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Antidiabetic and antioxidant activity of *Shorea tumbergia* Rox.

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ABSTRACT

To consider potentially new antihyperglycemic, hypolipidemic and antioxidant activity sources, ethanolic extracts from plant, *Shorea tumbergia* Rox belongs to the family Dipterocarpaceae, was investigated in alloxan-induced diabetic rats. To induce diabetes, alloxan was administered intraperitoneal (150mg/kg.i.p.b.w.). Effect of extract from plant on blood glucose levels of diabetic rats was determined at various time intervals. The biochemical parameters studied were serum glucose, triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL), insulin, Glycosylated hemoglobin (HbA_{1c}) levels. All these effects were compared with glibenclamide as a reference antidiabetic drug. In order to determine antioxidant activity of extract, liver tissue were homogenized in ice cold saline buffer and the assay of thiobarbituric acid reactive substance (TBARS), Superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) were performed for *in-vivo* activity. For *in-vitro* antioxidant activity DPPH scavenging method, reducing power method and hydrogen peroxide method are evaluated. Oral administration of *S.tumbergia* for 28 days resulted in a significant reduction in blood glucose levels, glycosylated hemoglobin and increased insulin levels. The lipid metabolites were significantly altered near to normal. And there is significant improvement in TBARS, SOD, GSH and catalase in liver tissues of alloxan induced diabetic rats when compared with untreated diabetic rats. For *in-vitro* activity the increase in extract concentration increase the absorbance at various wavelengths. IC₅₀ values are very much comparable to the standard drug ascorbic acid. *S.tumbergia* showed significant antihyperglycemic, hypolipidemic and antioxidant effects in alloxan induced diabetic rats.

Keywords: *Shorea tumbergia*, Antidiabetic, Antioxidant, Lipid profile.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. It is most common of the serious metabolic disease. The most common form of diabetes is diabetes mellitus, a chronic progressive, systemic condition of impaired carbohydrate metabolism. Its major manifestations include disorder metabolism and inappropriate hyperglycemia. In diabetes, oxidative stress has been found to be mainly due to an increased production oxygen free radicals and sharp reduction of antioxidant defense.

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. (Wild *et al.*, 2004). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. In spite of the presence of known

antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al.*, 2002). Treatment imposes economical burden (Ali *et al.*, 2008) and the documented incidence is quite ambiguous.

S.tumbergia is an endemic and globally endangered semi-evergreen tree species restricted to the southern Eastern Ghats upto 1000m, distributed in Seshachalam and veligonda hills in Cuddaph, Tirupati hills in Chittoor district, Andhra Pradesh. North Arcot and Chengalpattu district, Tamilnadu (Solomon Raju *et al.*, 2009). It is more prevalent at drier areas in non-teak mixed deciduous forest vegetation at an altitude of 300m amsl (Sandhya rani *et al.*, 2002). The tree trunk is used as flagpoles for temples. The stem is source of resin, which is used as incense and as a substitute in marine yards for pitch. It is also used in indigenous medicine as an external stimulant and a substitute for abietis; resina and Pix Burgundica of European Pharmacopoeias (Watt 1889). The plant extracts are used as a cure for ear-aches and leaf juice is used as ear drops for children. (Rao, 1998). Methanolic extract of leaves of this plant reported

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to have antinociceptive and anti-inflammatory activity (Jyothi *et al.*, 2008). Stem bark is reported to have antiulcer activity (Kenia *et al.*, 2004).

Materials and methods

Phytochemical screening

The alcoholic extract was screened for various phytoconstituents like steroids, alkaloids, tannins, flavonoids and glycosides by employing standard phytochemical tests (Trease *et al.*, 1983).

Plant material and extraction

S.tumbuggaia Rox. leaves are freshly collected during the month of August-September from foothills of Tirumala hills, Chittor. The Plant material was authenticated by Dr. Madavashetty, Department of Botany, S.V.University, Tirupathi. Voucher specimen was at Vaagdevi College of Pharmacy, Warangal. The leaves are shade dried, powdered and material was extracted with ethanol 1:1 v/v (0.5kg in 2 liter 95%ethanol) for 4hours reflex under heating water bath. The extract was dried under reduced pressure using a rotary vacuum evaporator. The % yield was 8.7%w/w and the extract was kept in refrigerator for further use.

