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INVESTIGATION OF ANTIDIABETIC ACTIVITY OF AERIAL PARTS OF *WALSURA PISCIDIA* ROXB IN HIGH FAT DIET (HFD) FED – STREPTOZOTOCIN (STZ) INDUCED DIABETIC RATS

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ABSTRACT

The present study was aimed to evaluate the antidiabetic activity of ethanolic extract of aerial parts *Walsura piscidia* Roxb (EEWP) on high fat diet (HFD) - Streptozotocin (STZ) induced diabetic rats. Male wistar albino rates were fed with high fat diet for a period of two week prior to administration of Streptozotocin 50mg/kg intrapeitonially. The long duration effect of the extract was investigated by treating diabetic rats with vehicle (0.5%mg/kg) EEWP (200 and 400mg/kg body wt) for the period of 28 days. The results of EEWP on fasting blood glucose and serum insulin levels were evaluated. In addition of body weight changes, lipid profile and serum biomarkers of liver and kidney utility were also studied. The results raise the roof that EEWP-400mg shows admirable anti-diabetic activity. The elevated serum urea and creatinine level decreased by treatment with the extract. Thus the results defend *Walsura piscidia* Roxb (EEWP) acquire an antidiabetic activity.

KEYWORDS: Anti-diabetic activity, Streptozotocin, *Walsura piscidia* Roxb, Ethanolic extract.

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INTRODUCTION

Diabetes mellitus (DM) is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 150 million people in 2000, which is expected to increase to 320 million by 2025[1]. By definition, diabetes mellitus is the most common metabolic disorder characterized by hyperglycemic, glucose urea and negative nitrogen balance, and it is mainly due to lack of insulin secretion in beta cells of pancreas and desensitization of insulin receptors for insulin[2]. The treatment of DM is based on parenteral insulin and oral anti-diabetic drugs. Oral hypoglycemic agents, currently used have serious side effect hence there is a need to search a newer anti-diabetic agents[3]. Management of diabetes without any side effect is still a challenge to the medical community that having high therapeutic efficacy with minimum side effect and easily available at low price. This may be fulfilled by treating DM with traditional medicine using as an anti-diabetic agents from medicinal plants[4]. The plant *Walsura piscidia* Roxb belongs to the family Meliaceae. It has comprised 10 species in India[5, 6] and is used extensively in traditional system of medicine and used by tribal peoples to treat various diseases like skin allergies, astringent, diarrhoea[7]. Considering the beneficial effects of herbs over allopathic treatment, there was an outlook for herbs which has a medicinal use in treating the DM. An attempt was made to study the beneficial effects of the plant *Walsura piscidia* Roxb against Streptozotocin induced diabetics in experimental rats. Thus the present study was aimed to study the hypoglycemic activity of ethanolic and aqueous extract of aerial parts of *Walsura piscidia* Roxb.

MATERIALS AND METHODS

Collection of plant materials

Walsura piscidia Roxb. was collected from Western Ghats and identified by Dr. V. Chelladurai, Rtd Senior Research Officer, Tirunelveli, Voucher specimens have been deposited at Gokula Krishna College of Pharmacy, Department of Pharmacognosy, Sullurpet, Nellore Dist, Andhra Pradesh (Voucher No. GKCP-25). The plant material was shade dried and blended into fine powder with a blender. About 200g of the powdered material was subjected to Soxhlet and exhaustively extracted with 80% ethanol for 20 hrs. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator. The extract obtained, was concentrated and dried under controlled temperature (40-50 ^{o}C).

Drugs and chemicals

Streptazotocin (STZ) was purchased from sigma Uldrich. Pioglitazone was a gift sample from Dr.reddy's laboratories. All the other chemicals were of analytical grade.

Animals

Healthy male Albino rats of Wistar strain (200-250g) were used in the study. The animals were kept under standard conditions and allowed for free access to food and water. The animals were housed for seven weeks in polypropylene cages prior to the experiment to acclimatize to laboratory conditions. Food pellets were with held overnight prior to dosing.

