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Full Length Research Paper

Antihyperglycaemic, antioxidative activities of a formulated polyherbal drug MTEC (Modified) in streptozotocin-induced diabetic rat

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Musa paradisiaca **belongs to the family of Musaceae and is distributed throughout India and Malaysia. The plant has medicinal value as antidiabetic agent. Seeds of** ***Tamarindus indica*** **of Caesalpiniaceae family used as traditional medicine for the management of diabetes mellitus found throughout India.** ***Eugenia jambolana*** **an evergreen tree of Myrtaceae family is used as traditional medicine for the management of diabetes in India.** ***Coccinia indica*** **of Cucurbitaceae family is distributed widely all over India and its medicinal value as antidiabetic agent is well recognized. The diabetic therapeutic efficacy of a formulated polyherbal drug (abbreviated form MTEC), constituted with n-hexane fractions of the hydromethanol extract of root of** ***M. paradisiaca*****, seed of** ***T. indica*****, seed of** ***E. jambolana*** **and leaf of** ***C. indica*** **at specific ratio was investigated in streptozotocin-induced diabetic rats. Streptozotocin-induced diabetic state was confirmed here by the monitoring of increased level of fasting blood glucose, decreased glycogen level in liver and skeletal muscle along with elevation in the activities of glucose-6-phosphatase and lactate dehydrogenase and diminution in the activities of hexokinase as well as glucose-6-phosphate dehydrogenase in liver, skeletal muscle and cardiac muscle. Oral administration of MTEC (modified) at a dose of 100 mg/5 ml of olive oil/Kg body weight daily to the diabetic rats significantly ($P<0.05$) corrected fasting blood glucose level, glycogen contents of liver and skeletal muscle along with said carbohydrate metabolic enzyme activities in liver, skeletal and cardiac muscles in diabetic rat towards the control levels. Diabetes mellitus induced oxidative stress was also corrected significantly ($P<0.05$) by the treatment of this polyherbal drug (modified MTEC) to the diabetic rat. Results of the present study support the therapeutic potentiality of this polyherbal drug MTEC for the management of diabetes.**

Key words: Antihyperglycaemic drug, polyherbal drug, diabetes mellitus, oxidative stress, carbohydrate metabolic enzymes.

INTRODUCTION

Diabetes mellitus, an endocrine and metabolic disorders characterized by chronic hyperglycemia produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidation that play role in the progression of the symptoms of diabetes (Giugliano et al., 1996). Several hypotheses have been postulated to explain the development of free radicals in diabetes include auto oxidation of glucose, enzymatic and non-enzymatic glycation of proteins with increased formation of glucose derived advanced glycosylation end products (AGEs), enhanced glucose flux through polyol pathway (Oberley, 1988) and reduction of anti-oxidant defence (Kuyvenhoven et al., 1999). Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run especially drug resistance is also noted (Rao and Apparao, 2001. So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern (Upathaya and Pandy, 1984; WHO, 2002).

Musa paradisiaca (Musaceae) is distributed throughout

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India and Malaysia. Several reports have claimed the medicinal values of different parts of *M. paradisiaca* (Kirtikar and Basu, 1991; Joshi, 2000). We have also reported the antidiabetic activity of the root of *M. paradesiaca* (Mallick et al., 2007[a]). Seed of *Tamarindus indica* (Caesalpiniaceae) is used as traditional medicine for the management of diabetes mellitus (Iyer, 1995; Maiti et al., 2004). *Eugenia jambolana* (Myrtaceae), an evergreen tree is used as traditional medicine for the management of diabetes (Kirtikar and Basu, 1991; Mallick et al., 2007[b]) in India. *Coccinia indica* (Cucurbitaceae) is distributed widely all over India and its medicinal value as antidiabetic was reported (Chopra, 1958; Mallick et al., 2007[a]).

These above four plants are used in folk medicinal practices to treat diabetes mellitus in remote villages of West Bengal state, India. Previously we have reported the antidiabetic activity of our formulated herbal drug known as MTEC taking the aqueous methanol extract of above four plants at a specific ratio (Mallick et al., 2007[c]). The present study was designed to develop a more potent MTEC with a low dose efficacy for the management of diabetes using the n-Hexane fraction of hydromethanolic extract of compounds of above four plant parts as this fraction is most bio-active in this purpose out of the fractions prepared using other organic solvents found from our pilot studies in this line. The philosophy of polyherbal drug over mono herbal drug has been adopted here as composite extracts of plant shows promising effects may be due to synergistic effect or herb-herb interaction (Kumari and Devi, 1993; Mallick et al., 2007[c]).

MATERIALS AND METHODS

Plant material and polyherbal drug formulation

Roots of *M. paradisiaca*, seeds of *T. indica*, seeds of *E. jambolana* and leaves of *C. indica* were used in this study. All the said plant parts were collected locally in June, and identified by a taxonomist in the Botany Department, Vidyasagar University, Midnapore, India. Voucher specimens (HPCH No-7, 1, 6, 8) were deposited in the Department of Botany, Vidyasagar University. After collection, the samples from each species were washed under tap water and dried in an incubator for two days at 40°C, crushed and powdered separately in an electric grinder. From these powders, 50 g of each species was separately suspended in 250 ml of aqueous-methanol (2:3, v/v) mixture that formed slurry and kept in an incubator at 37°C with intermittent stirring for the first 2 h. After 36 h, the mixture was filtered and the filtrate was dried under partial vacuum at 35-40°C and the pulps residues were collected. These dried hydromethanol extracted pulps were then subjected to fractionation with laboratory grade n-Hexane solvent. These fractions were then dried under partial vacuum at 35-40°C to collect the solvent free residue. MTEC, a polyherbal anti diabetic drug was then formulated using the solutes of n-hexane fraction of *M. paradisiaca*, *T. indica*, *E. jambolana* and *C. indica* at the ration of 2:2:1:1 respectively.

Animal care and selection

Normoglycemic Wistar strain male albino rats of three months of age about 150 ± 10 g in weight were used in these experiments. The animals were housed at an ambient temperature of 25 ± 2°C under a 12 : 12 h light-dark cycle and acclimated to these conditions for 15 days before use in experimental trials. All animals had free access to standard feed of rat and water *ad libitum*. The principles of laboratory animal care (NIH 1985) and instruction given by our institutional ethical committee were followed throughout the experiments.

Induction of diabetes mellitus

Rats, kept at fasting state for last 12 h, were subjected to a single intramuscular injection of streptozotocin (STZ) (Sisco Research Laboratories, Mumbai, India) at a dose of 35 mg/1 ml of citrate buffer/Kg of body weight/rat (pH:4.5). Diabetic condition (type I) was confirmed in fasting rats from blood glucose level more than 250 mg/100 ml determined after 72 h interval and then on the 7th day after day of injection. Out of twenty rats used for diabetes induction in the above said methods, eighteen rats fulfilled the above criteria and were selected for this experiment.

Animal grouping and treatment

Initial body weight of all the eighteen rats was recorded and rats were divided equally into following three groups. The duration of experiment was of 28 days after the confirmation of diabetic state as well as from the starting day of MTEC administration though the duration is of 35 days from the day of streptozotocin injection.

Group I (Control group) animals received a single intramuscular injection of citrate buffer (1 ml/Kg body weight/rat).

