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RESEARCH ARTICLE

Antihyperglycemic and Hypolipidemic Effect of *Azadirachta indica* Leaves Aqueous Extract in Alloxan-Induced Diabetic Male Rabbits

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ABSTRACT

Introduction: In the present study, the hypoglycemic potentials of *Azadirachta indica* (AI) were studied in rabbits.The animals were divided randomly into four groups: Normal control, diabetic control, and two diabetic treated with AI groups. **Method:** An aqueous extract of AI fresh leaves was administered daily (200 and 400 mg/kg, orally) for a period of 25 days to alloxan-induced diabetic rabbits. Fasting blood serum was analyzed for blood glucose, in the day 5, 10, 15, 20, and 25 (every 5 days), cholesterol, high-density lipo protein, low-density lipoprotein, and triacylglycerides levels in the day 25 (the past day of experiment). The alloxan-injected rabbits exhibited hyperglycemia, indicating their diabetic condition. **Result:** At a dose of 200 and 400 mg/kg, extract of AI was significantly effective in lowering serum glucose, and lipid profile levels in the AI-treated diabetic rabbits compared with the control diabetic rabbits. The present results indicate that AI possesses hypoglycemic and hypolipidemic potential. **Conclusion:** Thus, AI may be of great value in managing the hyperglycemia and some diabetic complications in human subjects.

Keywords: *Azadirachta indica*, diabetic rabbits, hypoglycemia, lipid profile.

INTRODUCTION

Diabetes mellitus (DM) is a group of heterogeneous metabolic disorders characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both.[1] Plants have been the major source of drugs for the treatment of DM in traditional medicine and other ancient systems in the world, and for a long time DM has been treated orally with herbal medicines or their extracts,[1,2] because plant products are frequently considered to be less toxic and more free from side effects than synthetic ones.[2] Furthermore, after the recommendations made by the WHO on DM, investigations on hypoglycemic agents from medicinal plants have become more important, and the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control DM.[1] Many herbs and plants have been described as possessing hypoglycemic activity when taken orally.[3] Some of these plants have also been pharmacologically tested and shown to be of some value in human diabetes treatment. *Azadirachta indica* (AI) is also known as neem, and its leaves have long been used in the traditional medicine as a treatment for DM and peptic ulcers. In the past two decades research on AI, has been intensified, and many agricultural and medical properties of the tree were rediscovered.[4]

There has been a tremendous interest in this plant as can be evidenced by the voluminous work on its different parts have been carried out by various groups, leading to the isolation and structure elucidation of more than 135 compounds.[5] AI belongs to the family of Meliaceae.[6] It is one of the most useful medicinal plants.[7] Blood glucose lowering activity of AI seed oil and leaf extracts have been reported in various models of diabetic animals.[5-9] Ethanol extracts of neem leaves have also been shown to demonstrate anti-lipid peroxidative, antihyperglycemic, and anti-hypercholesterolemia activities as well as reduce serum triglyceride (TG) level in a diabetic rat model.[10] Chloroform extract produced attenuation of antiglycation non-enzymatic, increase level

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antioxidant enzymes, glucose-6-phosphatase, glucokinase (GK), hepatic glycogen, and insulin plasma levels.[11] In another study, aqueous extract of this plant increased body weight gain and decreased blood glucose in rats[12] whereas supplementation with leaf powder (1 g daily) in diabetic reduced symptoms of polydipsia, polyphagia, and headache.[13] Oral administration of nimbidin demonstrated the significant hypoglycemic effect in fasting rabbits.[14] Azadirachtolide possesses α-amylase and α-glucosidase inhibition, anti-hyperglycemic, and antilipidemic effect at dose of 50 and 100 mg/kg.[15] In fact, these two tetranortriterpenoids are known as active ingredients in AI that are in part responsible for the decrease the blood glucose in animal models.[15]

The possible mechanisms underlying the hypoglycemic activity of the aqueous leaf extract have also been discussed indicated that AI leaf extract, in itself, was found to have no action on the peripheral utilization of glucose or on hepatic glycogen, however, increase insulin release in rat pancreas.[16]

In the present study, we evaluated the antihyperglycemic and hypolipidemic effect of AI leaves aqueous extract in alloxan-induced diabetic rabbits.

