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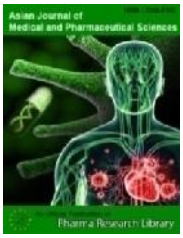

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Anti-diabetic activity of ethanolic extract of *Psychotria octosulcata* in streptozotocin and high fat diet induced diabetes in male wister rats.

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A B S T R A C T

Antidiabetic activity of *psychotria octosulcata* Linn. whole plant ethanolic extract-Shade dried and coarsely powdered plant (1 kg) was extracted successively with soxhlation method. The ethanolic extract of different doses of 200 and 400 mg/kg b. w and Pioglitazone (2 mg/kg), were evaluated for Antidiabetic activity using Strptozotocine (50mg/kg) induced Diabetic in rats. The biochemical parameters assessed were cholesterol, triglycerides, low density lipoproteins (LDL), and serum biomarkers of liver and kidney like SGOT, SGPT, Total proteine and serum creatinine. Test drug also shown significantly reduced glucose levels in diabetic rats. The extracts also showed reduction in the feed and water consumption of diabetic rats when compared with the diabetic control. The histopathological results of treated groups showed the regenerative/protective effect on β-cells of pancreas in diabetic rats. The current study revealed the antidiabetic potential of Artemisia amygdalina being effective in hyperglycemia and that it can effectively protect against other metabolic aberrations caused by diabetes in rats, which seems to validate its therapeutic traditional use.

Keywords: Antidiabetic activity, *Psychotria octosulcata*, Pioglitazone, Streptozotocine.

A R T I C L E I N F O

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1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The total number of people with diabetes is projected to increase from 171 million in 2000 to

366 million in 2030.Diabetes is best controlled either by diet alone and exercise (non-pharmacological), or diet with herbal or oral hypoglycaemic agents or insulin (pharmacological).[1] Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care.[2] The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Since the time of Charka and Sushruta many herbal medicines have been suggested for the treatment of diabetes mellitus. Example like the fresh juice from the bark of *Erythrina indica* (Papilionaceae) is used by the tribal for the treatment of diabetes.[3] One of such ethnomedicinal plant named *Psychotria octosulcata* was chosen for the study to evaluate its antidiabetic activity.[4] Plant species of the genus Psychotria (Rubiaceae) have been extensively studied, particularly due to the presence of bioactive alkaloids. Literature review showed that the extracts of many Psychotria species showed anti-inflammatory and analgesic activity.[5] As the plant was enriched with antioxidant constituents it may be used for relieving free radical induced pathogenesis. The whole plant of *Psychotria octosulcata* will be extracted with ethanol and investigated for its antidiabetic activity. the present investigation was under taken to evaluate the anti-diabetic activity of ethanolic extract of whole plant of *Psychotria octosulcata* in streptozotocin induced diabetic rats to confirm the pharmacological evidence in support of folklore claim.[6]

2. Experimental

Plant Material and Extraction

The whole plant of *Psychotria octosulcata* was collected from Seshachalam forest, Tirumala, Chitoor Dt and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chitoor Dt, A.P. The collected whole plant was immediately dried at room temperature for one month, powdered mechanically sieved (10/44) and stored in air tight containers. After collection of the plant, shade drying the whole plant of *psychotria octosulcata* were then blended in to fine powder with a blender and used for the aqueous and ethanol extracts. Ethanol extract was extracted by using soxhlet extractor for 18-20 h. The extract obtained, was concentrated under reduced pressure at controlled temperature (40-50°C) and finally made powdered.[7]

Experimental animals:

Male Wistar albino rats (130-160gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22±2°C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized at least 7days to the laboratory conditions prior to experimentation. Tap water and standard food pellets were provided ad libitum. Food pellet was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of Guide for the care and Use of Laboratory animals.

Phytochemical analysis: The ethanoilc extract of whole plant of *psychotria octosulcata* was subjected to different chemical tests separately for the identification of various active constituents.

