



Effect of *Ichnocarpus frutescens* extract on antihyperglycemic, antihyperlipidemic and antioxidant status in streptozotocin-induced diabetic rats

Deepak K Dash^{1,3}, Tirtha Ghosh², Veerendra C Yeligar³, K Muruges³, Siva S Nayak¹, Bhim C Maiti³ and Tapan K Maity^{3,*}

¹College of Pharmaceutical Sciences, Mohuda, Berhampur, Orissa, India; ²Institute of Pharmacy and Technology, Salipur, Orissa-754202, India; ³Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India

SUMMARY

The present study was carried out to investigate the antihyperglycemic, antihyperlipidemic and antioxidant effect of chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* (CEIF and MEIF) in streptozotocin (STZ)-induced diabetic rats. Administration of CEIF and MEIF orally at the dose of 200 mg/kg and 400 mg/kg body weight resulted in significant ($P < 0.01$) reduction in blood glucose levels. The body weights were significantly ($P < 0.001$) reduced in STZ-induced diabetic rats when compared to normal rats while the extracts significantly ($P < 0.01$) prevented the decrease in body weight in the CEIF and MEIF treated rats. The study was further undertaken to evaluate the antioxidant and antihyperlipidemic potential of CEIF and MEIF in STZ-induced diabetic rats. The increased levels of lipid peroxidation in the liver tissues of diabetic rats were significantly reverted back to normal levels and a significant increase in the activity of antioxidant enzymes such as superoxide dismutase, catalase and the level of reduced glutathione in the liver of diabetic rats after the treatment with CEIF and MEIF was noticed. These results clearly indicate that CEIF and MEIF exhibit significant antihyperglycemic, antihyperlipidemic and *in vivo* antioxidant activity in STZ-induced diabetic rats and the results were found to be in a dose dependent manner.

Key words: *Ichnocarpus frutescens*; Streptozotocin; Diabetes; Antihyperglycemic; Antihyperlipidemic; Antioxidants

INTRODUCTION

Diabetes is one of the major degenerative disease in the world today. According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 171 million diabetics worldwide and this is likely to increase to 366 million or more by the year 2030. Statistical analysis

about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (Boyle *et al.*, 2001; Wild *et al.*, 2004). Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes (Harris *et al.*, 1998).

Clinically diabetes mellitus is the most important disease involving the endocrine pancreas. Its major manifestations include hyperglycemia (Georg and Ludvik, 2000; Nyholm *et al.*, 2000), hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy, neuropathy and cardiovascular

*Correspondence: Tapan K Maity, Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India. Tel: +913324146074; E-mail: jutkmaity@yahoo.com

disorders (Gandjbakhch *et al.*, 2005). Non-insulin dependent diabetes mellitus (NIDDM) is caused due to inadequate release of insulin from the pancreatic β -cells or insensitivity of target tissues to insulin. A variety of orally active hypoglycemic agents are frequently used to manage the glucose intolerance of NIDDM. However, the effectiveness of these drugs is limited and suffers from a variety of side effects including hypoglycemia (Vikrant *et al.*, 2001). There is an increasing evidence indicating that oxidative stress produced under hyperglycemia can cause or lead to insulin resistance and diabetes complications (Matsuoka, 1997). Moreover, several studies have shown that antioxidant ameliorates a number of altered physiological and metabolic parameters that occur as a result of NIDDM diabetes (Kaneto *et al.*, 1999; Balasubashini *et al.*, 2004). Hence, compounds with both hypoglycemic and anti-oxidative properties would be useful anti-diabetic agents (Baynes, 1995).

There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Therefore, studies with plant extracts and isolated phytoconstituents are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently (Shukla *et al.*, 2000; Grover *et al.*, 2002).

The plant *Ichnocarpus frutescens* (Linn) R.Br. (Family-Apocynaceae) popularly known as "Dudhi", "Shyamalata" in Bengali "Black Creeper" in English and "Ananta", "Sariva" in Sanskrit is a large much branched twining shrub; young branches finely fulvous-tomentose (Kirtikar and Basu, 1998). It is locally called as *Botilai* and the plant is used by the local peoples of Mohuda, Berhampur, Orissa, India for simple fevers and to treat against liver disorder. The whole plant is used as tribal medicine in