Induction of diabetes in rats (chemical method)

Wistar albino rats of male sex were fed with high fat diet (HFD) that consists of 20% fat, 46% carbohydrate and protein (w/w). Two weeks later HFD fed rats were administered streptozotocin at a dose of 50mg/kg b wt intraperitonially and allowed to free access to food and water[8]. Fasting blood glucose levels were measured 3days after STZ administration. The rats with fasting glucose ≥ 200mg/dl were considered diabetic and selected for the study.

Treatment schedule

Animals were randomly divided into seven groups containing 6 animals in each group (n=6). Treatment will given for 28 days continuously. Group-I not received any drug it kept as normal control by giving 0.05%CMC,

Group-II rats were not treated with drugs and kept as diabetic control, Group-III animals were treated with Pioglitazone 2mg/kg, p.o, Group-IV and Group –V animals were treated with EEWP 200mg/kg and 400mg/kg, p.o.

Estimation of biochemical parameters

At the end of the experimental period, rats were fasted overnight and blood was collected by cardiac puncture. The serum samples were analyzed for various biochemical parameters lipid profile and serum biomarkers of liver and kidney. The serum insulin was measured by ELSIA kit. The rats were sacrificed by cervical dislocation and samples of pancreas, liver, kidney were collected immediately, stored in 10% formalin and send for histological assessment.

Statistical analysis

All values were expressed as mean ± SEM and were analysed by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test for all groups using Graph pad prism 5.0. The differences were considered as significant at p≤ 0.05.

RESULTS

Effect of EEWP 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 EEWP 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 EEWP 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.

Effect of EEWP on body weight

The body weights of STZ control animals were decreased compared to normal control animals on 7^{th}, 14^{th}, 21^{st}, 28^{th} days. Pioglitazone (2mg/kg, *p.o*), EEWP 200mg/kg and EEWP 400mg/kg treated animals showed an increase in body weights compared to STZ control animals on 7^{th} , 14^{th} , 21^{st} ,28^{th} days.

Effect of EEWP on triglycerides

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), EEWP 400mg/kg and EEWP 200mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum triglycerides levels compared to STZ control animals on 28^{th} day.

Effect of EEWP on total cholesterol

The STZ control animals were showed significant ($p < 0.001$) increase in serum total cholesterol levels compared to normal control animals on 28^{th} day. Pioglitazone (2mg/kg, po), EEWP 400mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum total cholesterol levels compared to STZ control animals on 28^{th} day. EEWP 200mg/kg treated animals were showed significant ($p < 0.05$) decrease on 28^{h} day compared to STZ animals.

Effect of EEWP 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), EEWP 200mg/kg and EEWP 400mg/kg treated animals were showed significant ($p < 0.001$) increase in serum HDL levels compared to STZ control animals on 28^{th} day.

Effect of EEWP on LDL

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), EEWP 400mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum LDL levels compared to STZ control animals on 28^{th} day. EEWP 200mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum LDL levels on 28^{h} day compared to STZ control animals.

Effect of EEWP on VLDL

The STZ control animals were showed significant ($p < 0.001$) increase in serum VLDL levels compared to normal control animals on 28th day. Pioglitazone (2mg/kg, *p.o*) treated animals were showed significant ($p < 0.001$) decrease in serum LDL levels where as EEWP 200 & 400mg/kg treated animals were showed significant ($p < 0.01$) decrease in serum LDL levels compared to STZ control animals on 28th day.

Effect of EEWP on serum SGPT

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

Effect of EEWP on serum SGOT

The STZ control animals were showed significant ($p < 0.001$) increase in serum SGOT levels compared to normal control animals on 28th day. Pioglitazone (2mg/kg, *p.o*)($p<0.05$), EEWP 200mg/kg and EEWP 400mg/kg treated animals were showed significant ($p < 0.001$) decrease in serum SGOT levels compared to STZ control animals on 28th day.

