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EVALUATION OF ANTI-DIABETIC ACTIVITY OF METHANOL EXTRACT OF *FLACOURTIA JANGOMAS* (LOUR) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was undertaken to evaluate the antidiabetic activity of methanol extract of *Flacourtia jangomas* (lour) in streptozotocin induced diabetic rats. In this study, the acute and subacute anti hyperglycaemic effect of the two different doses (200 and 400 mg/kg b.w.p.o) of plant extracts were investigated. Fasting blood glucose level, body weight and serum lipid profiles were evaluated in normal and diabetic rats. The liver and muscle glycogen level were also evaluated. Supplementation of this extract significantly reduces the fasting blood glucose level and increases the glycogen level as compared to diabetic control. In the extracts treated groups, serum lipid profile shows a significant improvement. For all the estimated parameters, the results of the extract treated groups were restored to the near normal level, thereby indicating good antihyperglycemic activity of the methanol extract of *Flacourtia jangomas* (lour).

KEY WORDS

Antidiabetic activity, *Flacourtia jangomas,* Streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025.[1] India has today become the diabetic capital of the world with over 32 million diabetics and this number is set to increase to 80 million by 2030.[2] Diabetes mellitus causes disturbances in carbohydrate, protein and lipid metabolism damaging multi organ systems, especially the kidneys, eyes, nerves, and blood vessels which lead to complications such as diabetic nephropathy, retinopathy, neuropathy and microangiopathy.[3]

In India around 25,000 plant-based formulations are used in traditional and folk medicine. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Although the therapeutic effects of many herbs has not been scientifically proven, research continues to identify the active ingredients that may one day form the basis of drugs to fight diseases like cancer, AIDS, diabetes mellitus, asthma, physiological disorders and many more chronic diseases.[4]

Flacourtia jangomas (lour) is a large deciduous shrub or a small spreading tree upto 9m in height.[5] It is found in the northern district of Uttar Pradesh, Assam, Manipur, and South India. The plant is used for a variety of astringent, acrid, sour, refrigerant, stomachic diarrhoea, inflammation, skin disease, jaundice, tumours, nausea, dyspepsia and diabetes in South Indian traditional medicine. The study was conducted to investigate the antidiabetic activity of *Flacourtia jangomas* (lour) in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant collection and identification: The plants of *Flacourtia jangomas (lour)* fruits was collected from local area in Manipur, Imphal. The plant materials was identified and authenticated by Mr.K. Devdutta Sharma, Msc (Agriculture) PGB, Department of Horticulture & S.C., Manipur.

Plant extract preparation: The fruits were cut into pieces and dried in shade and made powder. The powder was extracted using methanol as a solvent in Soxhlet apparatus (60-70ºC). The extract was evaporated to dryness 45 ^{0}C. The methanolic extract of *Flacourtia jangomas* (MEFJ) yielded a thick blackish brown semi-solid residue. These extracts was suspended in distilled water and used for further studies.[6]

Phytochemical Screening [7,8]*:* The extract was subjected to preliminary phytochemical screening by the methods previously described by Kokate and Jayaraman J.

Animals**:** Healthy adult wistar rats (150-250g) of either sex were obtained from the animal house of C.L.Baid Metha College of Pharmacy, Chennai. Housed individually in polypropylene cages, maintained under standard conditions (12-h light and 12-h dark cycle; 25±5 ^{0}C; 35-60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Limited., Bangalore) and provided water *ad libitium.* The experimental protocol was approved by the Institutional Animal Ethics Committee of CPCSEA.

Acute oral toxicity study [9]: The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of rats and observed for signs of behavioral, neurological toxicity and mortality for 14 days.

***Effect of MEFJ on normoglycaemic and glucose induced hyperglycaemic* rats (NG-OGTT)**[10]: The effect of MEFJ on normoglycaemia and hyperglycaemia were determined by using a commercial glucometer and test-strips (Accuchek Sensor test meter).The blood glucose levels were determined in the following time pattern 30min and 60min to access the effect of the samples on normoglycemic animals. After the last measurement (at 60min) the rats were then loaded orally with 2gm/kg glucose and the glucose concentration are determined at 60, 90 and 210 min after glucose load.