MATERIALS AND METHODS

Plant material

Fresh and matured AI leaves were collected from Habilen city-Radfan, Lahij Governorate. The leaves were dried in the shade and grounded into fine powder. The powder (1 kg) was extracted thrice with double distilled water for 8 h in a percolator at room temperature, and then the solution filtered. Two concentrations were prepared allowing the following doses in terms of mg fresh material/kg body weight: 200 and 400 mg/kg.

Experimental animals

Forty male rabbits local strain (1300–1500 g), were used in this experiments, the animals were maintained in standard environmental conditions, and kept on a standard commercial diet with water *ad libitum*. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health, 1978 and were approved by the Animal Experiments Local Ethics Committee at the Zoo, Sana'a, Yemen.

Animals were acclimatized to laboratory conditions for 10 days before the starting the experiment, after the acclimatization period the animals were randomly divided into 4 groups as follows:

- Group 1: (n = 10) normal control animals, they orally received 10 ml normal saline once a day period of 25 days.
- Group 2: (n = 10) diabetic control, animals in this group were intraperitoneally (i.p.) injected with alloxan in dose 150 mg/kg to induce diabetes mellitus, and not treated with any type of treatment.
- Group 3: (n = 10) animals in this group were intraperitoneally (i.p.) injected with alloxan in dose 150 mg/kg to induce diabetes mellitus, and orally treated with 200 mg/kg of AI leaves aqueous extract once a day period of 25 days.
- Group 4: (n = 10) animals in this group were intraperitoneally (i.p.) injected with alloxan in dose 200 mg/kg to induce diabetes mellitus, and orally treated with 400 mg/kg of AI leaves aqueous extract once a day period of 25 days.

Inducing the DM

The animals were made artificially diabetic by intraperitoneally (i.p.) injection 150 mg/kg 10% solution of alloxan monohydrate in distilled water. Animals with a glucose level above 200 mg/dl were considered as diabetic. Alloxan was obtained from Avra synthesis private limited, India.

Blood glucose assay

Blood glucose was estimated using Blood Glucose test strips (Accu-Chek, Active, Roche-Swiss) after overnight fasting. Results are expressed as mg/dl.

Serum lipid profile assay

Serum total cholesterol, TG, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol were estimated using biochemical assay kits obtained from Avra synthesis private limited, India, according to manufacturer's instructions.

Statistical analysis

Mean of blood glucose level and lipid profile of 10 animals were expressed as mg/dl ± standard deviation, Student's *t*-test was used to check the significance $P < 0.05$.

RESULTS

Table 1 show that the decrease in the level of blood glucose was dose and time-dependent, the percent of decrease in 5th day in the animals that received AI in dose 200 mg/kg, was 11%, in compare to diabetic control, whereas in the animals that received AI in dose 400 mg/kg, was 16%, in compare to diabetic control. In the day 15, the decrease in blood glucose was significantly high $P < 0.05$, the percent of decrease in the animals that received AI in dose 200 mg/kg, was 25%, and the percent of decrease in the animals that received AI in dose 400 mg/kg, was 26%, in compare to diabetic control.

In the day 25, the decrease in blood glucose of treated groups was significantly high $P < 0.05$ in compare to diabetic control, the percent of decrease in the animals that received AI in dose 200 mg/kg and 400 mg/kg, was 36% and 41%, respectively, in compare to diabetic control.

Table 2 shows the lipid profile in the animals after 25 days of treatment by AI leaves aqueous extract, we noticed a significant decrease $P < 0.05$ in the level of cholesterol in the serum of animals treated by 200 mg/kg of extract, the percent of decrease was 14% in compare to diabetic control, while the percent of decrease in animals treated by 400 mg/kg of extract was 21% in compare to diabetic control.

We also noticed a significant increase $P < 0.05$ in the level of HDL (good cholesterol) in the serum of animals treated by 200 mg/kg of extract, the percent of increase was 2.4% in compare to diabetic control, while the percent of increase in animals treated by 400 mg/kg of extract was 31% in compare to diabetic control.

Our results clearly showed a significant decrease $P < 0.05$ in the level of LDL in the serum of animals treated by 200 mg/kg of extract, the percent of decrease was 23% in compare to diabetic control, while the percent of decrease in animals treated by 400 mg/kg of extract was 39% in compare to diabetic control.

TG level significantly decreased $P < 0.05$, in the serum of animals treated by 200 and 400 mg/kg, the percent of decrease was 8% and 12%, respectively, in compare to diabetic control.

