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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *ANOGEISSUS LATIFOLIA* WALL IN SWISS ALBINO RATS

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ABSTRACT

The Anti-Diabetic activity of methanolic extract of *Anogeissus latifolia* Wall (combretaceae) was investigated in Alloxan induced diabetic albino rats and extract was compared with the standard using Metformin. *Anogeissus latifolia* is widely used in traditional system of medicine to treat diabetes in India. The Methanolic extract (300mg/kg) of leaves was taken to evaluate the antidiabetic activity against normal and alloxan induced diabetic rats. Oral administration of the methanolic extract for 21 days resulted in a significant reduction in blood glucose level. The extract has decreased the level of serum cholesterol, triglycerides, creatinine, urea and alkaline phosphatase significantly, but the level of HDL and total protein were found to be increased after treatments. Thus the study has shown the antidiabetic action of *Anogeissus latifolia*, and should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

Key words: *Anogeissus latifolia*, Anti-Diabetic activity, Alloxan, Acute oral toxicity.

INTRODUCTION

Anogeissus latifolia DC belonging to combretaceae family is a large or moderate sized tree which is available in dry deciduous forests and available throughout India. The tree has been studied for antioxidant activity, hydrogen donating ability, nitric oxide, super oxide scavenging activity and hydrogen peroxide decomposition activity[2]. Leaves are opposite or sub-opposite. Bark is smooth with grey- white colour and exfoliating in irregular thin scales. A variety of substance which contributes to hepatoprotective activity has been identified in the extracts of *Anogeissus latifolia* which includes tannins[3], gallic acid, ellagic acid and

flavanoids such as leutin, quercetin which are known as potential antioxidants. The bark of the plant has also reported to have several biological activities such as anti ulcer, anti microbial and wound healing activities[4]. The hydroalcoholic extract of *Anogeissus latifolia* has reported to have chemoprotective activity in paracetamol induced toxicity in rat model. Thus, the present study was undertaken for the investigation of analgesic activity of methanolic extract of *Anogeissus latifolia*[5, 6].

Collection and authentification of plant materials: The plant material was collected from Srisailam hills and a specimen was dropped in the herbarium and the leaves were authenticated by Professor Dr. Madhavachetty, S. V. University, Trupathi. The collected powdered material was shade dried and pulverized.

Solvent used for extraction: Petroleum ether and methanol.

Preparation of the extract: The dried powder of *Anogeissus latifolia* leaf was defatted with petroleum ether (60-80ºc) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with methanol using same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Phytochemical Screening: The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Test for reducing sugars (Fehling's test): The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for anthraquinones: The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test): To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids: A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins: About 0.5 g of the individual extract was boiledin 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl3) was added and observed for brownish green or a blue-black coloration.

Test for alkaloids: 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test): To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

MATERIALS AND METHODS

Drugs and Chemicals: Alloxan monohydrate was purchased from Vijay enterprizes, Kanchanbagh. All other chemicals used were of analytical grade.

Experimental Animals

Male Wister albino rats of body weight 140g-180g were obtained from National Institute of Nutrition, Indian council of medical research, Hyderabad, India. The study was approved by

the Institutional Animal Ethical Committee (1447/po/a/11/ CPCSEA). The animals were housed in groups of six and maintained in a well-ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under controlled temperature (26 ± 1°C) and humidity (30%–40%). They were fed with a standard pellet diet, and water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days.

Experimental Induction of Diabetes

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150mg/kg body weight. Blood samples were collected before the administration of alloxan and after 5 days of alloxan administration. Diabetic state was confirmed when the blood sugar level was above 200mg/dl. The rats with moderate diabetes and hypolipidemia were used for the experiment.

Drug Administration

After seven days of alloxan inductions, the Methanolic leaf extract (100, 250 and 300mg/kg) was administered orally using intragastric tube.

TREATMENT PROTOCOL

After the induction of diabetes the rats were divided into 5 different groups of 6 rats each.

Group-I - Control rats received normal saline and fed on normal diet.

