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Anti-Hyperglycemic Effect of Aqueous and Ethanolic Extracts of Leaf and Stem Bark of *Alangium Salvifolium* (L.F.) Wang in Alloxan Induced Diabetic Rats

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Abstract

In present study anti hyper glycemic potential of aqueous and ethanolic extracts of *Alangiumsalvifolium* were estimated at dose of 250 mg/Kg body weight in alloxan induced hyperglycemic rats. The effect of aqueous and ethanolic extracts of *Alangiumsalvifolium* was estimated on 28 th day. A marked decrease in the blood sugar level ($p< 0.001$) was observed in hyperglycemic rats upon *Alangiumsalvifolium* treatment. The plasma insulin, blood glucose, glycogen content and total heamoglobin were also estimated. Preliminary qualitative analysis shows the presence of alkaloids, glycosides, tannins, phenolic compounds, triterpenoids, fixed oils, fats and flavonoids. The results suggest that aqueous and ethanolic extract of *Alangiumsalvifolium* have promising anti hyperglycemic action in alloxan induced rats.

Key words: *Ethanolic and Aqueous extract, Anti hyper glycemic, Alangiumsalvifolium.*

Introduction

Diabetes mellitus is a global problem, which has long been recognized in the history of medicine. Before the advent of insulin and hypoglycemic drugs, the major treatment involved the use plants. More than 400 plants are known to have been recommended and recent investigations have affirmed the potential value of some of these treatments. Present study aims to open avenues for the improvement of medicine use of indigenous plant *Alangiumsalvifolium* in the selected area for diabetes mellitus. Study aims the effect of aqueous and ethanolic extract of *Alangiumsalvifolium* on plasma insulin, blood glucose, glycogen content and total heamoglobin.

The plant *Alangiumsalvifolium* , Family: *Alangiaceae.*The root of the plant have been used in Skin diseases, Astringent, Anthelmintic, Purgative, Emetic,Diaphoretic, Antipyretic and Anti – tubercular properties[1-6]. Rootbark is an antidote for several poisons. Root bark is very bitter and is reputed as a cure for skin diseases. The stem bark of the plant exerts a biphasic action and possesses marked hypotension in higher dose .The leaves of the plant was used as Anti-protozoal , Hypoglycemic, spasmolytic**.** The leaves are applied as poultice in rheumatism.Fruits are sweet, cooling, and purgative and used as a poultice for treating burning sensation and haemorrhage.

Experimental

Plant material

The Plant *Alangiumsalvifolium* (L.f.) wang was collected from Alagarkovil Hills, Madurai during the month of May. Then was identified by Dr. Stephen Msc. Ph.D,Department of Botany, The American College, Madurai-2.

Preparation of the extract

The leaves and stem bark were cut off and dried in shade for 45 days. Then about 3 kg of the shade dried leaves and stem bark was made in to coarse granules. The dried powder material of *Alangiumsalvifolium*were defatted with petroleum ether in a soxhlet apparatus. The defatted powder material was obtained and furtherextracted with ethanol and aqueous for 72 hrs in soxhlet apparatus.The resulting semisolid mass was dried and used for phyto chemical analysis and hyper glycemic activity.Preliminary phyto chemical investigation[7-10] was carried out and results are tabulated 1.

Animals

Albino rats (180-220gm) of both sexes were obtained from the experimental facility of the college. Before the start and during the experiment they were acclimatized for a period of 2-3 days in

the new environment before initiation of experiment. Animals described as fasting had been deprived of food for at least 16 hr but had been allowed free access to drinking water.

