ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *IPOMOEA REPTANS* POIR AERIAL PARTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Summary

Diabetes mellitus is a metabolic disorder characterized by fast elevation of blood sugar level, along with other multiple complications and is a growing health concern worldwide. The present study was undertaken to evaluate the antihyperglycemic activity of methanol extract of aerial part of *Ipomoea reptans* Poir (Convolvulaceae) in streptozotocin (STZ) induced diabetic rats. After 14 days treatment with the methanol extract, 200 and 400 mg/kg b.wt. p.o., fasting blood glucose (FBG) level was found to be reduced by 56.16% and 65.7% respectively with respect to the initial FBG levels. Antioxidant activity of the extract was determined by measuring MDA, Catalase and glutathione (GSH) levels of liver, kidney and pancreas tissues. Apart from these, body weight, serum enzyme levels (SGPT, SGOT, ALP), total protein, triglyceride and total cholesterol level were also estimated. Results of the extract treated groups were compared with those of the diabetic control and normal animals. For all the estimated parameters, the results of the extract treated groups were restored to the near normal level, thereby indicating good antihyperglycemic and antioxidant activity of the methanol extract of *I. reptans*.

Key words: *Ipomoea reptans*, Diabetes, Streptozotocin, Antihyperglycemic.

Introduction

Diabetes mellitus is the most important disease involving the endocrine pancreas. It is characterized by inappropriate hyperglycemia and disordered metabolism [1]. The incidence of diabetes mellitus is a growing health concern worldwide, causing severe and costly complications including blindness, cardiac and kidney diseases [2]. Pharmacological means (insulin and synthetic oral hypoglycemics) as well as non-pharmacological means (diet and exercise) may be used in the management of this disease. However the obvious limitations of these management methods, like the cost factor and some of the serious side effects for oral hypoglycemics [3]; and for insulin therapy, insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment [4], necessitate a search for the antidiabetic agent from the arsenal of herbs available to man.

In recent years, herbs are effectively being tried in the variety of pathophysiological states. The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important [5].

Ipomoea reptans (Linn) Poir belongs to the family Convolvulaceae. The aerial part of this plant is an edible, green leafy vegetable, available in all over India as well as in many parts of Southeast Asia. It is an annual or biennial herb; stems are long, trailing on mud or floating, thick, hollow, rooting at the nodes, glabrous [6]. The leaves are good source of minerals and vitamins especially carotene. Its juice has purgative action. Leaves and stems are said to be cooling [7]. As per Yunani, it is useful in fever, inflammation, bronchitis, liver complaints etc. [6]. Literature survey disclosed its potent antioxidant property [8]. In diabetes, oxidative stress has been found to play an important role. Hence compounds with both antihyperglycemic and antioxidant properties would be the useful antidiabetic agents. So in the present study, methanol extract of *I.reptans* (MEIR) aerial parts has been studied for its antihyperglycemic potential on streptozotocin (STZ) induced diabetic rats.

Materials and Methods

Plant material

The aerial parts of *I. reptans* was collected in March 2007, from Khardah, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (PIR- 1) was retained in our laboratory for further reference.

Preparation of plant extract

The aerial parts were dried and powdered in a mechanical grinder. The powdered material was extracted by methanol using soxhlet apparatus. This extract was filtered and concentrated in *vacuo* and kept in a vacuum dessicator for complete removal of solvent. The yield was 17.25% w/w with respect to dried powder. Preliminary qualitative analysis showed the presence of polyphenols, flavonoids and saponins in the methanol extract. Aqueous suspension of MEIR was prepared using 2 % (v/v) Tween-80 and used for oral administration.

Animals

Male Wistar albino rats (200 g ± 20) were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The experiments were performed based on animal ethics guidelines of Institutional Animals Ethics Committee.