Drugs and chemicals

Alloxan was purchased from Sigma Chemicals, USA. Standard drug glibenclamide was procured from Cipla Ltd. Diagnostic kits used in this purchased from Span Diagnostics Ltd. India. All the other chemicals used were of analytical grade.

Animals

Wistar albino rats weighting 150-200g were procured from Mahaveer Enterprises, Hyderabad. (CPCSEA Regd.No:146/ 1999/ CPCSEA). They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animal experiments were designed and conducted in accordance with the guidelines of Institutional animal ethical committee (IAEC-VCOP, Warangal).

Acute toxicity studies

The acute oral toxicity study of the plant extract was carried out in adult Swiss albino mice of both sexes. This method was carried out according to OECD guidelines by adopting fixed dose method (Ecobichon, 1997). Four animals per treatment group and different dose range 5, 50, 300, 2000 mg/kg respectively, the animals were observed continuously for any change in autonomic or behavioral response for first 2 hours, then intermittently and at the end of 24 hours, the mortality was recorded.

Oral glucose tolerance test (OGTT)

Rats were fasted overnight and divided into five groups with 6 animals in each group. Group-I received distilled water, served as control. Group-II animals were treated with glibenclamide (0.5mg/kg p.o.) to serve as standard. Group-III to Group animals was treated with EDH in three different extract doses (100mg/kg,

200mg/kg, 400mg/kg and 800mg/kg b.w.). The groups control, standard and test were treated with drugs 30 minutes prior to the glucose load (2g/kg p.o.). Blood samples were collected at 30,60,90 and 120min after glucose loading, by retro orbital sinus and glucose levels were measured immediately after separation of serum (Leng *et al.*, 2004).

Experimental induction of diabetes in rats

Alloxan monohydrate was used to induce diabetes. Animals were allowed to fast for 16hr and were injected intraperitoneally (i.p.) with freshly prepared Alloxan monohydrate in normal saline in a dose of 150mg/kg. Rats with blood glucose level above 200mg/dl were considered to be diabetic and were used in this experiment.

Experimental design

After induction of diabetic the rats were divided into seven groups of six animals each.

Group 1: Normal rats.

Group 2: Diabetic rats treated with the vehicle solution (2% gum acacia)

Group 3: Diabetic rats treated with glibenclamide (5mg/kg).

Group 4: Diabetic rats given ethanolic extract of *S.tumbuggaia* (100mg/kg).

Group 5: Diabetic rats given ethanolic extract of *S.tumbuggaia* (200mg/kg).

Group 6: Diabetic rats given ethanolic extract of *S.tumbuggaia* (400mg/kg).

Group 7: Diabetic rats given ethanolic extract of *S.tumbuggaia* (800mg/kg).

The drugs and vehicle were administered orally by an intragastric tube daily for 28 days.

The body weight was calculated on 7, 14, 21 and 28days and blood was withdrawn from the retro orbital venous puncture under ether anesthesia using a glass capillary tube from 12 hours fasted rats. The blood sample were used for the biochemical analysis of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL).

Bio-chemical analysis

The blood samples were centrifuged at 5000rpm for 20 min and serum was separated and stored at -20°C until analysis was done. Samples were analyzed spectrophotometrically for blood glucose by GOD-POD method (Kaplan *et al.*, 1984), using commercial kit (Span diagnostics, India). TG (Kaplan and Lavernal, 1983) was estimated by GPO-POD method, TC (Herbert, 1984) was estimated by CHOD-PAP method, HDL was analyzed by kits (Roche diagnostics, Germany) LDL and VLDL-cholesterol using Friedevald's equation (Roche diagnostics, Germany). Glycosolated hemoglobin by Gould *et al.*, 1982 and serum insulin was estimated by ACS: 180 automated chemiluminescence system. On 28th day serum glycosylated hemoglobin and insulin

estimations were carried out at Vijaya diagnostic center, Hanamkonda, A.P.

