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Research Article

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTS OF *ALANGIUM SALVIFOLIUM* AND *PAVONIA ZEYLANICA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study is aimed to investigate the effect of ethanolic extracts of stem and leaves of *Alangium Salvifolium* (EEAS) and *Pavonia Zeylanica* (EEPZ) on blood glucose level in normal and streptozotocin (STZ) induced diabetic rats. Oral administration of EEAS and EEPZ (400 and 800mg/kg body weight) resulted in a significant reduction in blood glucose level. The effect was compared with a standard drug Glibenclamide. The results support the traditional usage of the plants of *Alangium Salvifolium* and *Pavonia zeylanica* by ayurvedic physicians for the control of diabetes.

Keywords: Alangium Salvifolium, Pavonia Zeylanica, Streptozotocin and Glibenclamide.

INTRODUCTION

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. There are two general types of diabetes mellitus: Type I diabetes, also called insulin dependent diabetes mellitus (IDDM), and is caused by lack of insulin secretion. Type II diabetes, also called non-insulin dependent diabetes mellitus (NIDDM), and is caused by decreased sensitivity of target tissues to the metabolic effect of insulin. This reduced sensitivity to insulin is often referred to as insulin resistance.

In both types of diabetes mellitus, metabolism of all the main foodstuffs is altered. The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result, blood glucose concentration increases, cell utilization of glucose falls increasingly lower, and utilization of fats and protein increases.[1]

Medical nutrition therapy, physical activity, pharmacotherapy, SMBG and patient self-management education-especially concerning decision making skills are essential for successful management of the metabolic aspects of diabetes mellitus. Although some patients with "early" type II diabetes may not need pharmacotherapy for a while, the progressive nature of the disease ultimately results in the requirement of drug therapy. Patient education about the disease and participation in medical care are the most important aspects of diabetes mellitus management. Without patient involvement and participation, even the "ideal" pharmacotherapy, dietary, or other interventions will fail. Patient education improves understanding of the disease, promotes optimal patient choices regarding diet, medication, and exercise, and facilitates decision making skills.[2]

In India, the diabetology practice is more of clinic or hospital based in nature. This means, unless otherwise the patients takes steps for their treatment, it is not possible to detect the unknown or rather undiagnosed diabetic patients in the community, earlier and also systematically, so that the cost burden of treating the complications of the disease, which is eating the health resources of even developed countries, is not possible to stop. This has got a major complication for the countries like India.[3]

Many Indian medicinal plants are reported to be useful in diabetes. However, search for new anti-diabetic drug continue. *Alangium Salvifolium* belongs to the family Alangiaceae. It is commonly known as sage leaved alangium, stone mango, hill sack tree and ancole fruit plant in English, nalla oodaga, oodaga chettu, aankolam and urgu in Telugu. It is a deciduous shrub or tree. It is commonly distributed in most parts of Chittoor district of Andhra Pradesh like Tirupati, Talakona, Chandragiri and Aragonda. The root bark is used for snake bite, cutaneous troubles, anthelmintic, astringent, purgative, diaphoretic and colic. Leaves are used in diabetes and the fruits are used as astringent, tonic and laxative, whereas the seeds are used in hemorrhage. *Pavonia Zeylanica* belongs to the family Malvaceae. It is commonly known as karubenda, china mutharapulagam, peramuthi and chittimulli in Telugu. It is very commonly distributed in farm fields, wastelands and rare in forest fringers, throughout the Chittoor district of Andhra Pradesh. Whole plant is used as febrifuge and anthelmintic .[4]

An extended literature review shows that an Anti-arthritic activity of bark extracts of Alangium Salvifolium Wang[5] and Anti-fertility activity of the stem bark of Alangium Salvifolium Wang in wistar female rats[6] has been reported. Larvicidal efficacy of medicinal plant extracts against Anopheles Stephensi and Culex quinquefasciatus[7] for Pavonia Zeylanica has been reported. However the plant is not scientifically explored for its anti-diabetic activity. Hence an effort has been made to screen the plants for anti-diabetic activity.

