Antidiabetic and antihyperlipidemic effects of ethanolic extract of *Salvadora persica* L. on alloxan-induced diabetic rats

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ABSTRACT

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities. Herbal medicines have been used for many years to overcome this global health problem. Therefore, the present study was undertaken to evaluate the antidiabetic and antihyperlipidemic effect of ethanolic extract of aerial parts of Salvadora persica in alloxan induced diabetic rats. Diabetes was induced by administration of alloxan monohydrate (120mg/kg, i.p.). Normal as well as diabetic albino rats were divided in to four groups receiving different treatments: vehicle (control), ethanolic extract (1g and 2g/kg b.w.) and standard drug tolbutamide (0.5g/kg b.w.) for 21 days. Fasting blood glucose and lipid parameters i.e. triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein and very low density lipoprotein were measured. The ethanolic extract produced significant reduction (p<0.01) in blood glucose and also had beneficial effect (p<0.01) on lipid profile in normal and diabetic rats at the end of the treatment period. The antidiabetic effect of the extract was similar to that observed for tolbutamide. Thus the present study reveals that the ethanolic extract of S. persica may be effective in controlling blood glucose level and in improving lipid profile in normal as well as diabetic rats.

Keywords: *Salvadora persica*, antidiabetic, antihyperlipidemic, alloxan, tolbutamide
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INTRODUCTION

Diabetes is a metabolic disorder affecting carbohydrate, fat and protein metabolism. Diabetes mellitus is mainly due to relatively low level of insulin production or an inability of the body to use insulin properly which in turn leads to hyperglycemia [1]. Type 2 diabetes mellitus is commonly associated with hyperlipidemia which is a significant risk factor the premature development of atherosclerosis and its cardiovascular complications [2]. Therefore, successful management of diabetes mellitus requires a drug that not only controls the glycemic level but also prevents the development of arthrosclerosis and other complications of diabetes. Approximately 171 million individuals worldwide had diabetes in the year 2000 and it is estimated that this will increase to 366 million by 2030 [3]. The current studies in India indicate that there is an alarming rise in prevalence of diabetes which has gone beyond epidemic form to a pandemic one. Presently India has got the largest number of diabetic patients and is being called as diabetic capital of the world [4].

The available therapies for diabetes include insulin and many oral hypoglycemic agents, such as biguanids and sulfonylurea. However, the use of oral drugs is limited due to adverse side effects including hematological and gastrointestinal reactions, hypoglycemic coma and disturbance of liver and kidney functions; in addition they are also not suitable during pregnancy [5]. So, with regard to the issue of socioeconomic burden of diabetes, discovery of more effective and without side effect therapies are necessary. Since ancient times, good data have been obtained from traditional medicines indicating usefulness of many herbal medicines. Ayurveda and other Indian literature

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mention the use of plants in treatment of various human ailments [6]. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. More than 100 medicinal plants are listed in the Indian system of medicines including folk medicines for the management of diabetes which are effective either independently or in combinations [7].

Salvadora persica L. (Kharijal) is a large, well-branched and evergreen shrub or a tree distributed in the dry and arid regions of India. *Salvadora persica* have a number of proven medicinal applications with almost all parts having pharmaceutical significance [8-9]. Around the world *S. persica* is more famous by the brand name of Miswak and the tree is referred as the toothbrush tree and extensively used as toothbrush or component of toothpaste [10]. The young branches and leaves are also favorite fodder for camels because of the high water content (15-36%). Oil from seed is used in rheumatic pain, diabetes and spleen and stomach disorders. The most vital aspect of oil is its constituency of low percentage of C8 and C10 fatty acids that are of great economic significance [11]. This plant holds strong antiulcer [12] antifungal [13], anti-parasitic, antiviral [14], and antibacterial [15] properties. Literature survey shows that stem decoction of *S. persica* possess hypoglycemic effects [16] in normal rats and it shows hypolipidemic activity on experimental hypercholesterolemia in rat [17]. But, thus far, no scientific reports are available regarding the antidiabetic and antihyperlipidemic activites of the ethanolic extract of aerial parts of this plant in alloxan induced diabetic rats. In present communication its role on glucose and lipid metabolism in alloxan diabetic rats is being reported.

