International Journal of Pharma and Bio Sciences V1(2)2010

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *SAPINDUS TRIFOLIATUS* LINN.

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ABSTRACT

The treatment of diabetes mellitus has been confined to use of oral hypoglycemic agents and insulin, due to having serious side effects. This leads to increasing demand for herbal products with antidiabetic factor with less side effects. This manuscript describes the antihyperglycaemic activity, *in vivo* antioxidant potential, effect on glycosylation of hemoglobin and *in-vitro* peripheral utilisation of glucose of the ethanolic extract of the aerial parts of *Sapindus trifoliatus*. The extract produced significant decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycemic rats both in the single dose as well as multiple dose experiment at the tested dose level and is comparable with the standard drug glibenclamide. It was observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal. The extract prevented significant elevation of glycosylated hemoglobin *in vitro*, with IC_{50} value being 12.5 µg/ml that is comparable with the reference drug α-tocopherol. Administration of the extract and glibenclamide significantly decreased the levels of TBARS, increased the content of GSH and increased the activity of SOD and CAT in liver of diabetic rats. The extract increased peripheral glucose utilisation in the diaphragm of diabetic rats *in vitro*, which is comparable with the action of insulin. Thus, the extract might have insulin like activity and the antihyperglycemic effect of the extract might be due to an increase in peripheral glucose consumption as well as protection against oxidative damage in alloxan induced diabetes.

KEYWORDS

Sapindus trifoliatus, Diabetes, Antioxidant activity; Antihyperglycemic activity.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. A worldwide survey reported that diabetes mellitus is affecting nearly 10% of The population every year[1].The treatment of diabetes mellitus in clinical practice has been confined to use of oral hypoglycaemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects[2]. This leads to increasing demand for herbal products with antidiabetic factor with little side effects. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus[3]. *Sapindus trifoliatus* L. (Fam. Sapindaceae) is a big tree distributed throughout India in all parts. The plant is reported to show vasoconstrictor and anti-inflammatory activity[4]. It has been reported that the plant contains tetracyclic triterpenoid saponins, bacosides A and B, hersaponin, alkaloids viz. herpestine and brahmine and flavonoids [4,5]. In folklore practice, some of the tribes of Orissa, India use the decoction of the aerial parts of the plant for the treatment

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of diabetes mellitus. In the present paper we report the antihyperglycaemic activity, *in vivo* antioxidant potential, effect on glycosylation of hemoglobin and *in vitro* peripheral utilisation of glucose of the ethanolic extract of the aerial parts of *Sapindus trifoliatus* using standard laboratory procedures.

EXPERIMENTAL

Plant Material

The plant was identified by the taxonomists Dr P.K.Sahoo,Botany Department, Utkal University, Vanivihar. After authentification the ripen fruits were collected in bulk amount at Forest Park, Bhubaneswar, Orissa, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder.

Preparation of extract

The powdered plant material (400 g) was defatted with petroleum ether (60-80 °C) and then extracted with 1.5 litre of ethanol (95%) in a soxhlet apparatus. The solvent was removed under reduced pressure, which obtained a greenish-black sticky residue (yield: 11.6% w/w with respect to dried plant material). The dried extract was stored in a desiccator till further study.

Animals Used

Wistar albino rats of either sex, weighing 180-250 g supplied by M/s Jena farm, Cuttack, Orissa, India were used. The selected animals were housed in acrylic cages in standard environmental conditions (25-30 °C). They were allowed free access to standard dry pellet diet (Lipton India,Mumbai,) and water *ad libitum*. All experiments were carried out as per the guidelines of the Institutional Animal Ethical Committee UDPS,Utkal University.

Drugs and chemicals used

Bovine serum albumin (Sigma chemical St. Louis, MO, USA), thiobarbituric acid, nitro blue tetrazolium chloride (NBT), hemoglobin (S.D.Fine, Mumbai, India), trichloro acetic acid (Merck Ltd, Mumbai, India), 5,5'-dithio bis-2- nitrobenzoic acid (DTNB) (S.D.Fine, Mumbai, India)were used. All the solvents were of analytical grade and purchased from local market.

Screening for antidiabetic activity

The method of Joy and Kuttan was followed[6]. The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before and 72 h after alloxan injection. The animals were considered diabetic when the blood glucose level was raised beyond 300 mg/dl of blood. This condition was observed at the end of 72 h after alloxan injection.

Effect on oral glucose tolerance in rats

After overnight fasting, an initial blood sample was taken from the tip of the tail of each rat of different groups under mild ether anaesthesia. Without delay a glucose solution (2 g/kg) was administered by a gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration[7] All blood samples were taken for the estimation of the blood glucose. Estimation of blood glucose was carried out with the haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a Johnson & Johnson ONE TOUCH blood glucometer.