Group-II - Diabetic control

Group-III - Diabetic rats received MEAL 100mg/kg b.w daily using an intragastric tube for 21 days.

Group-III - Diabetic rats received MEAL 250mg/kg b.w daily using an intragastric tube for 21 days.

Group-III - Diabetic rats received MEAL 300mg/kg body weight daily using an intragastric tube for 21 days.

Group-III - Diabetic rats received with standard drug Glibenclamide 100mg/kg b.w.

BIOCHEMICAL ANALYSIS

Estimation of Blood Glucose: Blood glucose was determined by the O-toluidine method[7].

Estimation of Total Cholesterol (TC): Total cholesterol level was determined by the commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman,India). It is based on (CHD_PAP) enzymatic methods.

Estimation of HDL-cholesterol: HDL-cholesterol level was determined by commercially available reagent kit(Erba Mannheim, Transansia biomed, Daman, India) based on phosphotungustate method.

Estimation of Triglyceride (TG): Triglyceride level was estimated by commercially available kit (ErbaMannheim, Transansia biomed, Daman, India). Its working is based on enzymatic colorimetric method. This reagent kit was made for *in vitro* quantitative determination of triglycerides in serum or plasma. Our study was carried out by serum[8].

Statistical Analysis

Data were expressed as mean±SE.Statistical analysis was done using one-way analysisof variance (ANOVA) followed by dunnett's t test comparison. Values were considered statistically significant when at $p<0.05$.

RESULTS

The blood glucose value on day 0 indicates the fasting blood glucose values levels in the experimental animals. The study revealed that there was no significant change ($p>0.05$) in the blood glucose levels in the groups administered with 100, 200 and 300 mg/kg and mg/kg b w of glibencamide after the 3rd day, Whereas, there was a significant reduction ($p<0.05$) on the blood glucose levels in the groups treated with the extract at dose extract concentrations of 300 mg/kg b w after the 6th day, when compared to the diabetic control group. The study also showed that administration of the extract at dose concentration of 100, 200 and 300 mg/kg and glibencamide 250 mg/kg b w, resulted to a significant decrease ($p<0.05$) in the blood glucose level after 9th, 12th, 15th, 18th and 21 day, when compared to the diabetic control group, with a maximum reduction ($p<0.01$) in the blood glucose levels recorded with the extract 300 mg/kg b w after the 21st day and Metformin after 15th, 18th, and 21st day when compared to the diabetic control group (Table 1).

DISCUSSION

Alloxan has been shown to induce free radical generation and cause tissue injury. The pancreas is especially susceptible to action of alloxan induced free radical damage. Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of langerhans, there by inducing hyperglycaemia. The results in present study indicate that Methanolic extract of *Anogeissus latifolia* MEAL leaf extract was found to reduce the glucose level in animals made diabetic with alloxan.

Table – 1 Effect of Methanolic extract of *Anogeissus latifolia* leaves *on* blood glucose levels in alloxan induced diabetes mellitus in rats.

Groups	Treatment	Dose mg/Kg	Fasting blood glucose level (mg/dL)							
			0 Day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day
1	Diabetic Control	1 mL D W	406.83±52.1	409.50±22.6	404.50±29.6	407.33±21.7	416.50±29.0	402.67±22.8	424.00±29.7	427.17±22.3**
2	Alloxan+ MEAL	100	403.50±33.1^{ns}	352.50±30.2^{ns}	315.17±22.9*	288.33±22.4*	258.83±24.2*	221.83±18.1*	200.67±18.4*	161.83±18.2**
3	Alloxan+ MEAL	200	407.97±40.7^{ns}	353.17±45.7^{ns}	280.67±38.2*	263.33±30.3*	231.17±27.7*	210.17±25.2*	174.33±19.2*	153.67±13.8
4	Alloxan+ MEAL	300	414.83±55.5^{ns}	303.50±34.6^{ns}	251.33±26.9*	213.50±20.7**	194.65±21.2*	169.83±13.6*	151.33±12.2**	133.83±10.4**
5	Glibencamide	100	412.33±40.8^{ns}	345.00±28.8^{ns}	299.17±22.3*	261.83±19.4*	264.33±16.4*	179.50±7.7*	160.17±12.2**	144.50±11.6**

