th edition, 2012) guidelines.

Qualitative phytochemical analysis of methanolic extract of *D. ovalifolia*: The methanolic extract of plant was subjected to qualitative analysis for confirmation of phytochemicals present by using standard methods (Shabi and Kumari, 2014).

Quantitative phytochemical analysis of methanolic extract of *D. ovalifolia*: Plant extract was subjected for estimation of secondary metabolites such as total polyphenolics, total flavonoids, and total alkaloids by the methods as described by Slinkard and Singleton (1977), Chang *et al*. (2002).

***In-vitro* Free radical-scavenging activity DPPH Assay:** Radical scavenging activity of methanolic extract of *Derris* ovalifolia against stable DPPH (2,2-diphenyl--2-picrylhydrazyl hydrate) was determined by using spectrophotometer. The following formula was used for calculation of radical scavenging activity.

Activity (%): [1-(Absorbance of sample - Absorbance of blank) / Absorbance of control] x 100

Alloxan induced diabetic model: Ninety healthy male / female Wistar rats weighing 167-260 gm were used. The rats were purchased from the Department of Pharmaceutical Sciences, Government College University, Faisalabad and were kept under standard conditions by maintaining temperature and humidity. The research study was conducted by following the guidelines on use and care of animals as approved by the animal ethical committee. All the rats were given diet and water ad libitum. Rats were divided randomly in to five groups each consisting of six rats each.

Group 1: Normal rats treated with water and diet. (Negative control)
Group 2: Diabetic rats, no treatment (Positive control)
Group 3: Diabetic rats on Standard treatment with Glibenclamide @ 10 mg/kg b.wt.
Group 4: Diabetic rats on treatment with methanolic extract of *D. ovalifolia* @ 400 mg/kg b.wt.
Group 5: Diabetic rats on treatment with methanolic extract of *D. ovalifolia* @ 600 mg/kg b.wt.

Intra peritoneal injection of Alloxan monohydrate was administered at dose of 150 mg/kg b.wt for induction of diabetes. Rats with blood glucose level $\geq$200 mg/dl were considered as diabetic and included for investigation in study. Different doses of water suspended methanolic extract and standard treatment of glibenclamide 10 mg/kg b.wt using normal saline as vehicle were administered on daily basis for the period 28 days.

At the end of study, rats were decapitated by cervical dislocation. Blood samples were collected in tubes duly centrifuged at 4000 rpm/min for 15 minutes in order to separate serum and stored at -15^{o}C for analysis.

Bio-chemical assay: Concentrations of leptin, glucose, insulin, amylin and glucokinase (GCK) level in serum were measured through corresponding commercially available diagnostic kits.

Statistical analysis: Data was expressed as mean ± SEM. Statistical analysis was carried using one-way analysis of variance followed by Dennett's post-hoc test (Graph pad prism, San Deigo, USA). $P<0.05$ was considered significant.

RESULTS

Qualitative phytochemical analysis of methanolic extract of *D. Ovalifolia*: *Derris ovalifolia* leaf and stem parts weighing 1800 gms was extracted with methanol to a final end product of 48.55 gm with a final percentage yield 2.6%. The qualitative screening confirmed the presence of phenolics, flavonoids, tannins and alkaloids in *D. ovalifolia* leaf and stem extract. Saponins were present in mild concentration, fixed oil was not detected.

Quantitative phytochemical analysis of methanolic extract of *D. ovalifolia*: The quantitative analysis of photochemical fraction of MEDO revealed the presence of highest concentration of alkaloids (12.5±0.73mgG^{1-}) and tannins (3.2±0.82 mgG^{1-}) while Saponins were present in lowest quantity (0.087±0.56mgG^{1-}) as shown in Table 2.

Radical-scavenging activity: The plant extract showed moderate/high antioxidant activity when compared with standard treatment of blank DPPH solution. The results of antioxidant activity are presented in Table 3.

Blood glucose: Significant elevation (P<0.001) of fasting blood glucose levels in untreated diabetic rats was observed in this study. Gradual reduction of fasting blood glucose levels was observed in glibneclamide (10 mg/kg b.wt.) treated rats over the period of the experiment (Fig. 1) and the final blood glucose at day 28 was close to 288.25±10.16 mg/dl. Treatment with *D. ovalifolia* (400 mg/kg b.wt. and 600 mg/kg) for 28 days significantly improved blood glucose level (327±10.2 mg/dl) compared to diabetic control rats (512.5±26.9mg/dl). The results were comparable with the blood glucose level of rats treated with glibenclamide (10 mg/kg b.wt.).