MATERIALS AND METHODS

Collection of Plant Material

The proposed plants material of fresh stem and leaves of Alangium Salvifolium and Pavonia Zeylanica were collected from Tirupati, Chittoor district of Andhra Pradesh, India. The species of the proposed study was identified and authentified by Dr.K.Madhava Chetty, Asst.Professor of Dept.of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Voucher specimens were deposited at Dept. of Pharmacognosy for further reference.

Extraction and phytochemical screening

The shade dried powder of the stem and leaves of plants was packed well in Soxhlet apparatus and was subjected to continuous hot extraction with ethanol after defatting with hexane until the completion of extraction. The extracts evaporated to dryness and kept in a desiccators till experimentation.

The extract was subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, alkaloids, flavonoids, carbohydrates, tannins and proteins.[8-10]

Animals

Wistar albino rats of either sex weighing between 200-250gms were used. They were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum.* All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines (1423/PO/a/11/CPCSEA).

Toxicity Study[11]

An acute toxicity study was performed to determine LD_{50} using different doses of the extracts according to the method described by Ghosh et al.

Effect of EEAS and EEPZ on Blood Glucose Levels in Normoglycemic rats[12, 13]

Animals were divided into six groups of six rats in each group.

Group-I: Animals received 1% NaCMC 2ml/kg body wt. per orally.

Group-II: Animals received EEAS 400mg/kg body wt. per orally.

Group-III: Animals received EEAS 800mg/kg body wt. per orally

Group-IV: Animals received EEPZ 400mg/kg body wt. per orally.

Group-V: Animals received EEPZ 800mg/kg body wt. per orally.

Group-VI: Animals received standard drug Glibenclamide 0.5mg/kg body wt. per orally.

In this study the entire groups of animals were fasted overnight and administered with respective drugs as per the mentioned dosage schedule. Blood glucose levels were determined at 0, 1, 2, 3 and 4 hours, after drug administration.

Effect of Blood Glucose Levels on Glucose fed Hyperglycemic Rats (Oral Glucose Tolerance Test)

The animals were divided into six groups of six rats in each group.

Group-I: Animals received glucose at a dose of 2gm/kg body wt. per orally.

Group-II: Animals received EEAS 400mg/kg body wt. in 1% NaCMC and glucose solution at a dose of 2gm/kg body wt. per orally.

Group-III: Animals received EEAS 800mg/kg body wt. in 1% NaCMC and glucose solution at a dose of 2gm/kg body wt. per orally.

Group-IV: Animals received EEPZ 400mg/kg body wt. in 1% NaCMC and glucose solution at a dose of 2gm/kg body wt. per orally. .

Group-V: Animals received EEPZ 800mg/kg body wt. in 1% NaCMC and glucose solution at a dose of 2gm/kg body wt. per orally.

Group-VI: Animals received standard drug Glibenclamide 0.5mg/kg body wt. and glucose solution at a dose of 2gm/kg per orally.

In this study, the entire group of animals were fasted and treated with above dosage schedule orally. EEAS, EEPZ and glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were determined at 0, 1, 2, 3 and 4 hours, after glucose administration.

Experimental induction of diabetes

For the induction of diabetes in rats, Streptozotocin solution (70mg/kg body wt. citrate buffer P^H4.5) is injected intraperitoneally. A rest period of two days is allowed for the blood glucose level to stabilize. During this period the animals used to have free access to both food and water. Blood sugar levels of the animals are determined, 48 hours after injection of STZ. The animals having blood glucose level more than 200mg/dL were selected for the experimentation.

Effect of EEAS and EEPZ on Blood Glucose Levels in Streptozotocin Induced Diabetic Rats

Different groups of rats were used to study the effects of EEAS and EEPZ. The rats were divided into seven groups each consisting of six rats.

