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Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Alangium lamarkii* in alloxan induced diabetic rats

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ABSTRACT

Alangium lamarkii (family-Alangiaceae) have been used, traditionally, in the treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleet, acute fever and lumbago. To investigate the antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of Alangium lamarkii leaves in alloxan (ALX) induced diabetic rats. Diabetes was confirmed after 5 days of single intraperitoneal injection of ALX (150 mg/kg) in albino Wister rats. MEAL (200 and 400 mg/kg) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. On the 15th day, overnight fasted rats were sacrificed and blood was collected for the determination of high density lipoproteins (HDL), low density lipoprotein (LDL), Triglycerides (TG). For in vitro antioxidant activity of MEAL, DPPH method was carried out. Methanolic extract of Alangium lamarkii leaves at doses of 200 and 400 mg/kg showed significant reduction is blood glucose, lipid profiles when compared to diabetic control group. We concluded that MEAL possesses antidiabetic, antihyperlipidemic and antioxidant activities.

Keywords: *Alangium lamarkii*, Alloxan, Antidiabetic, Antihyperlipidemic, Antioxidants, DPPH method.

INTRODUCTION

The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population [1]. Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine based disease. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy [2]. It is now well established that the hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications [3]. Disorders of lipid metabolism are manifest by increased plasma concentrations of the various lipid and lipoprotein fractions (total and LDL cholesterol, VLDL, triglycerides, chylomicrons). They results, predominantly, in the cardiovascular disease. The extract was given to correct abnormal lipid profiles and diminish vascular disease and its consequences.

Deposition of cholesterol in the arterial wall is central to the artherosclerotic process. Carriage of VLDL, remnant lipoprotein and LDL to arteries can thus be viewed as potential artherogenic. In the reverse process, HDL carries cholesterol away from the arterial wall and can be regarded as protective against artherosclerosis. Over production of VLDL in the liver raises plasma VLDL, remnant lipoprotein and LDL levels if the capacity to metabolise these

lipoproteins is compromised by either a primary and or secondary abnormality. Raised levels of LDL-cholesterol are associated particularly with risk of coronary heart disease, but it is increasingly clear that moderately raised concentrations of triglycerides, VLDL or remnants in the presence of low HDL-cholesterol may also be artherogenic.

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 5, 6-dioxyruacil) has been commonly utilized as an animal model of diabetes. Alloxan exerts its diabetogenic actions when administered intravenously, intraperitoneal or subcutaneously. The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreting cells (β-cells) [4] and also due to autoimmune destruction of the β-cells of the pancreas [5]. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes [6]. Plants are rich sources of antidiabetic, antihyperlipedemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenols [7].

Since generations, in India people are using the extracts and leachates of different herbs in order to stimulate and promote the growth of specific herbs. The example of *Alangium lamarkii* is one of them. The leaves are useful in treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleet, acute fever and lumbago [8]. In case of intense pain due to gout, the patients are advised by the healers to apply the Ankol leaves in affected parts. The leaves are also used in treatment of asthma. The leaves are dried and put on fire. The patients are advised to inhale the fumes. In the present study, we have evaluated the antidiabetic, antihyperlipedemic and antioxidant activities of methanol extract of whole plant of *Alangium lamarkii*.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Alangium lamarkii* are collected form Asansol, West Bengal, India. A herbarium sheet was prepared and it was identified and authenticated (CNH/35/2011/TECH II/446) by the Botanical Survey of India, Howrah, West Bengal, India. The leaves were dried in shade to avoid too many chemical changes occurring and made into a coarse powder. Methanol was used as solvent for extraction and extraction was performed in Soxhlet Apparatus.

Picture showing the *Alangium lamarkii* leaves

Preparation of extract

The air dried crushed leaves (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w/) of the residue, which was stored at 20ºC until use.

Treatment of animals
Healthy male and female rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioral veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within ± 20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA).

Sixty young adult male Wistar rats, weighting 120–150 g were obtained from the Institutional Animal House of Gupta College of Technological Sciences. The rats were housed in polyethylene cages in the Animal House. The rats were housed in polyethylene cages, allowed one week of acclimatization, and maintained on standard rat chow and standard laboratory conditions throughout the experiment.