Study on normoglycemic animals

After overnight fasting with free access to water, fasting blood glucose (FBG) level of each animal was determined at the beginning of the experiment. Animals in control group (Gr. I) received only the vehicle and the test group animals (Gr. II and III) were treated with the MEIR suspension (200 and 400 mg/kg b.wt). Blood sugar levels were determined at 30, 60, 120 and 180 min after the oral administration of test samples to assess the effect of the test samples on normoglycemic rats.

Study on glucose-loaded animals (Oral Glucose Tolerance Test, OGTT)

Overnight fasted normal rats were divided into four groups (n=6). First group received only vehicle, group II and III were given low and high dose (200 and 400 mg/kg b.wt) of MEIR and standard drug was given to group IV. The rats of all the groups were loaded with glucose (3 g/kg, p.o.) 30 min after the administration of the drugs or vehicle (for control). Blood glucose levels were measured at 30, 60, 120 and 180 min after glucose load to assess the effect of different doses of extract on blood glucose levels of the glucose loaded animals.

Induction of experimental diabetes

A freshly prepared solution of STZ (50 mg/kg) in ice-cold citrate buffer 0.1 M, pH 4.5 was injected intraperitoneally to the overnight fasted rats [9]. After 72 hrs of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level > 250 mg/dl were considered to be diabetic and were used for the study.

Study on STZ induced diabetic rats

The rats were divided into four groups (n=6). Treatment was made for 14 days. Group I: normal rats received only vehicle. Group II, III, IV and V contained STZ induced diabetic rats. Group II received only vehicle and served as STZ control group. Group III and IV were orally administered MEIR, 200 and 400 mg/kg b.wt. respectively; while Group V was treated with the reference drug, Glibenclamide (0.5 mg/kg).

Testing of FBG and body weight

The fasting blood glucose (FBG) level of each animal was monitored on days 0, 4, 8 and 15. Drop of blood was collected from the tip of the tail vein of each rat and FBG level was measured using One Touch Glucometer, Horizon, from Lifescan, Johnson and Johnson Company. Initial and final body weights were also recorded.

Estimation of biochemical parameters

On 15^{th} day blood samples were collected from the retro-orbital plexus of the rats and serum was separated for the biochemical estimations of serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) [10], alkaline phosphatase (ALP) [11], total protein

[12], total cholesterol and triglyceride [13]. All the analysis were performed by using commercially available kit from Span Diagnostics Ltd.

Evaluation of antioxidant properties

After collection of blood, all the animals were sacrificed by euthanasia. Liver, kidney and pancreas were collected for the estimation of the tissue malondialdehyde (MDA) [14], reduced glutathione (GSH) [15] and Catalase (CAT) [16] levels for the antioxidant study.

Statistical Analysis

Values were presented as mean ± S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. P values less than 0.05 were considered as statistically significant.

Results

Blood glucose level of normoglycemic study (NG) and OGTT were presented in Table.1, showing the effect in OGTT but no effect in NG results. FBG and change in body weight in the STZ induced 14 days study were summarized in Table.2.1, 2.2, indicating MEIR as equipotent as the reference drug, Glibenclamide.

Table.1. Effect of MEIR on blood glucose level in normal and glucose-loaded rats

(Values are Mean±S.E.M.; n=6 in each group)

Test Model	Groups	Blood glucose concentration (mg/dl)				
		0 h	½ h	1 h	2 h	3 h
NG	Control	82.33±0.92	80.33±0.72	79.17±1.74	80.67±0.67	80.50±1.29
	MEIR (200 mg/kg)	80.17±0.95	78.83±2.15	81.50±1.84	81.33±2.97	80.33±1.45
	MEIR (400 mg/kg)	83.33±2.01	81.50±1.52	78.67±0.96	81.17±2.12	78.17±0.95
OGTT	Control	77.00±1.24	108.50±2.67	121.00±2.37	103.33±2.54	80.50±1.34
	MEIR (200 mg/kg)	80.17±1.58	102.17±1.80$^{\#}$	117.00±1.90	102.83±1.20	81.50±0.85
	MEIR (400 mg/kg)	82.00±1.92	98.14±1.02$^{\#}$	105.33±1.69$^{\#}$	94.83±1.30$^{\#}$	76.83±1.07
	Glibenclamide (0.5 mg/kg)	79.00±1.03	96.17±0.75$^{\#}$	102.33±1.05$^{\#}$	89.17±1.01$^{\#}$	70.33±1.09$^{\#}$