Induction of diabetes mellitus [11,12,13]: A freshly prepared solution of STZ (50 mg/kg) in ice-cold citrate buffer 0.1 M, pH 4.5 was injected intra peritoneally to the overnight fasted rats. 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 hrs of STZ administration, the rats showing blood glucose level more than 200 mg/dl were considered to be diabetic and were used for the study.

The rats were divided into 5 groups of 6 rats each.

Group I - Normal control animals receives distilled water p.o.
Group II - Animals received STZ 50 mg/kg i.p.
Group III - Diabetic animals received MEFJ 200mg/kg p.o.
Group IV - Diabetic animals received MEFJ 400mg/kg p.o.
Group V - Diabetic animals received Glibenclamide 0.5mg/kg p.o.

Acute antidiabetic effect of MEFJ on blood glucose level [10,14]: After single-dose, fasting blood glucose was estimated from the tail vein

prior and after the administration of the MEFJ at 30, 60, 90, 120min and 240 min time interval.

Sub-acute antidiabetic effect of MEFJ on changes in body weight [15,16]: In multiple-dose study, the same groups were continued with the same dose of vehicle, MEFJ and glibenclamide once daily for 21days. Changes in the body weight were measurement on 1st, 7th, 14th and 21st day of treatment.

Sub-acute antidiabetic effect of MEFJ on blood glucose level [17,18]: FBG in the blood was measured at 24hr after the previous dose on 0th, 7th, 14th and 21st day by using a commercial glucometer.

***Biochemical determination*:** After 21days of treatment, overnight fasted rats were sacrificed and blood was collected from retro-orbital plexus using micro capillary technique and serum was separated. The biochemical estimation of serum triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol were estimated by using Span diagnostics kit. The liver and muscle glycogen were estimated by the method of Caroll et al.[19]

***Histopathological study of pancreas*:** The whole pancreas from each animal was removed after sacrificing the animal and was put in 10% formalin solution and immediately processed by the paraffin technique. Section of 5µ thickness were cut and stained by haematoxylin and eosin (H&E) for histological examination.

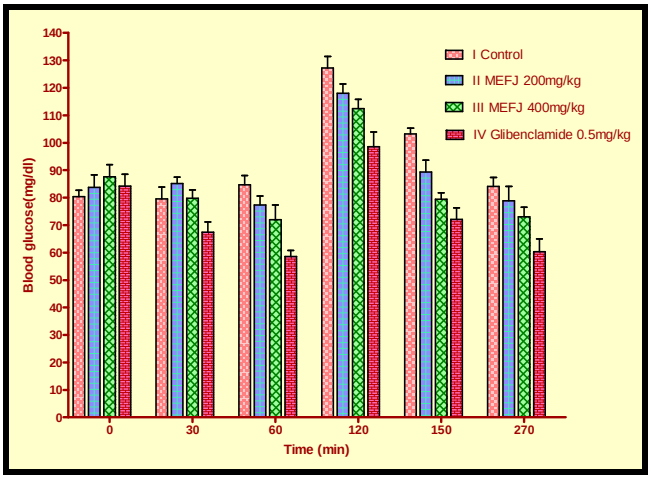
***Statistical analysis*:** Data were statistically evaluated by one- way ANOVA, followed by Dunnett's t test. The values were considered significant when $P < 0.05$.

RESULTS

***Phytochemical screening*:** MEFJ showed the presence of various phytochemical constituents such as carbohydrate, flavonoid, flavones, tannins, protein, phenol and glycoside.