Phytochemical Screening
The concentrated extracts were used for preliminary screening of various phytoconstituents *viz*. carbohydrate, amino acid, alkaloids, tannins and flavonoids were detected by usual methods prescribed in standard tests. [9]

Chemicals
Chemical used in the study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH), methanolic extract of the plant (3mg), Quercetin was used as standard. LDL-Cholesterol kit (Cogent), Triglycerides kit (Accurex Biomedical Pvt.Ltd), HDL-Cholesterol (Accurex Biomedical Pvt. Ltd), Alloxan (Mercks)

Induction of diabetes
The animals were fasted for 12 h prior to the induction of diabetes as described by Joy and Kuttan (1999) with slight modification. ALX freshly prepared in 0.5% Tween 80 was administered intraperitoneally (i.p.) at single dose of 150 mg/kg. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of ALX. Rats with blood glucose level of above 200 mg/dl were considered to be diabetic and used for the studies.

Experimental design
The rats were randomized into five groups comprising of six animals in each groups as given below. Solvent/MEAL (200 and 400 mg/kg) was administered orally using an intra-gastric tube once daily for 15 days.

Group I: normal control rats, received 0.5% Tween 80.
Group II: diabetic control received ALX in single dose (150 mg/kg. i.p.).
Group III: diabetic rats received MEAL (200 mg/kg/day. p.o.), 5 days after ALX treatment.
Group IV: diabetic rats received MEAL (400 mg/kg/day. p.o.), 5 days after ALX treatment.

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 2 and 3 h after solvent/MEAL (200 and 400 mg/kg) administration and serum glucose was estimated by enzymatic glucose oxidase method. Percent reduction in serum glucose was calculated with respect to the initial level. Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance.

For this purpose, overnight fasted rats were fed glucose (2 g/kg) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation. On 15th day of the study, blood samples were collected for biochemical estimations. Later animals were sacrificed and liver was removed, cleaned and washed in ice-cold normal saline for biochemical study.

Biochemical analysis
LDL-Cholesterol kit (Cogent), Triglycerides kit (Accurex Biomedical Pvt.Ltd), HDL-Cholesterol (Accurex Biomedical Pvt. Ltd) was estimated using standard enzymatic kits spectrometrically.

In-vitro antioxidant activity
DPPH solution was prepared by taking 20mg of DPPH in 20 ml of Methanol and from that stock solution 800µl was taken and volume was adjusted with 20 ml of methanol. Then dried extract 3mg was taken and dissolved in 30 ml of methanol so the concentration became 1mg/10ml.From this stock solution different concentration of 10, 20, 30, 40, 50 and 60µgm/ml was taken in different test tubes and volume adjusted with methanol. Then 3ml of DPPH was

taken in each test tubes and 200µl was added to it from the above stock solutions. The test tubes are kept at dark for 30mins. The absorbance was measured at 517nm using methanol as blank and DPPH as Control. The same procedure is applied for measuring standard Quercetin [10].

$$\text{Antioxidant activity} = OD_{control} - OD_{sample} / OD_{control} \times 100$$

Statistical analysis

Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-test formultiple comparisons. $p < 0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

From the Phytochemical study, it has evaluated the presence of alkaloid, amino acid and steroid in leaves**.**

Antidiabetic effect of MEAL

Table 1 reveals that the effect of MEAL on blood glucose level of diabetic rats during single dose study. MEAL (200 and 400 mg/kg) was given orally to the different groups and showed significant ($p < 0.001$) percentage reduction in glycemia when initial value of same group. Table 1 shows the effect of MEAL on blood glucose levels of diabetic and animals after the daily treatment (200 and 400 mg/kg) for 15 days. Showed significant ($p < 0.001$) percentage falls in blood glucose levels with the doses of 200, 400 mg/kg of MEAL as compared to diabetic control group.

