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Anti-hyperglycemic and antioxidant activity of stem bark of *Polyalthia longifolia* var. angustifolia

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Abstract

The present study was undertaken to evaluate the antihyperglycemic and antioxidant activity of methanolic extract of stem bark of Polyalthia longifolia var. angustifolia (PL). The plant extract was found with low toxicity (LD_{50} >3 g/kg). The methanolic extract of PL at the dose levels of 200 and 300 mg/kg b. wt. produced a significant decrease in fasting blood glucose (FBG) level by 48.83 and 53.68 % respectively with respect to initial FBG level after 21 days treatment. In the Oral Glucose Tolerance Test (OGTT), this extract at the dose levels of 100, 200, and 300 mg/kg decreased glycemia at 60 min and the effect persisted until 120 min ($p < 0.05$) after glucose loading, which showed impaired glucose tolerance. The elevated level of SGPT, SGOT and ALP in the diabetic control group reflected the significant alteration of liver function by STZ induction and was found to be equipotent to Glibenclamide in restoration of the elevated enzyme levels to normal. The elevated lipid levels (Triglyceride and total cholesterol) were restored to near normal in this extract. The extract at 100 µg/ml concentration showed maximum scavenging of the radical cation in ABTS observed up to 54.79 % followed by scavenging of stable radical DPPH (75.36 %), Nitric oxide (57.25 %) and Super oxide dismutase (78.40 %) at the same concentration. The IC_{50} values of this extract in these models were calculated as 77.07, 46.84, 88.54 and 40.91 respectively at 1 mg/ml concentration. For all the estimated parameters, the results of the extract treated groups were restored to the near normal level, thereby indicating good antihyperglycemic and antioxidant activity of the methanolic extract of Polyalthia longifolia var. angustifolia.

Keywords : Antihyperglycemic, antioxidant, free radicals, Polyalthia longifolia var. angustifolia

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INTRODUCTION

Diabetes mellitus is the most common heterogeneous metabolic disorder, which currently affects an estimated 143 million people worldwide and its incidence is increasing steadily with changes in life styles [1]. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major cause of morbidity and death [2]. Several oral hypoglycemic agents are the primary forms of treatment for diabetes. However prominent side effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side effects [3]. Investigations have related etiology of diabetes to oxidative stress [4, 5]. Improvement in *in-vitro* antioxidant status has been reported to sensitize the insulin receptor and stimulate the secretion of insulin from beta cells of Islets of Langerhans in pancreas of streptozotocin (STZ) induced diabetic rats [6]. Thus plant based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management [7, 8]. However a limited number of the plant species have been studied and validated for their hypoglycemic properties using diabetic animal models and in clinical studies using human subjects [9].

Polyalthia longifolia var. angustifolia Thw. (Annonaceae), is a small medium-sized tree with linear-lanceolate leaves, 1 to 1.5 cm broad, occurring in Sri Lanka and now grown in tropical parts of India on road side and garden for their beautiful appearance [10]. The plant is similar to *Polyalthia longifolia* var. pendula, which is an important drug used as febrifuge and tonic. [11]. The diterpenes, alkaloids, steroid and miscellaneous lactones were isolated from its bark [12,13,14,15,16,17].The stem bark extracts and isolated compounds were studied for various biological activities like antibacterial, cytotoxicity and antifungal activity [18,19, 20]. We have recently reported significant antidiabetic activity of the methanolic extract of *Polyalthia longifolia* var. pendula [21] and antidiabetic activity of the chloroform extract of similar species, *Polyalthia longifolia* var. angustifolia (PL) [22]. The methanolic extract of stem bark of PL has not earlier been reported for its antihyperglycemic and antioxidant activity. The objective of the present investigation is to evaluate the antihyperglycemic activity of the part of this plant in STZ induced diabetic rats and study of its *in-vitro* antioxidant effect.

MATERIALS AND METHODS

Plant material

The bark of *Polyalthia longifolia* var. angustifolia was collected from Bhubaneswar, Orissa, India, in the month of November 2008. The plant was identified by Dr. P. C. Panda, Senior Scientist, Regional Plant Resource Center, Bhubaneswar, Orissa. A voucher specimen (No. SPS-2) has been preserved in the department of our University for further reference

Preparation of plant extract.

