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EVALUATION OF ANTIDIABETIC ACTIVITY OF *PICRORHIZA SCROPHULARIIFLORA* PENNELL IN HIGH FAT FED DIET WITH STREPTOZOTOCIN INDUCED TYPE II DIABETIC RATS

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ABSTRACT

High energy-intake is a major factor revolved in type 2 diabetes. The objectives of this study is to set up a suitable animal model, which is similar to the human type 2 diabetes, 6 - 8 weeks old Sprague Dawley rats weighing 220 - 250 gm were consumed high fat diet (HFD) of 20% fat, 46% carbohydrate and 20% protein (w/w). Two weeks later the animals were given with intraperitonial injection of streptozotocin (STZ) (35mg/kg body weight). The purpose of this study was to examine the effect of repeated oral administration of the ethanolic extract of rhizomes of *Picrorhiza scrophulariiflora* Pennell at a dose of (250 and 500 mg/kg) on fasting blood glucose levels and lipid metabolism in streptozotocin induced type II diabetic rats. After 21 days of repeated oral administration of 500mg of *EEPS* extract produced a significant decrease on fasting blood glucose, triglyceride, total cholesterol, LDL levels ($p<0.05)(p0.01)$ in high fat-STZ induced type II diabetic rats, on the other hand there was significant increase in HDL levels ($p<0.05$). At the end of the experiment, animals were sacrificed under euthanasia, liver homogenate was prepared and the homogenised content was analysed for anti-oxidant activity. We conclude that the ethanolic extract of rhizomes of *Picrorhiza scrophulariiflora* (500 mg/kg) exhibits anti diabetic potential along with potent lipid lowering effect after repeated oral administration.

INTRODUCTION: Diabetes mellitus is a principal cause of morbidity and mortality in human populations [1]. It is a syndrome which is characterized by hyperglycemia, polydipsia and polyuria and causes complications to the eyes, kidney, and nerves. It is also associated with an increased incidence of cardiovascular risks [2]. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups in the same country [3]. The objective of the study is to develop a suitable type II diabetic rat model that would closely mimic the natural history of the disease events (from insulin resistance to beta cell dysfunction) as well as metabolic features of human type II diabetes. The general strategy is using high-energy diet feeding for a period with the purpose to induce mild insulin resistance at first, and then an injection of a low dose of STZ to make partial dysfunction of beta cell for suppressing the insulin secretion, which works as a compensation to insulin resistance with the result of persistent hyperglycemia. This increase can be attributed to many factors, including a stressful lifestyle as well as improper dietary habits. Type II diabetes mellitus is an increasingly common disorder of carbohydrate and lipid metabolism [4].

The main characteristics are insulin resistance, the failure of peripheral tissues (liver, muscle and adipose tissue) to respond to physiologic doses of insulin, and dysfunction of pancreatic beta cell to properly secrete insulin in response to elevated blood glucose level. Recent studies have shown that lots of genes are involved in insulin resistance and hyperglycemia, whereas molecular mechanism underlying type 2 diabetes is still not completely clear. This is of economic concern as the disease requires life-long treatment and is also associated with high morbidity from the resulting complications.

All forms of diabetes mellitus are due to a decrease in the circulating concentration of insulin (insulin deficiency) or a decrease in the response of peripheral tissue to insulin (insulin resistance) [2]. These abnormalities lead to alterations in the metabolism of carbohydrates, lipids, ketones, amino acids; the central feature of the syndrome is hyperglycemia. Insulin plays a role in regulating both glycogenolysis and gluconeogenesis in liver [5].

MATERIALS AND METHODS:

Preparation of ethanolic extract of *Picrorhiza scrophulariiflora*: The rhizomes of *Picrorhiza scrophulariiflora* were air dried in shade, The dried rhizomes were powdered and weighed quantity of the powder material (970 g) was passed through sieve number 20 and subjected to hot solvent extraction in soxhelt apparatus using ethanol, at a temperature range of 50-60˚c. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40˚C under reduced pressure in a rotary vacuum evaporator. The ethanol extract yielded brownish semi-solid residues, weighing 140.0g (14.0%) and the ethanol extract was preserved by refrigeration for further use.

Animals: Adult Sprague Dawley rats (200-250g) of either sex were obtained from the animal house in C.L.Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water was provided ad libitum till beginning of the experiment. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute Toxicity Studies [6]: Healthy adult wistar albino rats of either sex, starved overnight were divided into five groups (n = 6) and were orally fed with the ethanolic extract of PS in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight [7]. The animals were observed continuously for 2 h under the following profiles: [8]

(i) Behavioral profile: Alertness, restlessness, irritability, and fearfulness.