Acute oral toxicity study: The acute oral toxicity study was performed as per the Organisation for Economic and Cooperation and Development (OECD) 423 guidelines. Six female rats (nulliparous and non pregnant; 130-160 gm bwt) were divided into two groups (3 per group) ie., control and test groups. Control group received 0.5 % carboxy methyl cellulose as vehicle at a dose of 10ml/kg b wt while the test group received an oral dose of 2000mg/kg b wt of ethanolic extract of whole plant of *Psychotria octosulcata* [EEPO] (10ml/kg b wt in 0.5% CMC).[8]

Experimental Design:

All the animals were randomly divided into five groups with six animals in each, serving as normal (nondiabetic), diabetic control, diabetic treated with two different doses extracts, and diabetic reference control, that is, pioglitazone. pioglitazone was given at a dose of 2 mg/kg of body weight. The oral administration of crude extracts (extract with different concentrations (200&400mg/kg) was continued once daily at the same time for 28 days. Body weight and blood glucose levels were estimated on the 0th, 7th, 14th 21st and 28th day of treatment.[8]

Biochemical parameters

Plasma glucose levels were estimated by withdrawing blood samples from retro orbital plexus prior to dosing day 1 and then at regular intervals of day 7, 14, 21 and 28th respectively all groups of animals. The body weight, food and fluid intake of all groups of animals were monitored on a daily basis for 28 days at regular time. Samples were withdrawn by cardiac puncture into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum and plasma.[9] Serum samples were utilized for further biochemical estimation parameters After 28 days, from the sacrificed rat's liver was harvested and immediately frozen in fluid nitrogen for biochemical estimation and pancreas was isolated for histopathological studies.[10] The total cholesterol, blood glucose, HDL-cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol from the serum and serum biomarkers of liver and kidney like SGOT, SGPT, Total proteine and serum creatinine by making use of standard kits.[11]

Histopathology:

The rats were sacrificed by cervical dislocation and samples of pancreas were collected immediately, stored in 10% formalin and send for histological assessment. These were tabulated in results.[12]

Statistical analysis: All the data was expressed as mean ± SEM. Statistical significance between two or more groups was tested using one way analysis of variance (ANOVA) followed by the Tukey's test using statistical software package, Graph Pad Prism; version5.03. Statistical significance was set accordingly.

3. Results and Discussion:

Preliminary phytochemical screening

The data corresponding to table1 describes the preliminary phytochemical investigation report of EEPO. Phenols,

flavonoids. saponins, phytosterols, steroids and terpenoids are present in EEPO.[13]

Table 1: Phyto-chemical screening of aerial parts extract of *Walsura piscidia* Roxb

S No	Constituent	Ethanolic extract
1	Alkaloids	-ve
2	Glycosides	+ve
3	Saponin glycosides	+ve
4	Flavonoids	+ve
5	Tannins	-ve
6	Steroids	+ve
7	Triterpenoids	+ve
9	Phenols	+ve
10	Proteins	-ve
11	Carbohydrates	-ve

+ve sign indicates presence; - ve sign indicates abscence

Acute oral toxicity study: The EEPO treated rats throughout the study. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs of toxicity shown by the animals and these disappeared with in 24 hrs of extract administration.[12] During the study there were no significant changes in body weights of treated rats compared to control group.[13] Further there were no gross pathological abnormalities in both control and treated rats. Thus the LD_{50} value was found to be greater than 2000mg/kg b.wt. with reference to the Globally harmonized system of classification and labeling the chemicals, *psychotria octosulcata* can be classified as Category -5 and this provides the relevance for protecting human and animal health.[14]

Antidiabetic activity

Effect of EEPO on Body Weight

Effect of EEPO on fasting blood glucose levels

The fasting blood glucose levels were significantly ($p < 0.001$) increased in STZ control group on 7^{th}, 14^{th}, 21^{st}, 28^{th} days compared to normal control animals. Piogiltazone (2mg/kg, po) and EEPO 400mg/kg treated animals showed significant ($p < 0.001$) decrease in fasting blood glucose levels compared to STZ control group on 14^{th}, 21^{st}, 28^{th} days. In EEPO 200mg/kg treated animals were showed less significant ($p < 0.01$) on 14^{th} day and showed more significant ($p < 0.001$) on 21^{st}, 28^{th} days compared to diabetic control animals.[15]

Lipid profile

Effect of EEPO on triglycerides and total cholesterol

The STZ control animals were showed significant ($p < 0.001$) increase in serum triglycerides levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/kg, po), EEPO 400mg/kg and EEPO 200mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum triglycerides and Cholesterol levels compared to STZ control animals on 28^{th} day.[16]

Effect of EEPO on high density lipoprotein (HDL)

The STZ control animals were showed significant ($p < 0.001$) decrease in serum HDL levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/k, p.o), EEPO 200mg/kg and EEPO 400mg/kg treated animals were showed significant ($p < 0.001$) increase in serum HDL levels compared to STZ control animals on 28^{th} day.[17]