Freshly collected stem barks were washed under tap water, dried under shade and powdered with the help of mechanical grinder. The course powder is then defatted with petroleum ether (40-60^{0}C) followed by extraction with methanol in a Soxhlet apparatus for 48 h. The extract was filtered and concentrated to dryness under vacuum .The yield was 14.25% w/w with respect to dried powder. Tween-80 (5% v/v) was used as vehicle to suspend the extract. The extract so

obtained was subjected to qualitative chemical investigation using standard methods [23] and was screened for antihyperglycemic activity.

Animals

The male Wistar albino rats (220-250 g) and albino mice of both sexes (20-25 g) were purchased from the animal house of Siksha 'O' Anusandhan University, Bhubaneswar, India. They had free access to standard rat pellets and water *ad libitum* and maintained under standard condition at a temperature of 25 ± 2º C, with a 12/12-light/dark cycle and 35-60% humidity. The conditions in the animal house and the study protocols were approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) vides registration no. 1171 / C/ 08 / CPCSEA.

Study on normoglycemic animals

The rats were kept fasting for overnight with free access to water. The fasting blood glucose (FBG) level of each animal was determined at the beginning of the experiment. Animals in control group (Gr. 1) received only the vehicle, Gr. II received Glibenclamide and the test animals (Gr. III, IV and V) were treated with the suspension of methanolic extract of PL (100, 200 and 300 mg/kg b. wt). Blood sugar levels were determined at 1h, 2h, 4h and 8h after the oral administration of test samples to assess the effect of the test samples on normoglycemic rats.

Study on glucose- loaded animals (Oral Glucose Tolerance Test, OGTT)

The overnight fasted normal rats were divided into five groups (n=6). First group received only vehicle, standard drug (Glibenclamide) was given to group II. Group III, IV and V were given low and high dose (100, 200 and 300 mg/kg b. wt) of the suspension of methanolic extract of PL. The rats of all the groups were loaded with glucose (2 gm/kg, p.o) 30 min after the administration of the drugs or vehicle for control. Blood glucose levels were measured at 30, 60, 90 and 120 min after glucose load to assess the effect of different doses of extract on blood glucose levels of the glucose loaded animals.

Acute toxicity studies

The lethal dose (LD_{50}) of the plant extract was assessed by using albino mice of either sex, weighing 20-25 g to determine the dose. The animals were fasted overnight prior to the experimental procedure. Different doses of the extract were administered by the intra-peritoneal route. The LD_{50} was calculated according to Miller and Tainter [24]. 1/10th of lethal dose was taken as a screening dose [25]. Results were expressed as mean ± SD. The data were statistically analyzed by one-way ANOVA, followed by Dunnet's t-test. P values less than 0.05 and 0.01 were considered to be significant.

Induction of experimental diabetes

The Wistar albino rats were kept fasting for 24 h and thereafter diabetes was induced by intra-peritonial injection of STZ (Sigma Chemicals Co. USA) freshly dissolved in citrate buffer (pH 4.5) immediately before use. STZ was given at a dose of 65 mg/kg body weight [26]. In order to avoid the STZ induced hypoglycemic mortality, 5% glucose solution was given for 24 h to STZ treated rats [27]. After 72 h of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level > 250 mg/dl were considered to be diabetic and were used for the study.

Study on STZ induced diabetic rats

The rats were divided into five groups (n=6). Treatment was made for 21 days. Group I served as diabetic control group which received saline water (2 ml/kg b. wt), Group II received standard drug (Glibenclamide, 10 mg/kg b. wt) and group III, IV and V were orally administered methanolic extract of PL (100, 200 and 300 mg/kg b. wt. respectively).

Testing of FBG

The fasting blood glucose (FBG) level of each animal was monitored on days 0, 2, 4, 8, and 21. A drop of blood was collected from the tip of the tail vein of each rat and FBG level was measured using one touch Glucometer, Advanced Micro-draw Glucose Monitoring System (Hypo guard).