Table 1: Effect of methanolic extract of *Alangium lamarkii* on blood glucose level in ALX-induced diabetic rats

Groups	Treatment	Blood glucose level (mg/dl)			
		0 day	1st day	10th day	15th day
I	Control	66.75 ± 1.14	70.23 ± 1.32	55.16 ± 3.6	62.48 ± 2.5
II	Diabetic control (DC)	176.24 ± 22.12	195.24 ± 32.32	205.25 ± 5.05	193.43 ± 20.05
III	DC + MEAL (200 mg/kg)	182.4 ± 13.9	175.1 ± 21.9*	103.44 ± 7.01***	109.4 ± 1.5***
IV	DC + MEAL (400 mg/kg)	185.5 ± 24.4	156.2 ± 2.7***	92.23 ± 6.06	95.7 ± 3***

The data are expressed in mean ± S.E.M. n = 6 in each group.
** P < 0.05 compared with corresponding value of diabetic control animals.*
*** P < 0.01 compared with corresponding value of diabetic control animals.*
**** P < 0.001 compared with corresponding value of diabetic control animals*

Table 2: Effect of methanolic extract of *Alangium lamarkii* on lipid profiles in ALX-induced diabetic rats

Groups	Treatment	Tryglyceride	LDL	HDL
I	Control	80.0± 0.01	8.56±0.01	335.0±8
II	Diabetic control (DC)	85.0± 5	14.47 ± 1.05	250.38 ± 4.05
III	DC + MEAL (200 mg/kg)	83.0± 1	9.24±0.1**	200.0±7**
IV	DC + MEAL (400 mg/kg)	82.3 ± 0.02***	9.0±0.09***	197.0±8**

The data are expressed in mean ± S.E.M. n = 6 in each group.
** P < 0.05 compared with corresponding value of diabetic control animals.*
*** P < 0.01 compared with corresponding value of diabetic control animals.*
**** P < 0.001 compared with corresponding value of diabetic control animals*

Table 3: Optical Density and % inhibition of the methanolic extract at different concentrations

Sl. No	Concentration of Extract (µgm/ml)	Optical Density (nm)	% Inhibition
1.	0	1.099	-
2.	10	1.062	3.36
3.	20	0.972	11.55
4.	30	0.953	13.28
5.	40	0.872	20.65
6.	50	0.820	25.38
7.	60	0.798	27.38

Table 4: Optical Density and % inhibition of the standard at different concentrations

Sl. No	Concentration of Quercetin (µgm/ml)	Optical Density (nm)	% Inhibition
1.	0	1.099	-
2.	10	1.075	2.18
3.	20	1.032	6.09
4.	30	1.027	6.55
5.	40	0.842	23.38
6.	50	0.789	28.20
7.	60	0.668	39.21