(ii) Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait.

(iii) Autonomic profile: Defecation and urination.

After a period of 24 and 72 h they were observed for any lethality or death.

Oral glucose tolerance test (OGTT) [9]: The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. Rats divided into three groups (n = 6) were administered drinking water, Ethanolic extract of *Picrorhiza scrophulariiflora* at the dose of 250 and 500 mg/kg, respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 60 and 120 min of glucose administration and glucose levels were estimated using a glucose oxidase- peroxidase reactive strips and a glucometer (One touch horizon glucometer).

Induction of diabetes mellitus in experimental animals [10] (Benny K H *et al*., 2005): Experiments were performed in adult Sprague Dawley rats (n=36) of either sex, aged 6–8 weeks and weighing 220–250g. The animals were housed under standard environmental conditions (23±1 ◦C, with 55±5% humidity and a 12 h light/12 h dark cycle) and the rats were fed with High fat fed Diet (HFD) consisting of 20% fat, 46% carbohydrate and 20% protein (w/w).

After 2 weeks the animals fed with HFD were administered with low dose of streptozotocin (35 mg/kg, in 0.1 M citrate buffer, pH 4.5) intraperitoneally to induce diabetes mellitus.

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. The development of diabetes was confirmed after 72 hours of the streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 42 animals subjected for diabetes induction, 4 animals were died before grouping and two animals were omitted from the study, because of sub diabetic condition (108mg/dl) and (112mg/dl). The remaining 30 animals 6 groups of 6 animals were formed and used for the experimentation. In the present study, glibenclamide (0.5 mg/kg bw) and metformin (500 mg/kg bw) were used as the standard drug.

Experimental Design: Animals were divided into 6 groups of six rats each. The extract was administered for 21 days. Group I: normal control rats administered CMC (2%); Group II: diabetic control rats administered CMC (2%); Group III: diabetic rats were administered EEPS extract (250 mg/kg); Group IV: diabetic rats administered EEPS extract (500 mg/kg); Group V: diabetic rats administered glibenclamide (0.5 mg/kg); group: VI: diabetic rats were administered metformin (500mg/kg) [11]. The effects of EEPS extract in normal and diabetic rats were determined by measuring fasting plasma glucose levels, serum lipid profiles, glycated hemoglobin levels [12] and initial and final changes in body weight. Fasting plasma glucose was estimated on days 7, 14, and 21 of extract administration. Serum lipid profiles and glycated hemoglobin levels (turbidic inhibition immunoassay) were measured by biochemistry autoanalyser manufactured by Aspen technologies. On day 21st day when the animals were sacrificed, histopathological examinations of pancreas, liver and kidney were carried out at Madras University, Chennai, India.

Statistical Analysis: Datas were statistically evaluated by using one way ANOVA, followed by Dunnet's comparision test using 5th version of Graphad prism computer software. The values were considered significant when $p < 0.05$.

RESULTS: Acute toxicity studies revealed that the non-toxic nature of the ethanolic extract *picrorhiza scrophulariiflora*. There were no lethality or toxic reactions found at any doses selected until the end of study. EEPS at the dose of 500mg/kg b.w/p.o showed significant ($p<0.01$) increase in body weight when compared to negative control shown in **table 1**. The EEPS at a dose of 500mg/kg b.w/p.o produced a significant reduction in blood glucose levels in normal rats after 60 min of administration ($p<0.01$). Glucose loaded animals treated with EEPS (500 mg/kg/p.o) showed a significant ($p<0.01$) reduction in blood glucose level after 60 min shown in **table 2**. The effect of the EEPS (500mg/kg b.w/p.o) on fasting blood glucose showed significant ($p<0.01$) reduction when compared to diabetic animals shown in **table 3**. EEPS (500mg/kg bw/p.o) showed significant reduction in glycosylated hemoglobin levels, serum lipid profiles when compared to diabetic animal, whereas HDL, total protein, levels were increased significantly which shown in **table 4**.