Experimental design

In the pilot studies lasting for 4 weeks ,albino wistar rats were made diabetic by a single IP injection of alloxan monohydrate (150 mg/kg) in saline [11].Alloxan is commonly used to produced diabetic mellitus in experimental animals due to its ability to destroy the beta cells of pancreas possibly by generating excess reactive oxygen species such as H_2O_2,O_2, and HO [12].The development of hyperglycemia in rats was confirmed by plasma glucose estimation 72 h post alloxan injection. The rats with fasting plasma glucose level of 75mg/dl were included in the study. In the experiment a total of 30 rats (24 diabetic surviving rats, six normal rats) were used .The rats were divided in to 5 groups, Group-1 served as normal control,

Group-2 served as diabetic control, Group-3 served as positive control received glibenclamide (600 µg/kg) in aqueous solution daily using intragastric tube for 21 days[13]. Group-4 served as treatment control received ethanolic extracts of *Alangiumsalvifolium*(l.f.) wang (eeas) at a dose of 250mg/kg dissolved with normal saline, daily using intra gastric tube for 28 days. Group-5 served as treatment control received aqueous extracts of A*langiumsalvifolium* (l.f.) wang (aeas) at a dose of 250mg/kg dissolved with normal saline, daily using intra gastric tube for 28 days.

After 28 days of treatment ,blood was collected retro orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes.Blood was collected in fresh plasma tubes coated with EDTA, and Plasma was separated in a T8 electric centrifuge (Remi,Newdelhi) at 2000 rev/min for 2 min. Then animals were killed by decapitation ,liver tissue samples were collected for the assessment of plasma glucose ,hepatic glucokinase ,hexokinase ,glucose -6- phosphate and along with glycogen content in liver tissues.

Biochemical analysis

Estimation of blood glucose

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson based on glucose oxidase method[14].

Plasma insulin:

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit [15] with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of total haemoglobin and glycosylated haemoglobin:

Total haemoglobin was determined by the method of Drabkin and Austin [16] and glycosylated haemoglobin was determined by the method of SudhakarNayak and Pattabiraman[17].

Hepatic glucokinase and hexokinase activity

The liver was per fused with ice cold 0.15M KCL and 1mM EDTA solution and homogenized with twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1 ml Tris –HCl, PH 7.40 and glucose -6- phospahte dependent spectro photometric method [18]. The liver was homogenized with 40 times its weight of ice cold buffer (0.1citrate –KOH, pH6.5) and filtered through cheese cloth. Glucose -6-phosphatase activity was measured by phosphate release by the method of Marjorie.The calorimetric method for determination of phosphoricacidconcentration in the supernatant of the assay mixture was employed[19].

Glycogen content

The tissue sample was digested by hot concentrated 30% KOH and treated with anthrone reagent. Glycogen content was determined colorimetrically as glucose.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at $p<0.001$[20].

Results and Discussion

In all groups prior to alloxan administration, the basal levels of plasma glucose of the rats were not significantly different. However, 72hrs after alloxan administration , plasma glucose levels were significantly higher in the rats selected for the study. In contrast Non-diabetic control remained persistently glycemic throughout the study. Table 2 shows the effect of treatment with EEAS and AEAS respectively, on plasma glucose levels. In the treated groups G4 & G5 significant anti-hyperglycemic ($P<0.01$) effect was evident from the first week onwards, the decrease in plasma sugar was maximum on completion of the 4th week ($P<0.001$)in the group receiving 250mg/kg of EEAS and AEAS respectively.

In table 3 illustrates the levels of plasma insulin, total haemoglobin and glycosylated haemoglobin in normal and experimental animals in each group. In G2 treated animals plasma insulin, and total haemoglobin levels were decreased to 10.6 ± 0.85*, 6.12 ± 0.75* respectively and glycosylated haemoglobin level was increased in diabetic animals as compared to normal control animals. However the levels of plasma insulin, Hemoglobin and ,glycosylated hemoglobin returned to near normal range in diabetic rats treated with EEAS

and AEAS at 250mg/kg and diabetic rats treated with glibenclamide.

Glycogen Content

Glycogen content of liver tissues was estimated in control, diabetic control, positive control and treatment control groups as shown in table no 3.In diabetic controls hepatic glycogen content decreased significantly as compared to non-diabetic controls. Treatment with both extracts increase the hepatic glycogen content.

