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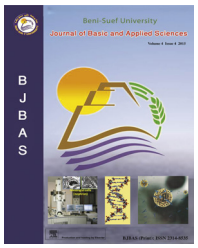

Full Length Article

Effect of *Commiphora mukul* gum resin on hepatic and renal marker enzymes, lipid peroxidation and antioxidants status in pancreas and heart in fructose fed insulin resistant rats

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ABSTRACT

This work aims to study the antioxidant efficacy of *Commiphora mukul* (*C. mukul*) gum resin ethanolic extract in high fructose diet (HFD) insulin resistant rats. The male Wistar albino rats were randomly divided into four groups of eight animals each; two of these groups (Control group [C] and Control treated with *C. mukul* [C + CM]) were fed with standard pellet diet and the other two groups (Fructose fed rats [F-group] and fructose fed with *C. mukul* treated group [F + CM]) were fed with high fructose diet (HFD) (66%). *C. mukul* gum resin ethanolic extract (200 mg/kg body weight/day) was administered orally to group C + CM and group F + CM. At the end of 60-day experimental period biochemical parameters related to antioxidant, oxidative stress marker enzymes and hepatic and renal marker enzymes of tissues were performed. The fructose fed rats showed increased level of enzymatic activities aspartate aminotransminases (AST), alanine aminotransminases (ALT) in liver and kidney and oxidative markers like lipid peroxidation (LPO) and protein oxidation (PO) in pancreas and heart. Antioxidant enzyme activities were significantly decreased in the pancreas and heart compared to control groups. Administration of *C. mukul* (200 mg/kg bwt) to fructose fed insulin resistant rats for 60 days significantly reversed the above parameters toward normal. In conclusion, our data indicate the preventive role of *C. mukul* against fructose-induced insulin resistance and oxidative stress; hence this plant could be used as an adjuvant therapy for the prevention and/or management of chronic diseases characterized by hyperinsulinemia, insulin resistance and aggravated antioxidant status.

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1. Introduction

Free radicals may play a pivotal role in the pathogenesis of a number of diseases, including diabetes mellitus (DM) (Feillet-Coudray et al., 1999). DM is a chronic metabolic disorder that continues to be a major health problem worldwide. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism (Dukworth, 2001). In addition to hyperglycemia, several other factors such as dyslipidemia or hyperlipidemia are involved in the development of diabetes related cardiovascular complications which are the major causes of morbidity and death (Markku, 1995).

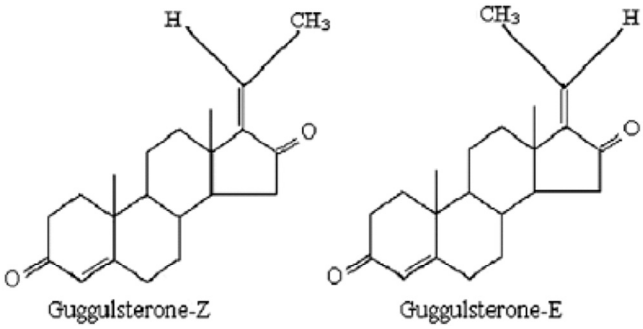
Rats fed with a high-fructose diet form a model of diet-induced insulin resistance, associated with hyperinsulinemia, hypertriglyceridemia and glucose intolerance (Thorburn et al., 1989). Recently, antioxidants are found to be effective in preventing a majority of the abnormalities induced by high-fructose diet (Faure et al., 1999).

Over the past few decades there has been increasing scientific and public interest in so called antioxidant hypothesis. Therefore, in addition to control of blood glucose levels, control of oxidative stress offers another avenue for the treatment of the disease. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of STZ.

Insulin sensitizers and antioxidants are found to be effective in preventing not all but at least a majority of the abnormalities induced by fructose (Faure et al., 1999). Current treatment with thiazolidinediones and particularly metformin does not adequately address the issue of IR (Hollenberg, 2003). Hence, there is a need to search for new agents with better efficacy and minimal side effects. Plants and herbs are mines of a large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs.

