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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF ANOGEISSUS LATIFOLIA WALL IN SWISS ALBINO RATS BY USING STREPTOZOTOCIN INDUCED DIABETIC MODEL

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ABSTRACT

The Anti-Diabetic activity of methanolic extract of Anogeissus latifolia Wall (combretaceae) was investigated in streptozotocin induced diabetic albino rats and extract was compared with the standard using glibenclamide. Anogeissus latifolia widely used in traditional system of medicine to treat diabetes in india. The Methanolic extract 300mg/kg of leaves were taken to evaluate the antidiabetic activity against normal and streptozotocin induced diabetic mice. Oral administration of the extracts for 21 days resulted in a significant reduction in blood glucose level. The extracts on serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatise levels were decreased. Significantly by all the extracts and glibenclamide but HDL levels and total protein were found to be increased after treatments. Thus this study shows that anogeissus latifolia has antidiabetic action and the extracts 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, streptozotocin, 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 in the month of June 2011 from Srichalam hills and a specimen was dropped in the herbarium and the leaves was authenticated by Professor Dr. Madhavachetty S. V. University, Trupathi. The collected powdered material was shade dried and pulverized.

Solvent used for extraction: Petrolium ether and methanol.

Preparation of the extract: The dried powders of leaf of Anogeissus latifolia were 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 with 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 indica tes 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: Streptozotocin, Glibenclamide,Tween-80,was purchased from BDH Chemicals, Poole, England. 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, hyderaded, india and the study approved by the ethical committee (1447/po/a/11/ CPCSEA). The animals were maintained at animal house and fed a standard diet. (Hindustan Lever, Mumbai, India) and water ad libitum.

Drug Administration

After seven days of Streptozotocin inductions, the Methanolic leaf extract was administered orally through intragastric tube at the following dose 100, 250 and 300mg/kg body weight.

Experimental induction of diabetes

The rats were injected intraperitoneally with Streptozotocin dissolved in sterile normal saline at a dose of 150mg/kg body weight. Blood samples were collected before the administration of Streptozotocin and after 5 days of Streptozotocin 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.

Animal Allotment

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 wasdetermined 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 1 estimated by commercially available kit (Erba Mannheim, 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.

Statistical Analysis: Data were expressed as mean±SE. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by dunnett's test comparison. Values were considered statistically significant when at $p<0.05$.

Table – 1 Effect of Methanolic extract of *Anogeissus latifolia* leaves on blood glucose levels in Streptozotocin 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 ±33.1ns	352.50 ±30.2ns	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 ±40.7ns	353.17 ±45.7ns	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 ±55.5ns	303.50 ±34.6ns	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 ±40.8ns	345.00 ±28.8ns	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 Streptozotocin induced diabetic rats

Group	Dose	Cholesterol	Triglycerides	Creatinine	Urea
Normal control	1 ml/kg	106±0.9309	81.00±1.983	0.933±0.4193	29.50±0.7638
Diabetic control		176.8±2.040	252±0.5774	1.417±0.07032	61.00±1.183
MEAL I	100mg/kg	155.3±1.145	135±0.5774	1.100±0.09661	50.50±0.7638
MEAL II	200mg/kg	146±1.065	133±0.5774	0.7500±0.7638	43.67±1.116
MEAL III	300mg/kg	135.2±1.515	138±0.7071	0.5500±0.07638	50.67±1.838
Glibencamide	100mg/kg	121±0.8944	99±0.5774	0.6500±0.07638	33.00±0.9661

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 Streptozotocin induced diabetic rats

Group	Dose	ALP	HDL	TP
Normal control	1 ml/kg	112±0.5774	30.00±0.5774	6.00±0.05773
Diabetic control		360.00±0.9309	25.00±0.9309	4.750±0.1057
MEAL I	100mg/kg	155.00±0.5774	41.83±0.6009	5.700±0.09661
MEAL II	200mg/kg	152.00±0.5774	43.00±0.5774	6.00±0.05773
MEAL III	300mg/kg	147.00±0.5774	45.17±0.6009	5.983±0.06009
Glibencamide	100mg/kg	106.00±0.8165	60.17±0.6009	6.050±0.07638

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 Streptozotocin induced diabetic rats

Group	Dose	0	5^{th}	10^{th}	15^{th}
Normal control	1 ml/kg	202.00±0.5774	202.8±0.6009	205.3±0.7601	209.0±0.3651
Diabetic control		203.00±0.3651	171.2±0.4773	157.8±0.6009	146.3±0.8819

MEAL I	100mg/kg	206.00±0.3651	177.5±0.7638	162.0±0.3651	152.2±0.7491
MEAL II	200mg/kg	205.00±0.5774	198.5±0.7638	191.0±0.3651	182.2±0.6009
MEAL III	300mg/kg	203.00±0.5774	201.4±0.4282	200.0±0.7303	190.0±0.5774
Glibencamide	100mg/kg	206.00±0.3651	202.2±0.6009	197.0±0.3651	191.0±0.3651

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

REFERENCES

1. K. Chandrasekaran* and L. Suseela “Analgesic activity of ethanolic extract of Pentatropis capensis and sarcostemma secamone. IJPBS, vol.2, issue. 4, 2011, 74-77.
2. K. C. Audichya, K. V. Billore, T. G. Joseph, *et al.* Roll of indigenous folk remedies for certain acute illness in primary health care. *Nagarjun*, 1983; 25:199-201.
3. Austin, D. F. and Bourne, G. R. (1992). *In vitro* cell development. *Biol Plant.*, 33:111-113.
4. G. J. Martinez. Ethnobotany- A method manual. Chapman and Hall, London, 1995; Pp268.
5. P. V. Orwa, Agroforestry database 2006; 4.0, Pp1-5
6. P. K.Warrier. Indian medicinal plants: A compendium of 500 species. 1994; 4:3811
7. Ravi , K.S Rajasekaran, S. Subramanian, Antihyperglycemic effect of Eugenia jambolana seed kernel on streptozotocin-induced diabetes in rats. Food chem. Toxicol, 2005, 43, 1433-1439.