Effect of EEWP 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 28th day. Pioglitazone (2mg/kg, *p.o*), EEWP 400mg/kg treated animals were showed significant ($p < 0.01$) increase in serum total protein levels compared to STZ control animals on 28th day. EEWP 200mg/kg is non-significant in improving protein levels when compared to disease control.

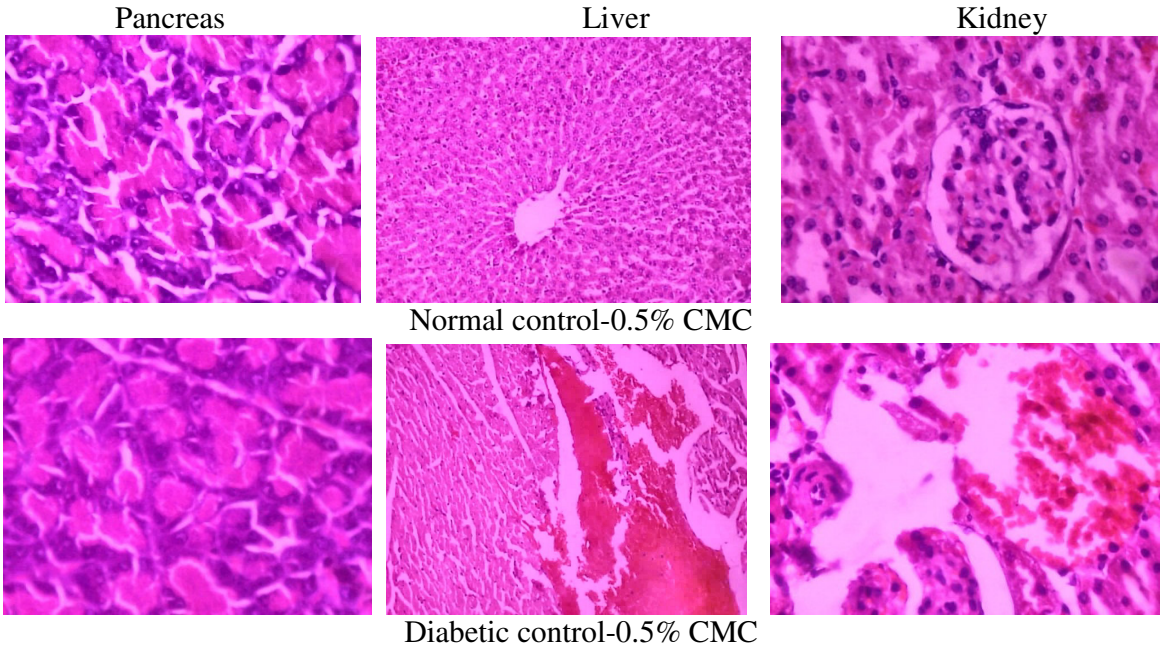
Histopathology

The histopathology slides of pancreas and liver of STZ control group showed damage in pancreatic and liver tissue and cause necrosis of beta cells when compared to normal group. Pioglitazone (2mg/kg, po), EEWP 200mg/kg and EEWP 400mg/kg treated animals were showed protection against damage of pancreatic and liver tissue compared to STZ control group