MATERIALS AND METHODS

Plant material:
Aerial parts (stem and leaves) of *S. persica* were collected from plants grown at CSSRI, Regional Research Station, Hissar. The plant material was authenticated at FRI, Dehradun (Voucher number: 10786, deposited at FRI, Dehradun). The aerial parts were shade dried and were finely powdered. The plant material was extracted with 70% ethanol using soxhlet apparatus. The ethanolic extract (EE) was concentrated under reduced pressure and finally dried in a vacuum desiccator.

Animals:
Healthy adult albino wister male rats weighing between 160-200g were obtained from the disease free small animal house, Haryana Agricultural University, Hissar (India). The animals were housed in clean, dry plastic cages under controlled conditions of light (12h: 12h light dark cycle), temperature (25 ± 2^0C) and humidity ($50 \pm 5\%$). The animals were fed on a standard pellet diet (Hindustan lever ltd., India)) and water was provided ad libitium. All the experimental protocols were approved by the Institutional Animal Ethic Committee of M. D. University, Rohtak.

Induction of diabetes mellitus:
Diabetes was induced in rats by induction of a single intra peritoneal injection of alloxan monohydrate (120 mg/kg b.w.). After 7 days animals with blood glucose level of 200-300mg/dl (mild diabetic) were used for the experiment. The diabetic animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

Experimental setup:
The experiment was performed in two steps:

Effect of ethanolic extracts of *Salvadora persica* on serum glucose level and lipid profile in normal fasted rats:
Four groups of normal rats, each group having six animals were used for the experiment. Group 1 served as control and received 1% w/v suspension of CMC (vehicle) in water at a dose of 10 ml/kg b.w. Group 2 and 3 received ethanolic extract of *S. persica* in 1% CMC at a dose of 1g and 2g/kg b.w. respectively and Group 4 was given standard drug tolbutamide at a dose of 0.5g/kg b.w. The drug was administered continuously for 21 days using infant feeding tube. Blood glucose was estimated before starting the experiment and weekly (7, 14, 21 days) up to the end of treatment period. Lipid profile was observed at the end of the 21-days treatment period.

Effect of ethanolic extracts of *Salvadora persica* on serum glucose level and lipid profile in alloxan induced diabetic rats:
Diabetic rats were also divided in to four groups. Group 1 (control) received 1% w/v suspension of CMC (vehicle) in water at a dose of 10 ml/kg b.w. Group 2 and 3 received ethanolic extract of *S. persica* at a dose of 1g and 2g/kg b.w. respectively and group 4 was given standard drug tolbutamide at a dose of 0.5g/kg b.w.The drug was administered continuously for 21 days using infant feeding tube. Blood glucose was estimated before starting the experiment and weekly (7, 14, 21 days) up to the end of treatment period. Lipid profile was observed at the end of the 21-days treatment period.

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Biochemical analysis:
Fasting blood glucose estimation was done by using ortho-toluidine method [18]. Total cholesterol (TCH) was determined by using Erba diagnostic Kit [19]. Serum Triglycerides (TG) were estimated using Ranbaxy Enzokit [20]. HDL (High density lipoproteins) cholesterol was measured by Erba diagnostic kit [21]. VLDL (Very low density lipoproteins) cholesterol was calculated as: Triglycerides/5 and LDL (Low density lipoproteins) cholesterol was calculated by the equation

LDL-cholesterol = Total cholesterol - (HDL + VLDL)

All these estimation were done using Erba Transasia auto analyzer.

Statistical analysis:
All the results were expressed as mean ± SD. The data was analyzed by one-way ANOVA followed by Tukey's test. The minimum level of significance was fixed at $p < 0.05$.