Estimation of biochemical parameters

The blood samples were collected on 22nd day from the retro-orbital plexus of the rats and serum was separated for the biochemical estimations of serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) [28], alkaline phosphatase (ALP) [29], total protein [30], total cholesterol and triglyceride [31]. All the analytical works were performed by using commercially available kit from Nicholus Piramal Diagnostic Research Centre.

Evaluation of *In-vitro* antioxidant properties

ABTS radical cation decolorization assay [32]

ABTS radical cation was produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 h before use. Different concentrations (12.5-100 µg/ml) of methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with methanol to make 1 ml. The absorbance was read at 745 nm and ascorbic acid was used as positive control. The experiment was carried out in triplicate.

DPPH radical scavenging activity

Free radical scavenging detection on thin layer chromatography (TLC) plates was performed using the diphenylpicryl-hydrazyl (DPPH) method [33]. One mg of the extract was weighed into a small test tube and 10 ml of methanol was added. The mixture was shaken together and with the help of capillary, spotted on the aluminum-coated plate about 10 mm away from the bottom of the plate. The point of the spot was clearly labeled and the plate was allowed to dry in air and developed in a trough containing the mobile phase (n-hexane: ethyl acetate, 9: 1). The above was allowed to dry and viewed in UV light at 365 and 254 nm. The fluorescent points were marked and the slide was sprayed with DPPH (1 µg/ml) solution in methanol. After this, the plate was left to dry and yellow coloration produced was noted. The evaluation of radical scavenging abilities of the extracts were carried out by UV spectrophotometric measurement using ascorbic acid as reference compound. Decrease in absorbance from that of freshly prepared 76 µL solution of DPPH in methanol in presence of different concentrations of plant extracts (12.5, 25, 50 75 and 100 µg/ml) were continuously recorded at 515 nm at 25 ºC. All the experiments were carried out in triplicate. The IC_{50} values were calculated following development of regression equations.

Scavenging of nitric oxide radical [34]
Nitric acid was generated from sodium nitropruside and measured by Griess' reaction. Sodium nitropruside (5mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations (12.5-100 µg/ml) of the methanolic extract dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 25ºC for 5 h. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1 % sulphanilamide, 2 % O-phosphoric acid and 0.1 % naphthyl ethylene diamine dihydrochloride). The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyle ethylene diamine was read at 546 nm. The ascorbic acid was used as positive control and the experiment was repeated in triplicate.

Scavenging of super oxide radical (Potassium superoxide assay) [35]
The scavenging activity towards the superoxide radical was performed by using alkaline DMSO method. Potassium superoxide and dry DMSO were allowed to stand in contact for 24 h and the solution was filtered immediately before use. The filtrate (200µl) was added to 2.8 ml of an aqueous solution containing NBT (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM). The reaction mixture was started by illuminating the reaction mixture with different extract for 150 seconds. Immediately after illumination, the absorbance was measured at 560 nm. Ascorbic acid was used as positive control.

Statistical analysis
Values were expressed as mean ± SD. Data were statistically evaluated by one-way ANOVA followed by Dunnet's t-test. P values less than 0.05 were considered to be significant. *: $p < 0.05$, **: $p < 0.01$ compared to control. Linear regression analysis was used to calculate the IC_{50} value.

RESULTS AND DISCUSSION

The phytochemical screening of methanolic extract of *Polyalthia longifolia* var. angustifolia (PL) revealed the presence of alkaloids, triterpenoids, flavonoids, steroids, saponin glycoside and tannins. The blood glucose level (FBG) of normoglycemic study and OGTT were presented in Table. I and II, showing the effect in OGTT but no effect in normoglycemic results.

TABLE- I: Effect of methanolic extract of *Polyalthia longifolia* var. angustifolia (PL) on blood glucose level in normal healthy rats.

