Key words: hypoglycemic activity, Dodonaea viscosa, alloxan diabetes

SUMMARY

Antidiabetic folkloric use of Dodonaea (D.) viscosa leaves was evaluated in normal and alloxan-diabetic rabbits. Blood glucose levels were determined after oral administration of 250 and 500 mg/kg of D. viscosa leaves and glibenclamide (5 mg/kg). These doses of the leaves significantly reduced blood glucose in normal and highly significantly in alloxan-diabetic rabbits. Glibenclamide could significantly reduce blood glucose levels in normal rabbits but not in alloxanized rabbits. It was also found that blood glucose levels of rabbits treated with aqueous: methanolic (A-M) extract of D. viscosa leaves representing 500 mg/kg body weight decreased highly significantly at 2, 4 and 6 h. Then oral glucose tolerance test was carried out in rabbits treated orally with A-M extract (representing 500 mg/kg of D. viscosa leaves and glibenclamide (5 mg/kg). Blood glucose of A-M extract treated rabbits was highly significantly decreased after oral glucose load. In addition, synergistic hypoglycemic effects of oral intake of A-M extract (representing 500 mg/kg) along with different doses of insulin were studied in diabetic rabbits. It was found that simultaneous administration of A-M extract and exogenous human insulin (3 units/kg body weight) reduced more potently the blood glucose levels of treated diabetic rabbits than those treated with the A-M extract only. Furthermore, oral administration of A-M extract of D. viscosa (250 and 500 mg/kg) continuously for 30 days produced highly significant reduction of blood glucose levels in diabetic rabbits as compared with controls. Simultaneously, plasma insulin levels of A-M extract treated rabbits were also found to have marked increased as compared with diabetic controls.

INTRODUCTION

Diabetes mellitus has been described as a metabolic disorder characterized by hyperglycemia and alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action (1). The disease has affected millions of people all over the world (2). Drugs of natural origin are considered to be less toxic and free from adverse effects than synthetic ones (3). Even though active compounds of many herbal drugs were unknown, they have been widely prescribed by
the practitioners of traditional medicines due to their minimal adverse effects and low cost (4). The traditional system of medicines, Ayurveda, Siddha and Unani, are based on the experience in the use of plant products in amelioration of many diseases (5). The World Health Organization (WHO) has estimated that 4 billion people (80% of the world population) use herbal medicines for some aspect of primary health care. Alternative treatment for diabetes has become increasingly popular during the last several years including medicinal herbs, nutritional supplementation and acupuncture (6).

Dodonaea (D.) viscosa, Linn., locally known as Sanatha, is an indigenous medicinal plant that has been used empirically for centuries as a hypoglycemic agent in the ethnomedicinal practices (7). However, as far as ascertained, no detailed scientific study seemed to have been carried out to assess the hypoglycemic activity of this abundantly wild grown plant. Therefore, the present study was conducted to determine the hypoglycemic activity of the dried and powdered D. viscosa leaves and their different extracts in normal and alloxan-induced diabetic rabbits.

MATERIAL AND METHODS

Plant material

Leaves of D. viscosa were collected from hilly areas of District Khoshab, Pakistan in the month of June 2009. D. viscosa leaves were identified and authenticated by Miss Naima Huma, Lecturer, Biological Sciences, University of Sargodha, Sargodha. The leaves were completely dried under the shade and powdered finely with a Chinese herbal grinder. The powdered material was stored in well-closed cellophane bags at 4 °C in a refrigerator.

Experimental animals

Healthy adult rabbits of a local strain (Oryctolagus cuniculus) weighing 1-1.5 kg were kept at animal house of the Department of Pharmacy, University of Sargodha, Sargodha. The animals were housed in stainless cages under standard laboratory conditions (light period: 8:00 am to 8:00 pm, 21±2 °C, relative humidity 55%, green fodder and water were available ad libitum).

Equipment

Glucometer (Optium Xceed, Abbot Laboratories, USA), Rotary Evaporator (Stuart, Bibby Steriline Ltd. UK), vortex mixture (Reax Top. Heldolph instrument, Germany), biochemical analyzer (Stat Fax 3300, Awareness Technology, Inc., USA), ELISA reader (Stat Fax 2100, Awareness Technology, Inc., USA), weighing balance (Shimadzu Corporation, Japan), lyophilizer (Christ alpha 1-4 LD, Germany), blood collection tubes, EDTA K₃ (BIO-VAC, Pakistan).