RESULTS

Antidiabetic effect of ethanolic extract:
The ethanolic extract of *S. persica* exhibited hypoglycemic effect in normal rats and antihyperglycemic activity in alloxan induced diabetic rats. The ethanolic extract at a dose level of 1g and 2g/kg b.w. in normal nondiabetic rats showed a significant decrease ($p<0.01$) in blood glucose level at the end of 2nd week and this was further lowered after 21 days of treatment (Table 1). The extract at a dose of 2g/kg b.w., showed the highest hypoglycemic effect, lowering the blood glucose from 83.36±1.56 to 56.72±1.21 at the end of treatment period. The effect was comparable with the standard drug tolbutamide which decreased the blood glucose level from 82.92± 2.37 to 51.26±1.56 after 21 days. In diabetic rats repeated administration of ethanolic extract at a dose of 1g and 2g/kg b.w. for 21 days produced a significant ($p<0.01$) dose dependent fall in blood glucose level as compared to vehicle treated group (Table 2). The effect of *S. persica* extract was similar to that observed for tolbutamide that had lowered the blood glucose from 286.56±6.2 to 202.77±8.21 after 21 days.

Table 1: Effect of ethanolic extract of *S. persica* on blood glucose level of normal rats

Groups (n=6)	Blood glucose level(mg/dl)			
	0 day	**After 7 days**	**After 14 days**	**After 21 days**
Control	80.26±2.42	81.47±2.67	78.42±2.72	79.04±1.92
Ethanolic extract (1g/kg)	80.14±1.53	73.42±1.95**	67.12±2.72**	63.48±1.83**
Ethanolic extract (2g/kg)	83.36±1.56	70.69±2.44**	62.74±1.67**	56.72±1.21**
Tolbutamide	82.92±2.37	68.56±1.62**	58.36±2.17**	51.26±1.56**

Values (mg/dl) are expressed as mean ±SD for groups of six animals each. Values are statistically significant at*p<0.05, **p<0.01 vs. control group on the respective day.

Table 2: Effect of ethanolic extract of *S. persica* on blood glucose level of diabetic rats

Groups (n=6)	Blood glucose level(mg/dl)			
	0 day	**After 7 days**	**After 14 days**	**After 21 days**
Control	288.57±4.12	292.32±7.46	296.71±9.26	298.91±7.2
Ethanolic extract (1g/kg)	281.11±6.39	260.26±7.13**	249.32±6.57**	238.72±7.42**
Ethanolic extract (2g/kg)	290.21±7.47	254.02±10.03**	237.11±8.86**	218.62±9.52**
Tolbutamide	286.56±6.2	234.47±9.42**	216.52±6.8**	202.77±8.21**

Values (mg/dl) are expressed as mean ±SD for groups of six animals each. Values are statistically significant at*p<0.05, **p<0.01 vs. control group on the respective day.

Antihyperlipidemic effect of ethanolic extract:
Oral administration of ethanolic extract at a dose of 2g/kg b.w. continuously for 3 weeks in normal rats led to a significant fall ($p<0.01$) in the level of triglycerides, total cholesterol, LDL and VLDL and improved ($p<0.05$) the HDL level at the end of the treatment period as compared to control group (Table 3). In diabetic control group there was a significant decrease in HDL cholesterol level and a significant elevation in total cholesterol, triglycerides and LDL cholesterol levels. Continuous administration for 3 weeks brought the levels of serum lipids to near normal levels in diabetic rats (Table 4). The triglycerides, total cholesterol, LDL, VLDL levels of diabetic control rats were raised to 114.92±2.13, 125.16±3.23, 78.82±3.57, 28.98±2.42 respectively whereas in ethanolic extract (2g/kg b.w.) treated diabetic rats these levels were lowered to 102.12±3.76, 96.43±3.92, 53.39±2.02, 20.42±1.06 respectively. The levels of HDL were decreased in diabetic rats to 17.36±1.76. Treatment with ethanolic extract regained the HDL level to 22.62±1.78.