Groups (n=6)	Dose (mg/kg)	Blood Glucose Level (mg/dl)					
		0h	1 h	2 h	4 h	8 h	F-value
Control	2 ml/ kg	90.46 ± 3.60	89.66 ± 2.58	91.5 ± 4.54	88.66 ± 2.73	91.5 ± 4.67	-
Glibenclamide	10	86.33 ± 4.27	81.83 ± 3.8*	77 ± 3.52*	73.33 ± 3.9*	86.66±3.66*	13.60*
PL	100	87.83 ± 4.16	87.5 ± 4.84	87.16 ± 3.18	88 ± 2.60	87 ± 3.68	0.74
PL	200	88.83 ± 4.49	86.16 ± 3.48	87.83 ± 3.31	87.66 ± 1.63	88.83 ± 3.65	0.60
PL	300	87.66 ± 4.76	86.83 ± 4.79	87.16 ± 3.65	89 ± 1.78	87.66 ± 3.14	0.28

Results expressed as mean ± SD (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet 's t-test. P values less than 0.05 were considered significant. Rats of all groups were loaded with glucose (2 g/kg p. o) 30 min after extracts, Glibenclamide and

water (p. o) *: p < 0.05; **: p < 0.01 (compared to control). PL: Methanolic extract of *Polyalthia longifolia* var. angustifolia.

TABLE- II: Effect of methanolic extract of *Polyalthia longifolia* var. angustifolia (PL) on blood glucose level in glucose-loaded healthy rats.

Groups (n=6)	Dose	Base value	Blood Glucose Level (mg/dl)			
			30 min.	60 min.	90 min.	120 min.
Control	2 ml/kg	85.66±7	137.33±6.34	146±3.34	140±4.69	90.16±3.6
Glibenclamide	10 mg/kg	88.16±6.11	120.83±4.53**	102.66±3.66**	91.16±3.43**	85.5±2.07**
PL	100 mg/kg	86.66±5.92	134.66±6.02	114.16±6.17**	102.66±4.36**	87.33±1.36*
PL	200 mg/kg	86±6.13	130.5±5.78*	111.83±3.76**	99±3.16**	85.83±2.31*
PL	300 mg/kg	88.66±5.35	124.83±4.75**	105.66±2.65**	95.83±1.72**	86.5±1.37*
F-value		0.280	9.10	107.52	175.43	3.97

Results expressed as mean ± SD (n=6). The data were statistically analysed by one-way ANOVA, followed by Dunnet 's t-test. P values less than 0.05 were considered significant. Rats of all groups were loaded with glucose (2 g/kg p. o) 30 min after extracts, Glibenclamide and water (p. o) *: p < 0.05; **: p < 0.01 (compared to control). PL: Methanolic extract of *Polyalthia longifolia* var. angustifolia.

The FBG level in the STZ induced 21 days study was given in Table. III indicating the extract of PL as equipotent as the standard drug, Glibenclamide.

TABLE-lll: Antihyperglycemic effect of methanolic extract of *Polyalthia longifolia* var. angustifolia (PL) in streptozotocin-induced diabetic rats after single dose and prolonged treatment.

Groups (n=6)	Dose (mg/kg)	Blood glucose level (mg/dl)					
		0h	2 h	4 h	8 h	21day	F-value
Control	2 ml/ kg	2.33±4.65	239±5.54	236.66±5.64	240.33±5.46	237.5±4.76	-
Glibenclamide	10	240±7.07	228±1.78*	199.83±2.92**	160.66±11.60**	102.66±11.46**	284.93**
PL	100	247.16±6.82	234.33±4.92*	217.66±4.22**	183.83±4.79**	167.16±4.95**	250.57**
PL	200	244.33±5.88	224.5±4.32*	194.33±4.71**	158.83±6.91**	125±4.73**	481.26**
PL	300	246.16±6.36	221±3.74*	186.33±4.96**	150.66±6.12**	114±4.60**	611.83**

Results expressed as mean ± SD (n=6}). The data were statistically analysed by one-way ANOVA, followed by Dunnet's t-test. P values less than 0.05 were considered significant. *: p < 0.05, **: p < 0.01 compared to control. PL: Methanolic extract of *Polyalthia longifolia* var. angustifolia.