TABLE 1: EFFECT OF EEPS ON BODY WEIGHT CHANGES IN HIGH FAT - STZ INDUCED DIABETIC RATS

Groups	Body weight in gms			
	0 day	7th day	14th day	21st day
Control	226.6±1.66	234.16±1.53	240.0±2.23	246.66±2.47
Diabetic	225.83±1.53	170.0±1.82	160.23±1.66	144.55±2.14
EEPS(250mg)	226.66±1.65	173.33±2.47^{ns}	180.5±3.35*^{b}	191.66±1.66*^{b}
EEPS(500mg)	226.6±1.66	170.0±2.58^{ns}	188.23±2.0**^{b}	208.33±1.05**^{b}
Glibenclamide (0.5mg)	226.60±1.66	174.16±1.53^{ns}	199.34±2.10***^{b}	219.16±1.54***^{b}
Metformin (0.5mg)	225.66±1.66	171.66±1.67^{ns}	197.32±1.53±**^{b}	216.66±1.65***^{b}

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's t test. n=6
a- Group II is compared with Group I. b- Groups III, IV, V, VI are compared with group II. ***P<0.001 **P<0.05,*P<0.01

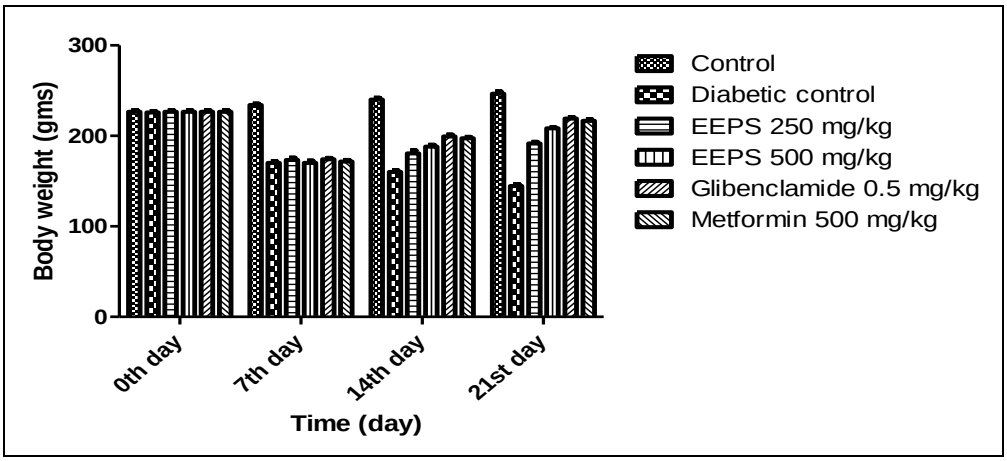

FIG. 1: EFFECT OF EEPS ON BODY WEIGHT CHANGES IN HIGH FAT - STZ INDUCED DIABETIC RATS

TABLE 2: EFFECT OF EEPS ON ORAL GLUCOSE TOLERANCE TEST IN NORMAL RATS

Groups	Blood glucose levels (mg/dl)				
	0 min	30 min	60min	90min	120min
Control	88.6±2.531	92.5±2.341	110.2±3.01	105.8±2.612	97.5±3.420
EEPS(250mg/kg)	90.1±2.603	89.7±1.964	102.5±2.631^{ns}	94.4±2.215^{*b}	89.1±2.604^{*b}
EEPS(500mg/kg)	92.4±2.812	88.2±3.408	99.6±2.540^{*b}	85.6±2.554^{**b}	79.3±3.151^{**b}
Glibenclamide (0.5mg/kg)	93.2±2.706	85.7±1.856	81.1±2.617^{**b}	74.3±3.125^{**b}	68.4±3.623^{**b}
Metformin (500mg/kg)	94.23±2.63	86.45±2.10	82.31±1.41^{**b}	76.32±2.87^{**b}	69.68±3.12^{**b}

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 , IV and V are compared with control animal values. *-p< 0.05, **-p< 0.01, ns-non significant.