Group II (Diabetic group) animals were made diabetic by a single intramuscular injection of STZ at a dose of 35 mg/1 ml citrate buffer/Kg body weight/rat.

Group III (MTEC treated group) animals were forcefully fed with MTEC by gavage at a dose of 100 mg/5 ml olive oil/Kg body weight/rat/day at fasting state on and from 8th day for 28 days.

Rats of group I and group II were subjected to forceful feeding of 5 ml of olive oil/Kg body weight/day for 28 days at the time of MTEC treatment to the animals of group III so that stress, if any, imposed on rat due to handling and feeding remain same in all the groups.

Starting from the day of extract administration to diabetic rats, fasting blood glucose levels in all the groups were measured by glucometer (Ascensia ENTRUST glucometer, Bayer, Germany) at every two days intervals and on the day of animal sacrifice. On the 29th day of the experiment (starting day from MTEC treatment or 36th day of experiment from starting day of streptozotocin injection), all the animals were sacrificed in light ether anaesthesia by decapitation after recording the final body weight and fasting blood glucose level. Blood was collected from dorsal aorta by syringe and the serum was separated by centrifugation at 3000 g for 5 min for the assessment of metabolic toxicity from serum samples. The liver, kidney, skeletal and cardiac muscle were dissected out and stored at -20°C for biochemical analysis of the carbohydrate metabolic enzyme activities along with the assessment of anti oxidative enzyme activities that is catalase (CAT), peroxidase (Px), superoxide dismutase (SOD), glutathione-s-transferase (GST) and for the quantification of the levels of conjugated diene (CD) and thiobarbituric acid reactive substance (TBARS) in the target tissues.

Measurement of hyperglycemic indices

Testing of fasting blood glucose level: Fasting blood glucose (FBG) level was measured using the glucometer (Ascensia ENTRUST glucometer, Bayer, Germany) by collecting blood from tail vein of all experimental and control animals at the initial time of experiment, every two days interval throughout the experiment and on the day of animal sacrifice.

Hepatic and skeletal muscle glycogen level: Hepatic and skeletal glycogen levels were measured according to the accepted standard protocol (Sadasivam and Manickam, 1996). In brief, hepatic tissue was homogenized in hot 80% ethanol at a tissue concentration of 100 mg/ml and then centrifuged at 8,000 g for 20 min. The residue was collected, dried over a water bath, and then extracted at 0°C for 20 min by adding a mixture of 5 ml water and 6 ml of 52% perchloric acid. The collected material was centrifuged at 8,000 g for 15 min and the supernatant was separated. From the recovered supernatant 0.2 ml was transferred in graduated test tube and made to 1 ml volume by the addition of distilled water. Graded standards were prepared using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of a working standard solution and volume of all these standards were made up to 1 ml using distilled water. Anthrone reagent (4 ml) was added to all test tubes and the tubes were then heated in a boiling water bath for 8 min. After that tubes were allowed to cool at room temperature and the intensity of the green to dark green color of the solution recorded at 630 nm. Glycogen content of the samples was determined from a standard curve prepared with standard glucose solution.

Estimation of hepatic, skeletal and cardiac muscle hexokinase, glucose-6-phosphatase activities: The enzyme activity was determined on the basis of reduction of NADP coupled with hexokinase which was measured spectrophotometrically at 340 nm (Chou and Wilson, 1975).

The liver glucose-6-phosphatase activity was measured according to standard protocol (Swanson, 1955). Tissue was homogenized in ice cold 0.1 M phosphate buffer saline (pH: 7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1 ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.5 M maleic acid buffer (pH: 6.5) were taken and brought to 37°C in water bath for 15 min. The reaction was stopped with 1 ml of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifugation at 3000 g for 10 min. The optical density was noted at 340 nm. The enzyme activity was expressed as mg of inorganic phosphate liberated per gm of tissue.

Biochemical assay of glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities in liver and skeletal muscle: The hepatic, skeletal and cardiac muscle glucose-6-phosphate dehydrogenase activities were measured spectrophotometrically (Langdon, 1966). One unit of enzyme activity is defined as that quantity which catalyses the reduction of 1 µM of NADP per minute. Activity of this enzyme was recorded by using glucose-6-phosphate as a substrate and absorbance was measured at 340 nm.

The L-lactate dehydrogenase enzyme activity was assessed spectrophotometrically as per the standard protocol (Anon, 1970). The assay mixture was kept into a 3 ml of cubette prepared using 0.1 ml of NADH solution, 0.04 ml of pyruvate solution, 0.36 ml of phosphate buffer and 2.4 ml of distilled water to make a total volume of 2.9 ml. From ice cold sample homogenate 0.1 ml was added to this assay mixture and the reaction velocity for the first minute was used to compute the enzyme activity. Enzyme activity was expressed as units of activity per mg of tissue.

Assessment of antioxidative enzyme activities

Biochemical assay of catalase and peroxidase activities: The activities of catalase of the hepatic, skeletal and renal tissues were measured biochemically (Beers and Sizer, 1952). For the evaluation of CAT activities, liver, kidney and skeletal muscles were homogenized separately in 0.05 M Tris-HCl buffer solution (pH: 7.0) at a tissue concentration of 50 mg/ml. These homogenized solutions were centrifuged at 10,000 g at 4°C for 10 min. To a spectrophotometric cuvette, 0.5 ml of 35 mM H_2O_2 and 2.5 ml of distilled water were mixed and the absorbance was measured at 240 nm. From sample supernatant 40 µl was added and the subsequent six readings were noted at 30 s intervals.

Activities of peroxidase of above said tissues were measured according to the standard method (Sadasivam and Manickam, 1996). Guiacol (20mM) was mixed with 0.1 ml of sample. In the presence of 0.3 ml of 12.3 mM H_2O_2, the time was recorded for an increase in the absorbance by 0.1 at 436 nm.

Estimation of superoxide dismutase (SOD) and glutathione-s-transferase (GST) activities: The SOD activities of the tissue samples were estimated by measuring the percentage inhibition of the pyrogallol auto oxidation by SOD according to the standard method (Marklund and Marklund, 1974). In a spectrophotometric cuvette, 2.04 ml of 50 mM TRIS buffer (pH: 8.2), 20 µl of sample and 20 µl of pyrogallol were taken and the absorbance was noted in spectrophotometer at 420 nm for 3 min period. One unit of SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50 %.

Activities of GST in the above tissue samples were measured spectrophotometrically (Habig et al., 1974) using CDNB (1-chloro-2, 4-dinitrobenzene) as a substrate. The assay mixture of 3 ml contained 0.1 ml of 1 mM CDNB in ethanol, 0.1 mM of 1 ml GSH, 2.7 ml of 100 mM potassium phosphate buffer (pH: 6.5) and 0.1 ml of supernatant of the tissue homogenate. The formation of the product of CDNB, S-2, 4-dinitrophenylglutathione, was monitored by measuring the net increase in absorbance at 340 nm against the blank. The enzyme activity was calculated using the extinction coefficient 6.9 $mM^{-1}Cm^{-1}$ and expressed in unit/mg of tissue.

Quantification of end products of lipid peroxidation i.e. thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD)

The liver, kidney and skeletal muscles were homogenized separately at the tissue concentration of 50 mg/ ml in 0.1 M of ice-cold phosphate buffer (pH: 7.4) and the homogenates were centrifuged at 10,000 g at 4°C for 5 min separately. Each supernatant was used for the quantification of TBARS and CD.