$^{\#}$ $p < 0.05$ when compared to glucose loaded control group animals; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Table.2.1. Effect of MEIR on FBG of control and STZ diabetic rats
(Values are Mean±S.E.M.; n=6 in each group)

Groups	Mean Serum FBG level ± S.E.M. (mg/dl)				
	Day 0	Day 4	Day 8	Day 15	% Change
Normal Control	82.67±0.99	81.33±2.09	80.00±1.13	83.17±1.38	0.73
Diabetic control	294.50±7.34[a^]	315.33±7.25[a^]	309.00±4.47[a^]	305.17±5.12[a^]	3.74
MEIR (200mg/kg)	284.00±6.58	268.50±7.66[b/]	212.17±8.24[b//]	124.50±2.19[b//]	-56.16
MEIR (400mg/kg)	286.00±9.17	231.83±10.97[b//]	159.33±11.58[b//]	98.00±2.84[b//]	-65.70
Glibenclamide (0.5 mg/kg)	293.67±3.67	235.50±6.76[b//]	172.50±2.54[b//]	96.17±2.57[b//]	-67.24

[a] when compared to normal control group, [^] $p < 0.001$
[b] when compared to diabetic control group, [/] $p < 0.01$, [//] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Table.2.2. Effect of MEIR on body weight of control and STZ diabetic rats
(Values are Mean±S.E.M.; n=6 in each group)

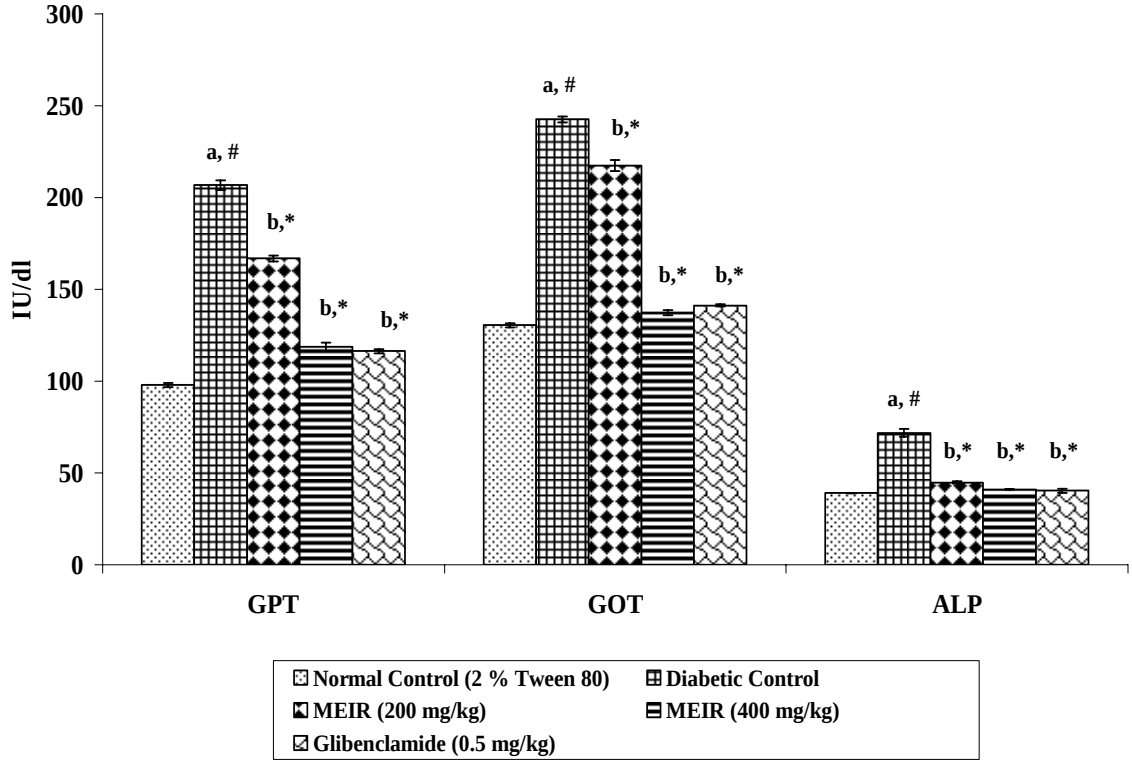
Groups	Body weight (g)		
	Initial	Final	Change
Normal Control	192.67±4.37	197.00±4.83	4.33±2.57
Diabetic control	203.33±5.84	177.83±5.00	-25.50±2.35[a#]
MEIR (200mg/kg)	200.67±5.65	184.50±5.09	-17.00±2.46[b*]
MEIR (400mg/kg)	196.33±4.83	179.33±5.60	-16.17±1.30[b*]
Glibenclamide (0.5 mg/kg)	196.00±3.31	182.00±3.43	-14.00±1.37[b*]