In vitro antioxidant studies

Estimation of DPPH scavenging activity

The hydrogen atom of electron donating abilities of the resultant compounds was measured from the bleaching of the purple-colored ethanol-solution of DPPH. This Spectrophotometric assay uses the stable radical DPPH as a reagent (Molyneux, 2004). 0.1mM solution of DPPH in ethanol was prepared and 0.1ml of this solution was added to 3ml of different concentration of the extract (5, 10, 20, 40, 60, 80 and 100µg/ml). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the extract was expressed as IC₅₀. The IC₅₀ value was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

Estimation by reducing power method

The reducing power of the compound was evaluated according to Oyaizu *et al* (1986). Different concentration of the extract (5-100µg/ml) were dissolved in distilled water and added with 2.5ml of 0.2M phosphate buffer (pH6.6), and 2.5ml of 1% of K₃Fe(CN)₆. The mixture was incubated at 50^oc for 20 min. 2.5ml of 10% TCA (Trichloro acetic acid) was added to the blend and centrifuged at 3000rpm for 10min. The upper layer of the solution (2.5ml) was assorted with distilled water (2.5ml) and FeCl₃ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated reducing power.

Estimation by hydroxyl radical scavenging activity

The scavenging ability for hydroxyl radical was measured according to the modified method of Halliwell *et al* (1987). Stock solution of EDTA (1mM), FeCl₃ (10mM), ascorbic acid (1mM), H₂O₂ (10mM) and deoxyribose (10mM) were prepared in distilled de-ionized water. The assay was performed by adding 0.1ml EDTA, 0.01ml of FeCl₃, 0.1ml of H₂O₂, 0.36ml of deoxyribose, 1ml of different concentrations of extract (5-100µg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50mM,pH 7.4) and 0.1ml of ascorbic acid added in sequence. The mixture was then incubated at 37^oC for 1hr. A 1.0 ml of incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% thio-barbituric Acid (TBA) in 0.025M NaOH containing 0.025% BHA (Butylated hydroxyl anisole) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % incubation of deoxy ribose degradation.

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

In vivo antioxidant studies

Preparation of liver post mitochondrial supernatant (Liver-PMS)

At the end of the study, animals were decapitated and cut open to excise the liver. The excised livers immediately and thoroughly washed with ice-cold physiological saline. The tissue of 100mg was homogenized in 1ml of 0.1M cold tris-HCl buffer (pH7.4) in a potter-Elvehjam homogenizer fitted with a Teflon plunger at 600rpm for 30 min. (Folch *et al.*, 1957). The homogenate was centrifuged at 10,000g for 20 min at 4^oc and the supernatant with firmly packed pellets were resuspended by homogenization in 100mM Tri-Hcl buffer containing 20%w/v glycerol and 0.1ml of 10mM EDTA, pH 7.4 (Naveen and Terkey *et al*, 2005). The post mitochondrial supernatant was used to assay TBARS, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity.

Estimation of lipid peroxidation (LPO) from liver PMS

LPO was induced and assayed in rat hepatic-PMS (Wright *et al.*, 1981). In 1ml of the reaction muddle, 0.58ml phosphate buffer (0.1 M, pH 7.4), 0.2ml of hepatic PMS (10%w/v), 0.2 ml ascorbic acid (100mM) and 0.02 ml ferric chloride (100mM) and was incubated at 37^oc in a shaking water bath for 1h. The reaction was clogged by the addition of 1ml TCA (10%, w/v), subsequently 1ml TBA (0.67%w/v) was added and all the tubes were kept in a boiling water bath for 20 min. The tubes were shifted to ice-bath and centrifuged at 2500×g for 10 min. The amount of malondialdehyde (MDA) formed in each of samples was assessed by measuring the optical density of the supernatant at 535nm allied with reagent blank without tissue homogenate. The molar extinction coefficient for MDA was taken to be 1.56×10⁵M⁻¹cm⁻¹.

Calculation = 3×absorbance of sample/50.156× (mg of tissue taken) = µM /mg tissue.

Estimation of Reduced glutathione (GSH) from liver PMS

Glutathione was assayed by the method of Jollow *et al.*, 1974. An aliquot of 1ml of hepatic PMS (10%w/v) was mixed with 1ml of sulphosalicylic acid (4%w/v) and centrifuged at 1200g for 5 min and filtered. From the filtrate, 0.1ml filtered aliquot, 2.7ml phosphate buffer (0.1M, pH 7.4) and 0.2ml DTNB (40mg/10ml of phosphate buffer 0.1M, pH7.4) in a total volume of 3.0ml. The yellow color developed was comprehended at 412 nm on a spectrophotometer.

Estimation of Super oxide dismutase (SOD) from liver PMS

Super oxide dismutase activity was estimated by Fridovich *et al.*, 1971 method. The reaction mixture consisted of 0.5ml of hepatic PMS, 1ml 50mM sodium carbonate, 0.4ml of 25µM NBT (Nitro blue tetrazolium) and 0.2ml, 0.1mM EDTA. The reaction was initiated by addition of 0.4ml of 1mM hydroxylamine-hydrochloride.