DISCUSSION

The increase in number of diabetic patients has motivated the scientists to find new methods to cure diabetes. Inspite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease[9].The present study is the preliminary assessment of the antidiabetic activity of the ethanolic extract of aerial parts of *Walsura piscidia* Roxb in HFD fed-STZ induced diabetic rats. STZ – induced diabetes is characterized by a sever loss in body weight. The decrease in body weight is due to the increased muscle destruction or degradation of structural proteins[10].when diabetic rats are treated with EEWP, they showed an improvement in bodyweight in comparisions to the disease control and standard Pioglitazone treated group, which signifies its protective effect in controlling muscle wasting i.e. reversal of gluconeogeneis. Moreover, the ability to protect body weight is seems to be result of its ability to reduce hyperglycemia. Number of plant have been reported for their hypoglycemic activity and the possible mechanism underlines could be an insulin secretion from β-cell of islets of langerhans or release of bounded insulin or their insulin like actions[11]. In diabetic control rats showed significant increase in fasting blood glucose levels compared to normal control, Pioglitazone (2 mg/kg, *p.o*) and EEWP (200 mg/kg and 400 mg/kg, *p.o*) extract treated animals showed a significant decrease fasting blood glucose levels compared to diabetic control rats. This antihyperglycemic action of *Walsura piscidia* may be due to the presence of flavanoids, triterpinoids, Polyphenols and coumarine. There is marked increase in serum triglycerides and total cholesterol observed in untreated diabetic rats compared to that of normal control. Elevations of plasma lipid levels in diabetes mellitus were well reported. Under

normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides to VLDL. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia and hypercholesterolemia. Pioglitazone (2 mg/kg, po) and EEWP (200 mg/kg and 400 mg/kg, po) extract treated animals showed that significant reduction of triglycerides, total cholesterol, VLDL, LDL levels compared to diabetic control animals. The diabetic control animals showed that increased levels of TG's, TC, VLDL, LDL due to deficiency in insulin. The effect of EEWP may be due to presence of flavonoids, triterpinoids and coumarine by enhancement of insulin release from existing β- cells and there by increased lipoprotein lipase activity. HDL levels were decreased in STZ control animals compared to normal control animals. HDL acts as reversal of cholesterol from peripheral tissues to liver with the help of cholesterol ester transfer protein (CEP) thereby reduce the increased cholesterol levels. Pioglitazone (2mg/kg, *p.o*) and EEWP (200 mg/kg and 400 mg/kg, po) treated animals showed significant increase in HDL. Diabetic rats showed significantly increased levels of urea and creatinine in the serum, which are considered significant markers of renal dysfunction[12]. In the present study the significant reduction in the levels of serum urea and creatinine in EEWP treated diabetic rats indicated that the extract prevented the progression of renal damage in diabetic rats. SGOT, SGPT are reliable markers of liver function. The liver was necrotized in STZ-induced diabetic rats. Therefore an increase in the activities of SGOT, SGPT might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of hepatotoxic effect of STZ[13]. Treating the diabetic rats with EEWPR reduced the activity of these enzymes in serum compared to the diabetic control group. There is no significant difference in total protein content. Histopathological observation also revealed that the alterations occurred in the architecture of pancreatic islets in STZ-induced diabetic rats. By oral administration of Pioglitazone (2mg/kg) , EEWP (200 and 400 mg/kg) for 28 days , the alterations were apparently reverted back to near normal.