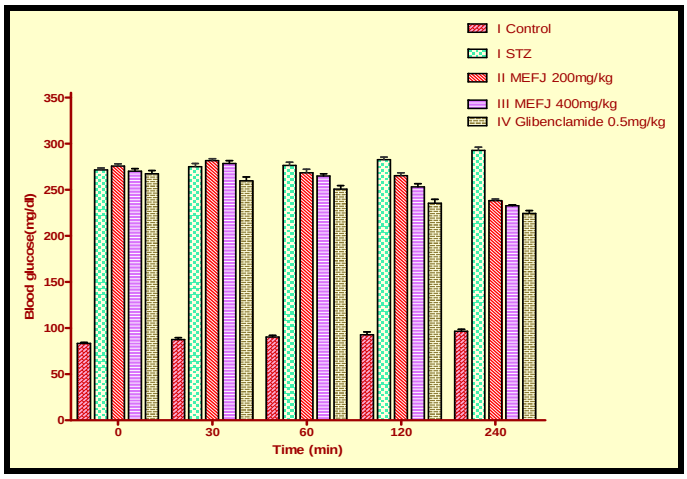
Effect of MEFJ on blood glucose levels in normoglycaemic and glucose induced hyperglycaemic rats [NG-OGTT]

The blood glucose level of control group increased when compared with glibenclamide treated group. The MEFJ at a dose level 200mg/kg did not exhibit significant hypoglycaemic effect but 400mg/kg reduced blood glucose. In glucose loaded (2gm/kg) rats, 200mg/kg reduced blood glucose level with less significance ($p<0.05$) but 400mg/kg reduced blood glucose significantly ($p<0.01$). Glibenclamide (0.5mg/kg) treatment showed significant ($p<0.01$) reduction in blood glucose levels in both normal and glucose induced hyperglycaemic rats. (Graph 1)



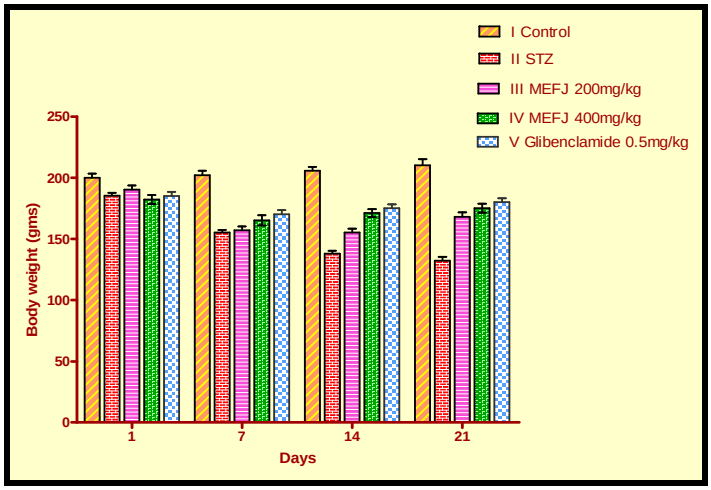
Graph 1: ***Effect of MEFJ on blood glucose levels in normoglycaemic and glucose induced hyperglycaemic rats [NG-OGTT]***

Antidiabetic activity screening in experimentally induced diabetic: A single-dose administration of MEFJ (200 and 400 mg/kg) showed a significant (P<0.01) reduction in blood glucose level (BGL) after 120min and 240min interval. Maximum reduction in BGL to 232.5 ± 2.2.6 mg/dl was seen at 240min after administration of 400mg/kg of MEFJ. Glibenclamide (0.5 mg/kg) also showed maximum reduction to 224.1±3.34 mg/dl at 240min. (Graph 2)