Single dose study

The animals were segregated into five groups of six rats in each. Group I and II rats were randomly selected from normal rats that received only distilled water and the extract (300 mg/kg, p.o.) respectively. Group III to Group V animals were selected from the alloxanised rats. Group III animals served as diabetic control. Group IV animals received glibenclamide (600 mg/kg) and group V was treated with the extract (300 mg/kg) in a similar manner. Blood samples were collected from the tip of tail of each rat under mild ether anesthesia at 0 h, 1 h, 2 h and 4 h after the administration of test samples and tested for glucose concentration as above.

Multidose study

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For multidose study, administration of test samples was continued for 10 days, once daily through oral route. Blood samples were collected from the tip of tail and the estimation of blood glucose was carried out as above on the 1, 3, 7 and 10 day of the drug administration. Body weights of all the animals were recorded just prior to 10th day for weight.

Determination of in vivo antioxidant activity

On the 10th day following study, the animals were deprived of food overnight and sacrificed by cervical dislocation. The livers were dissected out, washed in ice-cold saline, patted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris- HCl buffer and processed for estimation of lipid peroxidation by the method of Fraga *et al*. [8].

A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of glutathione by the method of Ellman *et al*. [9]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4 °C. The supernatant thus obtained was used for the estimation of SOD by the method described by Kakkar *et al*.[10] and CAT activity was measured by the method of Maehly *et al*.[11].

Determination of in vitro glycosylation of hemoglobin

The degree of glycosylation of hemoglobin *in-vitro* was measured colorimetrically as suggested by Fluckiger *et al*. [12]. Hemoglobin, 5gm/ml in 0.01 M phosphate buffer (pH 7.4) was incubated for 72h in presence of 2 g/100ml concentration of glucose in order to find out the best condition for hemoglobin glycosylation. The assay was performed by adding 1 ml of glucose solution, 1ml of hemoglobin solution and 1ml of gentamicin (20 mg/100ml) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature. The degree of glycosylation of hemoglobin in presence of different concentration of the extract and their absence was measured colorimetrically at 440 nm. α-tocopherol was used as standard.

Determination of peripheral consumption of glucose in vitro

The method of Chattopadhyay *et al*. was followed [13]. Peripheral glucose consumption was studied in rat diaphragm preparation from animals fasted for 36 h prior to the experiment. The animals were sacrificed by cervical dislocation and the diaphragms were quickly taken out; followed by dividing each diaphragm into four pieces. The pieces of diaphragms were incubated in the nutrient solution with constant oxygenation and shaking (90 cycles / min) at 37 °C for 90 min in accordance with the procedure. Glucose was added to a final concentration of 500 mg%. Each piece of diaphragm is incubated in 2.5 ml of glucose nutrient mixture.The results were expressed as glucose consumption per 10 mg of dry diaphragm (by subtracting glucose concentration after incubation from glucose concentration before incubation). The dry weight was determined after oven drying the diaphragm at 105 °C for 2 h.

Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnett's t-test. $P<0.05$ indicates significant difference between group means.

RESULTS AND DISCUSSION

Table 1 shows the blood glucose level of normal and experimental animals after oral administration of glucose (2 g/kg). Extract as well as standard drug treated animals showed more significant decrease in peak blood glucose level after 1 h. After 2 h, the extract treated animals tended to bring the values near normal. The results of Table 2 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycaemic rats in the single dose experiment at the tested dose level and is comparable with the standard drug glibenclamide. In the multi dose study (Table-3), the test extract constantly maintained significant reduction of the glucose level in diabetic rats throughout the experimental period suggesting the antihyperglycaemic property of the extract. Diabetes

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mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins[14]. In the present study, it was observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal. During diabetes the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated hemoglobin also decreases[15].Hence the estimation of glycosylation of hemoglobin is a well established parameter useful in the management and prognosis of the disease[16]

Table 1. Effect of ethanolic extract of *Sapindus trifoliatus* aerial parts (300 mg/kg, p.o.) on oral glucose tolerance test (OGTT) in normal and alloxan induced diabetic rats.

Groups	Treatment	Blood sugar level (mg/dl)				
		Fasting	30 min	60 min	90 min	120 min
I	Normal	75.00±0.77	149.83±2.32	176.83±2.09	125.17±2.83	80.16±1.83
II	Normal+Extract	74.83±0.79^{NS}	146.50±1.78^{NS}	181.17±2.39^{NS}	124.17±3.78^{NS}	85.67±3.18^{NS}
III	Diabetic control (Alloxan only)	250.33±3.10*	322.33±4.16*	374.17±5.16*	319.33±3.29*	317.83 ±2.67'
IV	Diabetic+Extract	77.50±1.50*	141.83±2.91*	176.17±3.52*	127.50±2.80*	87.50 ±1.43'
V	Diabetic+Glibenclamide	76.50±2.02*	151.56±3.45*	185.33±2.53*	126.83±2.46*	92.50±1.50*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with group I while Group IV and V are compared with group III.