Serum insulin, leptin, amylin and GCK levels: Serum level of different biochemical markers including, insulin, amylin GCK and leptin in alloxan-treated rats are shown in Fig. 2, 3, 4 & 5. A significant increase (P<0.05) in insulin level was found (Fig. 2) in treated groups as compared to positive control. The serum leptin level was reduced (0.45±0.12ng/dl) significantly (P<0.05) after administration of alloxan, the level was restored to normal (1.60±0.2 & 1.37±0.05ng/dl) in rats receiving methanolic extract of *D. ovalifolia* given at dose of 400 and 600 mg/kg b.wt. (Fig. 5). An ample decrease (P<0.05) in amylin and GCK levels was also observed in alloxan-induced hyperglycemic rats in comparison to negative control group whereas normal serum amylin and GCK levels were restored with treatment groups (Fig. 3 & 4).

Table 1: Proximate analysis of *D. ovalifolia*

Constituents	(%)
Ash	7.00±0.04
Crude Protein	21.03±0.56
Crude fat	8.05±0.73
Crude fiber	14.02±0.34
Total Carbohydrates	49.9±0.63
Moisture	8.70±0.29
Energy value (kJ/100g)	1503.66±8.70

Values are Mean ± standard deviation of triplicate determination (n=3).

Table 2: Quantitative phytochemical analysis of methanolic extract fraction of *D. ovalifolia*

Quantity	mg G^{-1}
Phenols	0.58±0.25
Saponins	0.087±0.56
Tannins	3.2±0.82
Alkaloids	12.5±0.73
Flavonoids	1.93±0.34

Values are Mean ± standard deviation of triplicate determination (n=3).

Table 3: DPPH free radical scavenging activity of methanolic extract of *D. ovalifolia*

Concentration (µg/ml)	% Inhibition	IC 50((µg/ml)
25	34.39	63.65
50	44.05	
100	67.193	
200	98.68	

Values are Mean ± standard deviation of triplicate determination (n=3).

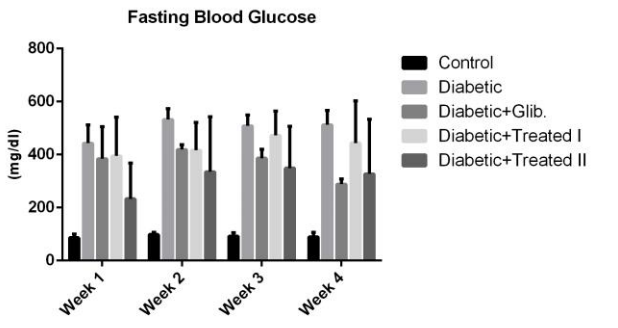

Fig. 1: Effect of methanolic extract of *D. ovalifolia* on fasting blood glucose level in experimentally induced diabetic rats.

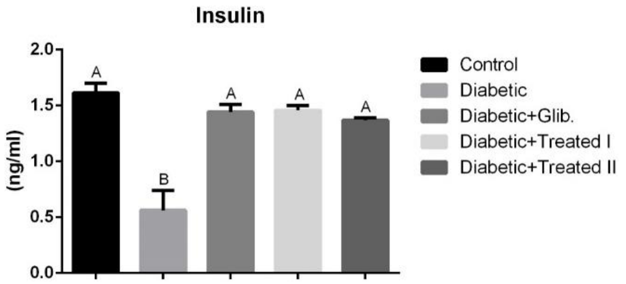

Fig. 2: Effect of methanolic extract of *D. ovalifolia* on serum insulin in experimentally induced diabetic rats.

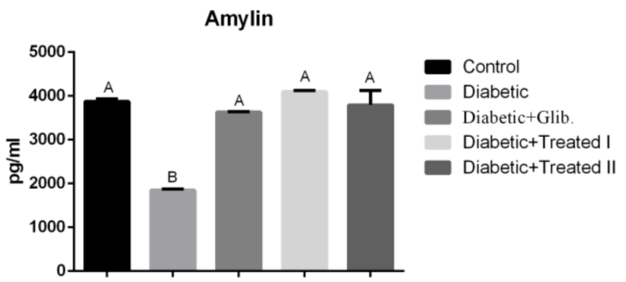

Fig. 3: Effect of methanolic extract of *D. ovalifolia* on serum amylin in experimentally induced diabetic rats.

