Research Article

Antihyperglycemic and antioxidant effect of *Allium sativum* aqueous bulb extract against alloxan-induced diabetic male *albino* rats

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ABSTRACT

Herbal drugs play an important role in health programs worldwide and there is a resurgence of interest in herbal medicines for the treatment of various ailments including diabetes mellitus. In both insulin-dependent (Type 1) and non-insulin-dependent diabetes (Type 2), an increased oxidative stress has been notified. In the present study, the folklore medicinal plant *Allium sativum* was selected to evaluate its antihyperglycemic and antioxidant effect against alloxan-induced diabetic male albino rats. The biochemical parameters such as blood glucose, insulin, and lipid profile (cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein (LDL) and very LDL, urea, uric acid, and creatinine) were analyzed. Finally, the antioxidant parameters such as superoxide dismutase, catalase, glutathione peroxide, and glutathione S-transferase also analyzed. The aqueous extract shows very good antidiabetic activity confirmed by biochemical parameters and histopathological analysis.

KEY WORDS: *Allium sativum*, Alloxan, Antioxidant, Diabetes

INTRODUCTION

Allium sativum is one of the important medicinal plants most widely used in our home. There are many studies proving that *A. sativum* for its biomedical properties. *A. sativum* is having very many biological activities such as antibacterial activity using different solvent extracts such as aqueous, ethanol, methanol, and chloroform against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Klebsiella pneumoniae, Actinobacillus pleuropneumoniae* serotype, and *Helicobacter pylori*.[1-5]The plant extract shows good antiviral activity against human rhinovirus, human cytomegalovirus (HCMV), parainfluenza virus Type 3, herpes simplex virus Type 1-2, vaccine virus, vesicular stomatitis virus, and influenza B,[6] it actvively involves the cardioprotective activity and it proved by various biochemical reactions such as stimulation of nitric oxide generation in endothelial cells.[7] and hypotensive through increasing nitric oxide synthesis,[8] angiotensin-converting enzyme-inhibiting activity,[9] antifungal activity against *Aspergillus niger*,[10] *Paracoccidioides brasiliensis*,[11] *Ascosphaera apis*,[12] *Botrytiscinerea*, *Trichoderma harzianum*,[13] and *Candidaalbicans*, *Candida tropicalis*, and *Blastoschizomyces capitatus*.[14]

Herbal plants are laying a major role in the antidiabetic activity which was proved in various studies. The plant parts such as leaves, root, flower, fruit, and stem are used for the antidiabetic activity shown in Table 1.

In this present investigation, we have used *A. sativum* aqueous bulb extract for the antidiabetic activity in alloxan-induced diabetic animals. In that, the biochemical parameters such as blood glucose, insulin, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein (LDL) and very LDL, urea, uric acid, and creatinine were tested and antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx), and glutathione S-transferase (GST)

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were investigated. Finally, the histopathology also analyzed.

MATERIALS AND METHODS

Plant Materials

The plant of *A. sativum* (*Allioideae*) was collected in and around Vellore district, Tamil Nadu. The plant materials were cleaned with distilled water, shade dried at room temperature, and authenticated. The shade dried plant materials were coarsely powdered separately in an electrical blender and stored at 5°C in an air-tight container for further use.

Aqueous Extract Preparation

About 100 g of the dried plant powder was taken separately and mixed with 500 ml of distilled water and then magnetically stirred in separate containers overnight at room temperature. The residue was removed by filtration and concentrated under reduced pressure in a rotary evaporator at 60 ± 10°C to get solid yield.

Experimental Animals

Adult male Wistar rats weighing around 180–220 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25 ± 2°C and 55–65% relative humidity. 12 ± 1 h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental Induction of Diabetes

Diabetes was induced to the animal by intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight [bw]) (SD Fine Chemical Ltd., Mumbai) in normal saline. After 48 h, blood was collected from the tip of the tail and the blood glucose was measured using Gluco Check Glucose estimation kit (Aspen diagnostic (P) Ltd., Delhi, India). When the level of blood glucose was >250–350 mg/dl, it was taken for the study. All animals were allowed to access water and pellet diet and maintained at room temperature in polypropylene cages.

Experimental Design

- Group I: Normal rats
- Group II: Control rats. Diabetes was induced to the animal with 150 mg/kg bw, of alloxan in normal saline by intraperitoneal injection.
- Group III: Diabetes-induced animals were administrated with the aqueous extract of *A. sativum* (250 mg/kg bw) orally using intragastric tubes for 45 days.
- Group IV: Diabetes-induced animals were administrated with the aqueous extract of *A. sativum* (350 mg/kg bw).
- Group V: Diabetes-induced animals were administrated with the aqueous extract of *A. sativum* (450 mg/kg bw).