Hepatic Enzymes

To establish diabetes, plasma glucose was determined 72 hrs after alloxan administration, only those mice with over 180mg%were included in the study. On the 28 day, hepatic enzymes (hexokinase, glucokinase and substrate (Glucose -6-phosphate) were estimated in all groups treated animals. The results have been complied in table-3, as compared to non-diabetic control values, mean levels of enzymes(hexokinase, glucokinase and substrate(Glucose -6-phosphate)values decreased in the diabetic controls. Treatment with both extracts increases these parameters nearby normal values.

Table 1: Preliminary phytochemical screening of the various extracts of *alangium salvifolium* (l.f.) wang

Consituents	PEEAS	EEAS	AEAS
Carbohydrates	-	+	+
Glycosides	+	+	+
Alkaloids	+	+	+
Phytosterols	-	+	-
Saponins	+	+	+
Fixed oils & fat	+	+	-
Tannins	+	-	-
Protein & Amino Acids	+	-	-
Flavonoids	-	+	+
Coumarins	+	+	+
Flavones	+	+	+

\+ - Indicate positive test results .
\- - Indicate negative test results.
These crude extract were also used for the exhibition of some selective pharmacological properties.

Table 2: The effect of 4 weeks treatment with extracts of EEAS and AEAS on glucose levels (mg%)in alloxan induced diabetic rats.

Groups	0 Day	14th day	28 day
Group I Normal control	59.16 ± 2.26	61.07 ± 2.2	60.4 ± 1.9
Group II Toxic control diabetic	155.4 ±4.06*a	182.6 ± 5.1*a	182.4 ± 3.2*a
Group III Positive control	159.3 ± 4.58	122.6 ± 3.6	88.4 ±2.10
Group IV Treatment control	164.4 ± 4.80*b	146.4 ± 4.2*b	106.5 ±4.6*b
Group V Treatment control	161.6 ± 3.80*b	138.6 ± 5.1*b	121.2 ±3.9*b

*values were expressed as mean ± SEM
*a-- Values were significantly different from normal control at P<0.001
*b-- Values were significantly different from diabetic control at P<0

Table 3: Effect of EEAS and AEAS on plasma insulin, Heamoglobin and glycosylated haemoglobin in normal and treated animals

Group	Plasma insulin µ/ml	Haemoglobingm/100ml	Glycosylated haemoglobin HbA_1%
Group I	24.6 ±1.55	12.25 ± 1.10	0.25 ± 0.04
Group II	10.6 ± 0.85* a	6.12 ± 0.75* a	0.96 ± 0.09* a
Group III	19.4 ±1.05	11.7 ± 1.06	0.37 ± 0.07
Group IV	15.6 ± 1.04*b	8.16 ± 0.75*b	0.50 ± 0.10*b
Group V	16.02 ± 1.40*b	8.42 ± 0.65*b	0.47 ± 0.12*b

*values were expressed as mean ± SEM
*a-- Values were significantly different from normal control at P<0.001
*b-- Values were significantly different from diabetic control at P<0.001

Table 4: Effect of EEAS and AEAS on enzymes in carbohydrate metabolism and Glycogen content in liver tissues

Group	Glycogen µg/g(tissue)	Hexokinase µg/mg	G-6-P µg/mg	Glucokinase µg/mg
Group I	43.48 ± 2.61	0.194 ± 0.011	0.382 ± 0.060	24.20 ± 1.75
Group II	8.92 ± 1.56* a	0.084 ± 0.007* a	0.160 ± 0.010* a	5.17 ± 0.90* a
Group III	26.41 ± 4.8	0.146 ± 0.019	0.288 ± 0.020	16.40 ± 2.45
Group IV	22.45 ± 5.4* b	0.114 ± 0.005* b	0.224 ± 0.011*b	12.16 ± 1.87*b
Group V	20.45 ± 2.8* b	0.116 ± 0.008*b	0.234 ± 0.014*b	10.50 ± 1.45* b

*values were expressed as mean ± SEM
*a-- Values were significantly different from normal control at P<0.001
*b-- Values were significantly different from diabetic control at P<0.001

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