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ASSESSMENT OF ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACTS OF *PHYLLANTHUS ACIDUS* LINN AND *BASELLA RUBRA* LINN LEAVES AGAINST STREPTOZOTOCIN INDUCED DIABETES IN RATS

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KEYWORDS:

Antidiabetic, *Basella rubra*, ethanol extract, glibenclamide, *Phyllanthus acidus*, Streptozotocin.

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ABSTRACT

The antidiabetic potentials of ethanol extracts of *Phyllanthus acidus* Linn (Phyllanthaceae) (EEPA) and *Basella rubra* Linn (Basellaceae) (EEBR) were investigated against streptozotocin induced diabetes in rats. The shade dried leaves were extracted by soxhlation using ethanol. Phytochemical studies showed that EEPA and EEBR contain significant quantities of Flavanoids, Tannins, Triterpenoids and Phytosterols. Results of acute toxicity study suggested that extracts can be considered as category 5 as per OECD guidelines 425. Antidiabetic potentials of EEPA and EEBR at doses 100, 200 and 400 mg/kg b.wt. were studied on streptozotocin (STZ) induced diabetic rats for 21 days. The obtained results were compared with the results of control, diabetic control and standard (Glibenclamide) groups. Blood glucose levels, , Lipid profile, SGOT, SGPT and ALP were analyzed at 0, 7, 14 and 21 days of study. Glycogen content in the liver and skeletal muscle was estimated after 21 days. Ethanol extracts of selected plants showed significant reduction (**P<0.01) in blood glucose levels, Lipid level, SGOT, SGPT and ALP. In contrast, elevation of HDLC, liver and muscle glycogen levels was observed in dose dependent manner. The results suggested that EEPA and EEBR (96.13% and 93.6%) are having equipotent effect compare with Glibenclamide (97.8 %) at dose of 400 mg/kg.

1. INTRODUCTION :

Diabetes mellitus is a chronic endocrine disorder characterized by metabolic derangements of carbohydrate, fat and protein, there by develops complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy over the period of time. Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus [1]. In recent past, many medicinal plants possessing experimental and clinical antidiabetic activity that has been used in traditional systems of medicine [2]. Ayurvedic literatures like Charak Samhita and Sushruta Samhita, have reported the use of the grains for the management of diabetes mellitus [3]. In other hand, many synthetic hypoglycemic agents were introduced for maintenance of type 2 diabetes. Yet, the diabetes and the related complications continued to be major medical problem all over the world. The prevalence of diabetes mellitus is estimated to be more than 300 million by 2025. The WHO has recommended the evaluation of traditional plant treatments for management of diabetes as they are effective, less toxic with minimum or no side effects and are considered to be excellent candidates for oral therapy [4]. The disease is a major degenerative ailment in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [5].

Phyllanthus acidus (Phyllanthaceae) popularly commonly known as “star goose berry”, is a small tree cultivated as a fruit tree in many Asian countries. The plant parts are being used in Indian folk medicine. The leaves were reported to be useful to treat fever, piles, small pox and blood vomiting [6].

Basella rubra known as Malabar/Ceylon spinach is a climbing perennial plant belongs to Basellaceae family. The plant is used as Anthelmentic, demulcent, anti-inflammatory, antimalarial, antidiabetic and analgesic in Indian traditional system of medicine. It was reported that the leaves are effective as diuretic, demulcent and against to gonorrhea and urticaria [7]. Spinach extracts were considered to possess chemo and central nervous system protection, anticancer and antiaging functions [8].

However, the above plants are claimed to possess antidiabetic activity, but no scientific evidence is supported. Therefore, study of antidiabetic effect of *Phyllanthus acidus* and *Basella rubra* was undertaken to evaluate the potential of the activity against streptozotocin induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Materials

Streptozotocin was purchased from Sigma-Aldrich, India and the solution was prepared by freshly dissolving in citrate buffer (0.01 M, pH 4.5). Glibenclamide was procured from Cipla Ltd. All the other chemicals used were of analytical grade.

2.2. Plant material

The plants were authenticated by Dr. Madhava Chetty, Taxonomist, S.V. University, Tirupathi, India and the specimens have been preserved in our research lab (PRRM/JP/2008-83 and 84).

2.3. Extraction

The fresh leaves both plants were collected in the month of Nov 2008 from the surroundings of Tirupathi, India. They were washed with tap water, shade dried for two weeks and pulverized, sieved (10/44) and stored in air-tight containers. About 5000 g of powdered drugs were extracted individually with ethanol by using soxhlet apparatus until the phytoconstituents were completely exhausted. The ethanol extracts were evaporated through rotary evaporator (Buchi type, Mumbai, India) under reduced pressure at 40°C. and labeled as EEPA (523 g) and EEBR (465 g).