The change in absorbance was recorded at 560nm. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required inhibiting the reduction of NBT by 50%.

Estimation of catalase (CAT) from liver PMS

CAT activity was assayed by the Claiborne *et al.*, 1985. The assay mixture consisted of 1.95ml phosphate buffer (0.05M, pH 7), 1ml H₂O₂ (0.019M), 0.05ml of hepatic PMS (10%w/v). Changes in absorbance were recorded at 240nm for 2min with 60 seconds interval using a spectrophotometer (Model 106).

Statistical analysis

Data for various parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Tukey-Kramer test (Graph Pad Version 3.06, La Jolla, CA, USA). Values were considered statistically significant when at $P < 0.05$.

RESULTS

Phytochemical screening

Screening of the ethanolic extract of *S.tumbuggaia* leaves revealed the presence of alkaloids, triterpenes, phenols, anthraquinones and cardiac glycosides.

Acute toxicity studies

In the acute toxicity study even with the dose tested 2mg/kg no mortality was observed during the 24 hours period and the animals showed no stereotypical

symptoms associated with toxicity such as convulsions, ataxia and diarrhoea or increased diuresis.

Oral glucose tolerance test

In oral glucose tolerance test with *S.tumbuggaia* leaf extracts an increase in blood glucose levels at 30 min followed by decrease in blood glucose levels from 60 minutes onwards was observed at various doses. Whereas at 400mg/kg and 800mg/kg doses of ethanolic extract causes significant ($p < 0.001$) decrease in glucose levels (Table.1).

Effect on body weight

All diabetic animals gain the body weight in 28 days experimental period. Whereas ethanolic extract of leaves of *S.tumbuggaia* treatment causes restoring the body weight within 21 days and maintained to normal upto 28 days (Table.5).

Effect on blood glucose levels in alloxan induced diabetic rats

Dose dependent hypoglycemic effects were however observed in the diabetic rats. The effect of alcoholic extract of the leaves of the *S.tumbuggaia* on serum glucose levels were shown in Table . Treatment for one 28days showed significant ($p < 0.05$) reduction of blood glucose with alcoholic extract of *S.tumbuggaia* at higher doses (400 and 800mg/kg) and glibenclamide respectively when compared with the values of untreated diabetic control animals (Table.2).

Table.1 Effect of alcoholic extract of *S.tumbuggaia* leaves on Oral Glucose tolerance in normal fasted rats (OGTT)

Group	Dose (b.w.) mg/kg	Blood glucose (mg/dl)(Mean \pm SEM)				
		Time(min) after glucose administration				
		0	30	60	90	120
Control(I)	-----	87.5 \pm 3.5	154.6 \pm 28.4	159.6 \pm 7.8	125.6 \pm 9.7	118.4 \pm 11.8
Glibenclamide(II)	5	86.6 \pm 3.6	126.5 \pm 19.5	107.6 \pm 10.5a	95.6 \pm 11.4a	75.4 \pm 3.6a
<i>S.tumbuggaia</i> (III)	100	88.23 \pm 1.6	128.34 \pm 4.5	132.4 \pm 3.6a	112.45 \pm 4.3	92.34 \pm 5.6a
<i>S.tumbuggaia</i> (IV)	200	87.36 \pm 2.4	132.45 \pm 4.2	104.5 \pm 5.6a	95.4 \pm 5.6a	72.86 \pm 5.7a
<i>S.tumbuggaia</i> (V)	400	84.3 \pm 4.8	129.4 \pm 5.8	104.76 \pm 4.8a	93.4 \pm 6.7a	76.5 \pm 3.6
<i>S.tumbuggaia</i> (VI)	800	83.4 \pm 3.6	132.4 \pm 6.7	107.5 \pm 5.6a	92.4 \pm 5.8a	72.3 \pm 5.7a

a= $p < 0.001$, when compared to control group, n=4

Table.2 Effect of alcoholic extract of *S.tumbuggaia* leaves on blood glucose levels