atropy, bleeding gums, cough, dysentery, stem decoction in fever, and root as antipyretic, demulcent, diuretic and hypoglycemic agent (Chatterjee and Pakrashi, 1995). Stalk and leaves is used in decoction in the treatment of skin eruptions. A decoction of the roots of Colocynth, Anantamul, Sariva (*Sanskrit*) and Hedyotis biflora prepared in the usual way is administered with the addition of powdered long pepper and *bdellium* in chronic skin diseases, syphilis, loss of sensation and hemiplegia (Nadkarni, 1982). The juices of flowers are traditionally used for the cure of diabetics (Bhandary *et al.*, 1995). Studies on chemical constituents of the plant revealed the presence of urosolic acid and kaempferol in the leaves (Khan *et al.*, 1995), lupeol, fridelin, β -sitosterol from stems (Lakshmi *et al.*, 1985) and quercetin from flowers of the plant (Singh and Singh, 1987).

After going through the literature available, it seems that no experimental work has been carried out to verify the claims of antihyperglycemic activity of *Ichnocarpus frutescens* and hence, we have evaluated the antihyperglycemic, antihyperlipidemic action of chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* in normal and STZ-induced diabetic rats. In addition to this, the effects of these extracts were evaluated on glutathione levels, related enzymes and lipid peroxidation, as oxidative stress is known to occur in diabetes.

MATERIALS AND METHODS

Plant material

The plant *Ichnocarpus frutescens* was collected from Mohuda forest area, Ganjam district, Berhampur, Orissa, India in the month of September. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Govt. of India, Shibpur, Howrah, India. A voucher specimen (NO.CNH/I-I (98)/2005/Tech.II/1448) has been preserved in our laboratory for the future references.

Extraction

The whole plant was dried under shade and then

powered with a mechanical grinder to obtain coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using petroleum ether (60-80°C), chloroform and methanol. Solvent elimination under reduced pressure afforded the petroleum ether extract (6% w/w), chloroform extract (2% w/w) and methanol extract (17% w/w) with respect to the dried plant material respectively.

Experimental animals

Studies were carried out using Male Wistar albino rats (150-200 g). 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 (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Drugs and chemicals

Streptozotocin was purchased from Sigma Chemical Co. Ltd. (St. Louis Mo., USA) and Tolbutamide (Hoechst Pharmaceuticals, Mumbai, India) was provided as a gift sample. Thiobarbituric acid (TBA), Nitro blue tetrazolium chloride (NBT), Phenazine methosulphate from Central Drug House, New Delhi, India and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), Reduced Glutathione (GSH) and the rest of the chemicals utilized were of analytical grade and were obtained from Sisco research laboratories, Ltd., Mumbai, India.

Induction of experimental diabetes

After one week of acclimatization, the rats were fasted for 16 h. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared

streptozotocin at a dose of (45 mg/kg, body weight) in 0.1 M citrate buffer (pH = 4.5) (Siddique *et al.*, 1987) in a volume of 1 ml/kg (Hamilton *et al.*, 1998; Murali *et al.*, 2002). The diabetic state was assessed in STZ-treated rats by measuring the non-fasting serum glucose concentration after 5th day of STZ administration. Only rats with serum glucose levels greater than 225 mg/dl were used in experiments (Cetto *et al.*, 2000; Mazumder *et al.*, 2005).

Experimental procedure

In the experiment, a total of 42 rats (6 normal; 36 STZ diabetic rats) were used. The rats were divided into 7 groups of 6 animals each. Group I: Normal untreated rats received normal saline solution (0.9% NaCl w/v, 5 ml/kg); Group II: Diabetic rats received streptozotocin (45 mg/kg; i.p.) once before the treatment; Group III and IV: STZ-treated diabetic rats were administered with 200 mg and 400 mg/kg of chloroform extract of whole plant of *Ichnocarpus frutescens* (CEIF); Group V and VI: STZ-treated diabetic rats were treated with 200 mg and 400 mg/kg of methanol extract of whole plant of *Ichnocarpus frutescens* (MEIF); and Group VII: STZ-treated diabetic rats were administered drug Tolbutamide as standard (10 mg/kg; p.o.) for 14 days.

The effects of CEIF and MEIF on STZ treated diabetic rats were determined by measuring blood glucose levels, food and fluid intake amount and changes in body weights (Kamtchouing *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. The liver was removed immediately, rinsed in ice cold normal saline, weighed and homogenized in 0.1 N Tris-HCl buffer (pH 7.4), and used for the estimation of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), super oxide dismutase (SOD) and catalase (CAT) activity.