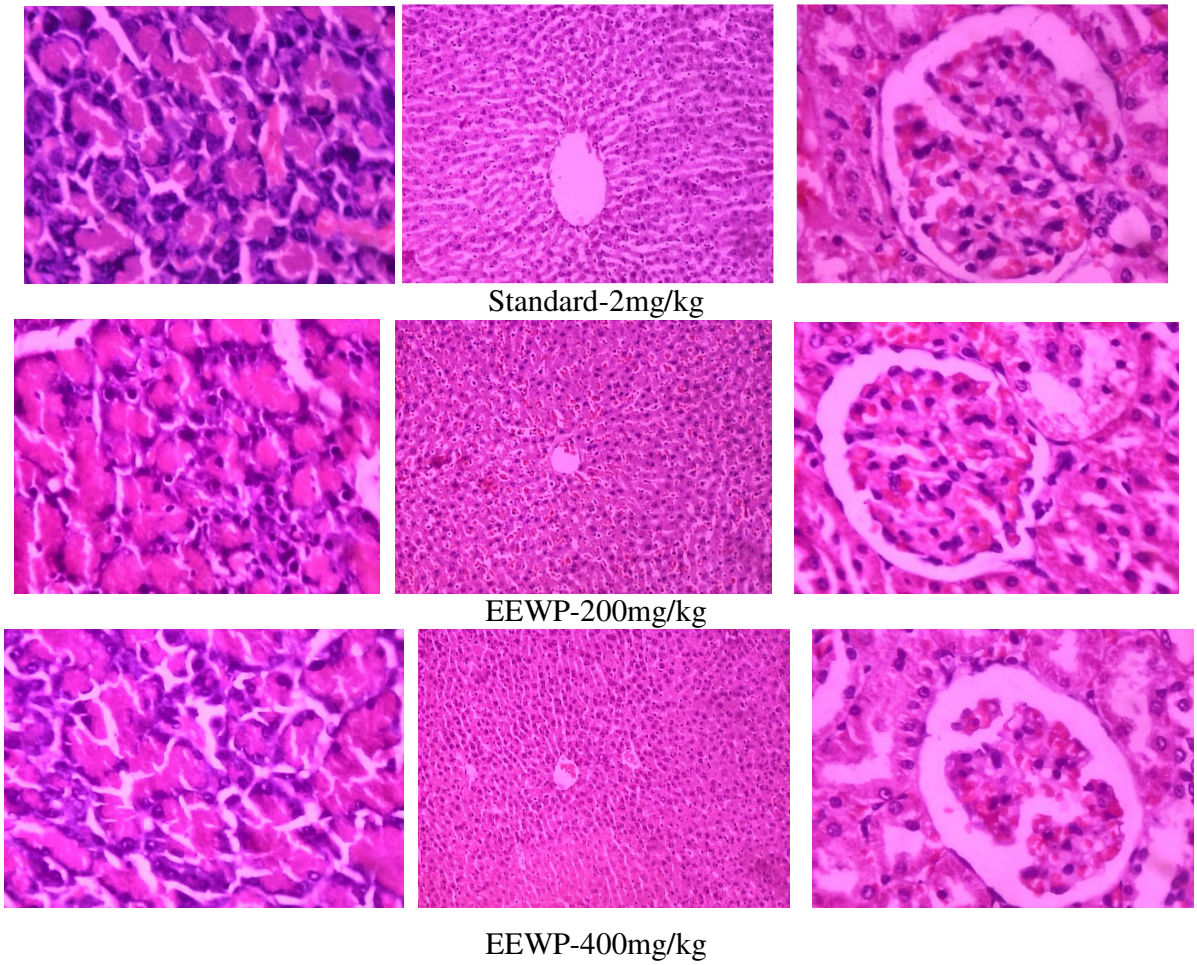


Figure 1
Photographs of histological examination of rats treated with EEWP & Standard