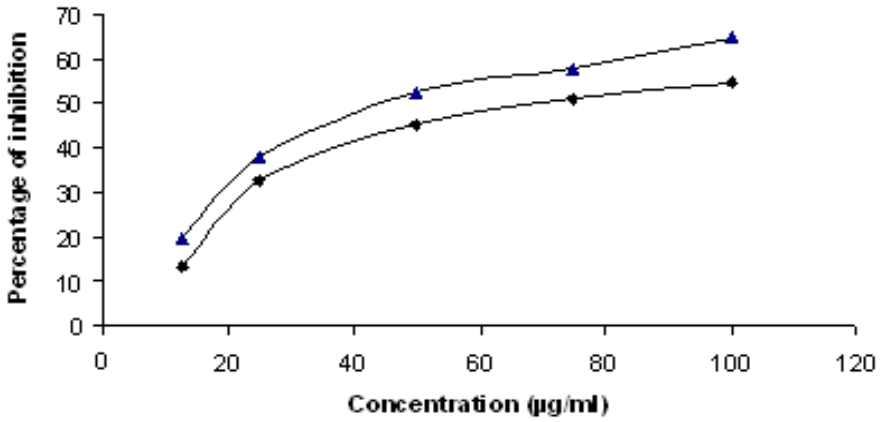
In case of biochemical parameter estimation, SGPT, SGOT, ALP, Total protein, Triglyceride and Total cholesterol were observed in the treated groups with respect to the diabetic control group and were presented in Table. IV.

TABLE-IV: Effect of methanolic extract of *Polyalthia longifolia* var. angustifolia (PL) on some biochemical parameters in control and STZ diabetic rats.

Groups	SGPT (IU/dl)	SGOT (IU/dl)	ALP (IU/dl)	Total protein (mg/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)
Diabetic control (2ml/kg)	213.33±4.41	249±6.03	89.5±4.54	5.16±1.94	213.16±5.11	252.83±6.17
Glibenclamide (10 mg/kg)	109.66±3.14**	139.16±4.30**	45.83±4.30**	7.16±1.47**	108.66±3.77**	123±5.93**
PL(100 mg/kg)	129±4.47**	159.16±3.65**	50.33±5.46**	4.83±2.22*	129.83±4.83**	145.16±5.34**
PL(200 mg/kg	120.16±4.16**	148.33±5.75**	48.83±5.74**	6.66±2.16*	120.33±4.32**	137.5±4.50**
PL(300 mg/kg)	116±3.40**	141.33±5.31**	46.5±4.67**	7±2.28**	111.5±4.80**	130.16±5.70**

Results expressed as mean ± SD (n=6). Treatment was done for 21 days. The data were statistically analyzed by one-way ANOVA, followed by Dunnet's t-test. P values less than 0.05 were considered significant. *: $p < 0.05$, **: $p < 0.01$ compared to control. PL: Methanolic extract of *Polyalthia longifolia* var. angustifolia.

Fig. l: Antioxidant activity of methanolic extract of *Polyalthia longifolia* var. angustifolia (12.5 µg/ml -100 µg/ml) and ascorbic acid in ABTS radical scavenging method. —▲—: Ascorbic acid —◆—: methanolic extract of PL



The methanolic extract of the plant was effective in the OGTT. A significant reduction of blood glucose level was observed at the dose levels of 100, 200, and 300 mg/kg of the plant extract when compared to glucose loaded control, which showed impaired glucose tolerance. Plasma glucose level reached a peak of 146 ± 3.34 g/l at 1 h after oral glucose loading (2 g/kg) in control rats. The PL at a dose of 100, 200 and 300 mg/kg produced a significant decrease of glycemia at

60, 90 and 120 min ($p < 0.01$) after glucose loading and the glucose lowering effect persisted until 120 min. In STZ induced diabetic rats, the methanolic extract of PL at the dose levels of 200 and 300 mg/kg showed a significant reduction (48.83 and 53.68 %) in fasting blood glucose level with respect to diabetic control group at the end of 21 days experimental period, indicating similar effect as the standard drug, Glibenclamide(57.22 %).