2.4. Phytochemical investigation

Phytochemical analysis on both extracts were carried out [9] to find out the presence of phytoconstituents viz flavonoids, phytosterols, phenolics, carbohydrates, tannins, triterpenoids etc.

2.5. Experimental animals

Female Wistar rats (nulliparous and non-pregnant) of 8 to 10 weeks old weighing 200–250 g supplied by National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3^{o}C and %RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water *ad libitum.* The experimental protocol was approved by the Institutional Animal Ethics Committee (Reg. No. 1423/PO/a/CPCSEA).

2.6. Acute toxicity studies

The studies were performed according to OECD Guidelines 425.

2.7. Oral Glucose Tolerance Test for EEPA and EEBR

Wistar rats of either sex were divided into eight groups and each group containing six animals. Group I animals served as normal control and received glucose (2 g/kg). Animals in group II,

received standard drug of glibenclamide (2 mg/kg) with glucose (2 g/kg). Animals in group III were treated with test EEPA (100 mg/kg) and glucose (2 g/kg), group IV were treated with test EEPA (200 mg/kg) and glucose (2 g/kg), group V were treated with EEPA (400 mg/kg) and glucose (2 g/kg), group VI were treated with EEBR (100 mg/kg) and glucose (2 g/kg), group VII were treated with EEBR (200 mg/kg) and glucose (2 g/kg) and group VIII were treated with EEBR (400 mg/kg) and glucose (2 g/kg).

The animals were fasted overnight and treated with above dosage schedule orally. The EEPA, EEBR and glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were assessed at 0 (before glucose challenge), 30, 90 and 150th min after glucose administration.

2.8. Antidiabetic activity on streptozotocin induced diabetic rats

About 70 animals were selected for the experiment out of which six animals were kept separately as normal control group (Group I). Remaining 64 animals were made diabetic by a single intraperitoneal injection of streptozotocin at dose of 50 mg/kg dissolved in citrate buffer (0.01 M, pH 4.5). The rats were provided with 5% glucose solution bottles in their cages for the next 24 h to prevent hypoglycaemia. The blood glucose levels were measured before and after 72 h of STZ injection to confirm the development of diabetes. Only those animals that showed blood glucose levels >250 mg/dL were separated and used for the study [10].

The rats were divided into nine groups each consisting of six animals. Group-I animals were served as control and were received 1%w/v sodium CMC, group-II animals were induced for diabetes with STZ (50 mg/kg, i.p.), group-III of diabetic induced animals were treated with glibenclamide at dose of 2 mg/kg, group-IV of diabetic induced animals were treated with EEPA 100 mg/kg, group-V of diabetic induced animals were treated with EEPA 200 mg/kg, group-VI of diabetic induced animals were treated with EEPA 400 mg/kg, group-VII of diabetic induced animals were treated with EEBR 100 mg/kg, group-VIII of diabetic induced animals were treated with EEBR at dose of 200 mg/kg, and group-IX of diabetic induced animals were treated with EEBR at dose of 400 mg/kg. The study was conducted for the period of 21 days by dosing once daily.

2.9. Biochemical analysis

Blood samples were collected from the animals prior to the treatment with above schedule and after 30 min of glibenclamide/ethanol extracts administration on 7th, 14th and 21st day.

Blood was obtained from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube and was centrifuged (2,500 rpm/10 min) to separate serum. The serum was used for biochemical analysis of blood glucose, total cholesterol (results are given in table5), triglycerides, HDL-cholesterol (results are given in table3), SGOT, SGPT(results are given in table 4) and ALP(results are given in table 6).

2.10 Collection of organs and analysis

After 21 days all the animals were euthanized by overdose of ether anaesthesia. Liver and skeletal muscle tissues were collected for the assessment of glycogen content. The glucose levels were estimated by commercially available glucose kits (Span Diagnostics Ltd, Surat, India) based on glucose oxidase method (results are given in table 5). The total cholesterol in tissues was estimated by the one step method [11] (results are given in table5). HDL-cholesterol level was determined by the commercially available reagent kit (Erba Mannheim, Transansia biomed and Daman, India) based on phosphotungustate method [12] (results are given in table 3). The serum triglyceride level was estimated by Enzymatic GPO [13] (results are given in table 3). Serum transaminase activity (results are given in table4) and ALP were measured (results are given in table 6) [14,15]. The glycogen content in the liver/skeletal muscle tissue was estimated [16] and the glycogen content was expressed as mg/g of tissue (results are given in table6).