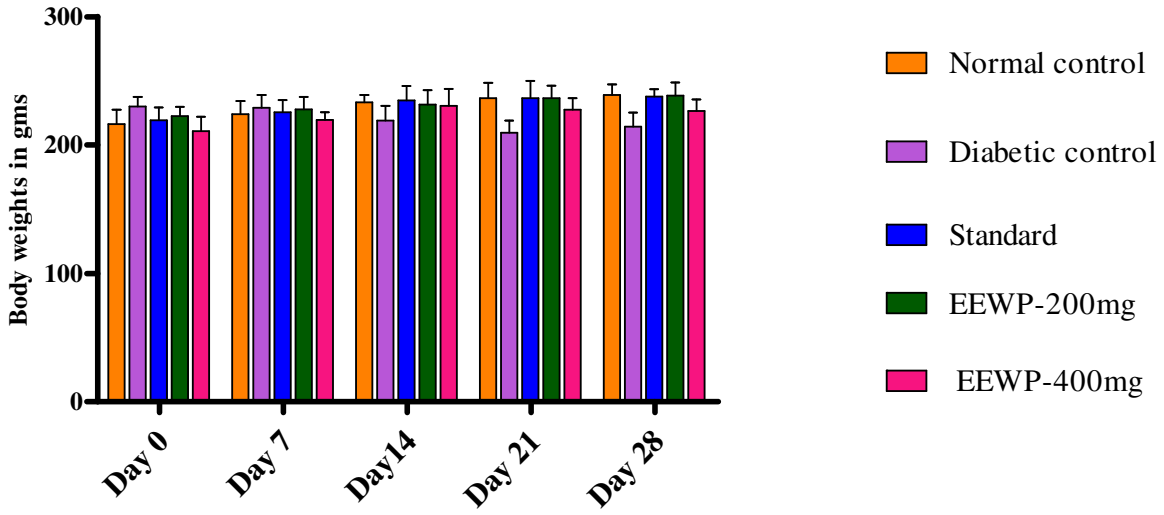


Figure 2
Effect of EEWP on Body Weight

Table 1
Effect of EEWP on fasting blood glucose

Group	Treatment (mg/kg)	Blood glucose (mg/dl)				
		0 Day	7th Day	14th Day	21st Day	28th Day
I	Normal	74.72±2.15	75.76±1.847	73.44±1.092	74.01±1.383	74.49±1.77
II	Diabetic control	262.80±3.07†	259.7±3.645†	258.8±3.130†	258.50±3.13†	257.1±1.41†
III	Pioglitazone-2mg/kg	273.60±3.24	95.87±2.382 ***	79.71±2.094 ***	74.73±1.115 ***	68.29±1.11 ***
IV	EEWP-200	275.9±3.25	235.0±5.574 ***	112.1±1.239 **	101.5±1.226 ***	96.93±1.846***
V	EEWP-400	263.2±4.55	214.1±3.091 ***	96..00±1.05 ***	81.56±1.345 ***	79.79±1.31 ***

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 2
Effect of EEWP 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	75.27±1.60	72.90±1.24	47.99±1.05	13.44±0.87	14.71±1.18
II	Diabetic control	104.80±1.23†	107.60±3.94†	31.30±0.66†	51.93±1.03†	21.13±1.26†
III	Pioglitazone-2mg/kg	83.32±1.24 ***	77.05±1.57 ***	44.67±1.46 ***	21.22±1.14 ***	16.65±1.17 ***
IV	EEWP-200	86.48±1.43 ***	82.29±1.51 **	41.01±0.91 ***	26.99±1.76 ***	18.79±0.69 **
V	EEWP-400	82.21±1.26 ***	80.76±2.34 ***	45.62±1.47 ***	21.60±0.94 ***	18.34±1.03 **

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 EEWP 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	268.2±6.75	62.43±4.52	52.58±3.27	0.68±0.029	6.98±0.329
II	Diabetic control	293.2±10.13†	73.03±2.90†	66.15±2.39†	0.82±0.039†	6.017±0.167†
III	Pioglitazone-2	280.3±5.30 *	79.09±1.77 **	57.68±2.66	0.63±0.036 ***	5.46±0.348 ***
IV	EEWP-200	158.0±6.30 ***	51.70±2.16 ***	59.01±3.33	0.54±0.031***	5.79±0.273 Ns
V	EEWP-400	196.9±6.72 ***	53.70±2.11 ***	52.24±2.39	0.51±0.035 ***	5.65±0.209 *

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.***

CONCLUSION

The present study revealed that ethanolic extract of *Walsura piscidia* Roxb shows potent antidiabetic activity against Streptozotocin induced diabetes in rats . The activity may be due to the presence of different phytochemical constituents like flavonoids, triterpenoids, polyphenos and coumarine etc and further studies needed to isolate the actual chemical constituents responsible for anti- diabetic activity, structural elucidation of that constituents and it's mechanism of action at cellular and molecular level.

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