DISCUSSION

The results of the present study indicated that AI leaves aqueous extract was an effective agent against alloxan-induced diabetes and also in preventing the gain of food and drink intake induced as a consequence of diabetes. A deficiency or insufficiency of insulin secretion or insulin resistance in the diabetic state usually causes a decrease in body weight gain[17,18] and increases in food intake, water intake,[19-21] in our experimental condition; the alloxan-induced diabetic animals also showed the same symptoms. However, administration of AI leaves aqueous extract slightly improved these physico-metabolic abnormalities. AI leaves aqueous extract, and its effective compounds could reduce blood glucose through increasing insulin secretion,[22,23] by modulating the pancreatic secretion. The results of the present study strongly suggest that AI leaves aqueous extract reduces blood glucose levels. This hypoglycemic effect of AI leaves aqueous extract could be also due to its ability to facilitate or enhance clearance of postprandial blood glucose in animals.[24] Interestingly, there was a decrease in the concentration of serum TG, cholesterol, and LDL, and an increase in HDL in treated rabbits. The mechanism by which AI leaves aqueous extract to induce a reduction in lipid concentration could be explained by stimulation of lipolysis[25] and fatty acid utilization[26,27] and/or suppression of hepatic fatty acid synthesis in rabbits.[28] It is well known that insulin activates lipoprotein lipase that hydrolyzes TG under normal conditions.[29]

Destruction of β-cells leads to depletion of plasma insulin, which results in hyperlipidemia[30] and hypercholesterolemia caused by derangement of metabolic abnormalities.[31]

We proved that repeated administration of AI leaves aqueous extract has a beneficial effect lowering hyperlipidemia associated with hyperglycemia.

Many studies showed that AI leaves aqueous extract caused a marked increase of hepatic glycogen content in experimental diabetic animals,[32,33] which indicates that rabbits cloud

Table 1: The level of blood glucose in mg/dl, in the various days of treatment by AI extract

Time interval	Treatments			
Days	Control	Diabetic control	(AI) 200 mg/kg	(AI) 400 mg/kg
0	96.20 ± 5.67	96.20 ± 4.44	97.20 ± 5.07	95.40 ± 6.31
5	93.60 ± 8.91	389.20 ± 11.17	344.20 ± 29.69	335.60 ± 18.15
10	98.60 ± 3.97	406 ± 10.77	322.40 ± 25.38	310.20 ± 19.16
15	94 ± 11.66	424 ± 26.71	318.40 ± 9.61	310.20 ± 10.06
20	96.2 ± 7.89	461.60 ± 10.97	310.20 ± 29.69	300.60 ± 18.15
25	98 ± 8.60	476.60 ± 14.47	301.80 ± 25.28	290 ± 17.00

Values are the mean of 10 animals'±SD, AI: *Azadirachta indica*. SD: Standard deviation

Table 2: Lipid profile after 25 days of treatment by AI extract

Type of lipids	Treatments			
(Mg/dl)	Control	Diabetic control	(AI) 200 mg/kg	(AI) 400 mg/kg
Cholesterol	45.91 ± 3.60	69.13 ± 2.58	59.45 ± 0.87	53.72 ± 4.00
HDL	17.31 ± 0.75	11.98 ± 0.44	12.27 ± 0.37	15.76 ± 0.92
LDL	18.77 ± 3.95	46.17 ± 3.09	35.06 ± 1.29	27.54 ± 3.99
TG	50.93 ± 0.61	58.22 ± 3.47	53.18 ± 0.48	51.52 ± 0.97

Values are the mean of 10 animals'±SD, AI: *Azadirachta indica*, HDL: High-density lipoproteins, LDL: Low-density lipoproteins, TG: Triglycerides. SD: Standard deviation

decrease hepatic glucose production (HGP) by increasing glycogen content.[34] In addition, AI leaves aqueous extract decreased G6Pase activity and increased GK activity in liver,[35-39] which indicates that there, could be an increase in hepatic glucose uptake and decrease in hepatic glucose release. The increased activity of hexokinase can promote glycolysis and increase utilization of glucose for energy production.[40] The administration of AI leaves aqueous extract to the diabetic increased the activity of hepatic hexokinase causing an increase in glycolysis and so decrease in blood glucose. Therefore, this study strongly suggests that AI leaves aqueous extract enhances hypoglycemic activity probably by reducing HGP through decreasing G6Pase activity and increasing GK activity.

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