2.11. Statistical analysis

Data were expressed as mean ± SEM, (n=6). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Values were considered statistically significant when at $p<0.05$.

3. RESULTS and DISCUSSION

3.1. Phytochemical studies

As per the results of phytochemical study (table 1), EEPA and EEBR have exhibited the presence of flavonoids, glycosides, phenolics, proteins, tannins, carbohydrates, saponins and phytosterols in appreciable amounts. The results are given in Table 1.

3.2 Acute oral toxicity studies

The EEPA and EEBR did not show any sign and symptoms of toxicity or mortality up to 2000 mg/kg. Thus, the extracts could be considered as category 5 as per OECD guidelines 425.

3.3. Effect on oral glucose tolerance

The blood glucose levels in the control group (Group I) were found to increase maximum levels within 30 min after glucose load and normal glucose levels were observed over a period of 150 min. In Group-II (glibenclamide treated group), Group V (EEPA 400 mg/kg treated group) and Group VIII (EEBR400 mg/kg, treated group) the blood sugar levels returned to normal within 30 min. Group III, IV, VI and VII showed significant decrease in blood glucose levels at 90 min. Results in table 2, suggested that the EEPA and EEBR have not decreased the blood glucose levels below normal levels.

Antidiabetic Activity of EEPA and EEBR:

3.3. Biochemical Analysis

As of Table 5, groups G-IV to G-VI (EEPA 100, 200 and 400 mg/kg) and G-VII to G-IX (EEBR 100, 200 and 400 mg/kg), have shown a dose dependent decrease in the serum glucose levels on 7^{th}, 14^{th} and 21^{st} day. G-VI and G-IX showed equipotent activity with G-III. The EEPA and EEBR at the dose of 400 mg/kg showed an efficient antidiabetic activity and its efficacy was found to be on par with glibenclamide. The plausible mechanism behind the antidiabetic potential of EEPA and EEBR could be due to the presence of flavanoids, phytosterols and tannins which would have increased the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway. Lowering of blood glucose level in Streptozotocinised rats after administration of the extracts indicated that the extracts possessed extra-pancreatic effects or regeneration of β-cells in pancreatic islets [17-21]. Lipid profile experimental results of EEPA and EEBR at 400 mg/kg have shown a significant decrease in TC (88.79 and 82.85%) , TG (89.29 and 84.03%), SGOT (89.3 and 85.2%), SGPT (95.1 and 93.2%), ALP (97.4 and 95.6%) and the progressive decrease in lipid levels were observed over 21 days period of treatment which was in dose-dependent manner and progressively increase in HDLC level (56.32 & 35.63%) was also noted during the study period. The devoid levels of lipids in serum could be due to the erratic affects of lipolytic compounds on adipose matter, majorly due to insulin. In general conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Hyper triglyceridemia and hyper cholesterolemia caused due to inactivation of lipoprotein lipase in insulin deficiency [22, 23]. The altered serum lipid profile was returned to normal after treatment with ethanol extracts. Liver and muscle glycogen contents were increased significantly over 21 days of treatment of ethanol extracts in a dose-dependent manner.

From the results obtained, that both ethanol extracts viz. EEPA and EEBR have shown effective antidiabetic activity against streptozotocin induced diabetic Wistar rats at the dose of 400 mg/kg. For comparison, the ethanol extract of *Phyllanthus acidus*, have exhibited a potential antidiabetic activity than the ethanol extract of *Basella rubra* on long-term treatment in Wistar rats. It could be due the presence of relatively more amounts of flavonoids, tannin and phytosterols.

In conclusion, EEPA and EEBR have demonstrated a significant antidiabetic potentials and which could be via restoration of the pancreatic functions, activation of the beta cells, decreased absorption of glucose and/or by phytochemical contents. However, further studied are required to assess the antioxidant properties, isolation and characterization of the bioactive compounds/enzymes responsible for antidiabetic activity and the establishment of the exact mechanism(s) of action.