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Table 3: Effect of ethanolic extract on lipid profile of normal rats after 21 days

Groups (n=6)	TG	TCH	HDL	LDL	VLDL
Control	91.62±1.20	84.16±2.52	20.36±1.41	45.88±1.22	18.32±1.94
Ethanolic extract (1g/kg)	74.29±2.73**	70.43±1.78**	21.24±1.64	36.57±1.81	16.41±1.23
Ethanolic extract (2g/kg)	64.19±2.14**	63.96±1.26**	23.12±1.08*	28.01±1.96**	12.83±0.62**
Tolbutamide	66.22±1.87**	64.12±1.31**	22.17±1.67	32.81±1.12**	15.12±1.14**

*Values (mg/dl) are expressed as mean ±SD for groups of six animals each. Values are statistically significant at*p<0.05, **p<0.01 vs. control group on the respective day.*

Table 4: Effect of ethanolic extract on lipid profile of diabetic rats after 21 days

Groups (n=6)	TG	TCH	HDL	LDL	VLDL
Control	114.92±2.13	125.16±3.23	17.36±1.76	78.82±3.57	28.98±2.42
Ethanolic extract (1g/kg)	110.18±3.49	116.54±3.12**	19.15±1.92	64.48±2.28**	24.86±1.36**
Ethanolic extract (2g/kg)	102.12±3.76**	96.43±3.92**	22.62±1.78**	53.39±2.02**	20.42±1.06**
Tolbutamide	106.13±3.16**	98.07±2.06**	21.36±1.23**	56.55±2.18**	24.05±1.03**

*Values (mg/dl) are expressed as mean ±SD for groups of six animals each. Values are statistically significant at*p<0.05, **p<0.01 vs. control group on the respective day.*

DISCUSSION

Alloxan a beta cytotoxin induces "Chemical diabetes" in a wide variety of animal species by damaging the insulin secreting cells of pancreas [22]. Literature sources indicate that alloxan induced rats become hyperglycemic [23-24]. Alloxan causes treatment duration and concentration dependent degenerative lesions of the pancreatic β-cells. The use of lower doses of alloxan produced a partial destruction of pancreatic β-cells even though the animals became permanent diabetic [25]. A numbers of pancreatic β-cells are able to survive in low dose alloxan treated animals and regeneration is possible [26].

The present study demonstrated hypoglycemic activity of *S. persica* ethanolic extract in normal as well as diabetic rats and the results were comparable with tolbutamide. It is well known that sulfonylurea (tolbutamide) act by directly stimulating β-cells of langerhans to release more insulin and these compounds are active in mild alloxan-induced diabetes. Similarly, the antihyperglycemic action of *S. persica* extract is able to regenerate of pancreatic cells which were partially destroyed by alloxan and potentiating of insulin from surviving cells of langerhans [27].

The antidiabetic activity of *S. persica* may be due to the presence of phytochemicals (flavonoids, tannins, glycosides, sterols, saponins) [28-29]. Plants that contain the active principals such as glycosides and flavonoids have antioxidant activity and are said to possess antidiabetic effect. Moreover, flavonoids are known to regenerate the damaged β-cells in alloxan induced diabetic rats [30]. Besides this, *S. persica* also contain several organic sulphur compounds [31] and it is well known that sulphur derivatives show hypoglycemic effects. In facts many plants containing sulphur are used traditionally as antidiabetic [32-33].

Hyperglycemia is accompanied with dyslipidemia [34] and represents a risk factor for coronary heart diseases. The higher lipid profile of diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones [35]. Under normal circumstances insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides. However in diabetic rats lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglycemia [36] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [37]. The dyslipidemia is characterized by increase in TCH, TG, LDL, VLDL and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with ethanolic extract of *S. persica*. High LDL levels are usually associated with arthrosclerosis. High HDL level reduce this risk. A possible mechanism of ethanolic extract may be due to the presence of flavonoides, which significantly increase LDL receptor mRNA levels, which, in turn increase hepatic uptake and degradation of LDL causing a decrease in serum LDL levels [38-39]. In this context, number of other plants has also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects [40-42]. Hence it can be concluded that *S. persica* has revealed significant antidiabetic and antihyperlipidemic effect owing to its ability to reduce level of blood glucose, total cholesterol, triglyceride, LDL and increasing HDL level. Further experimentation needs to be done with regard to fraction of extract, isolation and characterization to explore active constituents responsible for such activity and to elucidate the possible biochemical mechanism.

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