Group	Dose (mg/kg)b.w.	Blood glucose(mg/dl)(Mean \pm SD)				
		0 th day	7 th day	14 th day	21 st day	28 th day
Normal (I)	-----	86.7 \pm 3.12	88.3 \pm 4.5	87.4 \pm 3	87.3 \pm 4.3	88.4 \pm 5.6
Diabetic control(II)	----	210.34 \pm 4.2	217.8 \pm 8.4	234.8 \pm 9.4	248.9 \pm 12.3	269.9 \pm 19.3
Glibenclamide(III)	5	210.9 \pm 15.5	165.03 \pm 14.5a	109.83 \pm 4.47a	94.9 \pm 15.4a	84.9 \pm 16.8a
<i>S.tumbuggaia</i> (IV)	100	221.3 \pm 6.8	187.5 \pm 12.3a	156.56 \pm 5.6a	134.5 \pm 6.5a	102.45 \pm 5.6a
<i>S.tumbuggaia</i> (V)	200	223.4 \pm 7.9	176.45 \pm 4.9a	127.8 \pm 7.8a	98.4 \pm 5.8a	79.4 \pm 6.7a
<i>S.tumbuggaia</i> (VI)	400	256.4 \pm 5.8	201.45 \pm 12.3a	174.5 \pm 6.8a	125.6 \pm 23.3a	98.34 \pm 5.8a
<i>S.tumbuggaia</i> (VII)	800	237.8 \pm 7.8	203.5 \pm 14.3a	187.6 \pm 5.8a	123.5 \pm 20.4a	86.94 \pm 2.9a

a= $p < 0.001$, when compared to diabetic control group, N=6

Table.3 Effect of alcoholic extract of *S.tumbugaia* leaves on serum insulin and glycosylated haemoglobin

Group	Dose mg/kg(b.w.)	On 28 th day (Mean±SEM)	
		Insulin(μIU/ml)	Glycosylated haemoglobin (%)
Normal (I)	-----	11.5±2.6	4.05 ±0.403
Diabetic control(II)	----	10.7±1.7	12.29 ±1.210
Glibenclamide(III)	5	9.7±2.43***	3.95 ±0.621
<i>S.tumbugaia</i> (IV)	100	9.87±1.8	5.47±3.4
<i>S.tumbugaia</i> (V)	200	9.90±2.5	4.69±4.5*
<i>S.tumbugaia</i> (VI)	400	11.02±4.5	4.0800±3.9*
<i>S.tumbugaia</i> (VII)	800	10.9±4.5*	3.9700±4.1*

N=4; *p<0.05, when compared to diabetic rats

Table.4 Effect of alcoholic extract of *S. tumbugaia* on various lipid parameters

Groups	Treatment in days											
	HDL(mg/dl)				LDL(mg/dl)				VLDL(mg/dl)			
	7	14	21	28	7	14	21	28	7	14	21	28
Normal (I)	36.52 ±2.3	33.07 ±6.4	34.84 ±4.41	35.43 ±5.5	11.76 ±1.3	10.12 ±2.4	11.42 ±3.1	12.43 ±4.32	8.13 ±2.1	7.93 ±2.3	7.98 ±2.1	7.35 ±9.1
Diabetic (II)	29.19 ±1.34	16.24 ±1.02	13.91 ±2.2	10.46 ±2.33	156 ±16.8	214.36 ±32.4	226.59 ±20.9	232.92 ±22.1	24.09 ±3.2	25.12 ±4.5	27.9 ±2.8	29.8 ±3.5
Glibenclamide (5mg/kg) (III)	24.85 ±1.24a	35.42 ±2.43c	38.55 ±2.64c	41.38 ±3.4a	105.38 ±2.9a	45.96 ±3.1a	17.61 ±3.5a	3.04 ±2.01a	13.2 ±5.43a	11.1 ±0.98a	12.3 ±1.34a	9.8 ±2.34a
<i>S.tumbugaia</i> (100mg/kg) (IV)	25.17 ±1.86a	31.79 ±2.12	38.16 ±3.34c	38.5 ±5.5a	52.85 ±4.2a	33.53 ±9.7a	13.75 ±6.2a	5.20 ±2.1a	20.4 ±2.43a	14.1 ±2.53a	12.4 ±2.04a	8.54 ±2.1a
<i>S.tumbugaia</i> (200mg/kg) (V)	26.15 ±2.34a	31.98 ±3.45	37.54 ±4.45c	39.2 ±5.54a	115.97 ±4.5a	66.03 ±9.2a	35.64 ±4.6a	12.3 ±5.2a	17.3 ±4.23	14.5 ±0.98a	10.9 ±0.98a	7.9 ±0.98a
<i>S.tumbugaia</i> (400mg/kg) (VI)	25.18 ±4.5a	33.18 ±4.2c	39.54 ±5.34c	40.43 ±5.67a	99.14 ±4.33a	49.34 ±5.2a	23.04 ±4.3a	5.74 ±2.4a	19.1 ±0.23	15.9 ±2.3a	10.9 ±0.83a	8.23 ±0.9a
<i>S.tumbugaia</i> (800mg/kg) (VII)	22.13 ±2.3a	31.24 ±2.5b	34.56 ±3.45c	42.35 ±6.78c	102.3 ±4.55a	52.3 ±5.6a	33.6 ±3.56b	2.45 ±3.8a	18.4 ±0.45	12.3 ±3.4a	12.3 ±6.2a	7.89 ±0.56b