For the measurement of TBARS, the homogenate mixture of 0.5 ml was mixed with 0.5 ml of normal saline (0.9% NaCl) and 2 ml of thiobarbituric acid- trichloroacetic acid (TBA-TCA) mixture (0.392 g TBA in 75 ml of 0.25 N HCl with 15 g TCA). The volume of the mixture was made up to 100 ml by 95% ethanol and boiled at 100°C for 10 min. This mixture was then cooled at room temperature and centrifuged at 4000 g for 10 min. The absorbance of the whole supernatant was measured spectrophotometerically at 535 nm (Ohkawa et al., 1979).

Quantification of the CD was performed by a standard method (Slater, 1984). The lipids were extracted with chloroform methanol (2:1) followed by centrifugation at 1000 g for 5 min. The chloroform layer was evaporated to dryness under a stream of nitrogen. The lipid residue was dissolved in 1.5 ml of cyclohexane and the absorbance was measured at 233 nm to estimate the amount of hydro peroxide formed.

Biochemical estimation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)

GOT and GPT activities in serum were measured using specific kits (supplied by Span Diagnostics Ltd., Surat, India). The activities of these enzymes were expressed as relative units (Henry et al., 1960).

Histological study

The target tissue that is pancreas was dissected out from the same region of all the groups and cut into small pieces and then fixed in

Table 1. Effect of polyherbal drug MTEC on body weights in streptozotocin-induced diabetic rats

Groups	Body Weight (gm)	
	Initial	Final
Group I (Control group)	127 ± 6.5 [a]	132 ± 5.4 [a]
Group II (Diabetic group)	124 ± 2.1 [a]	106 ± 2.2 [b]
Group III (MTEC treated group)	123 ± 3.7 [a]	129 ±3.1 [c]

Data were expressed as Mean ± SEM, n=6. ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a, b, c) differ from each other significantly (p<0.05).

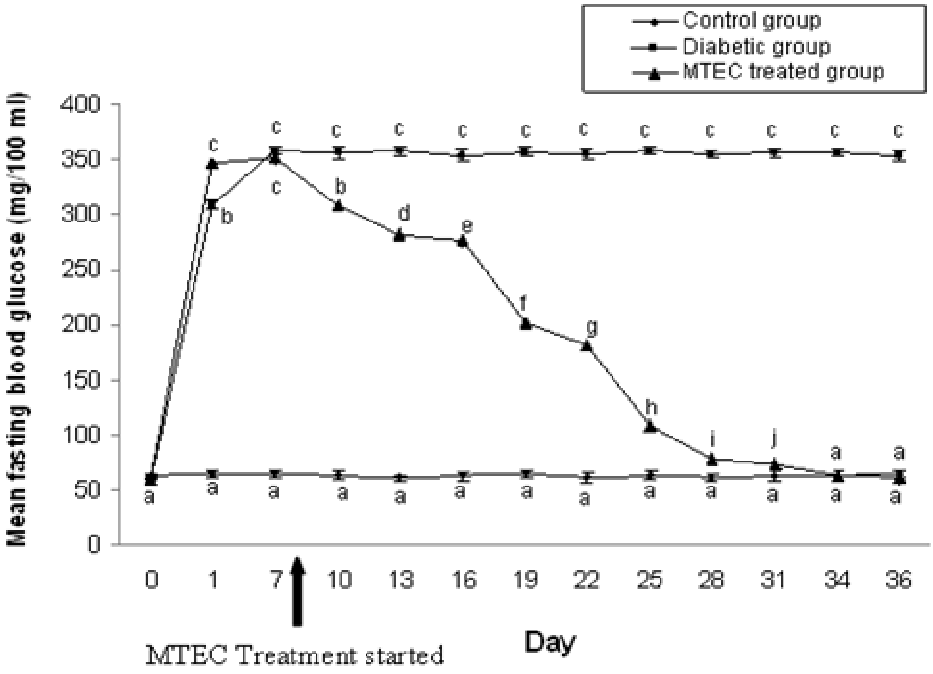

Figure 1. Protective effect of MTEC on mean fasting blood glucose level in streptozotocin-induced diabetic rats. Each line represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of line diagrams with different superscripts (a, b, c, d, e, f, g, h, i, j) differ from each other significantly at the level of p<0.05.

Bouin's fixative. Target tissue was subjected to paraffin embedding after proper dehydration process followed by section cutting in rotary microtome (4 µm thick). Sections were allowed to stain by heamatoxylin-eosin for microscopic examination in accordance with laboratory procedures. Count of Islets of Langerhans per pancreatic section and diameter of each islet was measured using AvarCap (Version-2.5; Aver Media TECHNOLOGIES Inc.) and DeWinter Calipro-3.0 Softwares.

Statistical analysis

All experimental trials were replicated three times. An analysis of variance (ANOVA) followed by multiple comparison two tail 't' test was used to compare in between the groups (Sokal and Rohle, 1997). Differences were considered significant at $p < 0.05$.

RESULTS

Body weight of the diabetic animals was decreased signi-flcantly in comparison with the control group rats (P<0.05). Treatment of MTEC to the rats of group III single time/day for 28 days resulted a significant (P<0.05) recovery of the said parameter though not to the level of control group (Table 1).

Treatment of MTEC to diabetic animals for 28 days resulted a significant (P<0.05) lowering of elevated fasting blood glucose level when compared with untreated diabetic animals (Figure 1).

Glycogen content in hepatic and skeletal muscle as well as the activities of hexokinase in hepatic, skeletal and cardiac muscles were decreased in the untreated diabetic group in comparison with the non-diabetic control group animals. The administration of this polyherbal drug MTEC to diabetic animals shows a significant (P<0.05) recovery in the glycogen content along with this carbohydrate metabolic enzyme activity towards the control level (Figures 2 and 3).

Side by side glucose-6-phosphate dehydrogenase

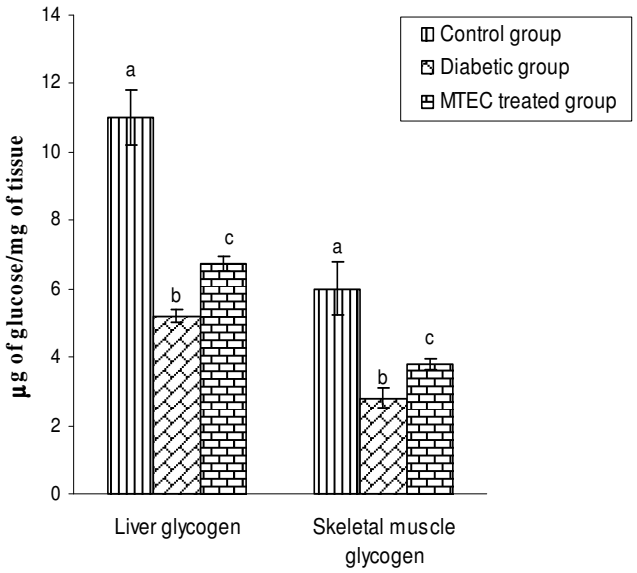

Figure 2. Effect of MTEC on glycogen content in liver and skeletal muscle in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' tests. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

