ORIGINAL ARTICLE

Evaluation of anti-diabetic activity of methanolic extract from the bark of *Atalantia monophylla* (*Linn.*) in alloxan-induced diabetic mice

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Objective: The objective of the present study was to evaluate the anti-diabetic activity of methanolic extract from the bark of *Atalantia monophylla* (*Linn.*) in alloxan-induced diabetic mice. **Materials and Methods:** Diabetes was induced in mice by injection of alloxan (200 mg/kg, i.p.). Diabetic mice were divided into different groups and methanolic bark extract of *Atalantia monophylla* (AMMt) was administered at dose ranges of 50–200 mg/kg, p.o for 14 days. Control group received normal saline (0.9%) for 14 days. Glibenclamide (4 mg/kg, p.o) was used as standard drug. Blood samples were collected from all the groups and analysed for serum glucose and lipid levels such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). AMMt was also tested for oral glucose tolerance test (OGTT) in normal fasted rats. **Results:** AMMt (100 and 200 mg/kg, p.o) showed a significant ($P<0.05$) reduction of serum glucose level in alloxan-induced diabetic mice as compared with diabetic control. AMMt (100 and 200 mg/kg) also showed a significant reduction in serum TC, TG, LDL and VLDL levels in alloxan-induced diabetic mice. In addition, AMMt (100 and 200 mg/kg, p.o) significantly increased serum HDL level as compared with diabetic mice. AMMt (100 and 200 mg/kg, p.o) significantly ($P<0.05$) increased the glucose tolerance in OGTT. **Conclusion**: The results obtained from the present study revealed the potential anti-diabetic activity of methanolic extract from the bark of *A. monophylla*.

Key words: Alloxan, anti-diabetic, anti-hyperlipidaemic, *Atalantia monophylla*

INTRODUCTION

Diabetes mellitus is a leading metabolic disorder characterised by fasting and/or postprandial state hyperglycaemia, resulting from defects in insulin secretion or action. It is well reported that diabetes mellitus is associated with a large number of macrovascular and microvascular complications such as obesity, hypertension, hyperlipidaemia, nephropathy and neuropathy.[1,2] A growing body of research has suggested that diabetes mellitus is increasing in an epidemic proportion throughout the globe, especially in India. Moreover, the prevalence of diabetes is expected to increase by more than two-fold worldwide and approximately 57 million Indians would be affected by this disorder in the year 2025, illustrating the severity and impact of the disorder on the quality of life.[3,4] Despite the steady increase in the number of anti-diabetic agents, the prevalence of the disorder remains stable, may be due to the inconsistent efficacy of currently available drugs. In addition, the currently available anti-diabetic drugs have a large number of adverse effects and high rates of secondary failure.[5] Therefore, this remains a grave need to develop and discover new therapy with a proper balance of risk to benefit that could be fruitful for the treatment of diabetes mellitus. In recent decades, many researchers have sought new plant products to treat diabetes mellitus, as they contain many bioactive substances with therapeutic potential.[4]

Atalantia monophylla Linn. (Rutaceae) locally known as "Manao Pee," is a shrub with brown bark and thorny branches and is distributed in Southeast Asia, East Bengal, South India and Ceylon.[6] In folk medicine, this plant is used for several medicinal purposes such as anti-rheumatic, anti-spasmodic, stimulant, in hemiplegia and for the treatment of paralysis.[6,7] The essential oil from the leaves has been reported for antimicrobial and strong inhibitory activities against some pathogenic fungi,[8] whereas decoction of the leaves is used for itching and other skin complaints.[6] However, information regarding the medicinal value of bark part of *Atalantia monophylla* plant is not explored extensively. In our previous study, we have been shown that the methanolic extract of bark exhibited