Chemicals and diagnostic kits

Glucose oxidase kits (Optium Xceed, Abbot Laboratories, USA), ALT and AST Kits (Fluitest, Biotechnologies AG, Germany), TC, TG, HDL, LDL-cholesterol kits (Fluitest, Biotechnologies AG, Germany), alloxan monohydrate (Research Organics, USA), chloroform (Labscan Asia Co., Ltd., Thailand), methanol (Merck Chemical Co., Germany), glibenclamide (CCL Pharmaceuticals (Pvt) Ltd., Lahore), gum tragacanth (Hi-Media Lab, USA), insulin (Regular Humulin, Lilly, USA).

Preparation of various extracts of D. viscosa leaves

Chloroform, methanol, aqueous:methanol (70:30) and aqueous extracts were prepared by cold maceration (8). The percentage yield was 15.4%, 16.7%, 19.5% and 17%, respectively. All the extracts except for aqueous were dried with the help of rotary evaporator and water extract by use of a lyophilizer.

Induction of experimental diabetes

Rabbits weighing 1-1.5 kg were made diabetic by injecting intravenous injection of 80 mg/kg of 10% alloxan monohydrate dissolved in isotonic saline (9). Control group received the same volume of saline solution. Eight days after injecting alloxan
monohydrate, blood glucose level of surviving rabbits was determined by the glucose-oxidase method. Rabbits with blood glucose level of 250-300 mg/dL were considered diabetic and were used in further study, as already used by Shani et al. (10) in their experiment.

**Preparation and administration of drug suspensions**

The amount of powdered leaves and extracts for each animal was calculated on body weight basis and triturated with about 10 mL of 2% aqueous gum tragacanth solution, and the final volume was always made up to 20 mL. Then the suspension was administered orally to each animal by using a stomach tube and disposable syringe (11). Glibenclamide was also administered after suspending in 2% aqueous gum tragacanth solution.

**Biochemical analyses**

Biochemical parameters such as blood glucose levels were measured by glucometer using glucose oxidized kit; total serum cholesterol, TG, HDL, ALT, AST were estimated by enzymatic test kit (Fluitest) using micro lab (Statfax 3300); LDL-cholesterol level was calculated by using the formula: \( \text{LDL} = \text{total cholesterol} - \text{HDL-cholesterol} - (\text{triglyceride}/5) \) (12); plasma insulin levels were estimated by ELISA kits using human insulin as a standard (13).

**Grouping of test animals**

**Hypoglycemic effects of powdered leaves of D. viscosa in normal rabbits**

Rabbits were divided into four groups of six animals each, normal control, powdered leaves treated (two groups) and glibenclamide treated. Group 1 animals served as untreated normal controls and were administered orally 20 mL of 2% gum tragacanth solution into water only. Groups 2 and 3 were given orally 250 and 500 mg/kg body weight of powdered D. viscosa leaves suspended in 2 per cent gum tragacanth aqueous solution, respectively. Group 4 animals were treated orally with 5 mg/kg body weight of glibenclamide (14). Blood glucose levels were checked at 0-, 2-, 4- and 6-h intervals after administration of powdered leaves and control drug, glibenclamide.

**Hypoglycemic effects of powdered D. viscosa leaves and glibenclamide in alloxan-induced diabetic rabbits**

The powdered leaves of D. viscosa significantly reduced blood glucose level in normal rabbits; therefore, hypoglycemic activity of powdered drug was also evaluated in alloxan-induced diabetic rabbits. Rabbits were fasted overnight and divided into five groups of six animals each, normal control, diabetic control, powdered leaves treated and glibenclamide treated. Group 1 animals served as untreated diabetic controls and were administered orally 20 mL of 2% gum tragacanth aqueous solution into water only. Groups 2 and 3 were administered orally 250 and 500 mg/kg body weight of powdered D. viscosa leaves. Group 4 was administered orally 5 mg/kg body weight of glibenclamide. In all groups, blood glucose levels were estimated at 0-, 2-, 4- and 6-h intervals after drug administration.