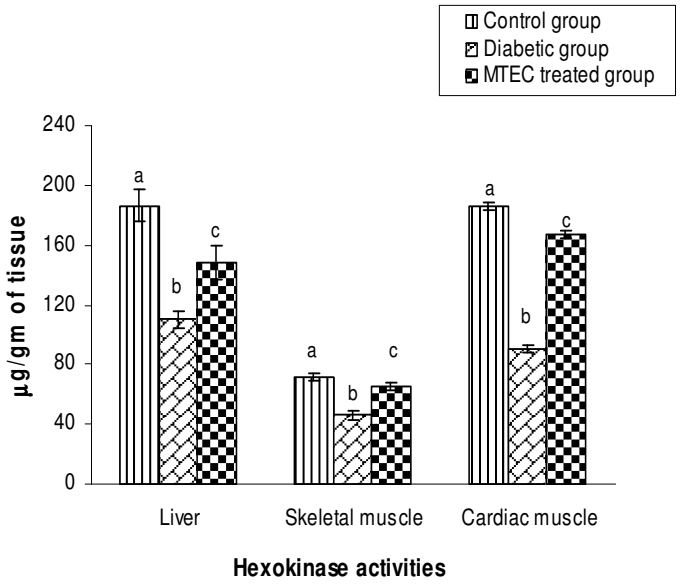

Figure 3. Corrective effect of MTEC on hexokinase activities in liver, skeletal and cardiac muscles in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

enzyme activities of hepatic, skeletal and cardiac tissues were also decreased significantly (P<0.05) in diabetic

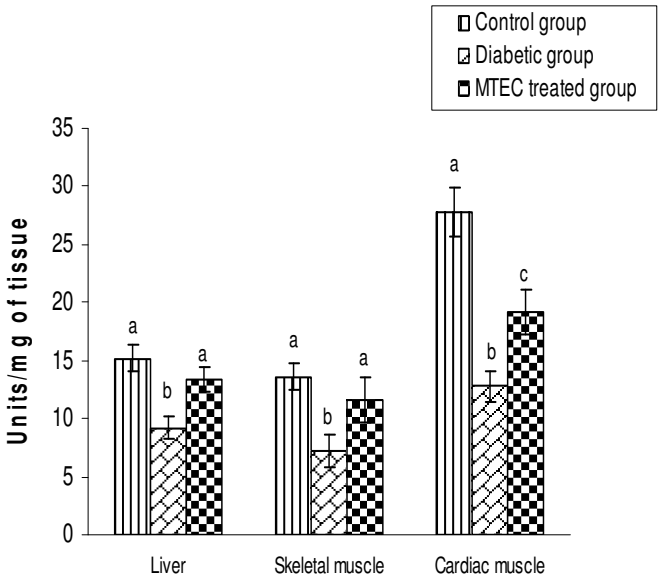

Figure 4. Correction of glucose-6-phosphate dehydrogenase activities in liver, skeletal and cardiac muscles after MTEC treatment in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n = 6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

group in comparison to the control group, which was returned significantly (P<0.05) towards the control levels after the treatment of MTEC to streptozotocin-induced diabetic rat (Figure 4).

Activities of glucose-6-phosphatase and lactate dehydrogenase enzymes in above said tissues were increased in streptozotocin-induced diabetic group in compare to the control group. From comparative analysis it has been indicated that MTEC treatment to the streptozotocin-induced diabetic group resulted a significant recovery towards the control group (P<0.05) (Figures 5 and 6).

Catalase, peroxidase, superoxide dismutase and glutathione-s-transferase activities in liver, kidney and skeletal muscles were decreased significantly (P<0.05) in diabetic group in respect to control group. After the treatment of this formulated polyherbal drug to streptozotocin-induced diabetic rat, the activities of these antioxidative enzymes were restored towards the control levels (Figures 7-10).

Levels of conjugated diene and thiobarbituric acid reacting substance, the end products of the lipid peroxidation, were increased significantly (P<0.05) in liver, kidney and skeletal muscle in diabetic group when compared to the control group. There was a significant (P<0.05) recovery in the levels of the above parameters in target tissues after treatment of MTEC to the diabetic group animals in compare to the untreated diabetic rats (Figures 11 and 12).

Activities of SGOT and SGPT were increased in

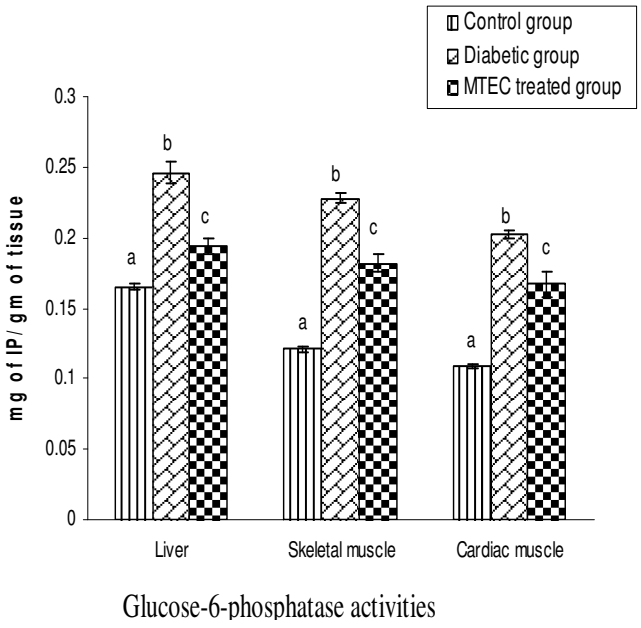

Figure 5. Ameliorative effect of MTEC on activities of glucose-6-phosphatase in liver, skeletal and cardiac muscles in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

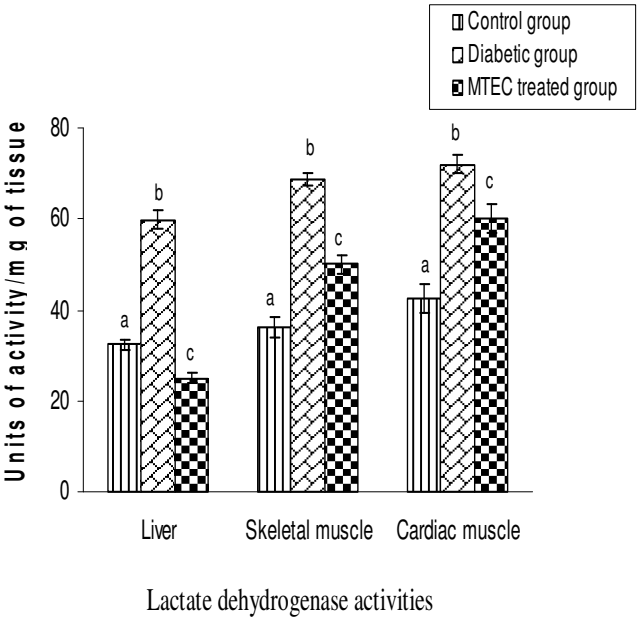

Figure 6. Effect of MTEC on lactate dehydrogenase activities in liver, skeletal and cardiac muscles in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of p<0.05.

diabetic group which compared to the control group. A significant (P<0.05) attenuation of the enzyme activities towards the control level was found after treatment of this polyherbal drug MTEC (Figure 13).

Diameter of pancreatic islets as well as count of islets were significantly decreased (P<0.05) in streptozotocin-induced diabetic group in respect to the control group. The values of these parameters were significantly restored towards the control group after MTEC treatment in diabetic rat (Table 2 and Plate 1A-1C).