Effect of EEPO on LDL and VLDL

The STZ control animals were showed significant ($p< 0.001$) increase in serum LDL levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/kg, p o), EEPO 400mg/kg treated animals were showed significant ($p<0.001$) decrease in serum LDL and VLDL levels compared to STZ control animals on 28^{th} day. EEPO 200mg/kg treated animals were showed significant ($p < 0.001$) and ($p< 0.01$) decrease in serum LDL levels on 28^{h} day compared to STZ control animals.[18]

Effect of EEPO on serum biomarkers of liver and kidney

Effect of EEPO on serum SGPT and SGOT

The STZ control animals were showed significant ($p < 0.001$) increase in serum SGPT and SGOT levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/kg, *p.o*) and ($p<0.05$), showed less significant ($p<0.01$) where as EEPO 200mg/kg and EEPO 400mg/kg treated animals showed good significant ($p < 0.001$) decrease in serum SGPT and SGOT levels compared to STZ control animals on 28^{th} day.

Effect of EEPO on total protein

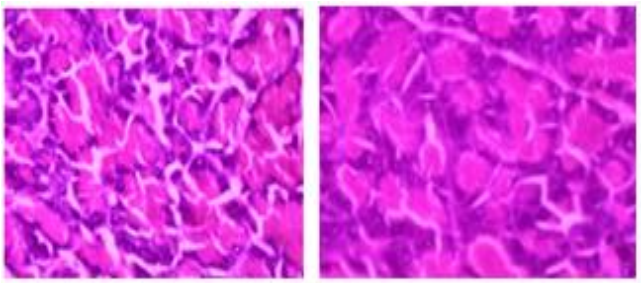
The STZ control animals were showed significant ($p < 0.001$) decrease in serum total protein levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/kg, *p.o*), EEPO 400mg/kg treated animals were showed significant ($p < 0.01$) increase in serum total protein levels compared to STZ control animals on 28^{th} day. EEPO 200mg/kg is non-significant in improving protein levels when compared to disease control.

Evaluation of EEPO on glycogen concentration in liver

Glycogen content in liver decreased to a better extent in diabetic control compared to that of normal (Table 5.10). EEPO during the dose of 200mg/kg & 400mg/kg b. w, during the final day of test, elevate the glycogen content of liver as much as 26.98± 1.25 and 35.83 ± 1.43 mg/g ($p<0.01$) whilst the standard pioglitazone registered glycogen content of 35.80 ± 1.38 for liver significantly ($p<0.001$ & $p<0.01$) .

Histopathology:

Histopathological changes of pancreas in EEPO and standard drug treated rats. Histopathological observation also revealed that the alterations occurred in the architecture of pancreatic islets in STZ-induced diabetic rats.[19] By oral normal. administration of Pioglitazone (2mg/kg), EEPO (200 and 400 mg/kg) for 28 days.[20]



Normal control (0.5%CMC) Diabetic group (0.5% CMC)

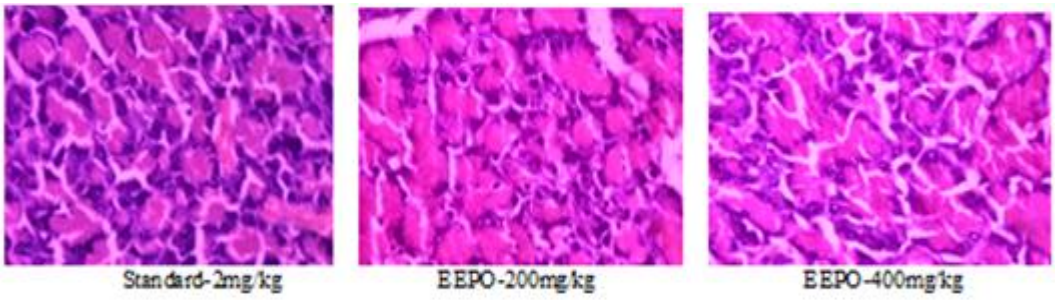


Figure1: Photographs of histological examination of rats treated with EEPO & Standard