[a] when compared to normal control group, [#] $p < 0.05$
[b] when compared to diabetic control group, [*] $p < 0.05$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

In case of biochemical estimation, improvement of serum enzyme levels, total protein and lipid profile were observed in the treated groups with respect to the diabetic control group as shown in Fig.1, 2 and 3 respectively.

Antioxidant status of liver, kidney and pancreas were shown in Fig.4.1, 4.2, 4.3. Significant increase in MDA levels was observed in diabetic control groups while these were restored to near normal by the supplementation of MEIR. GSH level and catalase activity were drastically reduced in STZ control group, which were increased to the normal level in the extract and Glibenclamide treated groups.

Fig .1. Effect of MEIR on serum GPT,GOT and ALP level in STZ induced diabetic rats

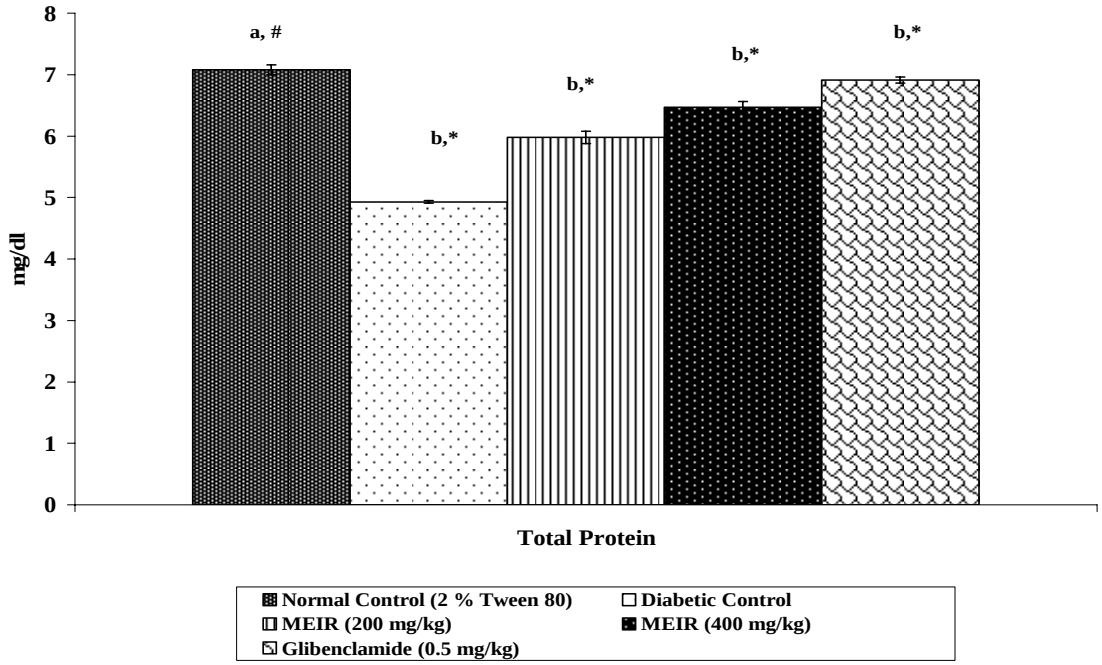


Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.001$

[b] when compared to diabetic control group, [*] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Fig.2. Effect of MEIR on Total Potein in STZ induced diabetic rats

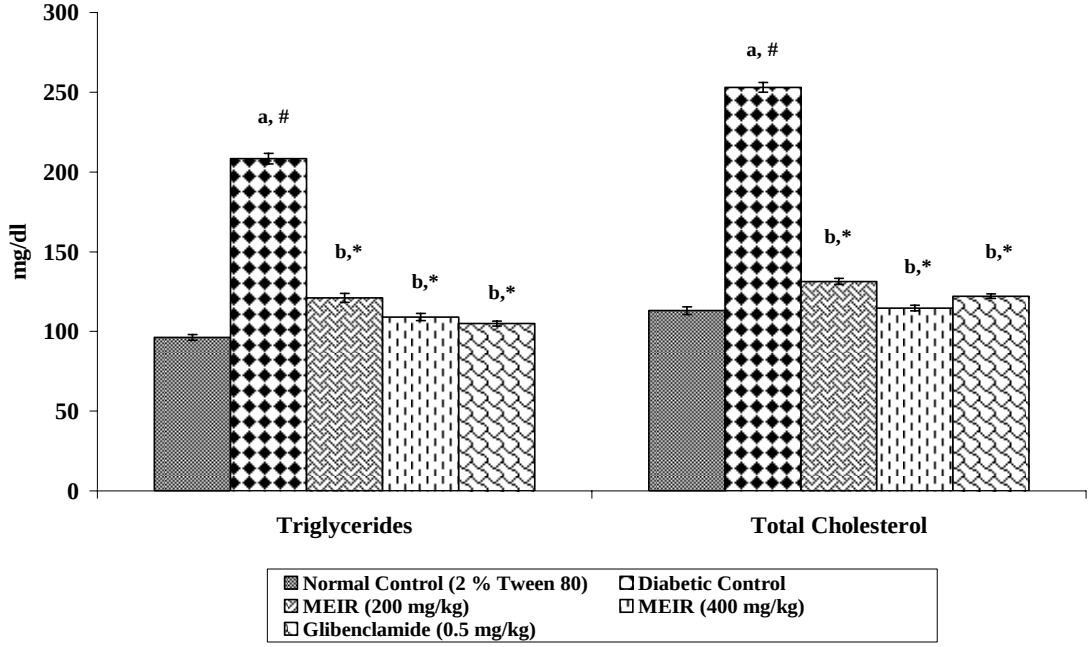


Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.001$

[b] when compared to diabetic control group, [*] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Fig.3. Effect of MEIR on Triglycerides and total cholesterol in STZ induced diabetic rats

