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Effect of *Berberis tinctoria* leaf (Berberidaceae) extract on antidiabetic, antihyperlipidemic and antioxidant status in streptozotocin induced diabetes in rats

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SUMMARY

The present study was carried out to investigate the antidiabetic and antioxidant effect of methanolic extract of Berberis tinctoria leaves (MEBT), in streptozotocin induced diabetic rats. Oral administration of MEBT extract (150 mg/kg and 300 mg/kg) for a period of 14 days. Blood glucose levels, body weight food and liquid intake were measured on every 5th day over a period of 14 days. In diabetic rats, MEBT at the dose of 150 mg/kg and 300 mg/kg body weight resulted in significant reduction in blood glucose levels. The study was further investigated to determine antioxidant and antihyperlipidemic potential of MEBT in streptozotocin (STZ) induced diabetic rats. These results suggest that the MEBT possess antidiabetic activity and is able to ameliorate biochemical damages in STZ induced diabetic rats and the results were found to be in a dose dependent manner.

Key words: *Berberis tinctoria*; Methanol extract; Streptozotocin induced diabetic rats; Antihyperglycemic activity; Antioxidant potential; Antihyperlipidemic effect

INTROUDCTION

Diabetes is a common endocrine disorder, affecting over 100 million people worldwide. The World Health Organization predicts that this number may increase fivefold in the near future. Complications are the major cause of diabetic morbidity and mortality. In India, diabetes affects 1 - 5% of the population. This disorder is from as early as 700 - dietary indiscretion 200 BCE, its two types are a genetically based disorder and one resulting from "dietary indiscretion." It affects several physical processes and organs, and its course may be modified by a variety of compounds, including alkaloids, glycosides, poly-saccharides, peptidoglycans, hypoglycans, glycopeptides, and terpenoids at numerous sites in the body.

The most common conventional treatment for diabetes is insulin, which has prominent side effects. Neither insulin nor other modern pharmaceuticals has been shown to modify the course of diabetic complications (Grover *et al.*, 2002). Many herbal products, including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where

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modern medicines alone fail (Anturlikar *et al.*, 1995).

The plant *Berberis tinctoria* Lesch (Berberidaceae) is a shrub, very variable in size and form, in the open often 2 to 3 feet high, but in the forest sometimes reaching a height of 15 feet with thick stem and long scandent branches bearing numerous slender leafy twigs (Fyson *et al.*, 1974). It is locally called as *Oosikala* and medicinally used by the Kurumbas, the Nilgiri tribe, for stomach-ache (root paste). The aqueous root paste along with honey is used as an antimicrobial agent against skin diseases. The wood, root bark and extract have been used in skin diseases, menorrhagia, diarrhoea, jaundice and infections of the eyes (Kirithikar *et al.*, 1975). The root and root bark of the plant is rich in alkaloid content, Berberine, the principal alkaloid.

There are no available reports on the anti-oxidant and antihyperlipidemic potential of *Berberis tinctoria* against diabetes. Therefore, we took the present study to determine antihyperlipidemic; anti-oxidant potential in streptozotocin (STZ) induced diabetic rats.

During the last two decades there has been an encouragement of plant drug research to fight with some communicable and non-communicable diseases. Diabetes is one of the chronic non-communicable diseases.

MATERIALS AND METHODS

Chemicals

Tolbutamide (Hoechst Pharmaceuticals, Mumbai, India) and STZ (Sigma Chemical Company, St. Louis, MO, USA) were provided as a gift sample. All other chemicals and reagents used were of analytical grade and were obtained from the following indicated commercial sources. Thio-barbituric acid, nitro blue tetrazolium (NBT), Nicotinamide adenine dinucleotide (NADH) (Loba Chemie, Mumbai, India), 5, 5-dithio *bis*-2-nitro benzoic acid (DTNB), reduced glutathione (GSH) (SISCO Research Lab, Bombay, India).

Plant material

The plant *Berberis tinctoria* was collected from Doddabetta forest area, Udhagamandalam, Nilgiri district, Tamil Nadu in the month of February 2004. It was identified by the Botanical Survey of India, Coimbatore. The voucher specimen number is BSI/SC/5/21/03-04/Tech.(1674). The underground root was separated from the aerial parts and the leaves was then dried under air, shade dried and mechanically powdered separately to obtain a course powder, which was then subjected to extraction.

Preparation of extract

The plant *Berberis tinctoria* was initially defatted with Petroleum ether (60 - 80°C) followed by chloroform and methanol, by the method of continuous hot extraction using a Soxhlet apparatus. The chloroform and methanol extract was distilled, and dried in vacuum and the yield is (2.5% w/w) and (12% w/w) respectively.

