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Anti diabetic activity of Ethanolic Extract of *Polyalthia Suberosa Roth in* Alloxan induced Induced rats

Saritha Chandra*, M. Divya Sigh, K. Madhu, D. Lakshmi Prasanna, P. Reshma Sai, V. Mary

Jagan's college of Pharmacy, Jangala Kandriga, Nellore, Andhra Pradesh, India

A B S T R A C T

The present study was conducted to investigate the anti-diabetic activity of *Polyalthia Suberosa Roth* and its synergistic effect with Glibenclamide in Alloxan induced diabetes in rats. *Polyalthia Suberosa Roth* at a dose of 200 and 400 mg/kg were selected for the investigation of antidiabetic activity alone with Glibenclamide. Diabetes was induced by s.c. injection of alloxan monohydrate (150 mg/kg) in healthy male albino wistar rats. Acute and sub acute Serum glucose were evaluated for the assessment of antidiabetic activity of *Polyalthia Suberosa Roth*. *Polyalthia Suberosa Roth* alone with Glibenclamide and decrease the serum glucose, cholesterol and increase the HDL levels. *Polyalthia Suberosa Roth* alone showed very significant antidiabetic activity on 10th day of the treatment and blood glucose level came to normal on 15th day of treatment.

Keywords: *Polyalthia Suberosa Roth*, anti-diabetic activity, Alloxan induced diabetes, Glibenclamide, serum glucose level

A R T I C L E I N F O

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***Corresponding Author**
Saritha Chandra
Jagan's college of Pharmacy,
Jangala Kandriga, Nellore,
Andhra Pradesh, India
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1. Introduction

Diabetes mellitus is a chronic major endocrine disorder and growing health problem in most countries and is characterized by hyperglycemia, hyperlipedimia, negative nitrogen balance and sometimes ketonemia[1]. Diabetes is one of stress related disorder. Diabetic subjects are shown to have increased oxidative stress and decreased anti oxidant levels. Antioxidants are claimed to work as antistress agents by decreasing oxidative stress[2]. Hence there is an increasing interest in herbal remedies. According to WHO herbal medicines are defined as finished, labeled

medicinal products that contain active ingredients, aerial or underground part of plants or other plant material or their combinations.[3] The annual herbal sales have skyrocketed and the global traditional market is growing at a rate of 7-15% annually. In the present scenario, herbal drugs are claimed for almost every disorder ranging from diabetes to rejuvenators.[4]Diabetes mellitus (DM) is the most common endocrine disorder. It affects more than 100 million people worldwide and its incidence is increasing steadily with changes in life styles.[5] it is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. Hyperglycaemia result from an absolute deficiency of insulin caused by pancreatic β-cell destruction or by a combination of peripheral resistance to insulin action and an inadequate secretary response by the pancreatic β-cells.[6] *Polyalthia Suberosa*, a plant drug of traditional systems of medicine in India i.e. Ayurveda and Siddha is used for the treatment of diabetes mellitus. An effort to pharmacologically evaluate the plant for its anti-diabetic and antioxidant property is done in the present study.[7]

2. Materials and Methods

Collection and authentication of plant material

The plant material (Leaves) of *Polyalthia Subarosa* was collected from tirumala hills and its authentication was done by an expert taxonomist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Thirupati, Andhra Praesh, India.

Preparation of Ethanolic extract of the leaf of *Polyalthia Suberosa* Roth. (EEPS): The collected plant materials were dried in shade for about 15 days, made in to coarse powder with the use of mixer grinder. Powdered plant material was extracted with and Ethanol in soxhlet apparatus for 24 hours.[8] The extracts were collected and concentrated under reduced pressure to a semisolid mass. The extracts obtained dried in desicator.[9] The dried extracts weighed and the percent yield was calculated.

Experimental animals

Inbred adult wistar albino rats (150-280 g) of either sex were obtained from animal house of Central Animal House Registration No: 769/2011/CPCSEA and the research work was carried out at same University. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided *ad libitum* throughout experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Fasting refers to that the animals were deprived of food for 16 hours but were allowed to free access for water. [10]

Preliminary phytochemical analysis:

The ethanolic extract of leaves were subjected to different chemical tests separately for the identification of various active constituents.[11]

Acute oral toxicity study

The acute oral toxicity study was done according to OECD 423 guide lines (Acute toxicity class method). A single administration of a starting dose of 2000 mg/kg bw/p.o, of EEPS was administered to 3 male rats and observed for 14 days.[12]

Experimental procedure [13]

Wistar rats of male sex weighing 150-280g were used for the study. The starting dose level of EEPS was 200mg/kg body weight p.o. As most of crude extracts possess LD50 value more than 2000mg/kg p.o. Dose volume was administered 0.5ml/100mg body weight to overnight fasted rats with 0.5%w/v SCMC. Food was with held for further 3-4 hours after administration of EEPS and observed for signs of toxicity. Body weight of rats before and after termination were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system and somato motor activity and behavior pattern were observed, and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