The methanolic extract of PL at several concentrations ranging from 12.5-100 µg/ml were tested for their antioxidant potential in different in-vitro method and the results are depicted in Figure I,II,III and IV.

Fig. ll: Antioxidant activity of methanolic extract of *Polyalthia longifolia* var. angustifolia (12.5 µg/ml -100 µg/ml) and ascorbic acid in DPPH radical scavenging method. —▲—: Ascorbic acid —◆—: methanolic extract of PL

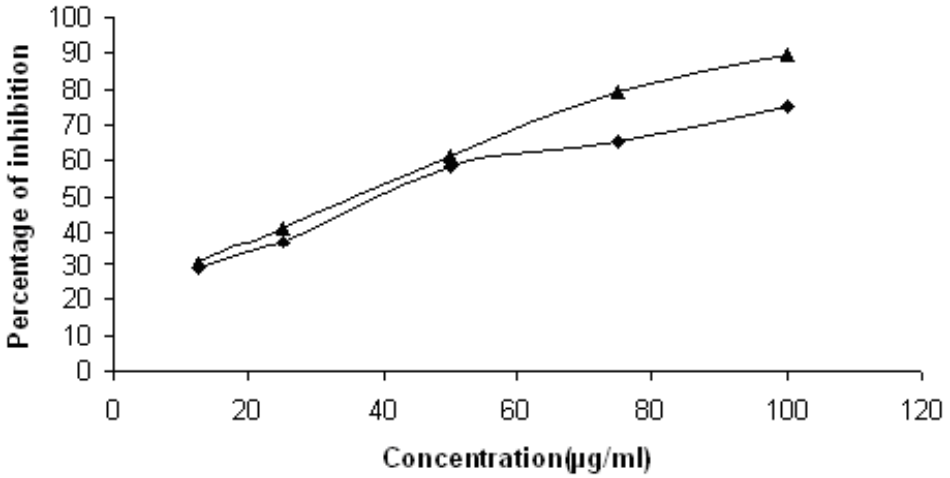


Fig. lll: Antioxidant activity of methanolic extract of *Polyalthia longifolia* var. angustifolia (12.5 µg/ml -100 µg/ml) and ascorbic acid in Nitric oxide radical scavenging method. —▲—: Ascorbic acid —◆—: methanolic extract of PL

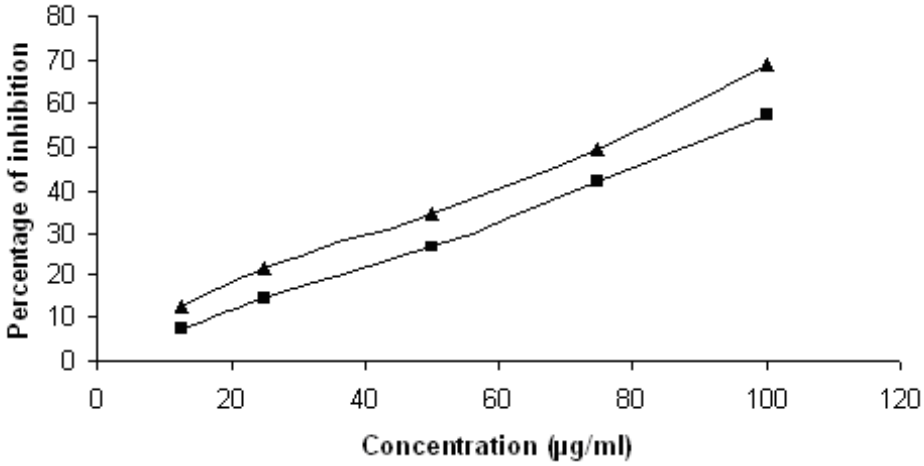
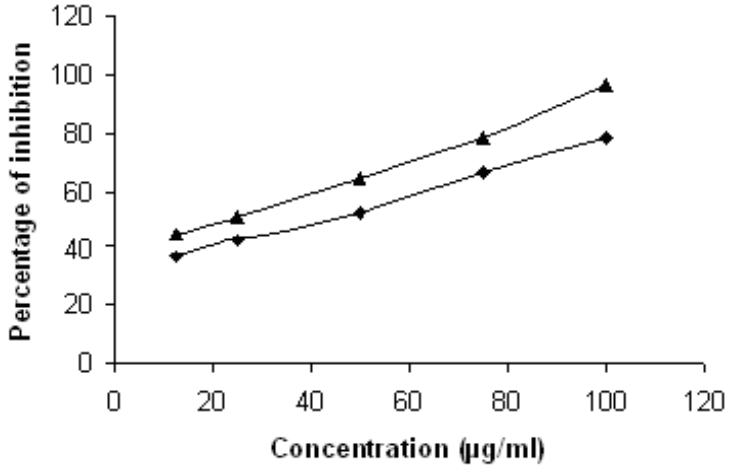