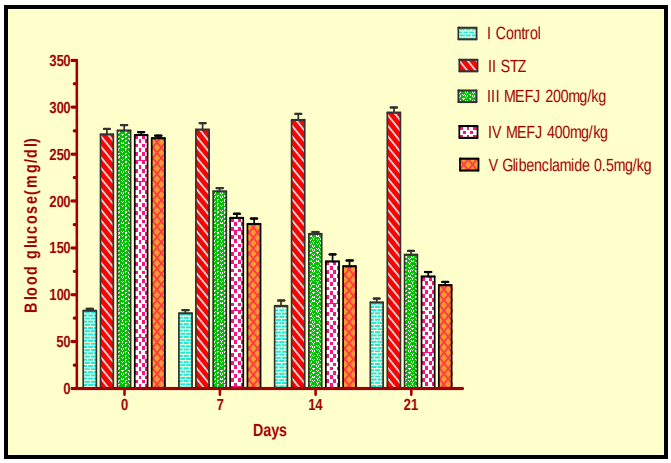
Graph 2: ***Effect of single dose treatment of MEFJ on blood glucose level in STZ induced diabetic rats***.

In the body weight measurement, normal vehicle control animals were found to be gained in their body weight but diabetic rats showed a significant reduction in the body weight, which reversed by MEFJ treated groups (200 and 400 mg/kg) and Glibenclamide (0.5 mg/kg) treated group during 21 days treatment. (Graph 3)



Graph 3: ***Effect of sub-acute treatment of MEFJ on body weight changes in STZ induced diabetic rats.***

On repeated administration of either vehicle, MEFJ or Glibenclamide for 21days, 200mg/kg showed significant decreased ($p<0.05$) and ($p<0.001$) in the blood glucose level at second and third week respectively. Both 400mg/kg and glibenclamide (0.5 mg/kg) produced a significant decrease ($p<0.05$) at first week, ($p<0.001$) at second and third weeks in blood glucose level. (Graph 4)



Graph 4: ***Effect of sub-acute treatment of MEFJ on blood glucose level in STZ induced diabetic rats.***

Serum cholesterol, serum triglycerides, serum LDL levels and serum VLDL were decreased significantly, but serum HDL levels was increased significantly in both standard and test drug. (Table 1)

Table 1.

Effect of MEFJ on Total Cholesterol, HDL-Cholesterol, Triglycerides, LDL and VLDL in STZ induced diabetic rats

Group	Treatment	Total Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Control (distilled water)	99.78±5.34	34.35±2.34	87.56±2.43	47.8±2.56	17.4±1.56
II	Diabetic control (STZ)	177.60±2.37[a]**	23.34±1.67[a] **	172.5±3.45[a] **	119.4±2.04[a] **	34.4±1.85[a] **
III	MEFJ (200mg/kg)+ STZ	128.6±2. 89[b]**	29.45±2.05[b]*	122.3±2.93[b] **	74.6±2.8[b] **	24.4±1.09[b] **
IV	MEFJ (400mg/kg)+ STZ	112.7±3.57[b]**	34.10±1.43[b]**	102.7±3.76[b]**	57.8±3.2[b] **	20.4±2.30[b] **
V	Glibenclamide +STZ	104.56±4.63[b]**	40.21±2.06[b]**	92.12±1.87[b] **	45.6±2.7[b] **	18.4±1.32[b] **

*n = 6, *p < 0.05, **p < 0.01, ns- non-significant, (one way ANOVA followed by Dunnett's 't' test)*
a- Group II is compared with Group I.
*b- Groups III, IV, V are compared with group II **P<0.01,*P<0.05*

Administration of the MEFJ (200 and 400mg/kg) increased liver and muscle glycogen level significantly (p<0.05) and (p<0.01) in respective group. Glibenclamide (0.5mg/kg) show significant (p<0.01) increase when compared to STZ induced diabetic animals. (Table 2)

Table 2.