Animals

Studies were carried out using Wistar albino rats (150 - 180 g) of Male sex were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Induction of experimental diabetes

After one week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced with a single i.p. injection of STZ at a dose of 65 mg/kg body weight. The STZ was freshly dissolved in

citrate buffer (0.01 M, pH 4.5) (Ozsoy-Sacan, 2000). The injection volume was prepared to contain 1.0 ml/kg (Hamilton *et al.*, 1998, Murali *et al.*, 2002). After 5 days, blood glucose levels were measured and the animals with a concentration of more than 225 mg/dl were taken for the investigation (Ewart *et al.*, 1975; Cetto *et al.*, 2000).

Experimental design

In the experiment, a total of 30 rats (6 normal; 24 STZ diabetic rats) were used. The rats were divided into five groups of six animals each. Group I: Normal, received normal saline solution (0.9% NaCl w/v, 5 ml/kg); Group II: Diabetic received Streptozotocin (65 mg/kg) once before the treatment; Group III: received MEBT (150 mg/kg); Group IV: received MEBT (300 mg/kg); and Group V: received Tolbutamide as reference standard (10 mg/kg) for 14 days.

The effect of MEBT on STZ induced diabetic rats were determined by measuring blood glucose levels, food and fluid intake amount and changes in body weights (Ewart *et al.*, 1975; Kamtchousing *et al.*, 1998). After 14 days of treatment, all the rats were decapitated after fasting for 16 h. The animals were dissected and a drop of blood from the heart was used for the estimation of blood glucose. Tissues (brain, heart, liver, and kidney) were removed and cleared off blood. They immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1N Tris-HCl buffer (pH 7.4), and used for the estimation of thio-barbituric acid reactive substances (TBARS), reduced glutathione (GSH), super oxide dismutase (SOD) and catalase (CAT) activity.

Acute toxicity study

Albino male mice of 10 animals per group and weighing between 20 and 25 g were administered graded dose of the methanol extract of *Berberis tinctoria* (MEBT). After administration of the MEBT the mice were observed for toxic effects after 48 h of treatment. The toxicological effects were observed in terms of mortality expressed, as LD_{50}. The number of animals dying during a period was noted (Ghosh, 1984). The LD_{50} of the extract was calculated by the method of Litchfield and Wilcoxon (Litchfield *et al.*, 1959) was found to be 2,089.20 mg/kg.

Measurement of blood glucose levels

At the beginning of the experiment and at 5-day intervals, body weight and blood glucose levels were measured. Blood samples were obtained by tail-vein puncture of the normal and STZ-induced diabetic rats on day zero (0), day 5, day 10 and on day 15. Blood glucose levels were determined using a glucometer (One Touch Ultra blood glucose monitoring system from Lifescan, Johnson and Johnson Company, Milpitas, CA).

Measurement of total cholesterol and triglyceride

On the fifteenth day blood samples (1 ml) were collected by tail-vein puncture under mild ether anesthesia in Eppendroff's tubes containing 50 micro liter of anticoagulant (10% trisodium citrate solution) from the normal and STZ - induced diabetic rats. Plasma was separated by centrifugation at 5,000 rpm for 10 min and analysed for total cholesterol and triglycerides using kits from Span Diagnostics Ltd., Surat, India.

Determination of *in vivo* antioxidants

Determination of TBARS: TBARS in tissues was estimated by the method of Fraga *et al.* (1981). To 0.5 ml tissue homogenate; 0.5 ml saline and 1.0 ml 10% TCA were added, mixed well and centrifuged at 3,000 rpm for 20 min. To 1.0 ml of the protein-free supernatant, 0.25 ml of thiobarbituric acid (TBA) reagent was added; the contents were mixed well and boiled for 1 h at 95°C. The tubes were then cooled to room temperature under running water and absorption measured at 532 nm.

Determination of GSH: GSH was determined by the method of Beutler *et al.* (1963) 0.2 ml of tissue

homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitating reagent (1.67 g of metaphosphoric acid, 0.2 g of EDTA disodium salt, 30 g sodium chloride in 1,000 ml of distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB (5, 5-dithio *bis* 2-nitro benzoic acid) reagent were added and read at 412 nm.