Fig. lV: Antioxidant activity of methanolic extract of *Polyalthia longifolia* var. angustifolia (12.5 µg/ml -100 µg/ml) and ascorbic acid in Superoxide anion radical scavenging method. —▲— : Ascorbic acid —◆— : methanolic extract of PL



It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in these models. The maximum percentage inhibition of PL in ABTS, DPPH, Nitric oxide and Super oxide dismutase models were found to be 54.79, 75.36, 57.25 and 78.40 respectively at 100 µg/ml concentration. The IC_{50} values of this extract in these models were calculated as 77.07, 46.84, 88.54 and 40.91 respectively at 1mg/ml concentration. On the comparative basis the extract showed better activity in quenching nitric oxide radical with an IC_{50} value of 88.54 µg/ml and ABTS with an IC_{50} value of 77.07 µg/ml. The LD_{50} value for PL was not less than 3 g/kg. This result suggests the less probability of toxicity of the plant.

This antihyperglycemic action may be attributed to the potentiation of pancreatic secretion of insulin from existing β cells of Islets of Langerhans or to the extrapancreatic mechanism like increased peripheral utilization of glucose through different enzymatic pathways. Since methanolic extract of PL did not exert any effect on normoglycemic animals, but significantly reduced the elevated blood glucose level, which implies that it acts through the extrapancreatic pathways rather than stimulating insulin secretion and results in antihyperglycemic effect without affecting normal blood glucose level, which may be beneficial in case of misdosing.

Diabetes is associated with alteration of plasma lipid and lipoprotein and consequently linked to increased risk of coronary heart disease [36]. Insulin deficiency and increased blood glucose level leads to hyperglyceridemia and hypercholesterolemia, as was found in the diabetic control group in the present study. This is may be due to uninhibited actions of lipolytic hormones on the fat depots and increased mobilization of free fatty acids from the fat depot. This excess fatty acid gets converted into phospholipids and cholesterol in liver. The elevated lipid levels were restored to near normal in the extract as well as reference drug treated groups.

The elevated levels of SGPT, SGOT and ALP in the diabetic control group reflect the significant alteration of liver function by STZ induction. The extract was found to be equipotent to Glibenclamide in restoration of the elevated enzyme levels to normal, implying the normal functioning of liver.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing [37]. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent diseases. The methanolic extract of PL showed increased scavenging activity against free radicals. It may be due to presence of more antioxidant principles. The phytochemical study on extract of PL has shown that flavonoids and tannins are abundant in this plant. Flavonoids and tannins have been reported to be antioxidative action in biological system, acting as scavenger of singlet oxygen and free radicals [38]. Hence the presence of these compounds in the methanolic extract of this plant may be contributory to its antioxidant activity. Several authors reported that flavonoids, tannins, alkaloids, terpenes are known to be bioactive antidiabetic principles [39, 40]. The antihyperglycemic effect of this plant extract may be due, in part to its flavonoids, alkaloids, tannins and terpenes.

CONCLUSION

The antihyperglycemic effect observed in the diabetic animals was in proportionate with its *in-vitro* antioxidant activity. Thus the glucose lowering activity of this plant extract may be attributed to its free radical scavenging action. Further studies are desirable to identify the active compounds.

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