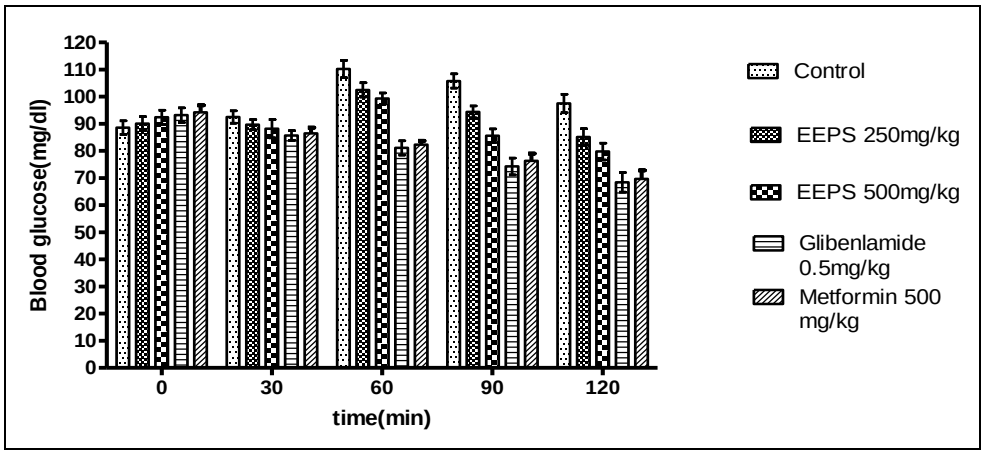

FIG. 2: EFFECT OF EEPS ON ORAL GLUCOSE TOLERANCE TEST IN NORMAL RATS

TABLE: 3 EFFECT OF SUB ACUTE TREATMENT EEPS ON BLOOD GLUCOSE LEVEL ON HIGH FAT - STZ INDUCED DIABETIC RATS

Groups	Blood glucose levels(mg/dl)			
	0th day	7th day	14th day	21st day
Control	78.0±1.82	82.33±1.42	85.33±1.22	90.0±0.86
Diabetic	286.66±3.58	292.16±3.62^{ns}	298.33±3.24^{ns}	305.83±3.26^{ns}
EEPS(250mg)	276.33±2.99	235.83±3.0^{ns}	188.83±2.18^{ns}	136.66±2.29^{*b}
EEPS(500mg)	277.83±2.84	213.56±^{*b}	163.16±2.44^{**b}	128.16±1.49^{**b}
Glibenclamide(0.5mg)	281.0±3.66	195.66±2.74^{**b}	148.5±1.72^{**b}	95.16±1.05^{***b}
Metformin(500mg)	279.5±3.54	210.5±2.17^{**b}	156.83±1.13$^{** b}$	99.17±1.07 ^{***b}

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's t test. n=6
a- Group II is compared with Group I, b- Groups III, IV, V and VI are compared with group II. ***P<0.001 **P<0.05,*P<0.01

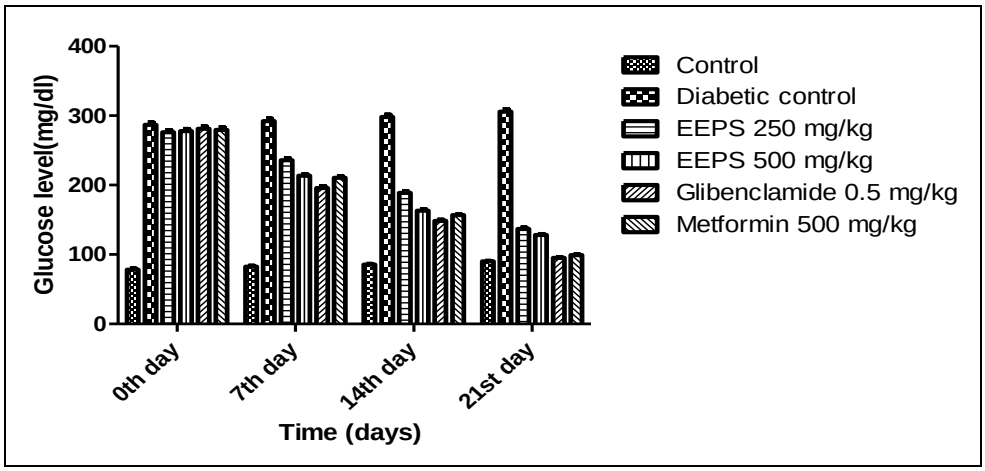

FIG. 3: EFFECT OF SUB ACUTE TREATMENT EEPS ON BLOOD GLUCOSE LEVEL ON HIGH FAT - STZ INDUCED DIABETIC RATS

TABLE 4: EFFECT OF EEPS ON VARIOUS PARAMETERS IN HIGH FAT DIET – STZ INDUCED DIABETIC RATS

Groups	TC (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	Protein (mg/dl)	Hb1Ac (GHb%)
Control	61.83±1.77	35.0±1.82	24.83±1.35	51.83±2.28	51.83±2.28	7.91±0.21	2.43±0.05
Diabetic	86.83±2.91	87.83±2.28	47.83±1.77	24.83±1.22	24.83±1.22	3.94±0.11	6.77±0.27
EEPS (250mg/kg)	79.33±2.23^{*b}	62.17±1.77^{***b}	41.67±1.14^{*b}	34.67±1.45^{**b}	34.67±1.45^{***b}	6.07±0.13^{**b}	5.01±0.12^{*b}
EEPS (500mg/kg)	72.0±1.52^{***b}	52.67±1.22^{***b}	33.67±1.11^{***b}	43.17±1.07^{***b}	43.17±1.07^{***b}	6.80±0.13^{***b}	3.40±0.17^{**b}
Glibenclamide (0.5mg/kg)	67.67±1.25^{***b}	46.50±1.47^{***b}	30.03±1.36^{***b}	49.50±0.95^{***b}	49.50±0.95^{***b}	7.14±0.11^{***b}	2.70±0.05^{***b}
Metformin (500mg/kg)	68.67±1.35^{***b}	47.67±1.02^{***b}	31.18±1.13^{***b}	48.33±1.39^{***b}	48.33±1.39^{***b}	7.13±0.13^{***b}	2.87±0.17^{***b}