Values are statistically significant compared to control group at * $p<0.01$; ns: not significant; Values are presented as mean ± S.E.M

Table 2 Effect of *Anogeissus latifolia* leaf extract on biochemical parameters in alloxan induced diabetic rats

Group	Dose	Cholesterol	Triglycerides	Creatinine	Urea
Normal control	1 ml/kg	146.8±1.79	83.17±1.01	0.58±0.09	20.8±1.07
Diabetic control	---	258.7±0.88*	255.5±1.54*	1.92±0.094*	79.5±0.88*
MEAL I	100mg/kg	156.0±0.89*	139.5±1.38*	0.69±0.008*	32.6±1.05*
MEAL II	200mg/kg	249.8±1.167*	138.7±2.06*	0.66±0.007*	36.5±1.05*
MEAL III	300mg/kg	147.7±2.14*	141.7±1.45*	0.63±0.07*	39.3±1.8*
Glibencamide	100mg/kg	120.2±1.42*	101.7±1.22*	0.42±0.01*	30.8±0.9*

Values are statistically significant compared to control group at * p<0.01 ns: not significant; Values are presented as mean ± S.E.M

Table 3 Effect of *Anogeissus latifolia* leaf extract on biochemical parameters in alloxan induced diabetic rats

Group	Dose	ALP	HDL	TP
Normal control	1 ml/kg	116±0.96	33.5±0.76	8.2±0.07
Diabetic control	---	363.3±1.25*	27.1±0.60*	3.86±0.08*
MEAL I	100mg/kg	157.5±0.76*	45±1.15*	4.6±0.05*
MEAL II	200mg/kg	155.5±0.76*	46.8±0.87*	4.7±0.57*
MEAL III	300mg/kg	150.5±0.76*	51.5±0.24*	4.9±0.57*
Glibencamide	100mg/kg	109.5±0.76*	64±0.96*	8.4±0.05*

Values are statistically significant compared to control group at * p<0.01; ns: not significant;

Values are presented as mean ± S.E.M

Table 4 Effect of *Anogeissus latifolia* leaf extract on body weight in alloxan induced diabetic rats

Group	Dose	0	5^{th}	10^{th}	15^{th}
Normal control	1 ml/kg	25.8±1.42	25.6±0.88	31.5±0.42	28±0.36
Diabetic control	---	30.5±1.14*	28.8±0.7*	27.1±0.47*	26±0.36*
MEAL I	100mg/kg	30.8±1.16*	31.3±0.91*	27.3±0.49*	26±0.57*
MEAL II	200mg/kg	32.3±1.25*	30.1±0.79*	28.3±0.49*	30±0.73*
MEAL III	300mg/kg	25.6±0.49*	24.3±0.8*	26±0.36*	27.8±0.54*
Glibencamide	100mg/kg	24.8±0.79*	28.3±0.71*	28.8±0.6*	30±0.57*

Values are statistically significant compared to control group at * p<0.01; ns: not significant;

Values are presented as mean ± S.E.M

The results from the study also indicate that MEAL leaf extract can reduce the levels of serum urea, serum creatinine, serum cholesterol, and increase the serum protein and confirms the possibility that the major function of the extract are on the production of vital issues including pancreas, thereby reducing the causation of diabetes in the experimental animals. Overall results showing the antidiabetic activity of MEAL leaves, the activity may be due to presence of chemical constituents like flavonoids, terpenoids in leaves. Therefore, alloxan-induced diabetes is one of the frequently used models for the study of IDDM in experimental animals. The present study evaluated the effects of MEAL on blood glucose levels in Alloxan-induced diabetic Wister rats. The mechanism by which the extract exert the hypoglycemic effect appear to be related to the presence of flavonoids among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituents in a group or as an individual responsible for the hypoglycemic activity of the plant extraction.

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