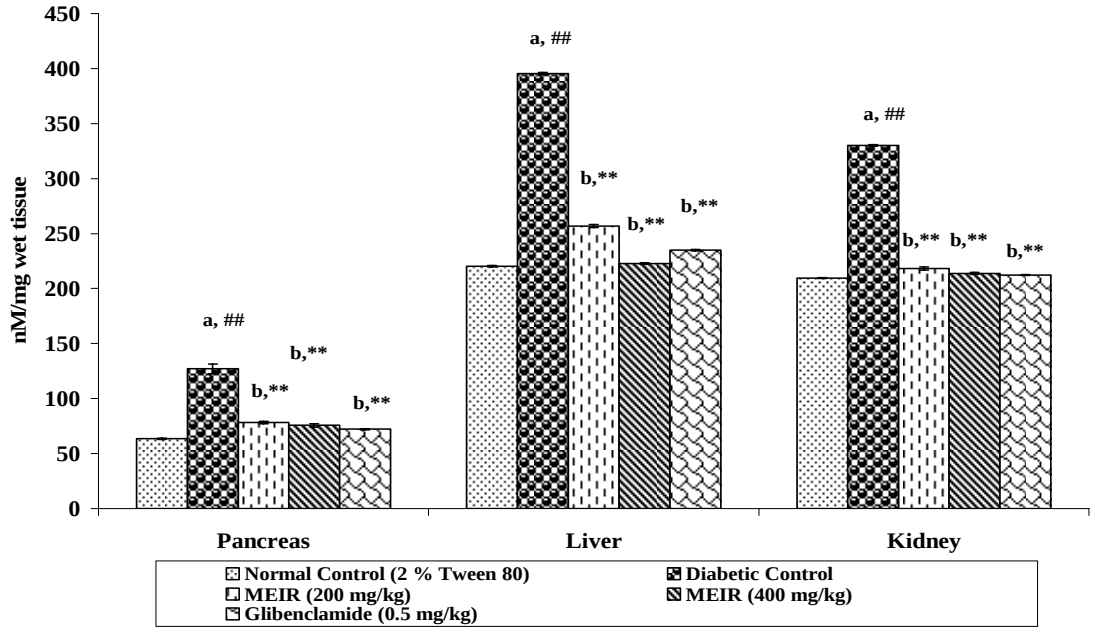


Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.001$

[b] when compared to diabetic control group, [*] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Fig.4.1. Effect of MEIR on MDA level in STZ induced diabetic rats

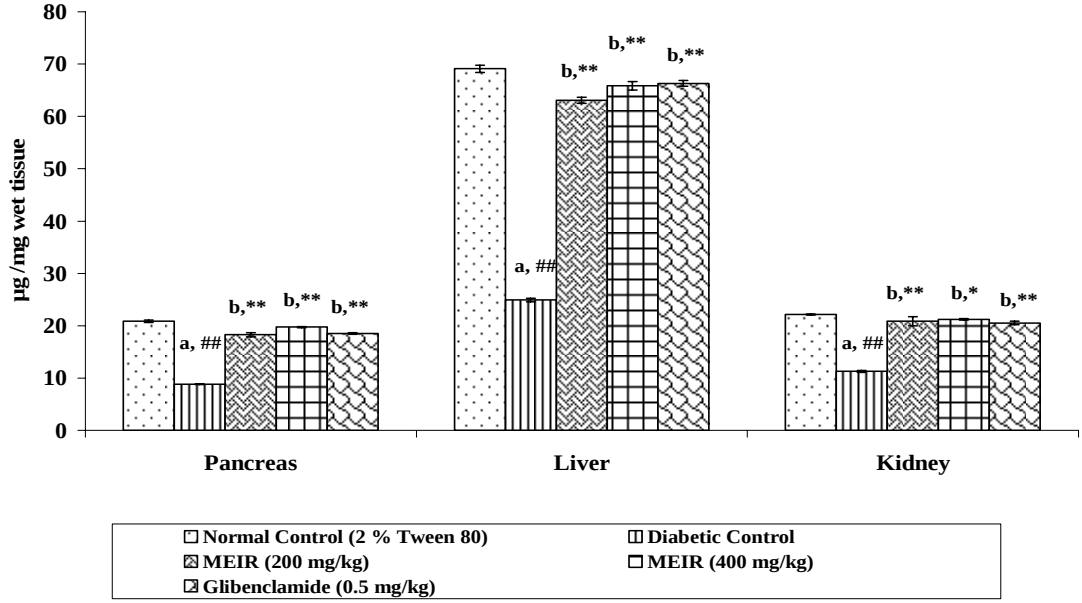


Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.01$, [##] $p < 0.001$,

[b] when compared to diabetic control group, [*] $p < 0.01$, [**] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Fig.4.2. Effect of MEIR on GSH level in STZ induced diabetic rats