Values are mean ± SEM. (n=6).

a=p<0.001 when compared to diabetic rats, b=p<0.05 when compared to normal control rats. c= p<0.05 when compared to diabetic rats.

Effect on Serum insulin and glycosylated haemoglobin

Diabetic rats recorded significant decrement in hemoglobin content and increase in glycosylated haemoglobin. Low doses of alcoholic extract (100mg/kg and 200mg/kg) were not able to induce significant changes in levels of glycosylated haemoglobin. Higher dose of extract (400mg/kg and 800mg/kg) have a significant (p<0.05) decrement in glycosylated haemoglobin contents. These results were comparable to glibenclamide treated rats. Insulin titer in the diabetic animals was significantly lower, while there was significant improvement (p<0.05) in EST 800mg/kg treated rats (Table 3).

Effect on Serum lipid profile

Serum triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels were found to be increased where as HDL-cholesterol levels were found to be decreased in alloxan treated diabetic rats. Treatment with ethanolic extract of *S.tumbugaia* produced a significant (p<0.05) reduction in elevated TG, TC, LDL, VLDL within 21 days in diabetic rats. There was significant (p<0.05) increase in HDL levels within 28 days (Table 4& 5).

Table.5 Effect of alcoholic extract of *S.tumbugaia* on different lipid parameters

Groups	Treatment in days											
	Serum triglyceride (mg/dl)				Serum total Cholesterol(mg/dl)				Body weight (g)			
	7	14	21	28	7	14	21	28	7	14	21	28
Normal (I)	35.88 ±3.8	37.42 ±3.4	37.08 ±5.4	38.4 ±4.9	56.41 ±4.32	56.12 ±5.9	54.21 ±6.6	55.21 ±4.4	180.23 ±2.3	184.5 ±1.3	193.3 ±2.1	185.5 ±2.3
Diabetic control (II)	121.4 ±10.7	124.99 ±12.4	138.09 ±14.2	142.37 ±14.2	207.96 ±12.5	255.72 ±14.3	268.4 ±15.6	272.46 ±13.9	142 ±2.5	140.1 ±3.7	138.3 ±2.2	135 ±5.2
Glibenclamide (III)	60.9 ±4.4a	52.3 ±4.3a	50.4 ±3.2a	45.8 ±4.2a	143.43 ±11.2a	92.46 ±3.34a	68.46 ±4.3a	54.22 ±4.5a	140.4 ±4.1d	145 ±3.2d	151.3 ±3.5d	155.8 ±4.1d
<i>S.tumbugaia</i> (100mg/kg) (IV)	102.8 ±9.2	88.4 ±11.2c	64.3 ±10.3a	42.8 ±5.3a	98.4 ±3.5a	79.42 ±4.32a	64.3 ±5.3a	53.24 ±11.2a	134.6 ±2.8d	138.8 ±6.4d	142.9 ±5.2d	155.9 ±3.7d
<i>S.tumbugaia</i> (200mg/kg) (V)	85.2 ±9.8a	71.08 ±4.3a	53.4 ±3.2a	39.4 ±4.3a	159.42 ±4.3	112.48 ±5.12a	83.4 ±5.9a	59.4 ±10.3a	144.1 ±3.8d	139.5 ±1.4d	148 ±3.5d	156.7 ±2.8d
<i>S.tumbugaia</i> (400mg/kg) (VI)	94.3 ±5.3c	79.3 ±6.4c	54.2 ±3.4a	41.2 ±5.4a	143.42 ±5.2a	98.42 ±6.14a	73.4 ±9.8a	54.4 ±11.4a	145.3 ±2.3d	142.6 ±4.5d	147.6 ±3.8d	156.3 ±5.6d
<i>S.tumbugaia</i> (800mg/kg) (VII)	92.34 ±4.6a	78.4 ±6.9a	46.78 ± 5.67c	38.67 ±5.6a	123.45 ±5.6a	96.7 ±8.7b	74.5 ±10.3c	52.3 ±4.5a	154.6 ±4.5c	147.8 ±3.56a	123.4 ±5.6c	134.5 ±8.9c