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analgesic and anti-inflammatory activity.[9] In addition, methanolic bark extract showed potential anti-oxidant activity, a property dependent on the presence of high flavonoid content.[9] Considerable research has shown that plants rich in flavonoid content with anti-oxidant potential are known to be bioactive for the management of diabetes mellitus.[10] In addition, a growing body of data has reported that anti-oxidants are used as supportive therapy in the treatment of diabetes mellitus.[10] Furthermore, to our best knowledge, the anti-diabetic potential of bark part of *A. monophylla* has not been investigated. Thus, considering this finding mentioned above, the present study was designed to investigate the anti-diabetic potential of methanolic bark extract of *A. monophylla*.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (20–25 g) and male Wistar rats (200–250 g) used in the present study were obtained from Hissar Agricultural University, Haryana, India. The animals were kept under standard laboratory conditions (temperature 23±2°C and room humidity 60±10%) and maintained on 12:12 h light/dark cycle. The animals were fed with standard diet and filtered water ad libitum. All the experiments were performed as per the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani, India.

Chemicals

Alloxan was purchased from Sigma Chemical Co. (St Louis, MO, USA). The fine chemicals of analytical grade were purchased from the Spectrochem Chemicals Ltd., Mumbai, India. The commercially available kits were purchased from Coral Clinical System, Goa, India.

Plant Material and Preparation of Plant Extract

The bark of *A. monophylla* plant was collected from a hilly area of Tamil Nadu. The collected plant was authenticated at botanical survey of India, Ministry of Environment and Forests, Government of India.[9] The collected bark of *A. monophylla* was dried under shade for 10 days and then made into a coarse powder. Initially, 400 g of dried bark was defatted with petroleum ether (60–80°C) in soxhlet apparatus (continuous hot percolation process) and after complete extraction (46 h), the solvent was removed by distillation under reduced pressure and resulting liquid was dried using heating plate at 50°C to get semisolid residue. After the extraction with petroleum ether, the same plant material was dried and further extracted with chloroform (36 h) followed by methanol (75 h) until the extraction was complete. The methanolic bark extract was concentrated under reduced pressure and dried using heating plate at 60°C to get semisolid residue or respective residue.

Oral Glucose Tolerance Test

OGTT was performed in non-diabetic rats. The fasted rats were divided into 4 groups (n = 6/group). Group I: glucose load control group. Group II, III and IV rats received AMMt at a dose of 50–200 mg/kg body weight, respectively. The rats of treatment groups were loaded with glucose (2 g/kg, p.o.) 30 min after the administration of the AMMt. Blood samples (100–200 µL) were collected at 0 min before the glucose load and 30, 60 and 90 min after the glucose load by retro-orbital vein plexus puncture under mild ether anaesthesia. The serum was separated and the glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method.[11]

Induction of Diabetes Mellitus

Diabetes was induced with a single intraperitoneal injection of alloxan freshly dissolved in citrate buffer (50 mM/L, pH 3) at a dose of 200 mg/kg body weight. After 72 h, blood samples (100 µL) were obtained from the overnight fasted mice and their serum glucose levels were measured. The animals with a serum glucose concentration of more than 180 mg/dL were classified as diabetic and used for the study.

Experimental Design

Thirty-six male Swiss albino mice were used in this study. The mice were randomised and divided into six groups of six animals each.

Group I: Vehicle control: received normal saline
Group II: Diabetic controls
Group III: Diabetic + glibenclamide (4 mg/kg, p.o)
Group IV: Diabetic + AMMt (50 mg/kg)
Group V: Diabetic + AMMt (100 mg/kg)
Group VI: Diabetic + AMMt (200 mg/kg). The doses of AMMt and glibenclamide were chosen based on previous study, respectively.[9,12]

Blood Collection

The blood samples (500–750 µL) were collected by retro-orbital vein plexus puncture of anaesthetized mice. Blood samples were collected at the time of grouping of animals (basal reading) and at 1st, 7th, and 14th day of treatment. Blood was centrifuged at 3500 r.p.m. for 20 min and serum was separated for biochemical estimation.

Estimation of Serum Glucose and Lipid Profile

The glucose concentration was estimated by (GOD-POD) method[11] using commercially available kit. The absorbance and concentration of test and standard samples were noted against blank at 505 nm with an autoanalyser.

Estimation of Serum Lipid Profile

Serum total cholesterol and HDL were estimated by cholesterol oxidase-peroxidase (CHOD-POD) method[13] using commercially available kit.