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Table 1: Phytochemical analysis of EEPA& EEBR

Phyto-constituents	EEPA	EEBR
Alkaloids	-	-
Flavanoids	+++	++
Phytosterols	+++	++
Phenolics	++	++
Carbohydrates	+	+
Tannins	++	+
Triterpenoids	+++	++
Proteins	+	+
Glycosides	+	+

+ Positive, - Negative, EEPA= ethanolic extract of *Phyllanthus acidus,* EEBR= ethanolic extract of *Basella rubra***.**

Table 2: Effect of EEPA and EEBR on Oral glucose tolerance test

Group	Blood glucose levels (mg/dL)			
	Initial	30 min	90 min	150 min
I	83.67 ±0.33	139.2 ± 1.10	127.9 ±1.66	89.50 ± 1.05
II	79.83 ± 0.60	119.5 ± 0.76	86.0 ±1.50**	75.33 ± 1.3**
III	79.33 ± 0.61	131.0 ± 0.41	122.8 ±0.98 **	84.33 ±0.95*
IV	80.50 ± 0.71	126.7 ±1.74	100.7 ±1.02**	79.83 ±1.16*
V	79.67 ±0.55	123.3 ± 3.30	94.33 ± 0.66**	76.0 ±1.23**
VI	81.52 ± 1.178	132.13 ± 2.39	124.28 ± 2.89**	78.65 ± 2.8*
VII	79.54 ± 1.27	129.11±1.25	106.69 ± 0.739**	81.31 ± 2.697*
VIII	78.54 ± 1.407	125.11 ± 1.19	95.5 ± 0.397**	77.9± 0.622**

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values **P<0.01, *P<0.05.compared to positive control.

Table 3: Effect of EEPA and EEBR on serum levels of HDL-Cholesterol and Triglycerides

Group	HDL-Cholesterol				Triglyceride			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
I	56.2 ±1.2	56.6 ±1.5	55.6 ±1.2	55.9 ±2.1	88.2 ±5.3	89.1 ±5.6	90.3 ±4.6	89.3 ±4.6
II	46.6 ±2.6	48.9 ±1.6	46.6 ±1.3	47.2 ±1.3	136.3 ±6.5	139.6 ±6.4	137.6 ±5.4	136.9 ±5.5
III	45.4 ±1.6	48.5 ±1.6**	51.3 ±1.4**	53.9 ±2.5**	134.6 ±6.4	121.7 ±6.8**	104.2 ±5.9**	92.4 ±5.9**
IV	44.6 ±2.7	46.7 ±1.8*	47.8 ±1.2**	48.3 ±1.9*	134.9 ±4.5	130.2 ±4.6*	117.9 ±6.2*	103.7 ±6.1**
V	45.7 ±1.5	47.4 ±1.9*	49.2 ±1.6*	50.6 ±2.6*	133.2 ±5.9	129.8 ±6.9**	112.1 ±6.3*	95.8 ±6.5*
VI	44.9 ±1.6	46.1 ±1.3**	49.1 ±1.1**	52.1 ±2.4**	135.5 ±5.4	124.6 ±6.8**	108.4 ±6.4**	94.4 ±6.3**
VII	45.1 ±2.5	45.9±1.2* *	46.9 ±1.8*	47.5 ±1.9*	134.2 ±6.9	129.9 ±5.9*	119.7 ±5.8**	106.9 ±5.4*
VIII	44.3 ±2.4	46.3± 1.4*	47.1 ±0.9*	49.7 ±2.5*	135.6 ±5.6	128.7 ±5.7**	114.7 ±5.6*	96.8 ±5.6*
IX	44.8 ±1.9	45.7 ±1.0**	48.2 ±1.6**	50.3 ±2.7**	134.8 ±5.7	123.9 ±5.1**	111.3 ±5.4*	96.9 ±5.3**

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values **P<0.01, *P<0.05.compared to positive control

Table 4: Effect of EEPA and EEBR on serum levels of SGOT and SGPT

Group	SGOT				SGPT			
	Day 0	Day 7	Day 14	Day 21	**Day 0**	**Day 7**	**Day 14**	**Day 21**
I	33.3±2.4	34.22±2.5	35.5±2.4	34.5±1.8	35.5±2.5	36.75±3.5	37.5±2.4	34.6±0.1
II	73.5±2.2	74.2±1.6	74.6±2.6	73.8±1.5	134.9±4.6	135.3±4.2	133.6±3.5	134.6±3.5
III	73.9±2.3	61.5±2.4*	43.8±2.3**	36.1±1.6**	136.2±3.5	96.5±3.5**	65.5±2.6**	37.5±2.4**
IV	73.4±1.6	69.8±2.4**	51.6±2.4*	44.2±0.7*	134.1±3.8	109±4.6*	77.1±3.5*	45.9±2.8*
V	74.7±1.9	67.9±1.9*	47.7±1.6*	40.9±1.6*	134.6±4.1	106.9±3.2**	74.5±4.1*	42.7±4.2*
VI	72.8±1.8	63.9±2.3**	45.1±0.9**	38.7±1.4**	135.2±4.2	98.8±3.5**	69.8±3.6**	39.5±4.6**
VII	74.4±2.3	69.5±2.4*	53.9±0.8*	46.9±2.1*	133.6±4.3	112.9±4.6*	80.1±3.8*	46.6±2.9*
VIII	73.9±1.8	67.1±1.8**	51.8±2.4*	42.6±2.3*	134.1±3.5*	105.9±4.6*	76.9±2.9**	43.9±3.1*
IX	71.5±1.7	64.2±1.8**	48.9±2.6**	40.3±2.1**	135.9±3.8	99.4±3.8**	72.9±2.8*	41.4±3.5**