DISCUSSION

This study was designed to develop more potent and effective MTEC in respect to our previous formulated drug for the management of streptozotocin-induced diabetes.

In streptozotocin induced diabetic rat, the elevation in fasting blood glucose along with diminution in liver and skeletal muscle glycogen levels may be due to low levels of plasma insulin as proposed by our previous report (Maiti et al., 2004). This has been supported here by the diminution in the activities of hexokinase and glucose-6-phosphate dehydrogenase in liver, skeletal muscle and cardiac muscle as these enzymes are under the regulation of insulin (Czech, 1977; Weber, 1966). The activities of glucose-6-phosphatase and lactate dehydrogenase, which were increased in above tissues in diabetes are also in the same line as these are under positive control of insulin (Chang, 1972). Oxidative stress development in diabetic condition (Gupta et al., 1997; Mallick et al, 2007[c]) also proved here by the study of poor activities of peroxidase, superoxide dismutase, glutathione-s-transferase and catalase in liver, kidney and skeletal muscle tissues along with the increased levels of end products of free radicals like TBARS and CD.

Treatment of modified MTEC resulted the correction of above carbohydrate metabolic enzymes activities which may be due to elevation in plasma insulin level which has been supported here by histological observation of pancreas. Moreover, the oxidative stress sensors were corrected significantly after treatment of the polyherbal drug, MTEC. The dose of this modified MTEC is comparatively less than our previously formulated MTEC (Mallick et al., 2007[c]). Moreover, this modified MTEC has no general toxicity which has been indicated here from the body weight as well as serum GOT and GPT activities as these are the indicators of general toxicity (Ghosh, 2001).

Conclusion

From the results it may be concluded that polyherbal drug MTEC has potent antidiabetogenic efficacy in streptozotocin-induced diabetic rat by the correction of oxidative stress and carbohydrate metabolic enzymes activities.

Table 2. Effect of polyherbal drug MTEC on islet number and islet diameter in streptozotocin-induced diabetic rats.

Groups	Islet number (per field in 100X magnification)	Islet diameter (micron)
Group I (Control group)	18.2 ± 2.6 [a]	146.6 ± 2.6 [a]
Group II (Diabetic group)	6.7 ± 1.1 [b]	96 ± 1.3 [b]
Group III (MTEC treated group)	12.3 ± 1.7 [c]	131 ±1.1 [c]

Data were expressed as Mean ± SEM, n=6. ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a, b, c) differ from each other significantly ($p<0.05$).

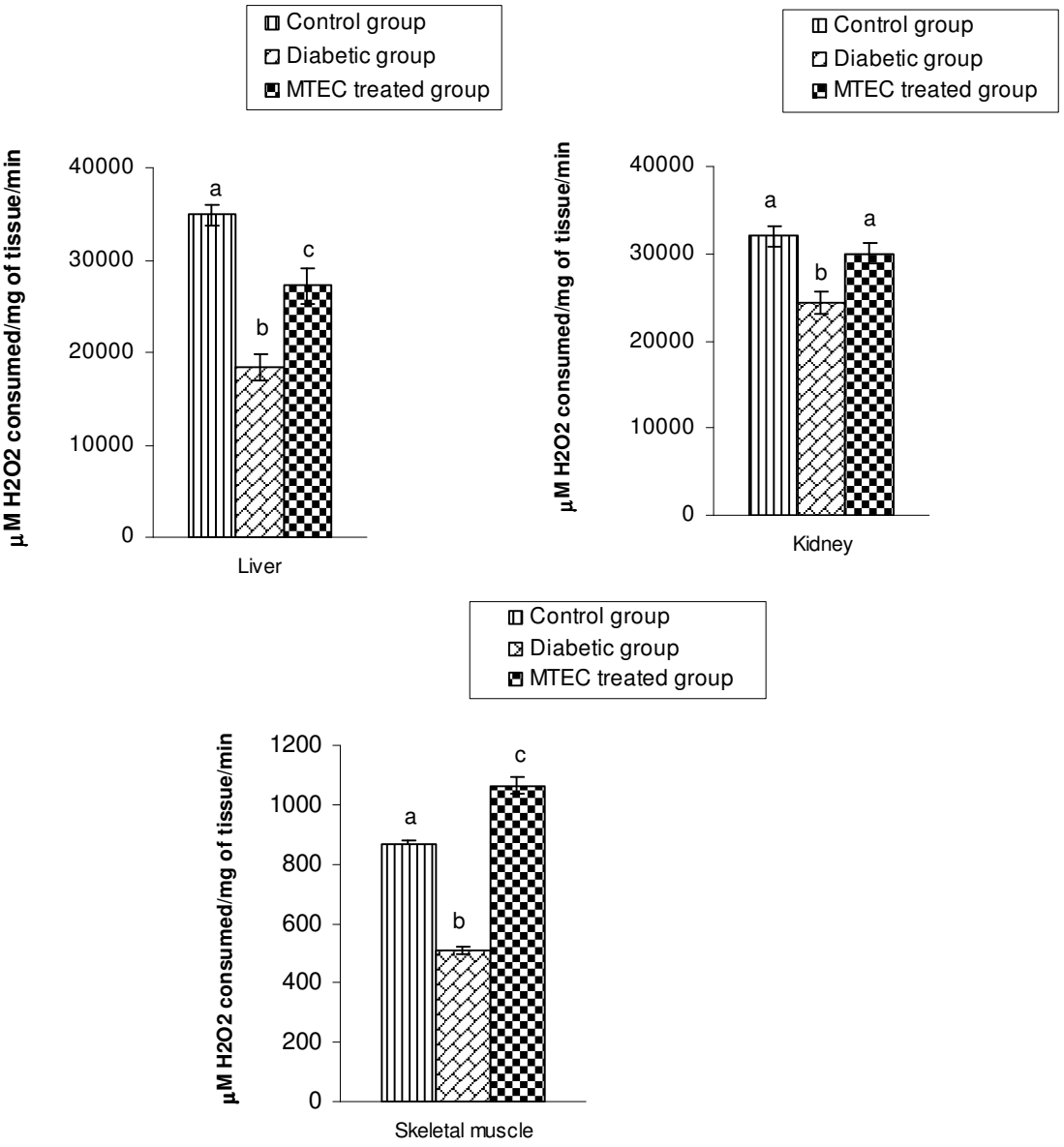

Figure 7. Protection of catalase activities in liver, kidney and skeletal muscle after MTEC treatment in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a,b,c) differ from each other significantly at the level of $p<0.05$.