Values are mean ± S.D. (n=6).

a= p<0.001 when compared to diabetic control

c=p<0.05 when compared to normal rats

d=p<0.001 when compared to normal rats

Table.6 Effect of alcoholic extract of *S.tumbugaia* on DPPH scavenging activity

Concentration (µg/ml)	%inhibition of DPPH peak area	
	Ascorbic acid	Ethanollic extraction of <i>S.tumbugaia</i>
5	18.34±0.340	15.60±0.010
10	26.87±0.560	18.56±0.990
20	34.56±0.230	23.40±0.020
40	56.78±0.060	34.50±0.050
60	78.90±0.080	48.67±0.060
80	87.60±0.090	65.40±0.080
100	100.00±0.088	79.50±0.045
IC ₅₀	25.20 µg/ml	50.70 µg/ml

Table.7 Effect of alcoholic extract of *S.tumbugaia* leaves on *in-vivo* antioxidant activity parameters

Groups	LPO (µM/mg tissue)	CAT (µM/mg tissue)	SOD(U/mg tissue)	GSH(µM/mg tissue)
Normal	0.0492 ±0.00062	0.0576 ±0.00168	0.03174 ±0.0077	0.185 ±0.0033
Diabetic	0.476 ±0.00522	0.0043 ±0.00014	0.432 ±0.0037	0.0405 ±0.00144
Glibenclamide (5mg/kg)	0.0663 ±0.00013a	0.0483 ±0.00178a	0.02843 ±0.0043a	0.1945 ±0.0045a
<i>S.tumbugaia</i> (100mg/kg)	0.0586 ±0.00132a	0.0386 ±0.00123a	0.03228 ±0.0188a	0.2028 ±0.173a
<i>S.tumbugaia</i> (200mg/kg)	0.0632 ±0.0032a	0.0683 ±0.0012a	0.04324 ±0.0043a	0.1724 ±0.2134a
<i>S.tumbugaia</i> (400mg/kg)	0.0598 ±0.00148a	0.0678 ±0.00178a	0.02953 ±0.0017a	0.1923 ±0.081a
<i>S.tumbugaia</i> (800mg/kg)	0.0567 ±0.00204a	0.0634 ±0.00124a	0.02453 ±0.00124a	0.1678 ±0.067a

The values are mean ± SEM. (n=6) ***p<0.001; when compared with diabetic rats

Effect on *In-vitro* antioxidant parameters

In DPPH radical scavenging method, reducing power method and hydroxyl radical scavenging method alcoholic extract of the *S.tumbuggaia* exhibited free radical scavenging ability or antioxidant activity in concentration dependent manner (Table.6).

Effect on *In-vivo* antioxidant parameters

Alloxan induced diabetic rats were found to have decreased GSH and catalase enzymes, where as increased TBARS and SOD levels in liver as compared to control. Treatment with *S.tumbuggaia* produced significant increase ($p<0.01$) in these enzyme levels and significant ($p<0.01$) decrease in TBARS and SOD levels (Table.7).

DISCUSSION

Present study was undertaken to demonstrate the effect of *S.tumbuggaia* leaf extract on lipid profile and antioxidant activity in alloxan induced diabetic rats. Alloxan causes diabetes through its ability to destroy the insulin producing β -cells of the pancreas (Lenzen and Panten, 1988). Diabetogenic effect of alloxan is due to excess production of reactive oxygen species (ROS) leading to cytotoxicity in pancreatic β -cells which reduces the synthesis and the release of insulin, (Jorns *et al.*, 1997). While effecting organs such as liver, kidney and haemopoietic system (Sabu *et al.*, 2002). Decreased antioxidant enzyme levels and enhanced lipid peroxidation have been well documented in alloxan-induced diabetes (Roy *et al.*, 2005).