Effect of MEFJ on the liver and Muscle glycogen in STZ induced diabetic rats

Group	Treatment	Liver glycogen (mg/g wet tissue)	Muscle glycogen (mg/g wet tissue)
I	Control(distilled water)	50.0±2.13	8.21±0.54
II	Diabetic control (STZ)	17.56±0.72[a] **	2.62±0.30[a] **
III	MEFJ(200mg/kg)+STZ	22.50±0.98[b] *	4.65±0.36[b] *
IV	MEFJ(400mg/kg)+STZ	35.53±0.57[b] **	6.18±0.33[b] **
V	Glibenclamide +STZ	42.62±0.76[b] **	7.15±0.27[b] **

*n = 6, *p < 0.05, **p < 0.01, ns- non-significant, (one way ANOVA followed by Dunnett's 't' test)*
a- Group II is compared with Group I.
*b- Groups III, IV, V are compared with group II **P<0.01,*P<0.05*

Histopathological study of pancreas

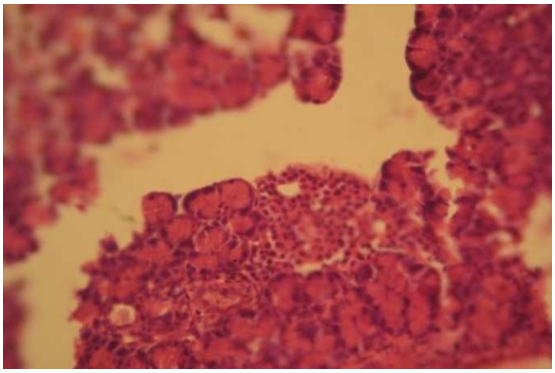


Fig: A

Fig-A: ***The Pancreatic islets of langerhans of normal rat show normal islets and acini.***

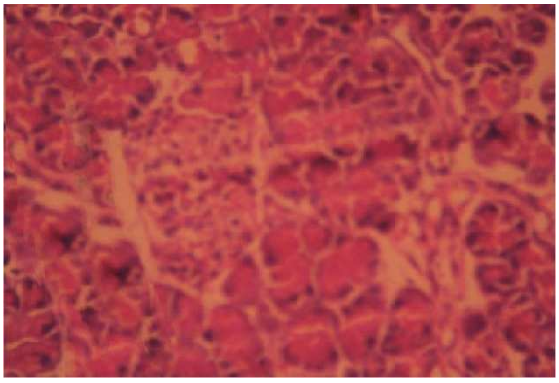


Fig: B

Fig-B: ***Streptozotocin induced diabetic pancreatic islets shows damaged and atrophic islet with acni***

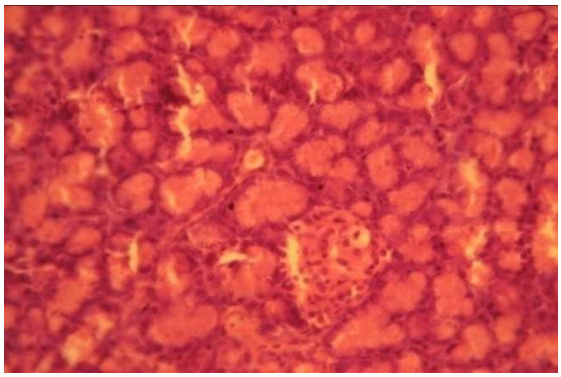


Fig: C

Fig-C: ***MEFJ (200mg/kg) treated pancreatic islets show small pancreatic islet cells.***

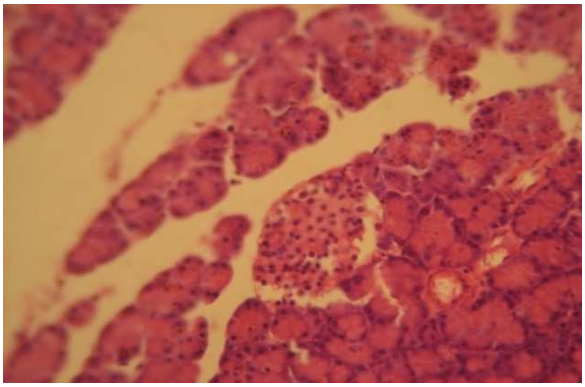


Fig: D

Fig-D: ***MEFJ (400mg/kg) treated pancreatic islets show hyperplastic islet with acni.***