Assay of SOD: The activity of SOD in tissue was assayed by the method of Kakkar *et al.* (1954). The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 M), 0.1 ml phenazine methosulphate (186 mM), 0.3 ml NBT (300 mM), 0.2 ml NADH (780 mM) and approximately diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 s, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

Assay of CAT: Catalase was assayed according to the method of Maehly and Chance (1954). The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1 - 4°C and centrifuged at 5,000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H_2O_2 and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

Statistical analysis: Data were expressed as Mean ± standard error of the mean (S.E.M.) for 6 rats in each group. The biochemical parameters were analysed statistically using one-way ANOVA, followed by Dunnett's multiple comparison test (DMRT). The minimum level of significance was fixed at $P < 0.05$.

RESULTS

Effect of MEBT on blood glucose levels

The blood glucose levels were measured in normal and STZ-induced diabetic rats. The results were shown in Table 1. There was a significant increase in blood glucose levels in STZ-induced diabetic rats (Group II). Administration of MEBT at a dose of 150 mg and 300 mg/kg. Body weight and tolbutamide (10 mg/kg) significantly ($P < 0.01$) decreased blood glucose levels in STZ induced rats (Group III, IV and V). The results were found statistically significant in a dose dependent manner.

Effect of MEBT on total cholesterol and triglyceride

The effects of MEBT at a dose of 150 mg/kg and 300 mg/kg on plasma total cholesterol and triglycerides were shown in the Table 1. MEBT

Table 1. Effect of MEBT extract on glucose level in STZ induced rats

Group	Dose (mg/kg)	Glucose Level (mg/dl)			
		0	5th Day	10th Day	15th Day
Normal-I (0.9% NaCl w/v)	-	84.1 ± 1.2**	85.0 ± 1.4**	85.3 ± 2.0**	85.2 ± 2.1**
STZ (Diabetic Control)-II	65	312.18 ± 2.0	312.32 ± 19	312.31 ± 22.1	312.20 ± 21.8
MEBT-III	150	310.20 ± 1.0*	262.16 ± 4.2**	145.72 ± 16.2**	124.23 ± 13.7**
MEBT-IV	300	311.21 ± 2.8*	260.42 ± 3.2**	130.62 ± 4.8**	117.23 ± 10.2**
Tolbutamide-V	10	312.6 ± 3.0*	252.1 ± 1.8**	125.12 ± 1.2**	112.56 ± 8.9**

Values are mean ± S.E.M. 6 animals in each group (n = 6). *$P < 0.05$, Values are considered statistically significant. *$P < 0.05$, **P < 0/01,When compared to diabetic control (STZ).

significantly reduced cholesterol and TGL levels. A significant increase in the cholesterol ($P < 0.01$) and TGL ($P < 0.01$) levels was observed in the diabetic group. In MEBT treated rat a significant ($P < 0.01$) decrease in total cholesterol and triglyceride was observed when compared to diabetic control (Table 3).

Effect of MEBT on body weight, food and liquid intake

The body weight, food and liquid intake were measured and summarized in Table 2. The initial body weights were not same in normal and diabetic groups, whereas the final body weights were significantly ($P < 0.01$) decreased in diabetic control (Group I) when compared with Normal control (Group II). At the same time, MEBT treated diabetic rats (Group III and IV) showed significant increase in body weight compared that of diabetic control. Food and fluid intake amount were significantly ($P < 0.05$) higher in diabetic group than the normal (Table 1).

Effect of MEBT on the levels of TBARS and GSH

The concentration of TBARS and the GSH in tissues in experimental diabetic rats are shown in Fig. 1 and 2. There was significant elevation of TBARS in STZ-diabetic control rats when compared to normal rats. Adminstration of MEBT and

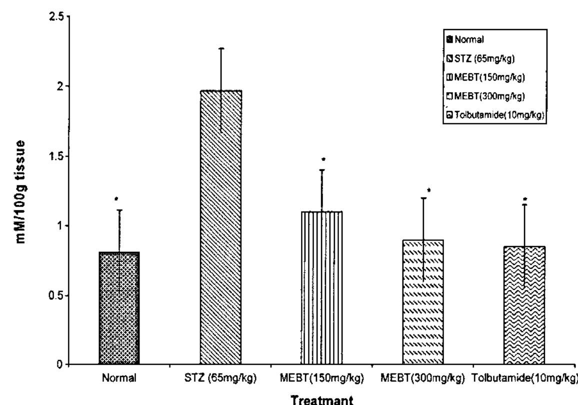

Fig. 1. Effect of MEBT on TBARS Level. $^{*}P < 0.05$, $^{**}P < 0.01$ Values are considered statistically significant when compared to diabetic control (STZ).