Table 2. Effect of single dose treatment of ethanolic extract of *Sapindus trifoliatus* aerial parts (300 mg/kg, p.o.) on blood glucose level in normal and alloxan induced diabetic rats.

Group	Treatment	Blood glucose level (mg/dl)			
		Basal value	1 h	2 h	4 h
I	Normal	76.33±0.71	76.17±0.65	75.83±0.95	76.17±0.79
II	Normal+Extract	76.17±0.87^{NS}	75.83±0.70^{NS}	75.17±0.75^{NS}	74.33 ± 0.71^{NS}
III	Diabetic control (Alloxan only)	349.67±2.95*	350.17±2.71*	349.83±2.62*	350.17± 2.79*
IV	Diabetic+Glibenclamide	343.17±5.12^{NS}	319.50±5.35*	298.83±3.91*	284.83 ± 3.65*
V	Diabetic+Extract	338.50±3.19^{NS}	289.33±4.89*	266.83±3.37*	246.83±3.20*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with group I while Group IV and V are compared with group III.

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Table 3. Effect of multiple dose treatment of ethanolic extract of *Sapindus trifoliatus* aerial parts (300 mg/kg, p.o., once daily) on blood glucose level and change in body weight after 15 days in normal and alloxan induced diabetic rats.

Group	Treatment	Blood glucose level (mg/dl)					Change in body weight (g)
		Basal value	Day 1	Day 3	Day 7	Day 10	
I	Normal	76.33 ± 0.71	76.17 ± 0.48	75.83 ± 0.40	76.17 ± 0.65	76.50 ± 0.56	(+) 9.83 ± 1.47
II	Normal + Extract	76.17 ± 0.87^{NS}	75.00 ± 0.73^{NS}	75.17 ± 0.54^{NS}	74.83 ± 0.60^{NS}	73.33 ± 0.67*	(+) 10.00 ± 0.97^{NS}
III	Diabetic control (Alloxan only)	349.67 ± 2.95*	356.83 ± 2.83*	353.83 ± 3.39*	354.33 ± 3.90*	354.17 ± 3.83*	(-) 8.83 ± 0.87*
IV	Diabetic + Glibenclamide	343.17 ±5.12^{NS}	264.33 ± 4.07*	235.83 ± 3.57*	219.33 ± 4.28*	205.33 ± 3.65*	(+) 8.83 ± 0.98*
V	Diabetic + Extract	338.50 ± 3.19^{NS}	219.67 ± 3.58*	209.33 ± 3.96*	200.33 ± 2.68*	186.50 ± 1.80*	(+) 9.16 ± 1.08*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with Group I while Group IV and V are compared with Group III.

Table 4. Effect of ethanolic extract of *Sapindus trifoliatus* on percent inhibition of hemoglobin glycosylation *in vitro*.

Group	Treatment	Blood glucose level (mg/dl)			
		Basal value	1 h	2 h	4 h
I	Normal	76.33 ± 0.71	76.17 ± 0.65	75.83 ± 0.95	76.17 ± 0.79
II	Normal + Extract	76.17 ± 0.87^{NS}	75.83 ± 0.70^{NS}	75.17 ± 0.75^{NS}	74.33 ± 0.71^{NS}
III	Diabetic control (Alloxan only)	349.67 ± 2.95*	350.17 ± 2.71*	349.83 ± 2.62*	350.17 ± 2.79*
IV	Diabetic + Glibenclamide	343.17 ± 5.12^{NS}	319.50 ± 5.35*	298.83 ± 3.91*	284.83 ± 3.65*
V	Diabetic + Extract	338.50 ± 3.19^{NS}	289.33 ± 4.89*	266.83 ± 3.37*	246.83 ± 3.20*

Values are Mean ± S.D. for n=3; r = regression co-efficient.

Our study gave a clear view that the ethanolic extract prevented significant elevation of glycosylated hemoglobin *in vitro*, with IC_{50} value being 12.5 µg/ml that is comparable with the reference drug α- tocopherol (Table 4). Further, since the non-enzymatic glycosylation of hemoglobin is an oxidative reaction[17], an antioxidant is expected to inhibit the reaction. The extract is thus expected to possess antioxidant activity.Lipid peroixidation is one of the characteristic features of chronic diabetes. Alloxan gives rise to dialuric acid, which undergoes oxidation and oxidative stress[21]. GSH also functions as free radical scavenger in the repair of radical caused biological damage[22,18] increased the content of GSH in liver of diabetic.Dialuric acid has been observed to stimulate lipid peroxidation *in vitro*. In this context, a marked increase in the concentration of TBARS was observed in liver of diabetic rats. Increased lipid peroxide concentration in the liver of diabetic animals has already been reported[19]. Administration of the exract and glibenclamide significantly decreased the levels of TBARS in diabetic rats (Table 5).