**Hypoglycemic effects of various extracts of D. viscosa leaves in alloxan-induced diabetic rabbits**

In this study, rabbits were fasted overnight and divided into nine groups of six animals each. Group 1 animals served as untreated diabetic controls and were administered orally 20 mL of 2% aqueous gum tragacanth solution. Groups 2, 3, 4 and 5 were administered chloroform, methanolic, aqueous: methanolic and aqueous extract of D. viscosa leaves in a dose representing 250 mg/kg of powder, respectively. Groups 6, 7, 8 and 9 were administered chloroform, methanolic, aqueous: methanolic and aqueous extract of D. viscosa leaves in a dose representing 500 mg/kg of powder, respectively. Blood glucose levels were estimated at 0-, 2-, 4- and 6-h intervals after the administration of extracts.
Hypoglycemic activity of aqueous:methanolic extract of D. viscosa leaves with and without different doses of insulin in alloxan-induced diabetic rabbits

The study was conducted to determine the synergistic effect of insulin and aqueous:methanolic extract on blood glucose levels of alloxan-induced diabetic rabbits (15). Rabbits were fasted overnight and divided into four groups of six animals each. Group 1 animals were administered 6 units/kg body weight of exogenous human insulin only. Groups 3 and 4 were administered 3 and 2 units of exogenous human insulin plus aqueous:methanolic extract representing 500 mg/kg of powdered D. viscosa leaves, respectively. Group 2 animals were administered aqueous:methanolic extract in a dose representing 500 mg/kg of powdered D. viscosa leaves only. Blood glucose levels were estimated at 0-, 1-, 2-, 3- and 4-h intervals after the administration of exogenous human insulin and aqueous:methanolic extract.

Oral glucose tolerance test (OGTT)

Rabbits were fasted overnight and divided into three groups of six animals each. Group 1 animals served as untreated normal controls and were administered orally 20 mL of 2% aqueous gum tragacanth solution. Groups 2 and 3 were administered orally the aqueous:methanolic extract (equivalent to 500 mg/kg of powder) and glibenclamide 5 mg/kg body weight, respectively. After 2 hours, glucose was administered orally (4 g/kg) to all three groups. Then blood glucose levels were estimated at 0-, 30-, 60-, 120-, 180-, 240-, and 300-min intervals. Percent change in blood glucose was estimated in comparison with the control group.

Fasting plasma glucose and plasma insulin levels

The study was conducted to investigate the effects of aqueous:methanolic extracts on blood glucose and plasma insulin levels of alloxan-induced diabetic rabbits over 30 days. Groups 1 and 2 served as untreated normal and alloxan-induced diabetic controls and were administered orally 20 mL of 2% aqueous gum tragacanth solution continuously for 30 days. Groups 3 and 4 were administered aqueous:methanolic extract (equivalent to 250 and 500 mg/kg powder) continuously for 30 days, respectively. Group 5 animals were administered 600 µg/kg body weight of glibenclamide continuously for 30 days (16,17). In groups 1-4, fasting plasma glucose levels were checked on days 0, 10 and 30. For estimation of plasma insulin levels, blood samples of all groups were collected in glass tubes treated with EDTA.

Statistical analysis

Analysis of variance technique (completely randomized design) was applied to test the significance difference at 5% and 1% significance level. Duncan’s multiple range (DMR) test was applied to check the difference among means using Minitab (15) and Microsoft Excel 2003.

RESULTS

Hypoglycemic effects of powdered leaves of D. viscosa in normoglycemic rabbits

As shown in Figure 1, the powdered leaves of D. viscosa in a dose of 500 mg/kg significantly reduced (P<0.05) blood glucose levels of treated rabbit at 6 hours. The standard drug, glibenclamide also significantly reduced (P<0.05) blood glucose levels of

Figure 1. Effects of powdered D. viscosa leaves and glibenclamide on blood glucose levels (mg/dL ± SEM) of normal rabbits at 0-, 2-, 4- and 6-h intervals.
normal rabbits at 6-h interval. There was no significant ($P>0.05$) change in blood glucose levels of group 1 receiving only 2% aqueous gum tragacanth solution.

**Hypoglycemic effects of powdered *D. viscosa* leaves and glibenclamide in alloxan-induced diabetic rabbits**

Data presented in Figure 2 clearly show that powdered leaves of *D. viscosa* decreased significantly ($P<0.05$) blood glucose levels of the treated group 2 at 2-, 4- and 6-h intervals, whereas in treated group 3 the hypoglycemic effects were significant ($P<0.05$) at 2 and 4 hours and highly significant at 6 hours ($P<0.01$). There was no significant ($P>0.05$) change in blood glucose levels of groups 1 and 4 treated with 2% aqueous gum tragacanth solution and glibenclamide, respectively.