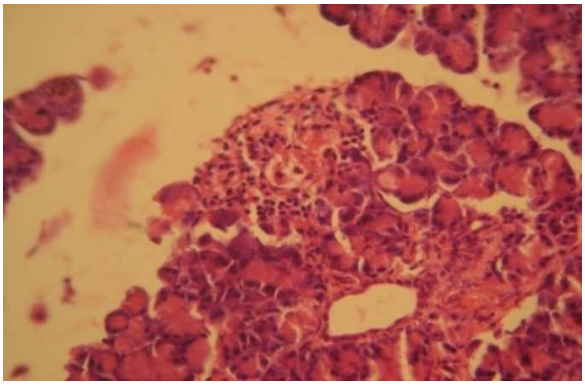


Fig: E

Fig-E: ***Glibenclamide (0.5 mg/kg) treated pancreatic islets shows preserved cells.***

DISCUSSION

The present study was undertaken to investigate the antidiabetic activity of methanol extract of *Flacourtia jangomas* (lour).in STZ induced diabetic rats. The MEFJ at a dose 200mg/kg and 400mg/kg did not significantly suppress blood glucose level in over night fasted normoglycaemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycaemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process.[20] However, the effect was significant when compared to standard drug glibenclamide. A single dose administration of MEFJ at a dose 200mg/kg and 400mg/kg significantly reduce blood glucose level at 120 and 240min. In the sub-acute study, glibenclamide treatment reduces blood sugar level from the first week to third week. Treatment with MEFJ at dose 200mg/kg and 400mg/kg significantly decreased the blood glucose level after the second and first week. At the end of the study a marked anti hyperglycemic effect was observed in the MEFJ treatment. Possible mechanism involved with suppressing blood glucose levels by MEFJ are 1) modulation glucose transport, 2) glucose disposal, 3) insulin secretion, which in turn control the hyper glycemic state.[21]

Induction of diabetes with 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 MEFJ 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 due to the improvement in insulin secretion and glycemic control.[22]

Lipid plays an important role in the pathogenesis of complications involved with diabetes mellitus. The elevated level of serum cholesterol and reduced level of serum HDL cholesterol in diabetic condition, poses to be a rises of factor for developing microvascular complication leading to atherosclerosis and further leads to cardiovascular diseases like coronary heart disease. The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase, insulin deficiency or insulin resistance may be responsible for dislipidimia.[23] Present study showed rat streptozotocin treated diabetic rats has abnormal lipid profile whereas the MEFJ treated group showed significant improvement in the lipid profile comparable to glibenclamide treatment group. This effect not only due to better glycemic control but could also been due to inhibition of the pathway of cholesterol synthesis and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver and reduce the serum LDL level.[24] Hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis. It is well known that hyperlipidemia has an association with atherosclerosis's and the incidence of atherosclerosis is vastly increased in diabetics.[24]

Insulin is the main regulator of glycogenesis in muscle and liver. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic condition or oxidative stress by diabetes may inactivate the glycogen synthetase. The marked reduction in liver and muscle glycogen level is observed (21 days) in streptozotocin induced diabetic animals. Treatment with MEFJ extract remarkably increased the glycogen level in liver and muscle. In the view of glycogen level, there may be three possible ways of antidiabetic action; one possible way may be increased insulin level by preventing the inactivation of the glycogen synthetase and by synthesize the glycogen synthatase.[25]

Histopathological studies, revealed in streptozotocin treated group necrosis of β-cells were confirmed. Glibenclamide treated group show preserved cytology. At 200mg/kg MEFJ treated rats shows small islet cells whereas 400mg/kg MEFJ treated rats shows an hyperplastic i.e, regulation of β-cells confirms the MEFJ at 400mg/kg.

CONCLUSION

From such information it may be stated that the antidiabetic activity of MEFJ may be by sensitize the insulin receptor or stimulation of insulin from β-cell of islets of Langerhans in pancreas of STZ induced diabetic rats.

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