Serum triglyceride was estimated by glycerophosphate oxidase-peroxidase (GPO-PAP) method by the addition of enzyme present in reagent kit.[14] The absorbance and concentration of test and standard samples were noted against blank at 505 nm with an autoanalyser. Serum VLDL and LDL concentrations were calculated according to Friedewald equation.[15]

Statistical Analysis

All values were expressed as mean ± S.E.M. The data obtained from various groups were statistically analysed using one-way ANOVA followed by Tukey's multiple comparison test in Graph pad prism 3. The *P* value < 0.05 was considered to be statistically significant.

RESULTS

Oral Glucose Tolerance Test

The effects of AMMt (50–200 mg/kg, p.o) on OGTT are summarised in Figure 1. Maximum serum glucose level was found at 60 min. in all groups after glucose load. The control group had a significant elevation in serum glucose level throughout the total measurement period, i.e., for 90 min, with respect to AMMt treatment group as shown in Figure 1. However, in the AMMt treated groups, blood glucose level although it reached the peak level within 60 min of administration of glucose but it almost resettled to the normal level by 90 min. The glucose level significantly ($P<0.05$) resettled close to the normal value in AMMt (100 mg/kg and 200 mg/kg) treated group. Moreover, at the dose of 200 mg/kg, the glucose level was significantly ($P<0.05$) less as compared with glucose loaded control rats throughout at 90 min, while at 100 mg/kg dose, the glucose level was significantly ($P<0.05$) less as compared to glucose loaded control rats at 60 and 90 min. However, no significant affect was observed at a dose of 50 mg/kg.

Alloxan-induced Diabetic Mice

Serum glucose and lipids level

Table 1 summarizes the serum glucose levels in normal and diabetic mice. There was a significant ($P<0.05$) increase in blood glucose levels in alloxan-induced diabetic mice as compared with vehicle control mice.

Table 2 illustrates the serum lipid levels in normal and diabetic mice. Serum levels of TC, TG, LDL and VLDL levels ($P<0.05$) were higher, whereas HDL levels reduced in alloxan-induced diabetic mice as compared with vehicle control mice [Table 2].

Effect of methanolic bark extract of A. monophylla on serum glucose level

AMMt at a dose range of 50–200 mg/kg decreased serum glucose levels in diabetic mice [Table 1]. The significant

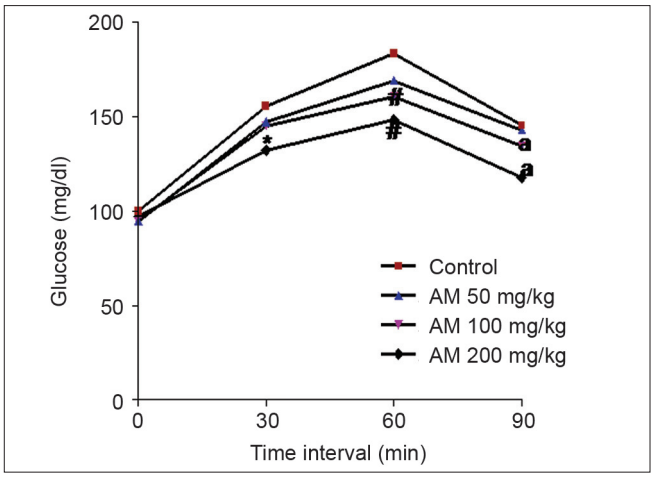

Figure 1: Effect of AMMt on blood glucose concentrations in fasting conditions at 0 min (pre-treatment) and 30, 60 and 90 min after oral glucose load in normal control rats, and rats treated with various doses of the methanolic bark extract. All values are represented as mean±SEM; **P*<0.05 vs. glucose loaded control animals at 30 min., #*P*<0.05 vs. glucose loaded control animals at 60 min., a*P*<0.05 vs. glucose loaded control animals at 90 min

Table 1: Effect of methanolic extract of *Atalantia monophylla* on serum glucose levels in alloxan-induced diabetic mice