Estimation of blood glucose levels

At the beginning of the experiment and at 5 day

intervals, the body weight and blood glucose levels were measured. Blood samples were collected from the tip of the tail vein of the normal and STZ-induced diabetic rats and the blood glucose levels were estimated using a glucometer (One Touch Ultra blood glucose monitoring system from Lifescan, Johnson and Johnson Company, Milpitas, CA). The results were expressed in term of mg/dl of blood.

Estimation of total cholesterol (TC) and triglyceride (TG)

At the end of the experiment (15th day) blood samples were collected from the tip of the tail vein in eppendroff's tubes containing 50 micro liters of anticoagulant (10% trisodium citrate solution) from the normal and STZ-induced diabetic rats. Serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for total cholesterol and triglycerides using kits from Span Diagnostics Ltd., Surat, India.

Estimation of *in vivo* antioxidants

Estimation of TBARS

The TBARS levels were measured as an index of malondialdehyde (MDA) production, determined by the method of Fraga *et al* (Fraga *et al.*, 1988). MDA, an end product of lipid peroxidation reacts with thiobarbituric acid to form a red coloured complex. The measurement of MDA levels by thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. 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.

Estimation of GSH

Reduced glutathione levels were estimated based on the ability of the SH group to reduce 5,5'-dithiobis-(2-nitrobenzoic acid) to form 1 mole of 2-nitro-5-

mercaptobenzoic acid per mole of SH. The method of Beutler and Kelly (Beutler and Kelly, 1963) was employed in the determination of GSH levels. 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this solution 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 reagent were added and read at 412 nm. The GSH concentrations of the samples were derived from the standard curve prepared using known amount of GSH.

Estimation of SOD

SOD activity was measured based on the modified method of NADH - Phenazinemetosulphate - nitroblue tetrazolium formazon inhibition reaction spectrophotometrically at 560 nm (Kakkar *et al.*, 1954). A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction/min/mg of liver tissue. The assay mixture contain 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 µm), 0.3 ml of nitro blue tetrazolium (300 µ m), 0.2 ml of NADH (750 µm). Reaction was started by addition of NADH. After incubation at 30°C for 90 s, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. The colour intensity of the chromogen in n-butanol layer was measured at 560 nm against n-butanol and concentration of SOD was expressed as units/g of liver tissue. Absorbance values were compared with a standard curve generated from known SOD.

Estimation of CAT

Catalase activity was measured based on the ability of the enzyme to break down H₂O₂. The method of Maehly and Chance (Maehly and Chance, 1954)

was employed in the estimation of CAT activity. 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 H₂O₂ and the enzyme extract. The specific activity of catalase is expressed in terms of units/g of liver tissue.

Statistical analysis

The experimental results were expressed as the Mean \pm S.E.M. for six animals in each group. The results were analysed statistically using one-way analysis of variance ANOVA, followed by Dunnett's multiple comparison test (DMCT). *P* values < 0.05 were considered as statistically significant.

RESULTS

Effect on general parameters

The body weight, food and liquid intake were measured and summarized in Table 1. The initial body weight was almost similar in normal, diabetic control and the extract treated diabetic rats whereas the final body weight were significantly (*P* < 0.001) decreased in diabetic control (Group II) when compared with normal control (Group I). At the same time, there was significant (*P* < 0.01)

increase in body weight of CEIF and MEIF treated diabetic rats (Group III to VI) when compared with diabetic control. The food and fluid intake were significantly (*P* < 0.001) higher in diabetic control group when compared with the control group (Table 1).

Effect of CEIF and MEIF on blood glucose levels

The changes in blood glucose levels on the treatment of normal and diabetic rats with CEIF and MEIF and diabetic rats treated with tolbutamide were presented in Table 2. The diabetic rats showed a significant (*P* < 0.001) increase in the blood glucose level when compared with control group. Oral administration of CEIF and MEIF in the dose of 200 and 400 mg/kg and tolbutamide in 10 mg/kg, body weight to diabetic rats significantly (*P* < 0.01) decreased the elevated blood glucose levels to near normal (Group III to VII). The results were found statistically significant in a dose dependent manner.