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values **P<0.01, *P<0.05.compared to positive control.

Table 5: Effect of EEPA and EEBR on serum levels of TC and glucose

Group	TC				Glucose (mg/dL)			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
I	103.3 ±4.5	102.1 ±5.3	103.1 ±5.6	101.2 ±5.6	115.27 ±3.5	113.4 ±2.5	112.4 ±2.5	114.23 ±1.9
II	206.6 ±5.6	204.5 ±6.5	205.3 ±6.4	205.6 ±5.4	272.69 ±4.5	295.6 ±3.5	324.1 ±2.6	351.5 ±1.8
III	204.6 ±6.1	171.4 ±4.5*	139.7 ±5.9**	109.5 ±3.8**	281.45 ±5.6	209.5 ±3.4**	135.2 ±3.5**	119.5 ±1.7**
IV	206.2 ±4.5	192.6 ±5.6*	173.4 ±6.8*	143.5 ±6.7*	285.36 ±3.5	247.5 ±2.4*	169.3 ±0.9*	146.3 ±2.6*
V	205.6 ±5.5	181.5 ±5.6*	153.6 ±6.1**	134.2 ±7.6*	287.69 ±4.9	235.1 ±2.5*	161.1 ±1.5**	134.9 ±2.4*
VI	204.9 ±6.3	175.4 ±7.3**	142.7 ±6.8**	112.9 ±7.2**	279.25 ±5.6	221.5 ±3.4**	146.3 ±1.7**	123.4 ±2.6**
VII	205.3 ±7.5	195.7 ±5.9*	177.5 ±6.4**	149.8 ±6.4**	281.69 ±5.4	255.4 ±3.8*	171.2 ±2.6*	151.6 ±2.7**
VIII	206.5 ±6.4	185.6 ±4.9**	159.3 ±6.7*	139.9 ±6.3*	279.56 ±4.8	241.7 ±3.9*	164.5 ±2.5*	137.7 ±2.6*
IX	204.2 ±8.4	177.2 ±8.9**	149.1 ±5.7**	119.1 ±3.7**	274.58 ±4.7	226.7 ±3.2**	157.4 ±2.7**	129.3 ±2.4**

Each value represents the mean ± SEM. n=6 number of animals in each group. Values **P<0.01, *P<0.05 compared to positive control.

Table 6: Effect of EEPA and EEBR on ALP, Liver glycogen and Muscle glycogen

Group	ALP				Glycogen (mg/g)	
	Day 0	Day 7	Day 14	Day 21	Liver	Muscle
I	289.5±11.2	292.6±10.2	292.6±10.6	291.3±10.5	54.2±1.2	8.9±1.7
II	841.6±15.6	839.6±9.5	842.2±9.5	836.1±9.6	17.6±2.1	3.4±1.3
III	845.9±12.5	520.6±9.6*	356.5±9.6**	298.9±9.4**	51.6±1.9**	7.8±2.4**
IV	845.1±13.4	544.1±10.5**	381.3±10.2*	320.5±11.3**	32.4±1.8*	4.1±2.1*
V	843.6±14.3	540.2±11.2*	375.2±10.6*	317.9±11.3*	42.3±2.4**	5.9±1.6*
VI	842.9±11.2	531.5±13.2**	362.9±12.5**	305.1±14.6**	49.6±2.2**	7.1±1.5*
VII	845.6±10.9	551.9±11.9*	391.6±9.4**	327.8±12.5*	31.6±2.3*	3.9±1.6*
VIII	844.5±16.5	542.1±12.3*	379.3±11.3*	321.6±9.4*	39.5±3.1*	5.2±1.8*
IX	844.1±12.1	534.6±10.2**	367.1±12.5**	315.1±10.2*	46.2±2.8*	6.9±2.4**

Each value represents the mean ± SEM. n=6 number of animals in each group. Values **P<0.01, *P<0.05.compared to positive control.