RESULTS AND DISCUSSION

The aqueous bulb extract of *A. sativum* was administrated to alloxan-induced diabetic animals (250, 350, and 450 mg/kg bw) orally. A separate group was maintained for each dose levels. In the aqueous bulb extract of *A. sativum* administrated animals, the

Table 1: Different plants used in antidiabetic activity[15]

Plant name	Part used
Aframomum melegueta	Leaves
Aloe barbadensis	Leaves (aqueous and ethanolic extract)
Azadirachta indica	Leaves (ethanolic extract)
Aegle marmelos	Leaves
Basella rubra	Leaves
Bougainvillea glabra	Leaves
Coccinia indica	Leaves (ethanolic extract)
Cassia occidentalis	Leaves
Ipomoia digitata	Root
Tectona grandis	Root (methanolic extract)
Pseudarthria viscida	Root (ethanolic extract)
Ginseng	Root (methanolic extract)
Anthocephalus indicus	Root (ethanolic extract)
Abrus precatorius	Seed (chloroform and methanol extract)
Eugenia jambolana	Seed (ethanolic extract)
Brassica juncea	Seed (aqueous extract)
Opuntia dillenii	Fruit
Phyllanthus emblica	Fruit (aqueous extract)
Blighia sapida	Fruit
Catharanthus roseus	Whole plant (dichloromethane: methanol extract)
Allium cepa	Whole plant (ethanolic extract)
Salacia oblonga	Whole plant
Phyllanthus niruri	Whole plant (methanolic extract)

Table 2: Effect of the treatment of alloxan-induced diabetic rats with aqueous plant extracts of folklore medicinal plants on blood glucose and insulin

Experimental groups	Blood glucose (mg/dl)	Insulin (µU/ml)
Normal	75±1.70	25.92±1.60
Alloxan-induced diabetic animal	374±5.16*	11.20±1.0*
A. sativum 250 mg/kg.bw	254±2.18*	20.89±1.2*
A. sativum 350 mg/kg.bw	250±2.64*	19.11±1.91*
A. sativum 450 mg/kg.bw	247±3.46*	18.60±2.15*

Values are mean of six individual observations in each group Mean±SD. "P" denotes statistical significance. *P denotes statistical significance of ANOVA to test the difference between the experimental groups. *A. sativum*: *Allium sativum*, bw: Body weight

increased levels of plasma glucose and lipid profile were decreased significantly when compared to the levels in control animals (Group-II) [Table 2].

The decrement of the blood glucose and lipid profile was dose dependent. The serum insulin levels showed a significant depletion in diabetic animals when compared to that of normal animals. On administration of the aqueous extract of the bulb of *A. sativum,* a significant augmentation of the serum insulin was recorded and was dose dependent [Table 3].

The level of urea, uric acid, and creatinine in control and experimental group of rats is presented in Table 4. In diabetic rats, all three tested renal markers urea, uric acid, and creatinine were significantly increased when compared to normal control Group I animals.

Administration of *A. sativum* extract reversed these changes to near normal levels such as urea, uric acid, and creatinine when comparison to the diabetic control group. The diabetic liver tissue of rat revealed depletion of antioxidant enzymes, namely SOD, CAT, GST, and GPx when compared to that of normal group. After treatment with 250, 350, and 450 mg/kg bw extract, increased activity levels of SOD, CAT, GST, and GPx when compared to that of normal animals were seen [Table 5].

Histological studies were also carried out in the control and experimental animals. The alloxan-induced control animals showed a significant shrinkage in the size of the islets of Langerhans and necrosis of β-cells population when compared to that of normal animals. After administration of plant extract in different dose levels, the damaged pancreatic cells revealed normal cell architecture [Figure 1].