Groups	Serum glucose (mg/dL)		
	1st day	7th day	14th day
Normal	89.6±4.94	87.66±4.25	86.5±3.21
Diabetic	228.98±7.42*	224.81±9.74*	216.3±3.21*
Glibenclamide (4 mg/kg)	180.32±6.49*	125±8.22#	99±5.89#
AMMt (50 mg/kg)	219.81±8.48*	199.83±5.5	187.1±8.51
AMMt (100 mg/kg)	225.30±4.84*	185.4±10.12#	151.3±9.57a,b
AMMt (200 mg/kg)	221.23±12.3*	172.8±10.17#	124.9±7.27a,b

All values are represented as mean±SEM; **P*<0.05 vs. Normal control animals. #*P*<0.05 vs. Diabetic control animals at 7th day, a*P*<0.05 vs. Diabetic control animals at 14th days. b*P*<0.05 vs. 7th day treatment value at 14th day

($P<0.05$) effect on serum glucose level was found at a dose of 100 and 200 mg/kg body weight, observed on 7th and 14th day of treatment. Moreover, the most pronounced decrease in serum glucose was observed on Day 14 at a dose of 200 mg/kg. In addition, the positive control glibenclamide also significantly decreased the serum glucose level in diabetic mice as compared with diabetic control mice. However, AMMt at a dose of 50 mg/kg failed to reach the level of significance as compared with diabetic control mice.

Effect of methanolic bark extract of A. monophylla on serum lipids level

Table 2 demonstrates the effect of the AMMt on serum lipid levels. Treatment of diabetic mice with AMMt (100–200 mg/kg) produced a significant reduction in serum levels of TC, TG, VLDL and LDL, whereas simultaneously increased the HDL level as compared with diabetic control mice. The effect observed in our study was dose dependent and time dependent. Moreover, glibenclamide treatment

Table 2: Effect of methanolic extract of *Atalantia monophylla* on serum lipid levels in alloxan-induced diabetic mice

Lipid	Days	Groups					
		Normal	Diabetic	Glibenclamide (4 mg/kg)	AMMt (50 mg/kg)	AMMt (100 mg/kg)	AMMt (200 mg/kg)
Total cholesterol	0th	93.98±4.13	96.88±2.69	96.45±5.33	98.02±2.18	95.0±3.62	94.45±4.53
	7th	98.70±4.72	170.04±3.8*	122.25±6.87#	160.32±5.41	146.95±3.67#	132.25±4.07#
	14th	97.82±7.02	167.8±7.38*	107.5±3.08a,b	155.5±7.74	128.4±2.48a,b	110.5±4.03a,b
Triglyceride	0th	89.90±7.65	89.52±6.26	87.8±3.59	83.32±6.18	84.8±3.29	84.8±3.29
	7th	83.47±4.07	195.7±7.19*	137.52±8.57#	180.07±6.69	151.42±5.37#	141.42±5.37#
	14th	91.92±4.19	188.1±5.07*	106.42±9.12a,b	172.4±6.81	127.82±5.82a,b	110.62±4.32a,b
HDL	0th	51.23±2.87	47.58±1.98	48.63±2.4	54.24±2.89	50.57±2.1	49.36±2.1
	7th	52.17±2.09	30.15±1.46*	40.77±5.7#	33.71±3.05	40.50±5.6#	42.67±8.1#
	14th	49.15±3.09	28.74±2.01*	55.57±4.9a	34.47±2.98	47.89±1.4a	54.27±2.9a
LDL	0th	24.17±3.9	31.43±2.7	26.33±5.6	25.31±4.2	27.66±2.3	28.13±4.1
	7th	29.85±2.7	100.75±1.8*	51.46±2.9#	90.6±3.5	76.17±3.3#	61.46±2.9#
	14th	30.30±3.1	101.46±4.2*	28.21±5.1a,b	88.75±4.4	58.1±3.1a,b	32.46±4.8a,b
VLDL	0th	17.98±1.53	17.90±1.24	15.69±0.21	16.67±1.08	16.96±1.05	16.96±0.78
	7th	16.68±0.82	39.14±1.43*	22.19±0.77#	36.01±1.33	30.28±1.31#	28.25±1.07#
	14th	18.38±0.83	37.62±1.01*	19.21±0.96a,b	34.48±0.60	25.56±1.26a,b	22.12±1.16a,b