In oral glucose tolerance test, at 90 and 120 min, a significant decrease in the blood glucose levels was observed in treated rats as compared with control rats. From the OGTT data, it is clear that administration of EST at the doses 400mg/kg and 800mg/kg effectively prevented the increase in serum glucose level without causing a hypoglycemic state. Antidiabetic activity of *S.tumbuggaia* may be through the stimulation of surviving β -cells of islets of langerhans to release more insulin. A number of other plants have been observed to exert antidiabetic activity through insulin-release stimulatory effects, such as *Tinospora cord folia* (Stanley *et al.*, 2000).

Administration of alloxan increased serum glucose levels when compared to normal animals and also induced persistent diabetes mellitus in rats. Our investigation also indicates the efficacy of alcoholic extract in decrease of blood glucose levels in normal and alloxan induced diabetic rats. Diabetic rats treated with the EST showed restoring of body weight as compared to the diabetic control, which may be due to its effect in controlling muscle wasting (Whitton *et al.*, 1975). The concentration of lipids, such as cholesterol, TG, LDL-cholesterol were significantly increased, where as HDL-cholesterol was decreased in the diabetic rats than normal rats. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of derangements in metabolic and

regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids (Rajalingam *et al.*, 1993). Further it has been reported that diabetic rats treated with insulin shows normalized lipid levels (Pathak *et al.*, 1981). Diabetic rats treated with EST and glibenclamide also normalized lipid levels. Thus, the results indicate that EST also may possess insulin like action by virtue of the ability to lower the lipid levels. These results are similar to earlier reports observed with the other plant (Kaleem *et al.*, 2006).

Glycosylated hemoglobin has been found to be increased over a long period of time in diabetes (Bunn *et al.*, 1978). During diabetes, the excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin (Alyassin *et al.*, 1981). The rate of glycation is proportional to the concentration of blood glucose (Sheela *et al.*, 1992). In the present study, the diabetic rats had shown higher levels of HbA_{1C} compared to those in normal rats. Treatment with EST and glibenclamide showed a significant decrease in HbA_{1C} levels in diabetic rats that could be due to an improvement in glycemic status. The EST increased serum insulin level significantly, indicating that they might have insulin secretaogues activity, which in turn controls the hyperglycemia state of type-2 diabetes.

Lipid peroxidation is one of the characteristic features of chronic diabetes and lipid peroxidation mediated tissue damage has been observed in diabetic conditions (Feillet-Coudray *et al.*, 1999). Hyperglycemia generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage (Hunt *et al.*, 1988). Increased concentrations of lipid peroxides in the liver are reported to decrease cytochrome P450 and cytochrome b5 activities, which may affect the drugs metabolizing activity in chronic diabetes (Levin *et al.*, 1973). Glutathione (GSH), a tripeptide normally present in high concentrations intracellularly, constitutes the major reducing capacity of the cytoplasm (Lu *et al.*, 1999) and protects the cellular system against the toxic effects of lipid peroxidation (Nicotera *et al.*, 1986). The diabetic animals in the present study registered lowered levels of GSH reflecting its increased utilization owing to oxidative stress while, a significant elevation of GSH levels in *S.tumbuggaia* administered to diabetic rats coincided with a significant decline in lipid peroxidation. It appears that the effect of *S.tumbuggaia* on GSH could be at low levels by increasing the biosynthesis of GSH or by inhibiting its utilization by reducing oxidative stress. The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen (Robinson *et al.*, 1998), while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals (Brioukhanov *et al.*, 2004) In the present investigation both these enzymes registered low levels of activity in diabetic controls indicating diabetes-induced stress. Such a decline in these enzyme activities has also been reported earlier (Selvam *et al.*, 1990). When *S.tumbuggaia* administered to the diabetic animals improved both SOD and CAT activities

substantially, reflecting the antioxidant potency of plant extract. The effects on antioxidants (GSH, SOD, CAT and LPO) were found to be lower than those of glibenclamide administered diabetic animals.

CONCLUSION

The present study reveals that the *S.tumbugaia* had antihyperglycemic, hypolipidemic and antioxidant agent. The bioactive component(s) responsible for the

observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the leaf extracts. Our phytochemical screening reported that the presence of flavonoid in extracts compounds which might be the constituents responsible for these activities. Further identification and isolation of these constituents may be fruitful.

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