Pharmacological study:

Induction of diabetes mellitus in experimental animals:

Adult inbred male wistar albino rats (32 numbers) of either sex were overnight fasted and received a freshly prepared solution of alloxan, (S.d.fine chemicals Ltd), (150 mg/kg) in distilled water injected intraperitonially in a volume of 1 ml/kg .After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. Normal rats (6 numbers) received 1ml distilled water as vehicle. The development of diabetes was confirmed after 48 hours of the alloxan injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 32 animals subjected for diabetes induction, 6 animals died before grouping and two animals were omitted from the study, because of sub diabetic condition (118mg/dl) and (122mg/dl). Of the remaining 24 animals,4 groups of 6 animals were formed and used for the experimentation.[14]

Determination of the blood glucose levels

Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (Accuchek Ltd) based on glucose oxidase method.

Effect of EEPS on normoglycaemic and glucose fed-hyperglycaemic rats [NG-OGTT][15]

A combined methodology is preferred for the activity assessment of extract in order to avoid wasting animals; there are some modifications incorporated in the time pattern for blood glucose level determination. After overnight fasting (16 h) the blood glucose level of rats were determined and then were given the test samples and standard. The animals were divided in to four groups of 6 rats in each.

Group I: Animals received distilled water.

Group II: Animals received glibenclamide 0.4mg/kg b.w /p.o.

Group III: Animals received EEPS 200mg/kg b.w/ p.o.

Group IV: Animals received EEPS 400mg/kg b.w /p.o.

Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following pattern: 30 and 60 min to access the effect of test samples on normoglycaemic animals. The rats were then loaded orally with 2g/kg

glucose and the glucose concentrations were determined at 60, 90 and 210 min after glucose load.

Effect of sub-acute treatment of EEPS on changes in body weight in Alloxan induced diabetic rats [16]

The animals were divided in to 5 groups .Group I consists of normoglycaemic rats. The remaining 4 groups consisted of 6 Alloxan induced diabetic rats.

Group I: Normal control animals receiveddistilled water,

Group II: Alloxan (150 mg/kg b.w) induced animals received distilled water,

Group III: Alloxan (150 mg/kg b.w) induced animals received glibenclamide 0.4 mg/kg b.w/ p.o for 14 days,

Group IV:Alloxan (150 mg/kg b.w) induced animals received EEPS 200 mg/kg b.w/ p.o for 14days,

Group V: Alloxan (150 mg/kg b.w) induced animals received EEPS 400 mg/kg b.w p.o for 14 days.

The above mentioned treatment schedule was followed for the respective group of animals for 14 days. Changes in the body weight was on 0 , 10th and 15th day of treatment.

Effect of sub-acute treatment of EEPS on blood glucose level in Alloxan induced diabetic rats[17]

The animals were divided in to 5 groups .Group I consists of normoglycaemic rats. The remaining 4 groups consisted of 6 Alloxan induced diabetic rats.

Group I: Normal control animals received distilled water,

Group II: Alloxan (150 mg/kg b.w) induced animals received distilled water,

Group III: Alloxan (150 mg/kg b.w) induced animals received glibenclamide 0.4 mg/kg b.w/ p.o for 14 days,

Group IV: Alloxan (150 mg/kg b.w) induced animals received EEPS 200 mg/kg b.w /p.o for 14days.

Group V: Alloxan (150 mg/kg b.w) induced animals received EEPS 400 mg/kg b.w/p.o for 14 days.

The above mentioned treatment schedule was followed for the respective group of animals for 14 days. Blood samples were collected from overnight fasted animals on 0th, 10th and 15th day to estimate blood glucose levels using glucometer.

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dunnet test using computer based fitting program (Prism graph pad 5.3). Statistical significance was set accordingly.

3. Results and Discussions

Preliminary phytochemical analysis of ethanolic extract of leaf of *Polyalthia Suberosa* Roth: The result of preliminary phytochemical analysis of ethanolic extract of leaf of *Polyalthia Suberosa* Roth. showed the presence of flavonoids and steroids is shown in **Table No 1.**

Acute oral toxicity study: The acute oral toxicity study was done according to OECD 423 guide lines (Acute toxicity class method). A single administration of a starting dose of 2000 mg/kg bw/p.o, of EEPS was administered to 3 male rats and observed for 14 days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. The results are shown in **Table No. 2.**

Effect of EEPS on blood glucose levels in normoglycaemic and glucose induced hyperglycaemic rats. [NG-OGTT][18]:

The EEPS at a dose level 200 mg/kg b.w/p.o did not exhibit significant hypoglycaemic effect in fasted normal rats after 30 minutes of administration and a high dose of 400mg/kg b.w/p.o reduced blood glucose in normal rats significantly after 60 min of drug administration ($p<0.01$). In the same group of rats which are loaded with glucose (2gm/kg b.w/p.o) after 60 min of drug administration a low dose of 200mg/kg bw reduced blood glucose level with less significance ($p<0.05$) but a high dose of 400mg/kg/b.w reduced blood glucose significantly ($p<0.01$) .The standard drug glibenclamide (0.4 mg/kg b.w/p.o) treatment showed significant reduction in blood glucose levels in both normal and glucose induced hyperglycaemic rats ($p<0.01$). Results are shown in **Table No. 3** and **Figure No. 1.**

Effect of sub acute treatment of EEPS on body weight changes in Alloxan induced diabetic rats[19]

The EEPS at oral dose level of 200mg/kg do not show significant improvement in the body weight of Alloxan induced diabetic rats on 10th day of the treatment and shows a slight significance in the body weight improvement on 15th day ($p<0.05$). An oral dose of 400mg/kg b.w shows significant improvement in the body weight of Alloxan induced diabetic rats on 10th day and 15th day of treatment ($p<0.01$). The standard drug glibenclamide (0.4 mg/kg b.w /p.o) also produced significant improvement in body weight of Alloxan induced diabetic rats.($p<0.01$) .Results are shown in **Table No. 4** and **Figure No. 2.**

Effect of sub-acute treatment of EEPS on blood glucose level in Alloxan induced diabetic rats[20]

In the sub-acute study, Alloxan induced diabetic rats were treated with EEPS 200mg and 400 mg/kg b w.t /p.o for duration of 14 days. Treatment with EEPS 200mg significantly ($p<0.01$) decreased the blood glucose level after 14th day onwards. Treatment with EEPS 500mg produced a significant ($p<0.01$) drop in blood glucose level after 10th day onwards. Treatment with glibenclamide 0.4 mg/kg produced a significant ($p<0.01$) decrease in blood glucose level after 10th onwards and thereafter. Results are shown in **Table No. 5** and **Figure No.3.**

Table 1: Phytochemical Screening of Ethanolic extract of leaf of *Polyalthia Suberosa* Roth**.**

S.No.	Constituents	EEPS
1.	Alkaloids	-Ve
2.	Carbohydrates	-Ve
3.	Protein	-Ve
4.	Steroids	+Ve
5.	Phenols	-Ve
6.	Tannins	-Ve
7.	Flavanoids	+Ve
8.	Gums and Mucilage	-Ve
9.	Glycosides	-Ve
10.	Sterols	-Ve
11.	Saponins	-Ve
12.	Terpenes	+Ve

Table 2: Acute oral toxicity studies (OECD 423 guideline)

S. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	EEPS	2g/kg	160	170	No signs of toxicity	Nil	Nil	14 days
2.	EEPS	2g/kg	164	175	No signs of toxicity	Nil	Nil	14 days
3.	EEPS	2g/kg	165	180	No signs of toxicity	Nil	Nil	14 days

Table 3: Effect of EEPS extract of whole plant on blood glucose in alloxan induced diabetic rats [NG-OGTT]

Groups	Test Sample (mg/kg)	Blood glucose levels (mg/dl)					
		0 min	30 min	60min (glucose load)	120min	150min	270min
I	Control	75.38±1.8	80.2±2.3	75.9±0.8	127.18±0.51	100.23±0.55	78.23±0.24
II	Std-0.4	73.11±2.7	50.9±1.7**	41.88±0.6**	90.8±0.3**	71.5±0.52**	55.8±0.52**
III	EEPS-200	65.5±1.2	70.2±2.3ns	61.85±0.71*	122.28±0.5ns	83.01±0.45*	62.46±0.65*
IV	EEPS-400	74.7±1.9	72.01±0.8ns	59.03±0.27**	110.15±0.5*	75.95±0.5**	60.6±0.52**

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. The blood glucose values of group II, III and IV are compared with control animal values. *-p< 0.05, **-p< 0.01, ns-non significant .

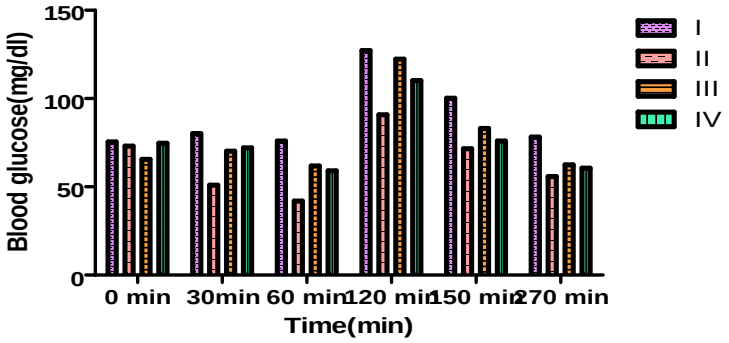

Figure 1: Effect of EEPS extract of whole plant on blood glucose in alloxan induced diabetic rats [NG-OGTT]