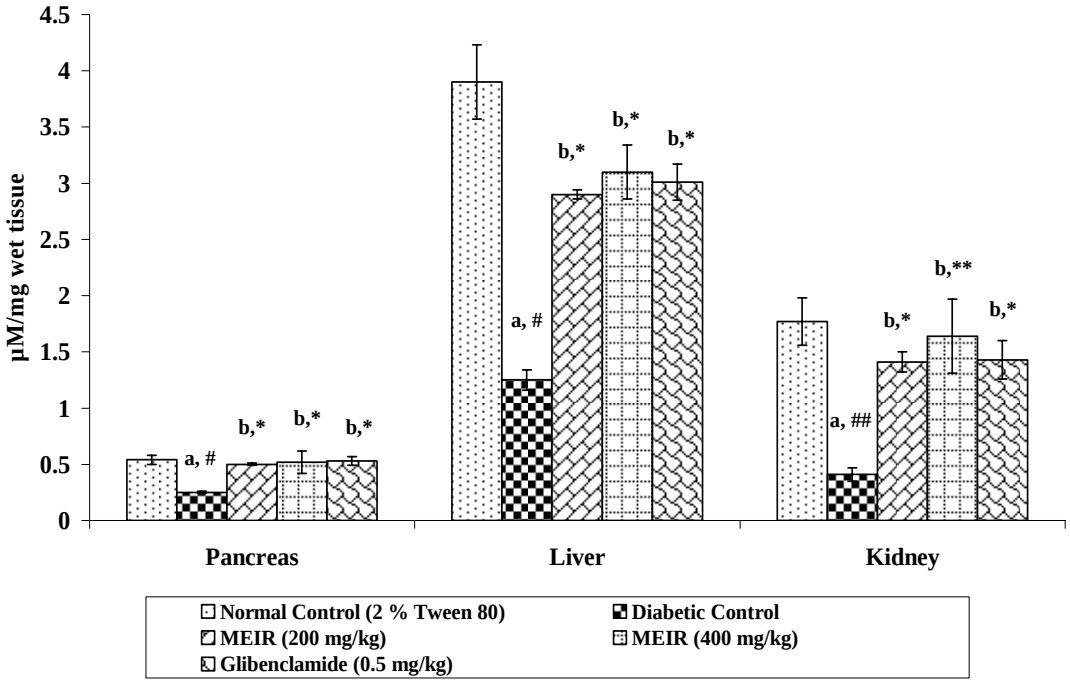


Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.01$, [##] $p < 0.001$,

[b] when compared to diabetic control group, [*] $p < 0.01$, [**] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Fig.4.3. Effect of MEIR on CAT level in STZ induced diabetic rats



Values are Mean ± S.E.M.; n=6 in each group.

[a] when compared to normal control group, [#] $p < 0.01$, [##] $p < 0.001$,

[b] when compared to diabetic control group, [*] $p < 0.01$, [**] $p < 0.001$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Discussion

Streptozotocin (STZ) is widely used for the induction of diabetes mellitus in experimental animals by the degeneration and necrosis of β cells of islet of langerhans of pancreas, which leads to the reduction in insulin release [17]. Due to the presence of glucose moiety in its structure, STZ can selectively enter the β cells via the low affinity glucose transporter GLUT2 in the plasma membrane. After entering into the cells, it exerts cytotoxicity by spontaneously decomposing into reactive methylcarbonium ions that alkylate DNA; and/or inducing free radical generation which target the DNA sugar moiety and result in DNA strand breakage [18].