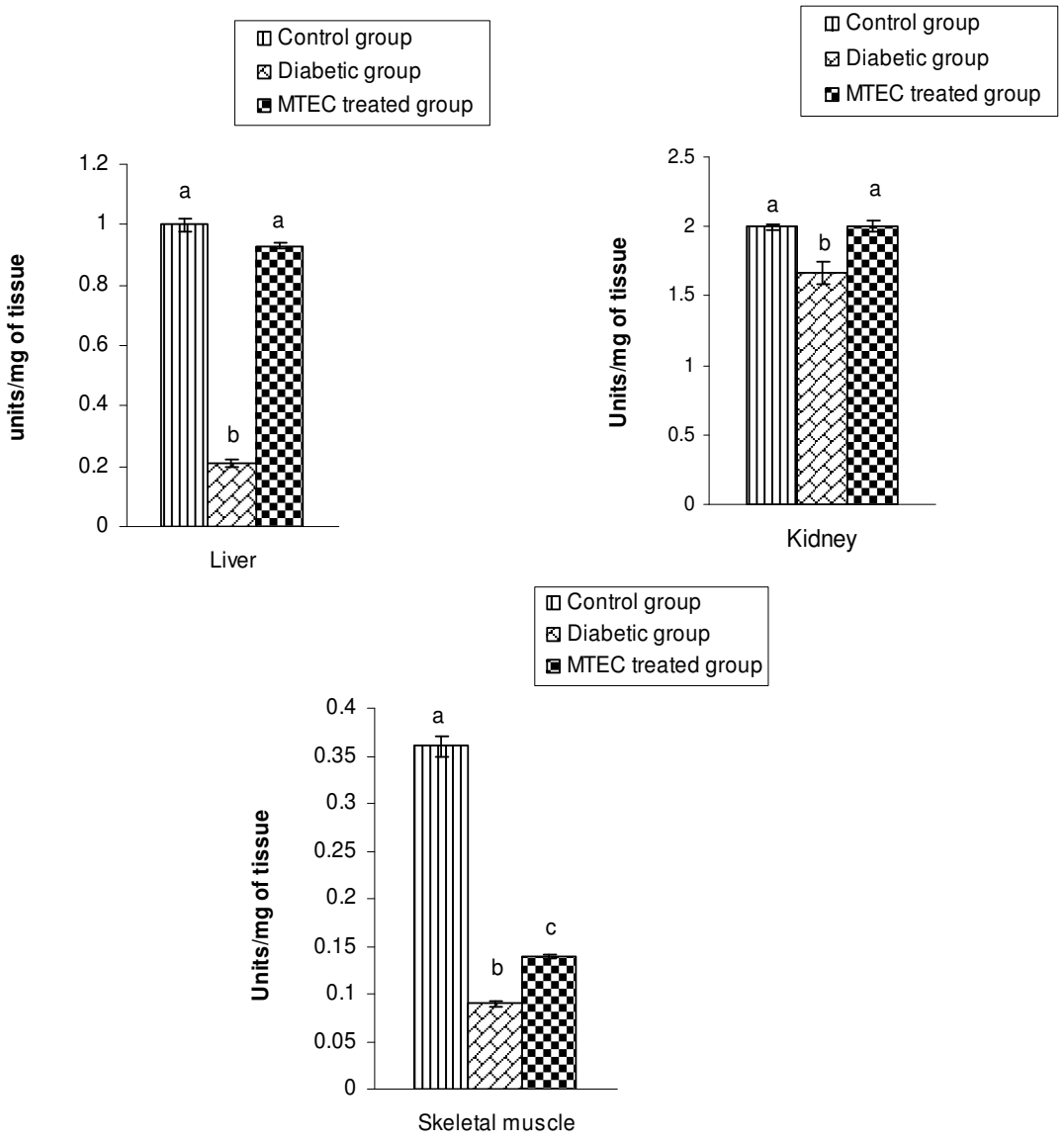

Figure 8. Peroxidase activities in liver, kidney and skeletal muscle in streptozotocin-induced diabetic rats and its correction after MTEC treatment. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c). differ from each other significantly at the level of $p<0.05$.

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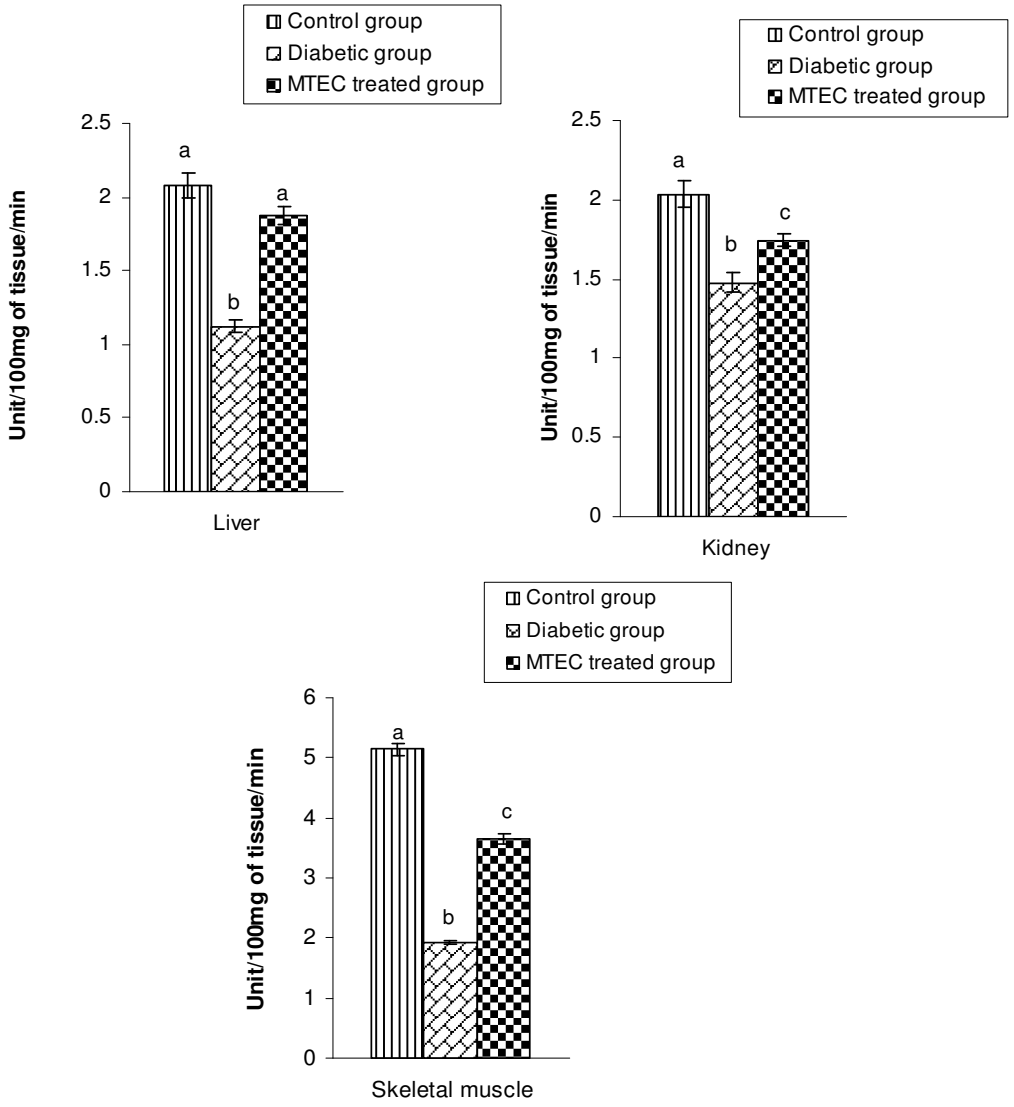

Figure 9. Effect of MTEC treatment on superoxide dismutase activities in liver, kidney and skeletal muscle in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparisons two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.05$.