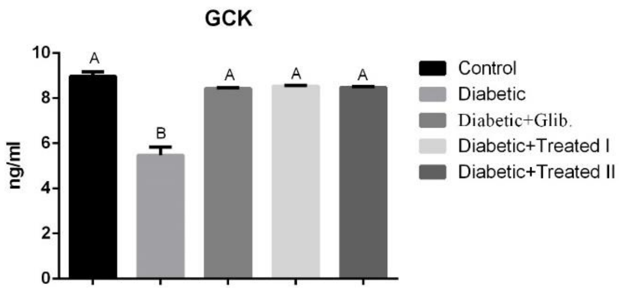

Fig. 4: Effect of methanolic extract of *D. ovalifolia* on serum glucokinase (GCK) in experimentally-induced diabetic rats.

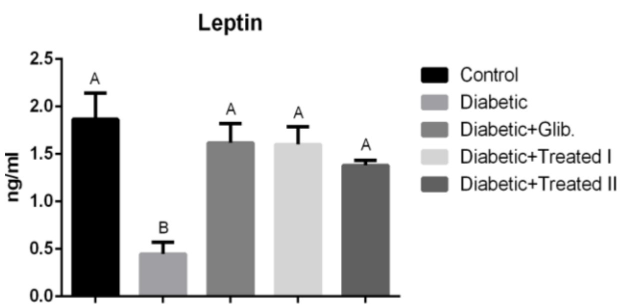

Fig. 5: Effect of methanolic extract of *D. ovalifolia* on serum leptin in experimentally induced diabetic rats.

DISCUSSION

From the analysis, results (Table 1) revealed that moisture contents of methanolic extract of *D. ovalifolia* (MEDO) are within satisfactory range of 6-15% and indicates extent of dryness of extract (Ehiabhi *et al.*, 2012). Total ash contents comprise of both physiological and non-physiological forms. The results revealed the presence of 7.0% ash content and indicative of diagnostic purity of plant extract. The plant extract contains crude proteins in proportion of 21.03%. The proteins are required essentially for biosynthesis of functional and structural components of body components. Similarly, crude fibers also help in the absorption of various trace elements in gut and assist in the removal of waste materials. It depicts nutritional value of a plant.

Free radical scavenging activity of MEDO was also estimated *in-vitro* by DPPH assay. The *in-vitro* DPPH assay showed that the MEDO exhibited free radical scavenging property over the range of 67.193%, which is in accord with the results reported by Škerget *et al.* (2009).

It has been reported in previous studies that alloxan-induced diabetes was described by loss in body weight, which is indicative of the fact that diabetes results in muscle wasting due to degradation of structural proteins (Rang *et al.*, 2003). Structural proteins are reported to plays important role in building body mass (Sunil *et al.*, 2011). The body weight of alloxan induced rats was increased significantly (P<0.01) both with administration of MEDO (400 and 600 mg/kg) and standard drug treatment glibenclamide as compared to negative control. The probable reason that the MEDO has lessened the reduction of body weight and breakdown of tissue protein, by the mechanism reversal of gluconeogenesis and control of glycogen level via glucose-6-phosphate dehydrogenase pathway in peripheral tissues (Sundaram *et al.*, 2014).

The increase in serum glucose level of alloxan-induced diabetic rats was depicted along with decrease in serum insulin level significantly in contrast to control group as reported in various research studies (Muzaffar *et al.*, 2019). The variation of initial and final blood glucose levels of MEDO at doses 400 and 600 mg/kg b.wt treated hyperglycemic rats revealed that the diabetes inducing effect of alloxan was decreased significantly during the period of treatment. This is indicative of fact that MEDO is effective in controlling the elevated blood glucose levels. The antihyperglycemic effect of MEDO at doses 400 and 600 mg/kg b.wt were significant (P<0.05) as compared to diabetic control and comparable with the standard treatment of glibenclamide 10 mg/kg b.wt after 28 days of treatment. The results are consistent with a previous study wherein antidiabetic activity of *Parkia biglobosa* aqueous seed extract produced considerable reduction in blood glucose level in streptozotocin induced diabetic rats (Ekperikpe *et al.*, 2019).

In the present study the results revealed that MEDO at dose 400 and 600mg/kg significantly (P<0.01) increased serum insulin level in diabetic rats up to 28 days of treatment as compared to negative control. Possible mechanism of action of anti-hyperglycemic activity of methanolic extract in diabetic rats may be due to increased insulin discharge from existing β cells as well as increased uptake of glucose into insulin sensitive peripheral tissues. This anti hyperglycemic effect might propose that the outcome may be due to reason increase in secretion of insulin from pancreatic β-cells i.e extra pancreatic and intra intestinal action of test extract (Day *et al.*, 1990). The effect may be contributed due to presence of various constituents contained in the extract including flavonoids and others having anti-diabetic and anti-oxidant activity. Studies have acknowledged that flavonoid compounds can be very valuable in improving the functioning of pancreatic β-*cells* along with utilization of glucose in tissues sensitive to insulin (Iftikhar *et al.*, 2018).