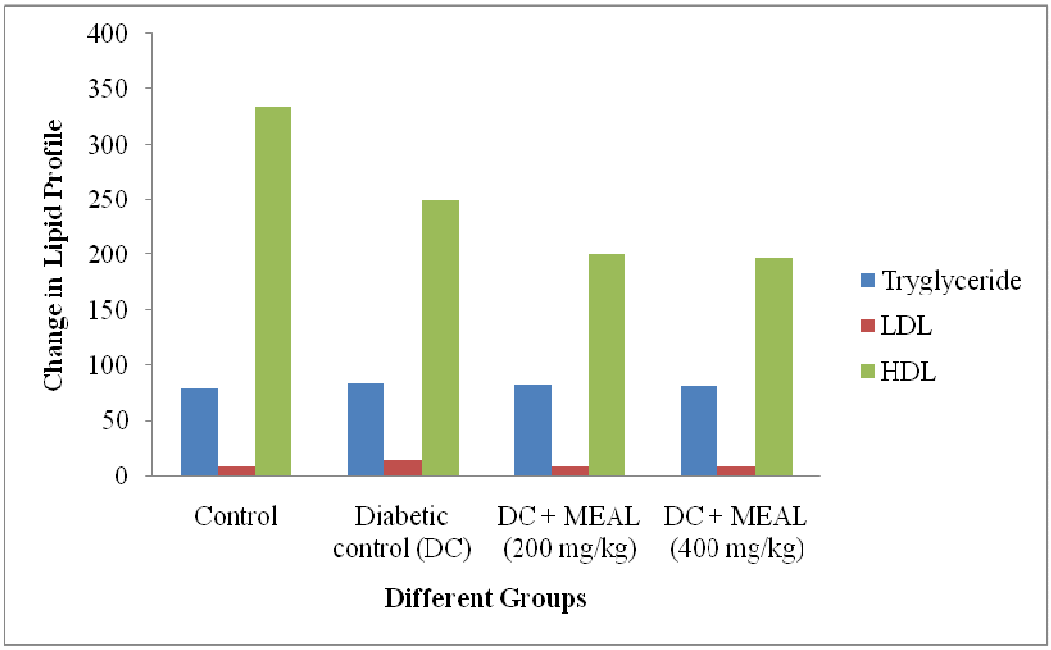

Fig.1. Effect of MEAL treatment on blood lipid level in diabetic rats. The change in lipid level with respect to the control dose

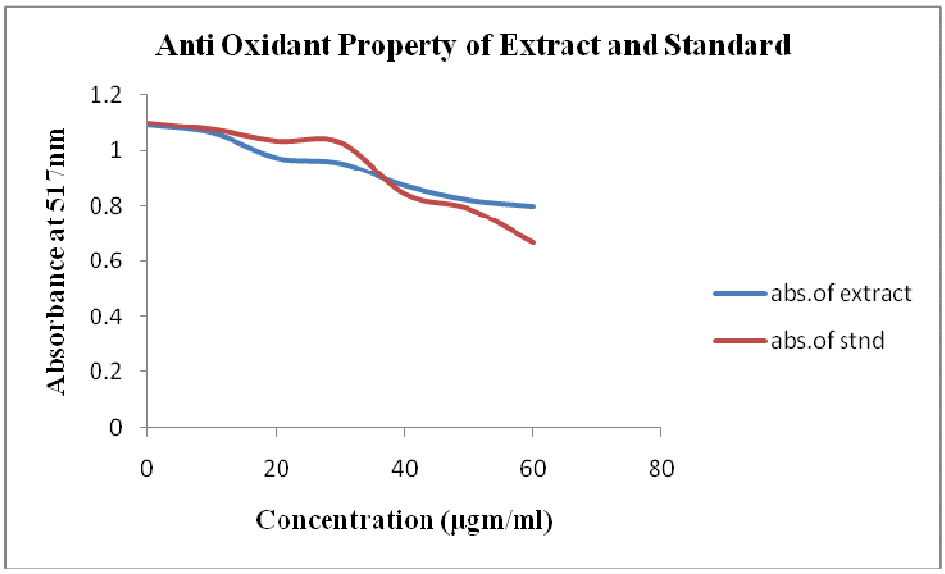

Fig 2. Antioxidant property of Extract and Standard (Quercetin)