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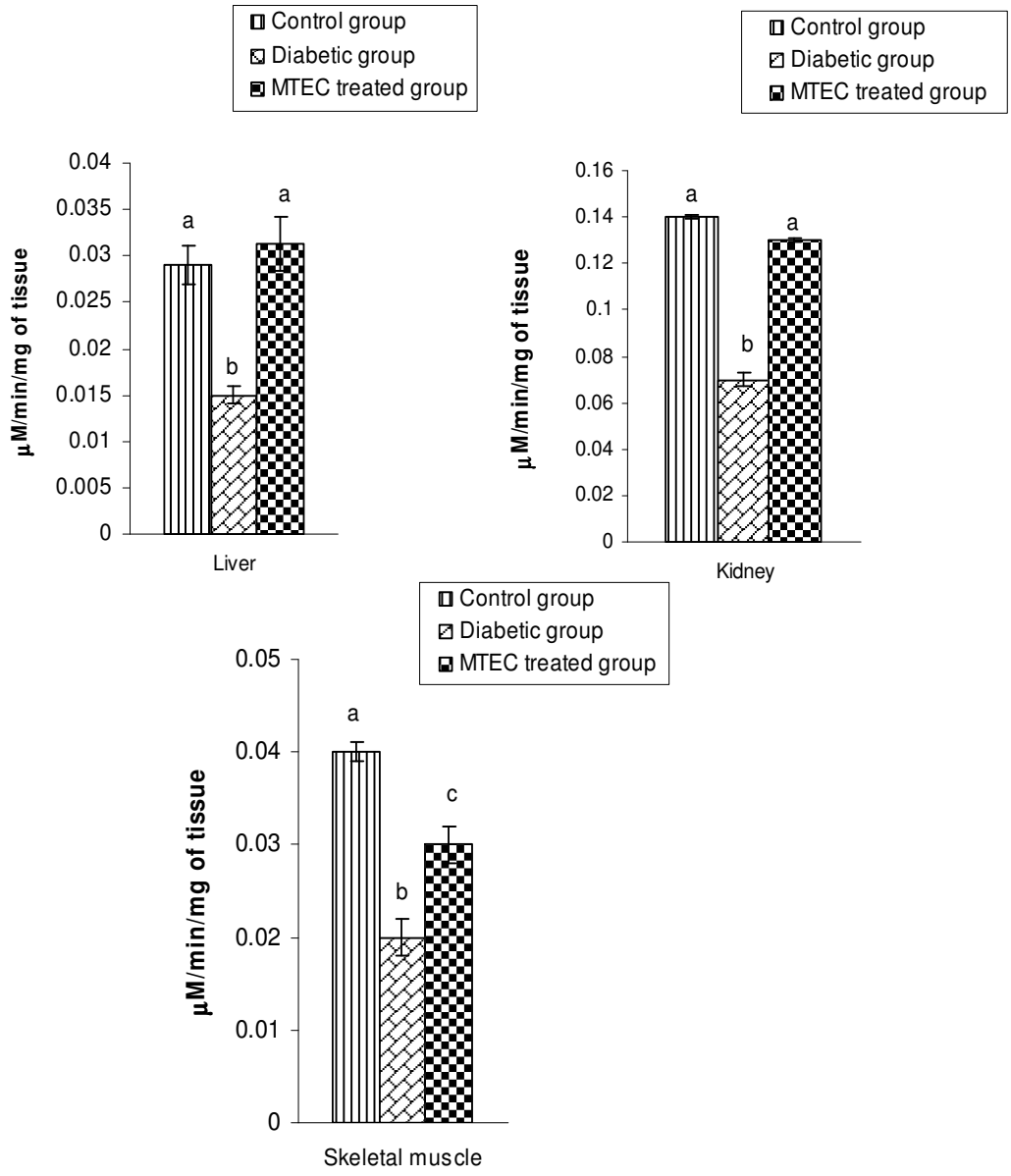

Figure 10. Correction of GST activities after MTEC treatment in liver, kidney and skeletal muscle in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.05$.

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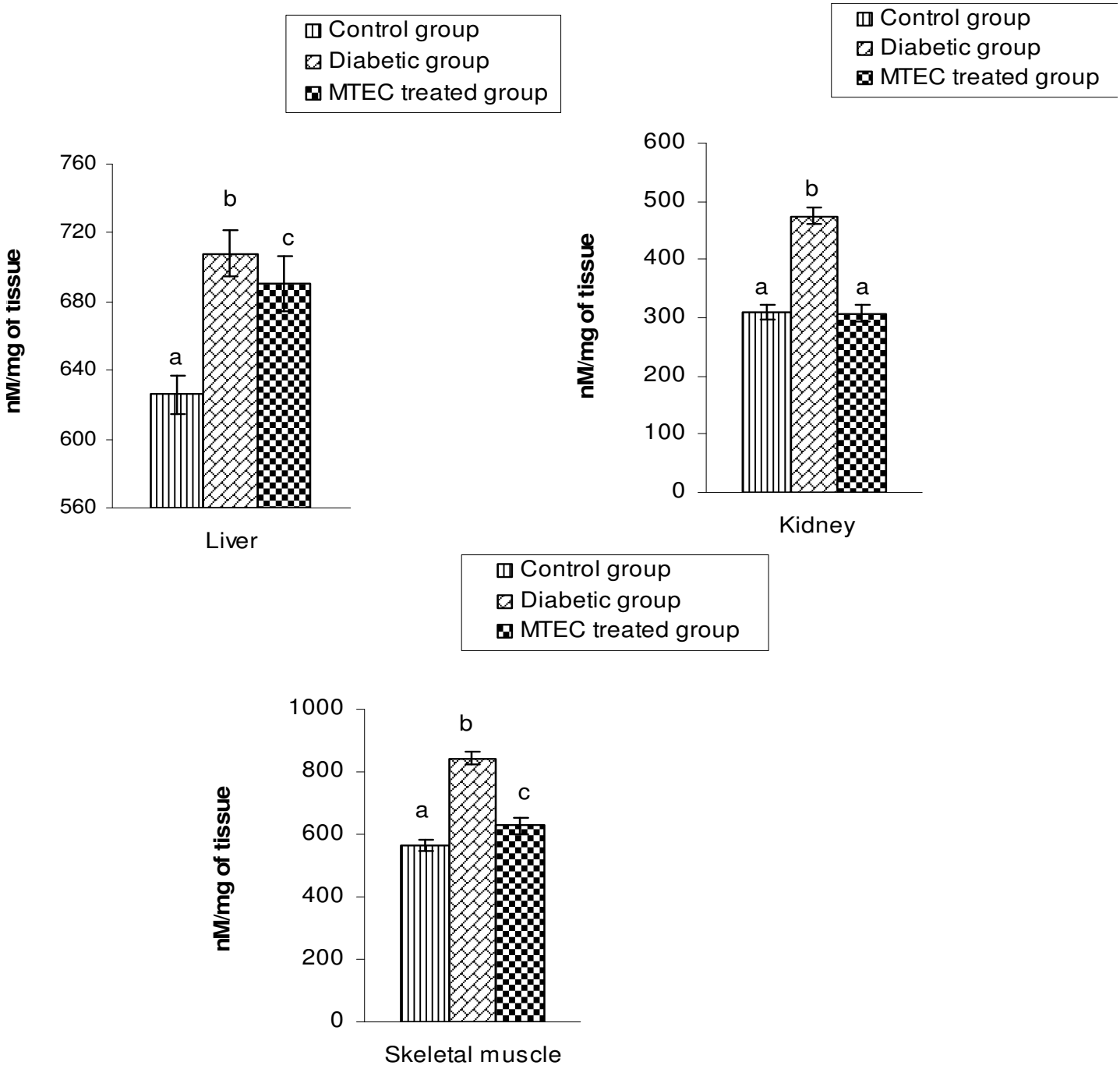

Figure 11. Effect of MTEC on conjugated diene levels in liver, kidney and skeletal muscle in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.05$.