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Table 5. Changes in the levels of TBARS and Reduced glutathione in liver of normal and diabetic rats.

Group	Treatment	TBARS[a]	Reduced glutathione[b]
I	Normal	2.32 ± 0.06	58.07 ± 1.27
II	Normal + Extract	2.07 ± 0.08[NS]	54.49 ± 1.39[NS]
III	Diabetic	5.27 ± 0.10*	27.30 ± 1.57*
IV	Diabetic + Extract	3.53 ± 0.11*	52.28 ± 0.88*
V	Diabetic + Glibenclamide		4.20 ± 0.17*
	48.47 ± 1.07*		

a=nmole of MDA/mg of protein; b = µg/ mg of protein; values are Mean ± S.D. for n=6; Group II and III are compared with group I while Group IV and V are compared with group III. *P< 0.05 = significant; NS =not significant.

Glutathione (GSH), a tripeptide present in all the cells is an important antioxidant[20]. Decreased glutathione levels in diabetes have been considered to be an indicator of increased rats (Table 5). The cellular radical scavenging systems include the enzymes such as superoxide dismutase(SOD), which scavenges the superoxide ions by catalysing its dismutation and catalase (CAT), a haeme enzyme which removes hydrogen peroxide[23]. Therefore, reduction in the activity of these enzymes (SOD, CAT) results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. Administration of ethanolic extract and glibenclamide increased the activity of SOD and catalase in diabetic rats .

Table 6. Changes in the activity of Catalase and Superoxide dismutase in liver of normal and diabetic rats.

Groups	Treatment	Catalase[c]	Superoxide dismutase[d]
I	Normal	68.71 ± 4.72	5.93 ± 0.70
II	Normal + Extract	66.52 ± 4.85[NS]	6.25 ± 0.79[NS]
III	Diabetic	40.78 ± 2.4*	4.22 ±
0.55* IV	Diabetic + Extract	52.26 ± 3.51*	5.74 ±
0.57* V	Diabetic + Glibenclamide	48.65 ± 4.85*	5.31 ±
0.57*			

c = µ mole of H_2O_2 consumed/ min / mg of protein; d = Units/ mg of protein; One unit of activity means enzyme reaction responsible for 50% inhibition of NBT per min. Values are Mean ± S.D. for n=6; Group II and III are compared with group I while Group IV and V are compared with group III. *P < 0.05 = significant; NS = not significant

Alloxan has been found to induce free radical generation and cause tissue injury. Since the extract showed *in vivo* antioxidative activity in normal and diabetic rats, improvement of the liver tissues and the subsequent increase in the uptake and utilisation of blood glucose might be the mechanism of action of this extract as antidiabetic agent. Alloxan causes irreversible destruction of pancreas β-cells. Thus, the antihyperglycemic activity might be due to extra pancreatic mechanism. Hence, the effect of ethanolic extract of *Sapindus trifoliatus* aerial parts on peripheral consumption of glucose was investigated. The result suggests that the extract produces an antidiabetic action mediated by an increase in peripheral glucose consumption in the rat diaphragm of diabetic rats, especially at a

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concentration of 600 µg/ml (Table-7). Insulin increased the peripheral glucose consumption in normal and diabetic rats. Thus, the extract might have an insulin like activity and the antihyperglycemic effect of the extract might be due to an increase in peripheral glucose consumption.

Table 7. Effect of ethanolic extract of *Sapindus trifoliatus* on *in vitro* peripheral glucose consumption in diaphragm of normal and diabetic rats.

	Glucose consumption (mg/10 mg of diaphragm dry weight)			
	Control	Ethanolic extract (µg/ml)		Insulin
		300 (µg/ml)	600 (µg/ml)	5 U/ml
Normal rats	0.47 ± 0.03	0.59 ± 0.05^{NS}	0.68 ± 0.05*	0.82 ± 0.06*
Diabetic rats	0.49 ± 0.03	0.64 ± 0.04*	0.78 ± 0.06*	0.90 ± 0.06*

Values were expressed as Mean ± SEM for n=6. *P<0.05 = Significant; NS = not significant when compared to control

The ethanolic extract of *S. trifoliatus* is reported to be rich in saponins. Saponins are reported to possess antidiabetic and antioxidant activity. Presence of saponins in the ethanolic extract was confirmed through our preliminary phytochemical screening also. Thus, the saponins in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in alloxanised diabetes.

ACKNOWLEDGEMENT

The authors are thankful to H.O.D,UDPS,UU for allowing us to carry out the research work successfully and his timely assistance helped us to complete the work within the stipulated time.

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