Effect of CEIF and MEIF on TC and TG

The changes in the total cholesterol and triglycerides were summarized in Table 2. Serum TC and TG levels were significantly (*P* < 0.001) elevated in both the STZ-induced diabetic groups in comparison to control. Supplementation of CEIF, MEIF and tolbutamide to diabetic rats resulted a significant (*P* < 0.01) diminution of these parameters and the

Table 1. Effect of CEIF and MEIF on body weight, food intake and liquid intake in rats

Groups	Dose (mg/kg)	Body weight (g)		Food intake (g/rat/day)	Liquid intake (ml/rat/day)
		Initial	Final		
Group I - Normal control (0.9 % NaCl w/v)	-	162.50 \pm 1.11	183.66 \pm 0.80	17.51 \pm 0.60	19.96 \pm 0.84
Group II - Diabetic control (STZ Induced)	45	166.33 \pm 1.14 ^c	144.83 \pm 1.19 ^a	23.60 \pm 0.46 ^a	34.07 \pm 0.97 ^a
Group III - CEIF + STZ	200 + 45	164.16 \pm 0.83 ^c	187.83 \pm 1.42 ^b	18.42 \pm 0.50 ^b	24.99 \pm 0.31 ^b
Group IV - CEIF + STZ	400 + 45	168.33 \pm 1.05 ^c	188.33 \pm 1.20 ^b	18.67 \pm 0.42 ^b	26.65 \pm 0.10 ^b
Group V - MEIF + STZ	200 + 45	164.16 \pm 1.53 ^c	183.00 \pm 1.09 ^b	18.11 \pm 0.31 ^b	21.63 \pm 0.56 ^b
Group VI - MEIF + STZ	400 + 45	170.83 \pm 0.83 ^c	192.00 \pm 1.00 ^b	19.61 \pm 0.29 ^b	24.09 \pm 0.39 ^b
Group VII - Tolbutamide + STZ	10 + 45	173.66 \pm 0.88 ^c	193.66 \pm 1.28 ^b	17.66 \pm 0.28 ^b	25.86 \pm 0.37 ^b

Values are mean \pm S.E.M. (n = 6) animals in each group. ^a*P* < 0.001, ^b*P* < 0.01 and ^c*P* < 0.05 were statistically significant when group II compared with group I and group III to VII compared with group II.

Table 2. Effect of CEIF and MEIF extracts on glucose level, total cholesterol and triglycerides levels in STZ-induced rats.

Group	Dose (mg/kg)	Blood glucose Level (mg/dl)		Total cholesterol (mg/dl)	Triglyceride (mg/dl)
		Initial	Final		
Group I - Normal control (0.9% NaCl w/v)	-	84.00 ± 1.21	85.50 ± 1.17	72.66 ± 1.34	100.96 ± 2.07
Group II - Diabetic control (STZ induced)	45	355.83 ± 1.49 ^a	334.00 ± 1.34 ^a	158.11 ± 1.56 ^a	188.73 ± 0.97 ^a
Group III - CEIF + STZ	200 + 45	345.66 ± 1.17 ^b	130.16 ± 0.87 ^b	110.95 ± 1.06 ^b	156.83 ± 1.52 ^b
Group IV - CEIF + STZ	400 + 45	343.83 ± 1.07 ^b	119.83 ± 1.22 ^b	101.00 ± 1.37 ^b	147.86 ± 1.5 ^b
Group V - MEIF + STZ	200 + 45	349.16 ± 0.94 ^b	112.16 ± 1.47 ^b	95.18 ± 0.96 ^b	144.15 ± 0.99 ^b
Group VI - MEIF + STZ	400 + 45	351.00 ± 1.46 ^b	109.50 ± 0.95 ^b	86.15 ± 0.90 ^b	138.23 ± 0.63 ^b
Group VII - Tolbutamide + STZ	10 + 45	354.33 ± 1.22 ^b	99.83 ± 1.53 ^b	78.98 ± 0.94 ^b	108.06 ± 1.61 ^b

Values are mean ± S.E.M. (n = 6) animals in each group. ^aP < 0.001, ^bP < 0.01 and ^cP < 0.05 were statistically significant when group II compared with group I and group III to VII compared with group II.

levels of these parameters were restored when compared to diabetic control.

Antioxidant parameters

Effects on hepatic TBARS levels

Changes in the concentration of TBARS in liver on treatment of diabetic rats with CEIF and MEIF and tolbutamide are illustrated in Fig. 1. There was a significant (P < 0.001) elevation in tissue TBARS during diabetes compared to corresponding control group. Administration of CEIF, MEIF (200 and 400 mg/kg) and tolbutamide (10 mg/kg) significantly