The values are expressed as mean ± SEM. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's t test. n=6
a- Group II is compared with Group I, b- Groups III, IV, V and VI are compared with group II. ***P<0.001 **P<0.05,*P<0.01

DISCUSSION: Streptozotocin is the most commonly used drug for the induction of experimental diabetes for both insulin- dependent diabetes mellitus and non-insulin dependent diabetes mellitus. Type II diabetes is associated with obesity, and obesity itself causes or aggravates insulin resistance which is found to be a major cause of type II diabetes. There is an increasing evidence that streptozotocin causes diabetes by rapid depletion of β-cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation.

It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic state [13]. In this study significant hyperglycemia was achieved within 48 hours after streptozotocin (35mg/kg b.w i.p) injection. Streptozotocin induced diabetic rats with more than 250mg/dl of blood glucose were considered to be diabetic and used for study.

The EEPS at a dose 250mg/kg/p.o and 500mg/kg/p.o did not significantly suppress blood glucose level in over night fasted normal animals after 1st, 2nd and 3rd hours of oral administration when compared with standard drug like biguanide classes of drugs such as metformin which is anti-hyperglycemic drug. They do not affect blood glucose in normal individuals [14].

The EEPS showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process. However the effect was significant when compared to standard drug glibenclamide and metformin.

Treatment with EEPS at dose 500mg/kg/p.o significantly (P<0.01) decreased the blood glucose level after first week. At the end of the study a marked anti

hyperglycemic effect was observed with the EEPS treatment. The possible mechanism involved in suppressing of blood glucose levels by EEPS are modulation glucose transport, glucose disposal, secretagogue insulin secretion and which in turn to control the hyper glycemic state [15]. Experimental induction of diabetes with High fat-fed diet with low dose of STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue protein. Diabetic rats treated with EEPS show an increase in the body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e reversal of gluconeogenesis and may also be to the improvement in insulin secretion and glycemic control [16].

In diabetes hyperlipidemia is found to be pathological state, in which elevated serum total cholesterol and reduced serum HDL cholesterol increase diabetic complications by accelerating cell death and atherosclerosis. In present study, treatment with EEPS at dose 250mg/kg and 500mg/kg significantly ($p<0.01$), decrease total cholesterol and significantly increase HDL levels. This effect not only due to better glycemic control but could also been due to that drugs direct action on lipid metabolic pathway. Hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis [17]. Lipid lowering activity of EEPS at the dose of 500mg/kg related to activity of standard drug metformin.

Glycated heamoglobin is formed throughout the circulatory life of RBC with the addition of glucose to the N-terminal of the hemoglobin beta chain. This process which is nonenzymatic reflects the average exposure of hemoglobin to glucose to an extended period. Glyco-heamoglobin is used as an indicator of metabolic control of diabetes.

In the present study, high fat-fed diet with STZ treated clear-cut abnormalities in the HbA_1C levels were evident from elevated level of normal glycated heamoglobin. EEPS at the dose of 500mg/kg treatment over 21 days significantly reduced the HbA_1C levels compared to the diabetic control.

In histopathological examination pathological changes of liver include congestion of portal triad with mild inflammation and sinusoidal congestion with fatty degeneration in the form of fat lake in diabetic control rats. The above pathological changes were reduced in rats treated with ethanolic extract of rhizomes of *Picrorhiza scrophulariiflora.*

So histopathological studies showed prominent islet cell hyperplasia and regeneration of islet cells show a proof for possible anti-hyperglycemic property of the EEPS.

Histopathological examinations of diabetic animals showed centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the liver of rats treated with high fat - STZ were of much intensity and which was recovered with the treatment of EEPS.

CONCLUSION: In conclusion, the sub-acute treatment of EEPS on high fat - STZ induced diabetic animals showed signicant increase in body weight, serum total protein, serum HDL cholesterol levels, whereas significant decrease in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol and creatinine levels were observed. Based on the results, ethanolic extract of rhizomes of picrorhiza scrophulariiflora at the dose of 500 mg/kg were effective and exhibit potent anti diabetic and anti oxidant activity as compared to 250 mg/kg.

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