Table 2. Effect of MEBT on body weight, food and liquid intake in rats

Group	Dose (mg/kg)	Body Weight (g) Initial	Body Weight (g) Final	Food intake (g/rat/day)	Liquid intake (ml/rat/day)
Normal (0.9 % NaCl w/v)-I	-	163.4 ± 1.1**	188.32 ± 2.4**	15.14 ± 2.5**	19.40 ± 0.98**
Diabetic (STZ)-II	65	180.2 ± 3.5	160.12 ± 2.2	30.20 ± 2.1	30.14 ± 1.2
MEBT 150mg/kg-III	150	165.1 ± 4.8*	189.41 ± 3.3**	19.91 ± 1.6*	25.67 ± 1.0*
MEBT 300mg/kg-IV	300	166.3 ± 3.1*	192.56 ± 3.2**	18.61 ± 1.6*	22.08 ± 1.2**
Tolbutamide-V	10	163.9 ± 3.5**	191.2 ± 2.9**	17.23 ± 4.5**	21.01 ± 1.1**

Values are mean ± S.E.M. 6 animals in each group (n = 6). $P < 0.05$ Values are considered statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to diabetic control (STZ).

Table 3. Effect of MEBT extract on glucose level, cholesterol and triglyceride levels in STZ induced rats

Group	Dose (mg/kg)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal (0.9 % Nacl w/v)-I	-	100.0±5.7**	97.55±2.33**
Diabetic (STZ)-II	65	148.12±12.7	185.67±12.45
MEBT-III	150	118.51±6.9*	147.67±8.88*
MEBT-IV	300	116.89±5.2*	146.89±5.6*
Tolbutamide-V	10	105.0±7.1**	108.32±10.97**

Values are mean ± S.E.M. 6 animals in each group (n = 6). $P < 0.05$ Values are considered statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to diabetic control (STZ).

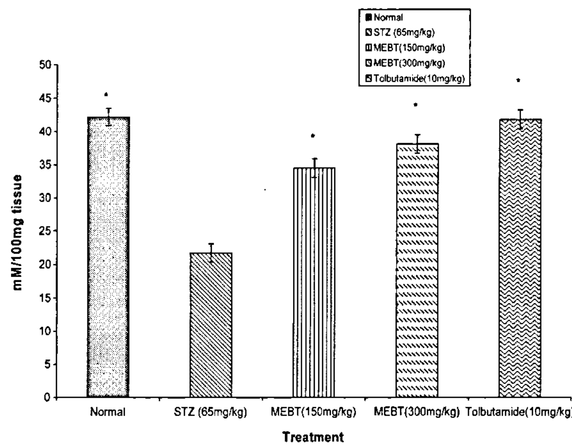

Fig. 2. Effect of MEBT on GSH Level. $^{*}P < 0.05$, $^{**}P < 0.01$ Values are considered statistically significant when compared to diabetic control (STZ).

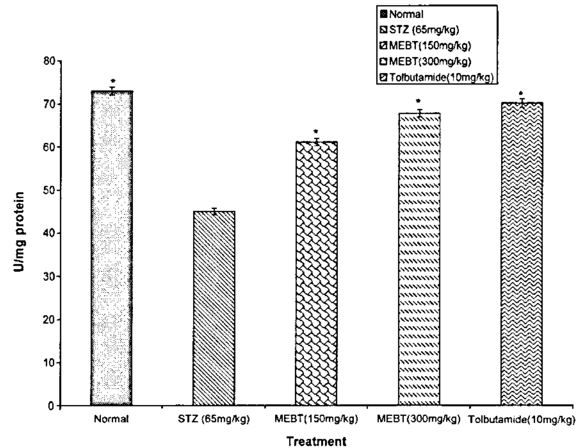

Fig. 4. Effects of MEBT on CAT levels. $^{*}P < 0.05$, $^{**}P < 0.01$ Values are considered statistically significant when compared to diabetic control (STZ).

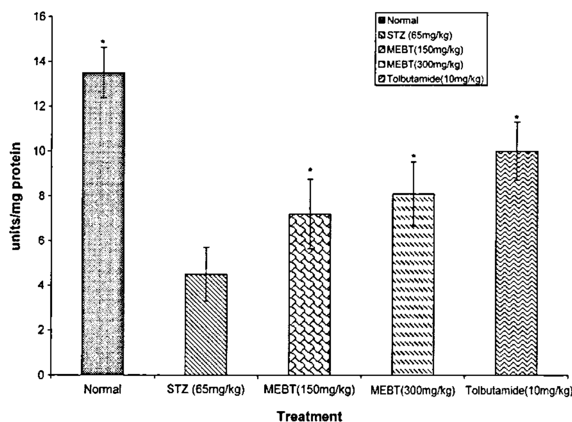

Fig. 3. Effect of MEBT on SOD levels. $^{*}P < 0.05$, $^{**}P < 0.01$ Values are considered statistically significant when compared to diabetic control (STZ).

tolbutamide showed significant decrease in the levels of TBARS in liver, when compared with diabetic group (II) diabetes in MEBT and Tolbutamide treated animals. There was a significant ($P < 0.01$) decease in the concentration of GSH in STZ-diabetic control group when compared with the normal. Administration of MEBT at the dose of 150 mg and 300 mg/kg body weight reduced the levels of GSH in liver, ($P < 0.05$) during diabetes.