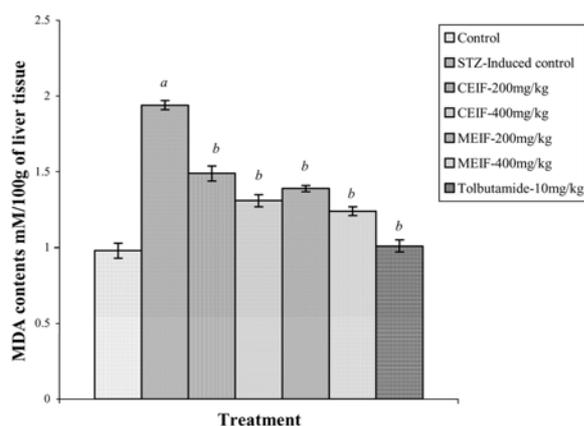


Fig. 1. Effect of CEIF and MEIF on TBARS levels in rat liver tissues. Values are mean ± S.E.M. 6 animals in each group (n = 6). ^aP < 0.001, ^bP < 0.01 and ^cP < 0.05 were statistically significant when group II compared with group I and group III to VII compared with group II.

(P < 0.01) decreased the level of TBARS in the liver tissue of diabetic rats and the effect was more pronounced in the group of rats treated with MEIF at dose of 400 mg/kg; body weight.

Effects on hepatic antioxidant enzymes and GSH

Changes in the concentration of reduced glutathione and the activities of superoxide dismutase and catalase in liver on treatment of diabetic rats with CEIF, MEIF and tolbutamide are shown in Figs. 2 - 4, respectively. There was a significant (P < 0.001) reduction in glutathione and the activities of liver superoxide dismutase and catalase in STZ-induced diabetic rats. Oral administration of CEIF, MEIF (200

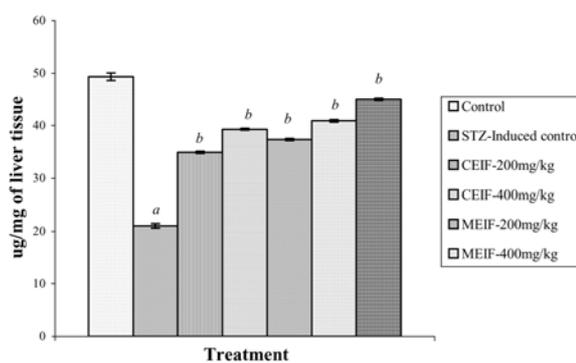


Fig. 2. Effect of CEIF and MEIF on GSH levels in rat liver tissues. Values are mean ± S.E.M. 6 animals in each group (n = 6). ^aP < 0.001, ^bP < 0.01 and ^cP < 0.05 were statistically significant when group II compared with group I and group III to VII compared with group II.

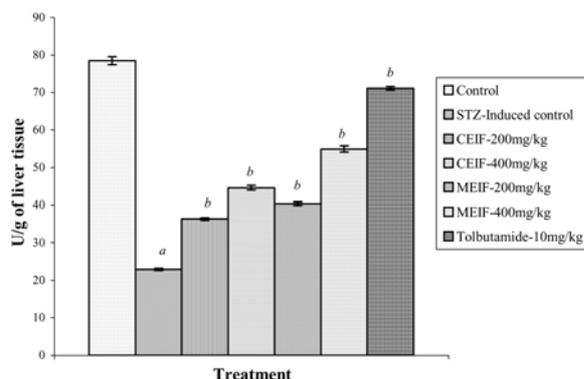


Fig. 3. Effect of CEIF and MEIF on SOD levels in rat liver tissues. Values are mean \pm S.E.M. 6 animals in each group ($n = 6$). ^a $P < 0.001$, ^b $P < 0.01$ and ^c $P < 0.05$ were statistically significant when group II compared with group I and group III to VII compared with group II.

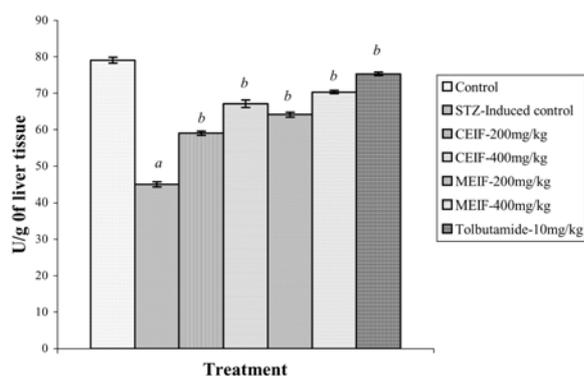


Fig. 4. Effect of CEIF and MEIF on CAT levels in rat liver tissues. Values are mean \pm S.E.M. 6 animals in each group ($n = 6$). ^a $P < 0.001$, ^b $P < 0.01$ and ^c $P < 0.05$ were statistically significant when group II compared with group I and group III to VII compared with group II.

and 400 mg/kg) and tolbutamide (10 mg/kg) to diabetic rats significantly ($P < 0.01$) increased the liver glutathione and restored the activities of liver superoxide dismutase and catalase to near normal status.