Group-I: Normal control animals received 2ml/kg of 1% NaCMC per orally for 15 days.

Group-II: Streptozotocin induced diabetic animals received 1% NaCMC 2ml/kg per orally for 15 days.

Group-III: Streptozotocin induced diabetic animals received EEAS 400mg/kg per orally for 15 days.

Group-IV: Streptozotocin induced diabetic animals received EEAS 800mg/kg per orally for 15 days.

Group-V: Streptozotocin induced diabetic animals received EEPZ 400mg/kg per orally for 15 days.

Group-VI: Streptozotocin induced diabetic animals received EEPZ 800mg/kg per orally for 15 days.

Group-VII: Streptozotocin induced diabetic animals received the standard drug Glibenclamide 2.5mg/kg per orally for 15 days.

All the group of animals received the treatment for 15 days. Blood samples were collected one hour after the drug administration and the day 5^{th}, 10^{th} and 15^{th} to determine the blood glucose level. For glucose determination, blood was obtained by snipping tail with sharp razor[14]. Then the blood glucose levels were determined by using Haemo-Glucotest (20-800R) glucose strips. This method, which permits the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method[15].

Statistical Analysis

Data obtained from pharmacological experiments were expressed as mean±SEM. The data were statistically analyzed by one way ANOVA followed by Dunnett's test.

RESULTS AND DISCUSSION

The preliminary phytochemical studies indicated the presence of alkaloids, flavonoids, terpenoids, tannins and carbohydrates. In acute toxicity study, the EEAS and EEPZ did not produced lethality up to the dose level of 5000mg/kg.

Effect of EEAS and EEPZ on blood glucose levels in normoglycemic rats showed the significant decrease in the blood glucose level at the doses of 800mg/kg. The results were shown in table-1. The mean blood glucose level maintained at 92.83 to 91.00 mg/dL at dose of 400mg/kg EEAS and 89.17 to 83.16 mg/dL at dose of 800mg/kg EEAS. The mean blood glucose level decreased from 92.00 to 90.33 mg/dL at dose of 400mg/kg EEPZ and 90.00 to 82.66 mg/dL at dose of 800mg/kg EEPZ.

Table 1: Effect of EEAS and EEPZ on Blood Glucose Level in Normoglycemic Rats

Groups		Blood Glucose Level (mg/dL)				
		0 hour	1 hour	2 hours	3 hours	4 hours
I	Normal	90.16±1.08	88.67±1.28	87.16±2.40	85.00±2.32	91.17±2.24
II	EEAS 400mg	92.83±1.83	83.16±1.85	84.50±2.28	76.33±1.89*	91.00±2.08
III	EEAS 800mg	89.17±2.07	83.50±2.04	79.83±1.60*	72.50±1.71**	83.16±1.96*
IV	EEPZ 400mg	92.00±1.41	83.83±1.51	82.66±1.38	75.17±2.53*	90.33±1.38
V	EEPZ 800mg	90.00±1.53	84.66±1.08	78.33±1.41**	72.16±2.43**	82.66±2.03*
VI	Standard	89.50±2.45	85.50±1.65	77.00±1.73**	71.50±2.04**	80.50±2.81**

*Values are expressed as Mean±SEM (n=6). * p<0.05, ** p<0.01. Statistical significant test for comparison was done by ANOVA , followed by Dunnett's test. The blood glucose values of group II to VI are compared with group I.*

Effect of blood glucose levels on glucose fed hyperglycemic rats (Oral Glucose Tolerance Test) results were shown in Table-2. The mean blood glucose level decreased from 80.00 mg/dL to 76.67 mg/dL at dose of 400mg/kg EEAS and 81.50 mg/dL to 74.50 mg/dL at dose of 800mg/kg EEAS. The mean blood glucose level decrease from 88.17 mg/dL to 77.00 mg/dL at dose of 400mg/kg EEPZ and 90.16 mg/dL to 74.16 mg/dL at dose of 800mg/kg EEPZ.