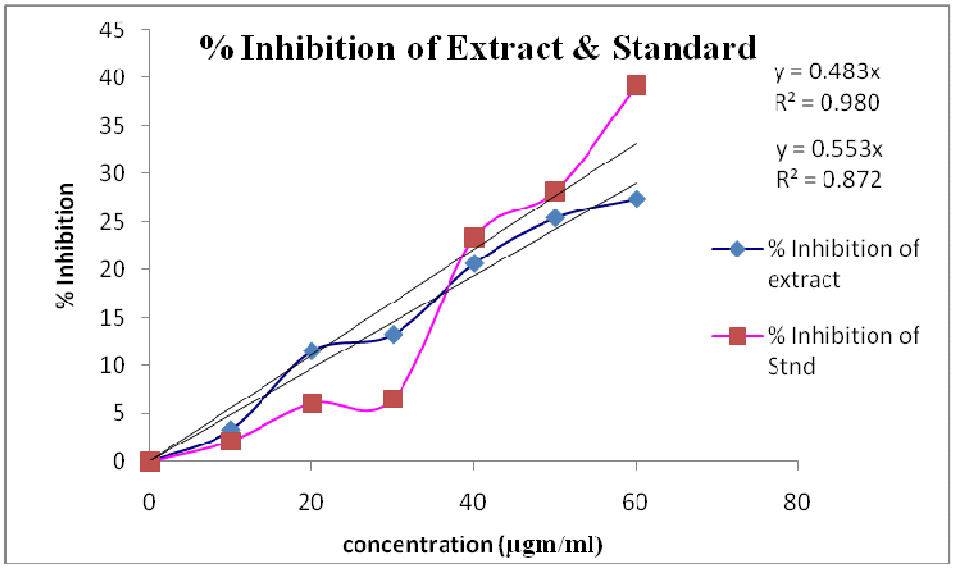

Fig 3. Percentage Inhibition of Extract and Standard

Antihyperlipidemia effect of MEAL

ALX treatment resulted in significant ($p < 0.001$) elevation of TG, LDL, and reduction of HDL levels as compared to the normal control rats. MEAL (200 and 400 mg/kg) and significant ($p < 0.001$) reduction in elevated TG, LDL and HDL level was restored respectively when compared to diabetic control (Table 2).

Antioxidant activity

The methanolic extract of *Alangium lamarkii* showed antioxidant activity. The DPPH scavenging activity as depicted by the percentage antioxidant activity varies proportionately with increasing concentration of the methanolic extract (103.51µgm/ml). However, the antioxidant property seems to be less compared to the standard antioxidant Quercetin (90.41µgm/ml), as evident from the IC_{50} values. Thus cyto-protective role of the methanolic extract of *Alangium lamarkii* is justified from the results, and its use in traditional medicine as a mediator of cellular protection receives force (Table 3 and 4)(Figure 2 and 3).

DISCUSSION

Diabetes mellitus is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease. Hyperlipidemia is a recognized complication of Diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition. The result of this present study clearly shows that *Alangium lamarkii* has a lipid lowering effects on serum triglycerides, total cholesterol and low-density lipoprotein cholesterol of Alloxan induced diabetic rats. *A. lamarkii* treatment also increase the serum level of High-density lipoprotein cholesterol termed “good cholesterol”. There is a substantial evidence that lowering the total cholesterol, particularly LDL-C level will lead to a reduction in the incidence of coronary heart disease which is still the leading cause of death in diabetic patients. [11]

Increased triglycerides and reduced HDL-C levels are the key characteristics of dyslipidemia in type 2 diabetes. Hypertriglyceridemia in type 2 diabetes can result from an increased hepatic very low-density lipoprotein (VLDL), overproduction and impaired catabolism of triglyceride-rich particles. The function of lipoprotein lipase, the key enzyme in removal and degradation of triglycerides is attenuated by both insulin deprivation and insulin resistance. [12]

Administration of the extract significantly reduced the plasma the LDL and Triglyceride levels ($P<0.01$) levels, one week after administration of the extract. But there is significant ($P<0.01$) rise in the plasma HDL levels even after administration of the extract.

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids (FFA) from the peripheral depots, since insulin inhibits the hormone sensitive lipase. Serum – FFA concentration are a result of the balance between the release from lipolysis, neosythesis and disposal and represent the major determinant of insulin effect on free fatty and oxidation and non-oxidative metabolism. [13]

Oxidative stress, altered lipid levels, and disturbances in glucose metabolism are important risk factors for diabetes, cardiovascular, oncologic and many other diseases. Diet undoubtedly plays a key role as chemopreventive agent against various diseases and optimizing the diet in both quality and quantity, has a preventive function. Fruit and vegetables are an invaluable source of many biologically active substances, including antioxidants. For this reason a diet rich in fruit and vegetable has a positive effect on reducing the incidence of these serious lifestyle diseases [14]. A. viridis, is used as vegetable, possess a wide ethnomedical history [15]. The diabetogenic agent alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which is toxic to pancreatic β cells because it can generate toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine [16]. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which generates free radicals due to auto-oxidation [18]. In the present work, involvement of free radicals in progression of disease and protective effects of *Alangium lamarkii* has been examined. Administration of MEAL for 15 days showed significant antidiabetic, antihyperlipidemic and antioxidant activities in ALX-induced diabetic rats.
Hyperlipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes which in turn leads to accumulation of lipids such TG and HDL in diabetic patients [19]. Our data were in line with notion as the ALX (150 mg/kg, i.p.) treated diabetic rats exhibited clear cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TG, HDL and reduction of LDL level. Treatment with MEAL for 15 days was sufficient to produce a significant reduction in the TG, HDL and significant increase in LDL levels in diabetic rats. These results indicate that MEAL has a lipid lowering effect on the diabetic rats.

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