Increased levels of serum insulin in diabetic animals

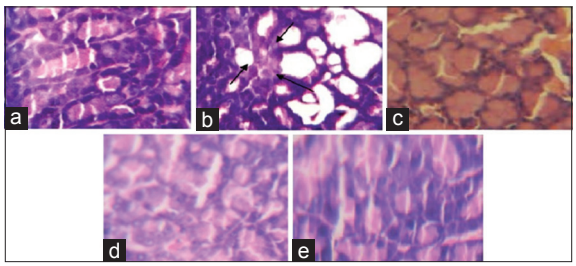

Figure 1: Pancreas of control and plant (*Allium sativum*) treated animal stained with hematoxylin and eosin (a) control (b) induced by alloxan (c) alloxan + 250 mg/kg bw, Cl treated (d) alloxan + 350 mg/kg bw, Cl treated (e) alloxan + 450 mg/kg bw, Cl treated

Table 3: Effect of the treatment of alloxan-induced diabetic rats with aqueous plant extracts of folklore medicinal plants on lipid profile

Experimental groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL	LDL	VLDL
Normal	72±1.3	85.6±2.46	31.1±2.11	32.0±2.16	17.12±2.13
Alloxan-induced diabetic animal	89±1.92*	102.2±2.96*	24.2±2.10*	40.4±2.92*	19.65±2.02*
A. sativum 250 mg/kg.bw	83.33±3.12*	93.00±2.76*	24.66±1.26*	36.48±2.58*	18.85±2.01*
A. sativum 350 mg/kg.bw	82.33±2.81*	95.05±2.72*	28.16±1.42*	35.16±2.06*	17.10±1.86*
A. sativum 450 mg/kg.bw	79.16±2.06*	88.1±2.63*	27.2±3.14*	30.06±2.18*	17.60±2.01*

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, *A. sativum*: *Allium sativum*, bw: Body weight. *represents statistically significant (P<0.001) results as compared to normal

Table 4: The effect of *A. sativum* aqueous bulb extract on urea, uric acid, and creatinine of control and alloxan-induced experimental diabetes in rats

Experimental group	Urea (mg %)	Uric acid (mg %)	Creatinine (mg %)
Normal	36.80±5.50	1.50±0.57	0.82±0.27
Alloxan-induced diabetic animal	63.50±7.53**	2.25±0.88**	2.01±0.73**
A. sativum 250 mg/kg.bw	57.80±6.28*	2.01±0.41*	1.85±0.26*
A. sativum 350 mg/kg.bw	45.38±7.88*	1.88±0.57*	1.55±0.57*
A. sativum 450 mg/kg.bw	40.83±5.80*	1.58±0.66*	1.01±0.22*

Values are mean of six individual observations in each group Mean±SD. *represents statistically significant (P<0.001) results as compared to normal. ** represents statistically significant (P<0.0001) results as compared to alloxan-induced diabetic animal. *A. sativum*: *Allium sativum*, bw: Body weight

Table 5: Diabetic rat-induced oxidative stress levels of SOD, CAT, GPx, and GST in the liver tissue of control and experimental animals

Experimental groups	SOD (mg/protein)	CAT (mg/protein)	GPx (mg/protein)	GSt (mg/protein)
Normal	5.55±0.25	25.76±1.02	4.57±0.11	4.12±0.10
Alloxan-induced diabetic animal	3.82±0.04*	18.11±0.85*	3.58±0.04*	3.45±0.10*
A. sativum 250 mg/kg.bw	4.20±0.11**	21.80±0.05**	4.12±0.05**	3.96±0.02**
A. sativum 350 mg/kg.bw	4.78±0.07**	22.10±1.06**	4.80±0.15**	3.95±0.08**
A. sativum 450 mg/kg.bw	5.03±0.13**	23.80±1.02**	4.94±0.01**	4.00±0.10**

SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxide, GST: Glutathione S-transferase, *A. sativum*: *Allium sativum*, bw: Body weight

treated with plant products are not an uncommon phenomenon as this effect has been reported with some other medicinal plants such as *Brassica juncea* and *Persea americana* (Thirumalai *et al*., 2011; Ezejiofor *et al*.). Administration of *A. sativum* extract at the dose of 450 mg showed a maximum decrease of urea, uric acid, and creatinine. The results of our study were similar to the previous findings of (Gondwe *et al*., 2008). The elevated levels of oxidative stress in diabetic animals are due to autoxidation of glucose, protein glycation, lipid peroxidation, and low activities of antioxidant enzymes (Giugliano *et al*., 1996).

CONCLUSION

The present study clearly indicates the antidiabetic potential of *A. sativum* aqueous extract on alloxan-induced diabetes in rats. In the biochemical parameters of glucose, insulin, and lipid profile, data very closely support the research work. The antioxidant parameters also very much supported the pharmacological potential of the plant extract. In future, we will use the plant extract and it is based on phytocompounds for the antidiabetic drug preparation.

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