Table 4: Effect of sub-acute treatment of EEPS on body weight changes in alloxan induced diabetic rats

Group	Treatment	Dose (Kg^{-1} bwt)	Body weight (gm)		
			0th Day	10th Day	15th Day
I	Control(Distilled water)	5 ml	192.45±1.24	196.92 ± 1.2	201.5 ±1.5
II	Disease control(Alloxan)	150mg	210.24±0.99	167.89±1.4**	153.5±0.7**
III	Standard(Glibenclamide+Alloxan)	0.4mg	183.13±2.64	185.47±3.2*	187.9±1.5**
IV	Test I (EEPS+Alloxan)	200mg	197.62 ± 4.3	166.6±5.4ns	173.2± 4.2*
V	Test II (EEPS+Alloxan)	400mg	189.92 ± 1.7	175.6 ±1.3*	187.6±4.1**

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test.The body weights of group II, III and IV are compared with control.*-p< 0.05, **-p<0.001, ns-non significant.

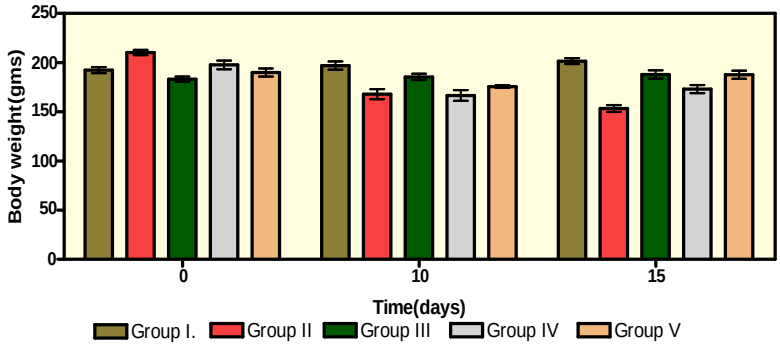

Figure 2: Effect of sub-acute treatment of EEPS on body weight changes in alloxan induced diabetic rats

Table 5: Effect of sub acute treatment of EEPS on blood glucose in Alloxan induced diabetic rats

Group	Treatment	Dose (Kg $^{-1}$ Body Weight)	Blood Glucose (mg/dl)		
			0th Day	10th Day	15th Day
I	Control (distilled water)	5 ml	76.27±1.27	79.27±1.93	82.35±9.7
II	Disease control (Alloxan)	150 mg	246.51±5.3	266.07±5.3**	289.42±5.23**
III	Standard (Glibenclamide+Alloxan)	0.4mg	231.12±4.8	168.65±4.2b**	108.74±2.51**
IV	Test I (EEPS+Alloxan)	200 mg	233±3.4	179.69±3.06*	132.67±4.1**
V	Test II (EEPS+Alloxan)	400mg	229.97±3.2	169.89±4.11**	118.24±4.67**

The values are expressed as mean ± SEM. Statistical significance test for comparision was done by ANOVA, followed by Dunnet's test. A-Group II is compared with Group I .b-groups III, IV, V are compared with group. **P<0.01, *P<0.05

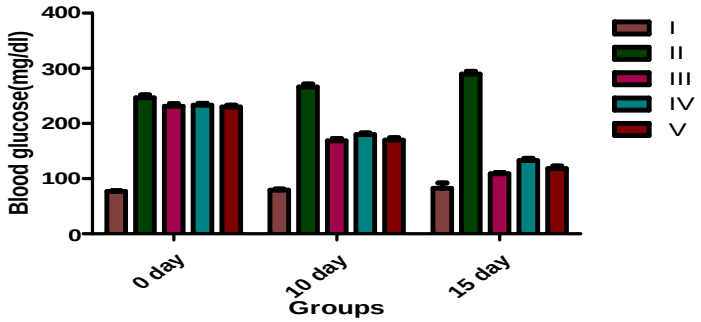

Figure 3: Effect of sub acute treatment of EEPS on blood glucose in Alloxan induced diabetic rats

4. Conclusion

The oral administration of the ethanolic extract of the leaves of plant *Polyalthia Suberosa Roth* on diabetic rats was useful for the treatment of diabetes induced by alloxan, because there were significant positive changes in the biochemical and physiological parameters related to carbohydrate, protein and lipids metabolism. The present studies indicated a significant anti-diabetic effect of the extract of leaves of *Polyalthia Suberosa Roth* and support its traditional usage in the control of diabetes and also concluded that the leaves extract of *Polyalthia Suberosa Roth* has strong effect on wound healing in albino rats**.**

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