**Figure 2. Effects of powdered *D. viscosa* leaves and glibenclamide on blood glucose levels (mg/dL ± SEM) of normal and alloxan-induced diabetic rabbits at 0-, 2-, 4- and 6-h intervals.**

**Hypoglycemic effects of various extracts of *D. viscosa* leaves (equivalent to 250 mg/kg of powder) in alloxan-induced diabetic rabbits**

As shown in Figure 3, blood glucose levels of groups 3 and 4 were significantly ($P<0.05$) lowered at 2- and 4-h intervals of methanolic and aqueous:methanolic extract administration, whereas blood glucose levels of group 5 were significantly ($P<0.05$) lowered at 2- and 4-h intervals, and highly significant at 6-h interval of aqueous extract administration. However, there was no significant ($P>0.05$) change in blood glucose levels of groups 1 and 2 receiving 2% aqueous gum tragacanth solution and chloroform extract, respectively (Fig. 7).

**Figure 3. Effects of chloroform, methanolic, aqueous:methanolic and aqueous extracts of powdered *D. viscosa* leaves (eq. to 250 mg/kg powder) and on blood glucose levels (mg/dL ± SEM) of alloxan-induced diabetic rabbits at 0-, 2-, 4- and 6-h intervals.**

**Hypoglycemic effects of various extracts of *D. viscosa* leaves (equivalent to 500 mg/kg of powder) in alloxan-induced diabetic rabbits**

In another experiment (Fig.4), blood glucose levels of group 6 were significantly ($P<0.05$) lowered at 2 h of chloroform extract administration. Blood glucose levels of group 7 were significantly ($P<0.05$) decreased at 2- and 4-h intervals after methanolic extract administration. There was a significant ($P<0.05$) reduction in blood glucose levels of group 8.
at 2 h and highly significant ($P<0.01$) at 4 and 6 h after aqueous:methanolic extract administration. Blood glucose levels of group 9 were significantly ($P<0.05$) decreased at 2, 4 and 6 h of aqueous extract administration.

Hypoglycemic effects of aqueous:methanolic extract of *D. viscosa* leaves with and without different doses of exogenous human insulin in alloxan-induced diabetic rabbits

The results presented in Figure 5 show changes of blood glucose levels in all treated groups. Blood glucose levels of groups 1 and 3 were found to be significantly ($P<0.05$) reduced at 1- and 2-h intervals and highly significantly reduced ($P<0.01$) after administration of exogenous human insulin and aqueous:methanolic extract plus insulin, whereas severe hypoglycemic effects ($P<0.01$) were observed in group 3 at all time intervals of blood glucose follow up. The administration of aqueous:methanolic extract alone to group 2 also produced highly significant ($P<0.01$) hypoglycemic effects at 2-, 3- and 4-h intervals.

Figure 5. **Effects of aqueous:methanolic extract of dried *D. viscosa* leaves alone and in combination with different doses of insulin on blood glucose levels (mg/dL ± SEM) of alloxan-diabetic rabbits at 0-, 1-, 2-, 3- and 4-h intervals.**

Oral glucose tolerance test (OGTT) and glycemic levels

Figure 6 shows changes in blood glucose levels in all groups after oral administration of glucose (4 g/kg). OGTT data revealed that blood glucose levels of untreated alloxan-diabetic group 1 increased significantly ($P<0.05$) over the 5-h period. However, in the aqueous:methanolic extract treated alloxan-diabetic group 2, significant ($P<0.01$) blood glucose decrease was observed from 30 min onwards. Better glucose tolerance was observed in group 3 treated with glibenclamide as compared with group 1.

Figure 6. **Effect of aqueous:methanolic extract of *D. viscosa* leaves and glibenclamide on oral glucose tolerance test of alloxan-diabetic rabbits.**

Figure 7. **Hypoglycemic activity of aqueous:methanolic extract of *D. viscosa* leaves in alloxan-induced diabetic rabbits on days 0, 15 and 30.**

Hypoglycemic effects of aqueous:methanolic extract of *D. viscosa* leaves in alloxan-induced diabetic rabbits for 30 days

As shown in Figure 7, there was no change in blood glucose levels of untreated normal and alloxan-diabetic groups 1 and 2 over 30 days. However,
significant (\(P<0.05\)) hypoglycemic effects were observed in group 3 on days 15 and 30. The hypoglycemic effects in group 4 were significant (\(P<0.05\)) on day 15 and highly significant (\(P<0.01\)) on day 30.

**Effect of aqueous:methanolic extract of *D. viscosa* leaves on plasma insulin levels of alloxan-induced diabetic rabbits**

Figure 8 shows plasma insulin levels in groups 3, 4 and 5 treated with aqueous:methanolic extract (eq. to 250 and 500 mg/kg of powder) and glibenclamide after 30 days to be significantly (\(P<0.01\)) higher as compared to untreated alloxan-diabetic group 2.