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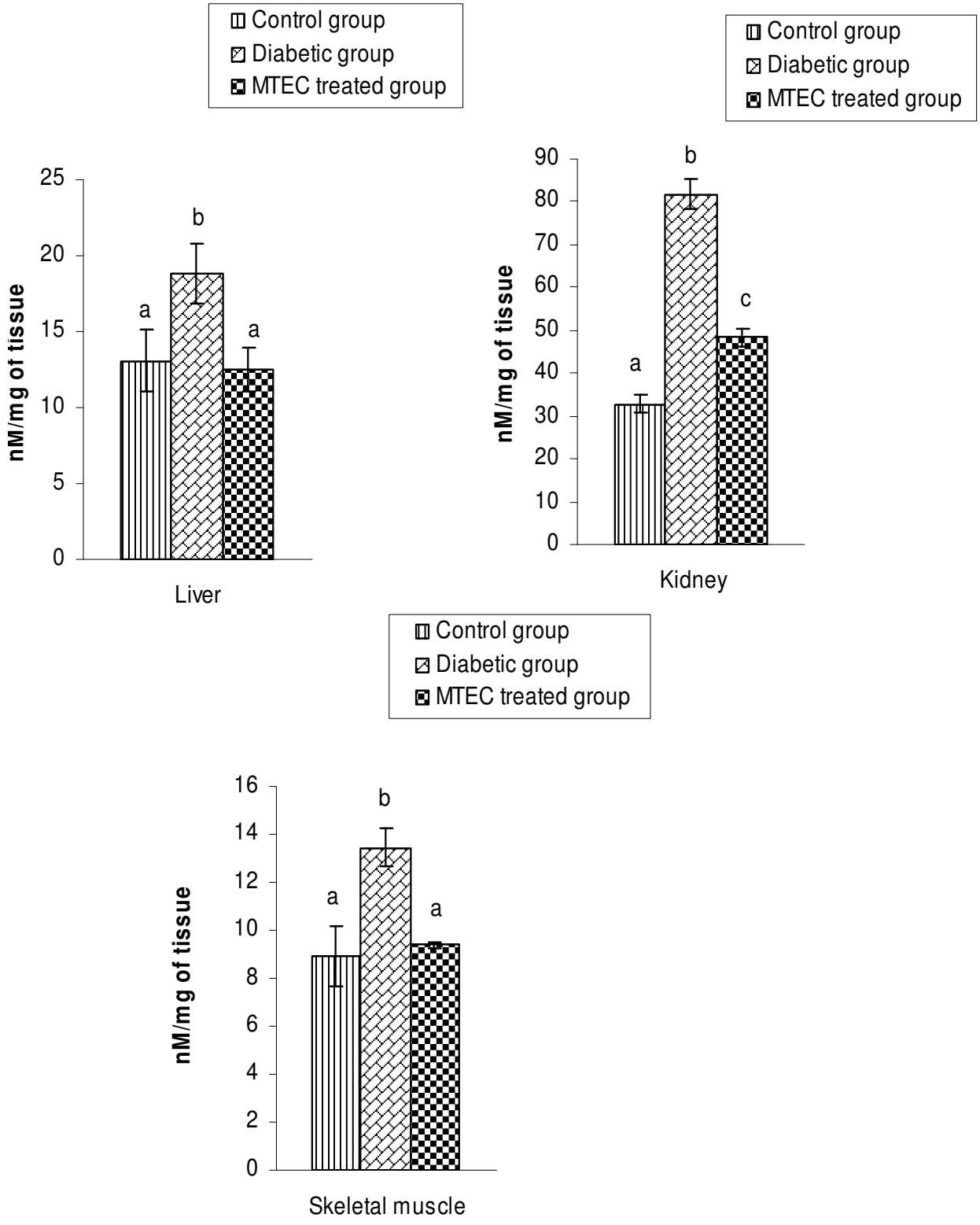

Figure 12. TBARS levels in liver, kidney and skeletal muscle in streptozotocin-induced diabetic rats and its amelioration after MTEC treatment. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.05$.

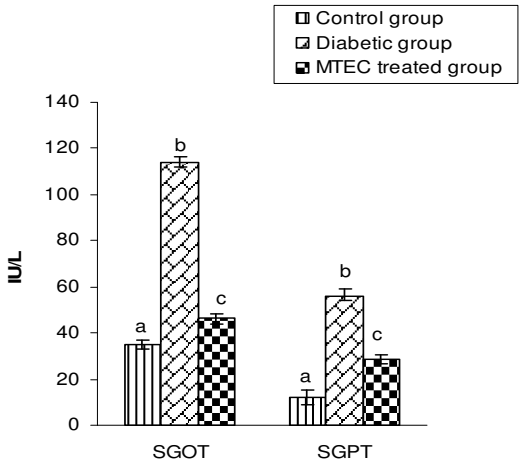

Figure 13. Protective effect of MTEC on serum GOT and GPT activities in streptozotocin-induced diabetic rats. Each bar represents Mean ± SEM, n=6 for each group. ANOVA followed by multiple comparison two tail 't' test. Values of bar diagrams with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.05$.

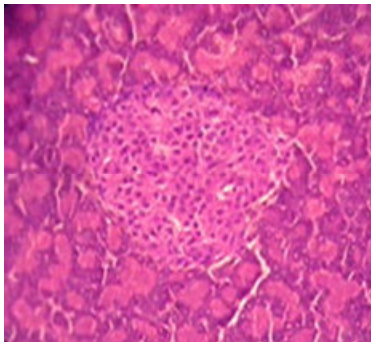

Plate.1a. Representative sample of pancreatic tissue of control rat focusing the size of normal islet in rat.

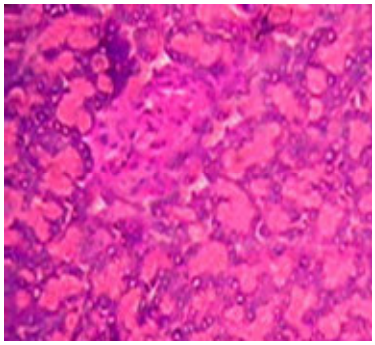

Plate.1b. Diminution in the diameter of islet in the representative pancreatic tissue sample of STZ-induced diabetic rat.

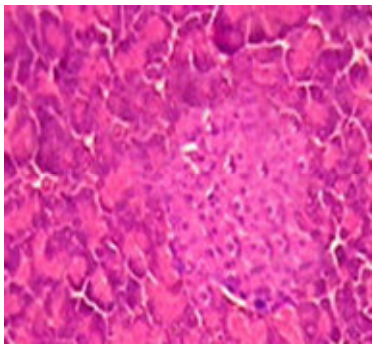

Plate.1c. Representative pancreatic tissue sample showing recovery in islet diameter after MTEC treatment in STZ-induced diabetic rat.

Plate1. Histology of Pancreas-H.E. Stain, X 400.