In the present study, MEIR did not show any effect on euglycemia of the rats, however, in OGTT, a significant reduction in blood glucose level was observed at a dose level of 400 mg/kg of MEIR, when compared to glucose loaded control, which showed impaired glucose tolerance. In STZ induced diabetic rats, MEIR showed a significant reduction (56.16 and 65.7 % for low and high dose respectively) in fasting blood glucose (FBG) level with respect to diabetic control group at the end of 14 days experimental period, indicating similar effect as the reference drug, Glibenclamide (67.24 %).

This antihyperglycemic action may be attributed to the potentiation of pancreatic secretion of insulin from existing β cells of islets or to the extrapancreatic mechanisms like enhanced transport of blood glucose to peripheral tissue, increased peripheral utilization of glucose *via* different enzymatic pathways. Since MEIR could not exert any effect on normoglycemic animals, but significantly reduced the elevated blood sugar level, it implies that it acts through the extrapancreatic pathways rather than stimulating insulin secretion and results in antihyperglycemic action without affecting normal blood sugar level, which may be beneficial in case of misdosing.

Induction of diabetes with STZ is associated with characteristic loss of body weight, mainly due to increased muscle wasting and due to loss of tissue proteins [19]. MEIR administration to STZ diabetic rats reversed the weight loss.

Diabetes is associated with profound alteration in the plasma lipid and lipoprotein profile and consequently linked to an increased risk of coronary heart disease [20]. Insulin deficiency and increased blood glucose level lead to hypertriglyceridemia and hypercholesterolemia, as was found in the diabetic control group in the present study. This is mainly due to the uninhibited actions of lipolytic hormones on the fat depots and increased mobilization of free fatty acids from the fat depot. This excess fatty acid gets converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins [21]. This elevated lipid level was however, restored to near normal in the extract as well as reference drug treated groups.

Elevated levels of the serum enzymes (SGPT, SGOT and ALP) in the diabetic control group reflect the significant alteration of liver function by STZ induction. Treatment of MEIR was found to be equipotent to Glibenclamide in restoration of the elevated enzyme levels to normal, implying the normal functioning of liver. Affected liver functioning also resulted in the decreased protein synthesis in diabetic rats while it was almost restored in the treated animals.

Oxidative stress plays a key role in the pathogenesis of diabetes mellitus by oxygen free radicals as well as due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides [22, 23]. Diabetic subjects may have a defective cellular antioxidant response against this oxidative stress generated by hyperglycemia, which can predispose to organ damage. Hence antioxidant therapy is beneficial for such diabetic patients. In our study, STZ induction caused elevation of MDA and decrease in GSH and CAT levels in liver, kidney and pancreas tissues.

In diabetes, hypoinsulinemia causes lipid peroxidation which in turn impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzyme and receptors and resultant products are harmful to the cells in the body [24]. MEIR treatment significantly reduced the MDA level of liver, pancreas and kidney tissue.

GSH has a multi-faceted role in antioxidant defence. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases [25]. In the present study, a significant increase in tissue GSH in extract treated diabetic rats with respect to diabetic control group indicates that MEIR can either increase biosynthesis of GSH or reduce oxidative stress leading to degradation of GSH or having both effects [26]. Antioxidant enzyme CAT is involved in the detoxification of hydrogen peroxides and thereby protects the tissue from highly reactive hydroxyl radicals [24]. Elevated CAT level was found in diabetic control group while its level was restored in the extract and Glibenclamide treated groups indicating that extract can reduce reactive oxygen free radicals and improve the activities of the antioxidant enzymes.

The present study shows that MEIR possesses significant antihyperglycemic properties and has the ability to reduce oxidative stress as well which may be useful to prevent diabetic complications. It can also improve hyperlipidemia due to diabetes. Therefore MEIR can be considered as a potential safe antidiabetic agent; however further studies are ongoing to establish its bioactive principle(s).

Acknowledgements

The authors are thankful to the University Grant Commission, New Delhi, India for providing financial assistance.

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