![Figure 8. Effects of aqueous:methanolic extract of *D. viscosa* leaves on plasma insulin level (\(\mu\)IU/dL ± SEM) of normal and alloxan-induced diabetic rabbits treated with aqueous:methanolic extract and glibenclamide after 30 days of treatment.](image)

**DISCUSSION**

The leaves of *D. viscosa* were found to significantly reduce blood glucose levels of both normal and alloxan-induced diabetic rabbits (Fig. 1). Previously, productions of hypoglycemic response in normal and diabetic animals have also been reported for many medicinal plants like *Berberis aristata*, *Acacia nilotica*, etc. (18,19). The glibenclamide which was used in the present study as a control drug also significantly (\(P<0.05\)) reduced blood glucose levels of normal rabbits (Fig. 1), but did not reduce significantly blood glucose levels of alloxan-diabetic rabbits (Fig. 2). It is well established that sulfonylureas, including glibenclamide produce hypoglycemic action in normal animals by stimulating pancreatic \(\beta\)-cells to release more insulin. However, these drugs do not produce hypoglycemia in alloxan-diabetic rabbits as their \(\beta\)-cell are completely destroyed (20).

Further, we determined hypoglycemic effects of the extracts of *D. viscosa* leaves in chloroform, methanol, aqueous:methanol and water solvents after their oral administration in doses equivalent to 250 and 500 mg/kg of powder in alloxan-induced diabetic rabbits (Figs. 3 and 4). Hypoglycemic activity of aqueous: methanolic extract was highly significant (\(P<0.01\)), indicating that the compound responsible for hypoglycemia is more extractable in this solvent than others. In diabetic patients, treatment is aimed to lower blood glucose to the near normal level (21). In the current study, a highly significant (\(P<0.01\)) fall in blood glucose levels was observed following administration of aqueous:methanolic extract of *D. viscosa* leaves to the alloxan-diabetic rabbits.

In addition, a synergistic effect of aqueous: methanolic extract of *D. viscosa* leaves was observed with different doses of insulin indicating that there are some biological principle(s) that may possess insulin-like action. Many of plant species including *Gymnema sylvestre* and *Momordica charantia* have been found in various studies to be beneficial when used as adjunct therapy in non-insulin dependent diabetes mellitus (22). It has also been reported that co-administration of aqueous extract of *Berberis lyceum* Royle results in severe hypoglycemia in alloxan-diabetic rabbits (15).

It has also been shown that the administration of *Caralluma sinica* to streptozotocin-induced diabetic rabbits significantly lowered plasma glucose levels as compared to diabetic control, after glucose load (23). In our investigation, OGTT has revealed the aqueous:methanolic extract of *D. viscosa* leaves to have a capacity to lower blood glucose levels (Fig. 6). OGTT showed that in alloxan-diabetic rabbits after glucose load, the blood glucose levels remained high even after 5 h. Moreover, the administration of
aqueous:methanolic extract of *D. viscosa* leaves was found to effectively prevent increase in the blood glucose level, which may be probably due to restoration of insulin response.

In separate experiments, the administration of aqueous:methanolic extract of *D. viscosa* leaves in different doses continuously for 30 days significantly decreased blood glucose level and also significantly increased plasma insulin level in treated rabbits. In experimentally-induced diabetes, alloxan causes destruction of β-cells of the islets of Langerhans, which results in massive reduction in insulin release (24). In our study, we observed a significant increase in the plasma insulin level when alloxan-diabetic rabbits were treated with aqueous:methanolic extract continuously for 30 days. The increase in the levels of insulin indicated that the hypoglycemic action exerted by the extract of *D. viscosa* leaves was due to the pancreatic release of insulin from the existing β-cells of the islets of Langerhans, i.e. direct insulinotropic effect.

In view of the above discussed studies, it has become evident that aqueous:methanolic extract of *D. viscosa* leaves had significant and consistent hypoglycemic effects in alloxan-induced diabetic rabbits. These results also suggest that the active principle(s) responsible for hypoglycemic action of *D. viscosa* leaves act perhaps due to stimulation of insulin release from the pancreatic β-cells in normal animals and in alloxan-induced diabetic animals; the hypoglycemic effects could be due to their direct insulin-like effect. However, further studies would be essentially required to elucidate the exact mechanism(s) of hypoglycemic, antihyperlipidemic and hepatoprotective activities of the aqueous:methanolic extract of *D. viscosa* leaves and to establish its efficacy and safety for further clinical use in diabetic patients.

**REFERENCES**