Table 2: Effect of Blood Glucose Levels on Glucose fed Hyperglycemic Rats (Oral Glucose Tolerance Test)

Groups		Blood Glucose Level (mg/dL)				
		0 hour	1 hour	2 hour	3 hours	4 hours
I	Glucose	83.83±1.92	144.83±2.52	113.50±4.14	101.17±3.52	86.00±3.39
II	EEAS 400mg	80.00±1.88	140.00±3.02	125.00±3.18	87.00±3.88*	76.67±2.39*
III	EEAS 800mg	81.50±2.23	150.00±2.34	131.16±4.56*	86.50±3.21*	74.50±1.54**
IV	EEPZ 400mg	88.17±2.56	137.17±3.26	125.67±3.63	88.66±3.13*	77.00±1.24*
V	EEPZ 800mg	90.16±3.58	151.33±3.12	131.33±4.59*	82.17±2.72**	74.16±1.25**
VI	Standard	79.50±1.41	154.83±2.57	131.33±3.05*	82.83±3.08**	71.66±2.20**

*Values are expressed as Mean±SEM (n=6). * p<0.05, ** p<0.01. Statistical significant test for comparison was done by ANOVA , followed by Dunnett's test. The blood glucose values of group II to VI are compared with group I.*

In the effect of EEAS and EEPZ on blood glucose levels in streptozotocin induced diabetic rats, the blood glucose levels were measured in first to seventh groups of the experimental rats in initial and at the 5, 10 and 15 days of treatments are given in table-3. STZ induced diabetic rats showed significant increase in the level of blood sugar. Oral administration of EEAS and EEPZ showed the significant decrease on blood sugar level in 10 to 15 days of treatment. The blood glucose level of diabetic animals significantly reduced from 296.17 mg/dL to 286.50 mg/dL at dose of 400mg/kg EEAS and 270.33 mg/dL to 172.33 mg/dL at dose of 800mg/kg EEAS. The mean blood glucose level decrease from 300.16 mg/dL to 233.83 mg/dL at dose of 400mg/kg EEPZ and 274.33 mg/dL to 166.50 mg/dL at dose of 800mg/kg EEPZ. These results were comparable with 2.5mg/kg of glibenclamide which shows significant reduction of blood glucose level from 267.66 mg/dL to 150.16 mg/dL on 15th day.

Table 3: Effect of EEAS & EEPZ on Blood Glucose in Streptozotocin Induced Diabetic Rats

Groups		Blood Glucose Level (mg/dL)			
		Initial	Day 5	Day 10	Day 15
I	Normal Control	78.83±1.19	84.83±3.16	82.50±2.42	81.00±1.51
II	Diabetic Control	276.50±2.23	279.00±4.52	306.33±3.15	312.67±1.45
III	EEAS 400mg	296.17±8.95	293.50±3.91	289.83±3.67*	286.50±3.17**
IV	EEAS 800mg	270.33±6.78	262.00±6.35*	252.17±5.71**	172.33±6.27**
V	EEPZ 400mg	300.16±8.44	292.67±3.18	288.50±2.49*	233.83±5.79**
VI	EEPZ 800mg	274.33±7.20	261.83±3.70*	226.66±4.69**	166.50±4.61**
VII	Standard	267.66±4.89	255.16±3.09**	219.67±5.15**	150.16±5.71**

*Values are expressed as Mean±SEM (n=6). * p<0.05, ** p<0.01. Statistical significant test for comparison was done by ANOVA , followed by Dunnett's test. The blood glucose values of group III to VII are compared with group II.*

CONCLUSION

The results of the pharmacological studies clearly demonstrates that the ethanolic extracts of stem and leaves of *Alangium Salvifolium* and *Pavonia Zeylanica* has significant anti-diabetic activity in streptozotocin induced diabetic rats. Thus the present study supports the traditional folklore.

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