The hormone leptin that is derived from adipose tissue and had been documented to be involved in the complications associated with diabetes and microvascular disorders (Katsiki *et al.*, 2018; Muzaffar *et al.*, 2019). The results revealed that a significant reduced level of leptin was observed in serum of diabetic rats that may be due to deficiency of insulin. However, the level was raised comparable in diabetic rats when treated with glibenclamide and both extracts given at dose of 400 & 600 mg/kg bw.t. This effect is contributed towards improvement of insulin sensitivity in hepatic and skeletal muscles.

Glucokinase (GCK) a glycolytic enzyme that regulates and causes secretion of insulin. It is found mostly in pancreas and liver of mammals. GCK acts as pancreatic β-*cells* sensor and modifies the secretion of insulin in response to blood glucose level (Matschinsky *et al.*, 1998). The increased blood glucose level causes activation of GCK, which bring about phosphorylation of glucose to G6P. That ultimately results in production of energy by Krebs cycle and generation of ATP in mitochondria. It represents a major part of activity of an enzyme hexokinase that imparts its role in uptake and utilization of glucose independent on insulin (Adewole and Ojewole, 2009).

The activity of GCK is directly associated with blood glucose level, it increases and decreases accordingly. The level of serum GCK in present study was decreased significantly (P<0.05) in alloxan-induced diabetic rats as compared to diabetic control group while glibenclamide and both plant extract fractions of 400 and 600 mg/kg b.wt restored the normal serum GCK level. The decrease in serum GCK levels in diabetic rats with high blood sugar might be due to fall in blood insulin level, reduced synthesis of GCK or ample increased degradation that occurs as a consequence of oxidative stress in diabetes mellitus (Matschinsky *et al.*, 1998). Both glibenclamide and plant extracts restored serum GCK (P<0.05) significantly at normal level which is suggestive that both plant extracts have insulin-releasing potential in diabetic rats.

Another hormone Amylin is known as diabetes associated peptide, co-secreted with insulin from β-*cells* of pancreas particularly after when food is ingested. Other actions include on cardiovascular system and bones. In the blood causes activation of specific receptors located in brain stems. It imparts its major role in regulation of glucose metabolism in healthy and diseased state in mammals. Principally interact with leptin and is useful in weight loss when given adjunct with other agents. Different factors that enhance the secretion of Amylin include glucose, arginine and fatty acids and follow secretion of insulin (Qi *et al.*, 2010) and also GLP-1 (Asmar *et al.*, 2010). While, decreased during fasting state. Variations in amylin concentration during meal is thought to reflect changes in secretion of β-*cells* and bring about its physiological effects on eating and energy homeostasis (Lutz, 2010).

In present, study amylin level was decreased significantly (P<0.05) in alloxan induced diabetic rats in contrast to negative control group whereas; the other groups treated with glibenclamide and 400 and 600 mg/kg b.wt of plant extract exhibited a significant rise (P<0.05) in amylin level. These results are consistent with previous study in which it was found that in streptozotocin induced rats loss of ability to secrete amylin was observed while stimulated with arginine and glucose resulted in discharge of both amylin and insulin in a similar pattern (Ogawa *et al*., 1990).

Conclusions: The results of present study have revealed the anti-hyperglycemic potential of *D. ovalifolia* (methanol extract) by showing significant reduction in alloxan-induced hyperglycemia and improvement in biochemical parameters including serum glucose, insulin, amylin, GCK and leptin. The present study also supported the presence of several phyto-constituents in *D. ovalifolia*, which might be responsible for its anti-hyperglycemic potential by increasing efficiency of pancreatic beta cells.

Acknowledgement: The authors are thankful to Director Institute of Pharmacy Physiology and Pharmacology, University of Agriculture Faisalabad, for providing experimental & technical facilities for the conduct of study.

Authors contribution: This manuscript is based on PhD thesis of first author. NB, US and MNF contributed to design and conduct the whole experiment. All other authors have contributed in performing analysis and writing the manuscript. All authors are involved in discussing the contents of manuscript and declare no conflict of interest.

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