All values are represented as mean±SEM; **P*<0.05 vs. Normal control animals. #*P*<0.05 vs. Diabetic control animals at 7th day, a*P*<0.05 vs. Diabetic control animals at 14th days. b*P*<0.05 vs. 7th day treatment value at 14th day. 0th day (basal value before treatment; HDL – High-density lipoprotein; LDL – Low-density lipoprotein; VLDL – Very low-density lipoprotein

also significantly decreased the serum levels of TC, TG, LDL and VLDL and increased HDL level.

DISCUSSION

Diabetes mellitus has been recognized as one of the most common metabolic disorders associated with common features such as hyperglycaemia and hyperlipidaemia. Alloxan is a β-cytotoxin diabetogenic agent, which induces diabetes by destructing the β-cells of the islets of pancreas, leading to a decreased insulin release and increased blood glucose level.[16] In accordance with the previous findings, the present study reports the significant increase in serum glucose level in alloxan-induced diabetic mice. The chronic administration (14 days) of the AMMt produced a decrease in serum glucose levels of diabetic mice. This effect may be due to regeneration of the β-cell following destruction by alloxan. The growing body of data suggested that to achieve maximum effect, therapy with plant products should be continued for a longer duration.[17] Considering this, AMMt was administered daily for 14 days, the period which may be produced a significant reduction in all the diabetic markers, and this effect was more potent as compared to acute dosing.[17]

In the present study we also investigated glucose tolerance test in normal rats. The AMMt significantly decreased the serum glucose levels in glucose loaded rats, and this information could be endorsed to the potentiation of the insulin effect of blood by increasing the pancreatic secretion of insulin from existing β-cells or its release from bound insulin.[18] In this context, a number of other plants have been observed to have similar pattern of hypoglycaemic effects.[18] Results on the insulin release from pancreas directly indicate that the anti-diabetic activity of *A. monophylla* may be through the release of insulin from the pancreas.

The rise in blood glucose is accompanied with the increase in TC, TG, and LDL and fall of HDL. Considerable research has been shown that abnormal lipid metabolism is a important predictor for diabetes mellitus.[19] Moreover, the present study data is in line with earlier observations that alloxan-induced diabetic animals are associated with hypercholesterolaemia and hypertriglyceridaemia.[20] Administration of AMMt showed a significant reduction in serum levels of TC, LDL, TG and VLDL, whereas a significant elevation in HDL levels. It is extensively reported that increased in the HDL level is accompanied by increased catabolism of VLDL and substitution of TG in the core of HDL with TC.[19]

The progress of diabetes is associated with the close relationship between the increased free radicals and decreased antioxidant potential.[21] Numerous studies have been shown that alloxan leads to the formation of reactive oxygen species and produces the cytotoxic effect on the pancreatic cells.[22] Further, there are various reports showing that experimental diabetic animals reveal high oxidative stress due to persistent rise in blood glucose level, which consequently deplete the activity of the anti-oxidative defence system and hence promote free radical generation.[23] It is well reported that a chemical entity with the free radical scavenging property and antioxidant potential may inhibit cytotoxic effects of alloxan on β cells of pancrease.[24] Furthermore, the herbal plants that have been found to be rich in flavonoid content with antioxidant potential show the hypoglycaemic activity.[25,26] In our previous study, we have shown that AMMt is rich in flavonoid content and exhibited potential antioxidant activity.[9] In this regard, the anti-diabetic effect of AMMt may be due to the presence of more than one anti-hyperglycaemic principle and their synergistic properties.

In conclusion, the methanolic bark extract of *A. monophylla* showed potential anti-diabetic effect in experimental diabetes. Further studies are being carried out in our laboratory to establish and clarify the detailed mechanism(s) underlying the anti-diabetic effect of methanolic bark extract of *A. monophylla*.

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