Table 2: Effect of EEPO on fasting blood glucose

Group	Treatment (mg/kg)	Blood glucose (mg/dl)				
		0 Day	7th Day	14th Day	21st Day	28th Day
I	Normal	74.32±2.15	74.96±1.73	72.57±1.39	75.21±1.39	74.94±1.24
II	Diabetic control	265.20±2.01†	260.2±4.56†	257.3±3.20†	257.40±3.16†	256.3±1.31†
III	Pioglitazone-2mg/kg	272.30±2.14	96.48±2.72 ***	78.61±2.04 ***	75.03±1.12 ***	67.99±1.05 ***
IV	EEPO-200	274.8±2.55	234.0±4.79 ***	113.4±1.92 **	102.8±1.26 ***	98.43±1.29***
V	EEPO-400	264.8±3.75	214.1±3.15 ***	95.30±1.15 ***	82.38±1.59 ***	78.93±1.81 ***

All values are expressed as mean ±SEM; †= p<0.001 compared to normal,*= p<0.05, **=p<0.01, ***=p<0.001 when compared to diabetic control.

Table 3: Effect of EEPO on lipid profile

Group	Treatment (mg/kg)	TGs(mg/dl)	TC(mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
I	Normal	74.97±1.50	73.40±1.14	48.09±1.13	14.04±0.63	15.31±1.19
II	Diabetic control	105.30±1.13 †	106.20±3.14 †	32.30±0.64†	50.13±1.23†	24.12±1.36†
III	Pioglitazone-2mg/kg	842.82±5.34 ***	76.55±1.37 ***	45.67±1.26 ***	22.02±1.54 ***	16.25±1.93 ***
IV	EEPO-200	87.45±1.93 ***	81.30±1.11 **	42.04±071 ***	27.10±1.37 ***	17.19±0.63 **
V	EEPO-400	81.30±1.73 ***	81.36±2.24 ***	45.52±1.63 ***	20.64±0.71 ***	17.34±1.04 **

All values are expressed as mean ±SEM; †= p<0.001 compared to normal. *= p<0.05, **=p<0.01, ***=p<0.001 when compared to diabetic control.

Table 4: Effect of EEPO on serum biomarkers of liver and kidney

Group	Treatment (mg/kg)	SGOT(IU/L)	SGPT(IU/L)	Urea(mg/Dl)	Creatinine (mg/Dl)	Total protein (gm/Dl)
I	Normal	267.1±5.35	63.23±2.02	51.48±2.17	0.62±0.020	6.97±0.239
II	Diabetic control	292.6±8.43†	71.02±1.94†	69.35±2.09†	0.85±0.029†	6.07±0.174†
III	Pioglitazone-2	273.3±3.30 *	65.19±1.94 **	57.43±2.16	0.62±0.056 ***	5.43±0.438 ***
IV	EEPO-200	157.0±3.30 ***	51.70±2.16 ***	58.04±2.53	0.53±0.012 ***	5.97±0.723 ns
V	EEPO-400	190.41±5.92 ***	54.60±1.81 ***	55.14±2.53	0.51±0.035 ***	5.34±0.291 *

All values are expressed as mean ±SEM; †= p<0.001 compared to normal.ns =non-significant;*= p<0.05; **=p<0.01;***=p<0.001 when compared to diabetic control.

Table 5: Effect of EEPO on liver glycogen

Treated groups	Liver glycogen (mg/gm of tissue)
Normal control (0.5 % CMC)	36.64 ± 1.42

Diabetic control (0.5 % CMC)	16.42± 1.25†††
Pioglitazone (2mg/kg)	36.09 ± 1.28***
EEPO (200mg/kg)	25.82± 1.35**
EEPO (400 mg/kg)	35.93 ± 1.85 **

All values are expressed as mean ±SEM; †= p<0.001 compared to normal.
ns = non-significant; *= p<0.05;**=p<0.01;***=p<0.001 when compared to diabetic control.

4. Conclusion

The anti diabetic activity of ethanolic extract of *Psychotria octosulcata* (EEPO) was evaluated against streptozotocin and high fat diet induced diabetes in wistar albino rats. Hence the results obtained in this present study indicates that *Psychotria octosulcata* have shown a good anti diabetic activity by decreasing serum glucose , lipid profile (Total Cholesterol, LDL, and Triglycerides) and liver parameters and increase in total protein, body weight and HDL . Thus, it may be concluded that the EEPO exerts its activity by enhancing the synthesis of endogenous antioxidants in *streptozotocin* and high fat diet induced diabetes. Histopathology of pancreas also supported the protective effects of *Psychotria octosulcata*.

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