Effect of MEBT on the activity of SOD and CAT

The activity of SOD and CAT in experimental animal tissues have been summarized in Fig. 3 and 4. There was a significant ($P < 0.05$) reduction in the activity of SOD in liver during diabetes. Administration of MEBT at 150 mg and 300 mg/kg body weight and Tolbutamide 10 mg/kg body weight increased the activity of SOD in liver to near normal.

DISCUSSION

Diabetic mellitus is characterized by a reduced capacity of the β-cells of the pancreas to release sufficient insulin to induce the activity of glucose metabolizing enzymes whether the cells are destroyed as in type 1 diabetes (IDDM) or intact as in type 2 diabetes (NIDDM) (Panneerselvam *et al.*, 2002). Oral administration of Methanol extract of MEBT has shown significant hypoglycemic effect against streptozotocin-induced diabetes in rats. The extract lowered significantly the levels of blood glucose, plasma thiobarbituric acid reactive substances and significantly increased the levels of GSH, SOD and CAT.

Nakakimura *et al.* (1980) have reported that the

concentration of lipid peroxidation increases in the kidney of diabetic rats and an increased level of TBARS is an index of lipid peroxidation. Our results show that in diabetic control animals the levels were high in plasma, due to increased lipid peroxidation. In MEBT and tolbutamide treated diabetic rats, the TBARS levels were low which may be due to the free radical scavenging action of active ingredients in *Berberis tinctoria* (Fig. 1).

GSH is a major endogenous antioxidant which counterbalance free radical mediated damage. It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions. Significant decrease in the levels of GSH has been observed in MEBT-treated rats when compared to STZ-induced diabetic rats (Fig. 2). The decrease in liver GSH levels represents increased utilization due to oxidative stress (Anuradha *et al.*, 1993).

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2•-), which damages the membrane and biological structures (Arivazhagan *et al.*, 2000). Catalase has been shown to be responsible for the detoxification of significant amounts of H_2O_2 (Cheng *et al.*, 1981). SOD and catalases are the two major scavenging enzymes that remove the toxic free radicals *in vivo*. Reduced activities of SOD and catalase in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (O2•-) and hydrogen peroxide (Searle *et al.*, 1981). MEBT and Tolbutamide treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT (Figs. 3 and 4).

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats (Riyad *et al.*, 1998; Tarfa *et al.*, 1998; Choi *et al.*, 1991; Sharma *et al.*, 1966) and significant increase observed in our experiment was in accordance to these studies. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides (Taskimen *et al.*, 1987). However, in insulin deficient subject; it fails to activate the enzyme and causes hypertrigly-ceridemia. MEBT and tolbutamide showed in a similar way by increased insulin production in STZ induced hyperglycemic animals and lowered the triglyceride levels by activation of enzyme lipoprotein lipase.

To our knowledge, this is the first study in which a follow up was made on the effect of Methanol Extract of *Berberis tinctoria* extract in the levels of glucose, lipid peroxides and non-enzymatic antioxidants in diabetic animals. In our study, we observed elevated levels of blood glucose in streptozotocin diabetic rats. Administration of MEBT decreases significantly the levels of blood glucose in diabetic rats. In addition, treatment of animals with MEBT caused a decrease in total cholesterol although this was less marked than decrease of triglycerides. Repeated administration of MEBT thus had a beneficial effect on the hyperlipemia associated with hyperglycemia.

Berberine can block K^+ channels on Colonic cell lines and that it also inhibits brain Na^+, K+, ATPase and various cation-dependent phosphohydrolases. As we mentioned Berberine is the principle alkaloid in *Berberis tinctoria*. The selection of Tolbutamide reduces the K^+ permeability of β-cells by blocking K_{ATP} channels causing depolarization, Ca^{2+} entry and insulin secretion. It may have the same mechanism action as mentioned above.

Thus, it may be concluded from the results of the present investigation that MEBT possess antidiabetic principle and can be useful for treatment of diabetes. Further studies to fractionate the active principle and to elucidate the exact mechanism of action are, therefore, required to be undertaken.

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