DISCUSSION

Diabetes is becoming a pandemic and despite the recent surge in new drugs to treat and prevent the condition; its prevalence continues to soar. Perhaps

the most worrying aspect of all is that the incidence is even reflected in children (Ludwig and Ebbeling, 2001). Many traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and layman to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer (Middleton *et al.*, 2000; Havsteen, 1984). The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β -cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics (Tiwari and Rao, 2002).

Streptozotocin is well known for its selective pancreatic β -cell cytotoxicity and has been extensively used to induce IDDM diabetes in experimental rat model (Papaccio *et al.*, 2000). STZ-induced diabetes causes a significant elevation in the level of blood glucose in rats. In the present investigation we have observed that the oral administration of CEIF and MEIF at the doses of 200 and 400 mg/kg; body weight to diabetic rats caused a significant decrease in the blood glucose level. At the present juncture, it is not possible to pinpoint the mechanism of antihyperglycemic action of the extracts of *Ichnocarpus frutescens*. However, it can be suggested that the antihyperglycemic effects may be due to presence of phytoconstituents in this plant. The change in body weight shows that rats treated with CEIF and MEIF has significant effect in minimizing the loss of body weight, which is caused during diabetes.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in STZ-induced diabetic rats (Chakrabarti *et al.*, 2003). Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides (Taskmen, 1987). However, in insulin deficient subject it fails to activate the enzyme and causes hypertriglyceridemia. CEIF, MEIF and tolbutamide exhibited a similar way by increasing insulin production in STZ-induced hyperglycemic animals and lowered the triglyceride

levels by activation of enzyme lipoprotein lipase. In addition, treatment of animals with CEIF and MEIF caused a decrease in total cholesterol although this was less marked than decrease of triglycerides.

Lipid peroxidation elevated in the circulation of diabetic rats, is one of the characteristic features of chronic diabetes (Feillet *et al.*, 1999). The increased free radicals produced, may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals (Levy *et al.*, 1999). Lipid peroxide mediated tissue damage has been observed in the development of both type I and type II diabetes mellitus. Insulin secretion is closely associated with lipooxygenase-derived peroxides (Metz, 1984; Walsh and Pek, 1984). The most commonly used indicator of lipid peroxidation is TBARS (Lyons, 1991). The increased lipid peroxidation in the tissues of diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and hydroperoxides in the liver and kidney of diabetic rats (Stanely *et al.*, 2001). In our study, the hepatic TBARS level was significantly lowered in the CEIF, MEIF and tolbutamide treated rats compared to the STZ-induced diabetic rats which may be due to the free radical scavenging action of active ingredients in *Ichnocarpus frutescens*.

Glutathione, a tripeptide present in millimolar concentrations in all the cells, is an important antioxidant (Lu, 1999) 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 (Anuradha and Selvam, 1993). We have registered a decrease in liver GSH level in diabetic rats. Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress (McLennan *et al.*, 1991). Similar results have been reported in STZ-induced diabetic rats (Matkovic *et al.*, 1998). Oral administration of CEIF, MEIF and tolbutamide significantly increases the content of

liver glutathione in the diabetic rats.

SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. The superoxide anion has been known to inactivate CAT, which is involved in the detoxification of hydrogen peroxide (Baynes, 1995). SOD scavenges the superoxide anion to form hydrogen peroxide hence diminishing the toxic effects caused by this radical. The reactive oxygen free radicals could inactivate and reduce the hepatic SOD and CAT activities (Wohaieb and Godin, 1987). In our study, it was observed that the extracts caused a significant increase in the hepatic SOD and CAT activities of the diabetic rats. This shows that the extracts can reduce reactive oxygen free radicals and improve the activities of the hepatic antioxidant enzymes.

In conclusion, our results show that the chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* not only possesses antihyperglycemic and antihyperlipidemic properties but also exhibits antioxidative effect. The mechanisms by which it elicits these effects may be multiple. Some of the constituents present in the extract may decrease the levels of lipid peroxidation products by scavenging free radicals like superoxide anion. Further studies should be undertaken to identify the active antihyperglycemic compounds and investigate the mechanisms of antihyperglycemic and antioxidant actions of the *Ichnocarpus frutescens*.

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