Traditional (India) uses of *Commiphora mukul* (CM) are for its anti-inflammatory, antispasmodic, carminative, emmenagogue, hypoglycemic, alternative, antiseptic, and astringent, a thyroid stimulant, anthelmintic and antihyperlipidemia properties. It is an important herb used also in the treatment of several degenerative disorders in modern medicine and established as a hypolipidemic drug (Ulbricht et al., 2005). Ayurvedic medicines containing gum guggul often contain guggul in their names, such as in Shunthi-guggul and Yogaraja guggul. All the formations used for traditional Ayurvedic treatments for obesity contained gum guggul among its herbal ingredients.

Guggulipid, an ethyl acetate extract of the resin of plant *C. mukul* is an established hypolipidemic agent. The hypolipidemic effect of guggulipid and guggulsterone has been consistently demonstrated in various animal species, including rat, mouse, rabbit, chicken (Baldwa et al., 1981), domestic pig (Khanna et al., 1969), dog and monkey (Dixit et al., 1980). In addition, it was found that guggulsterone treatment increased lipolytic enzyme activity as well as receptor-mediated catabolism of LDL (Chander et al., 1996). Two stereo isomers, E- and Z-guggulsterone (cis- and trans-4, 17(20)-pregnadiene-3, 16-dione, respectively), are the most important constituents studied in detail for their therapeutic potential from *C. mukul*. Various studies have been conducted to understand and illustrate the mechanism of action and potential of guggulsterone as a therapeutic agent using synthetic E and Z isomers (Ichikawa and Aggarwal, 2006).



The mechanisms implicated for lipid-lowering effect of guggulipid are stimulation of hepatic lipases and receptor mediated catabolism of low-density lipoproteins, and suppression of hepatic cholesterol biosynthesis (Nityanand and Kapoor, 1973). Guggulsterones inhibited cholesterol synthesis in the liver via antagonism of the forsenoid X receptor and the bile acid receptor (Nagarajan et al., 2001). A number of clinical trials have been conducted to evaluate the hypolipidemic effect of guggulipid. Most of these studies were carried out in India and one in the United States. Consistent with the preclinical data, most of these studies demonstrated polipidemic activity of guggul or guggulipid with an average of 10–30% and 10–20% decrease in total cholesterol and triglyceride, respectively. With proven hypolipidemic efficacy in rats, guggulsterone was used as a positive control to assess the hypolipidemic activity of other chemical compounds (Kumari and Augusti, 2007). Our previous work demonstrated that *C. mukul* gum resin ethanol extract has antidiabetic activity in streptozotocin (STZ)-induced insulin-dependent diabetes mellitus and fructose-induced insulin resistance in animals (Ramesh et al., 2011, Ramesh and Saralakumari, 2012). We are presently experimenting with the fructose-enriched diet rat model, and the aims of the present work were to characterize its efficacy against fructose-induced alterations in antioxidant enzymes, oxidative stress and hepatic and renal marker enzymes.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acids and pyrogallol were obtained from the Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and solvents were of analytical grade and procured from Sisco Research Laboratories (P) Ltd., Mumbai, India.

2.2. Plant material

Ethanolic extract of *C. mukul* gum resin (CMEE; brown, dry powder with Lot No. L5111031) was obtained from the manufacturers and exporters of herbal extracts, Ms Plantex Pvt. Ltd.,

Vijayawada, Andhra Pradesh, India. Procedure followed by the firm for the preparation of extract is as follows: the plant was identified by Dr. K Narasimha Reddy, Taxonomist, Laila Impex R&D Center, Vijayawada. The collected plant sample (resin) was washed thoroughly with tap water, dried at room temperature away from sun light, cut into small pieces, and then powdered. Ethanolic extract was prepared by cold maceration of gum resin powder in ethanol for 7 days. The extract was filtered, concentrated under reduced pressure and finally dried in vacuum desiccators. Herb-to-product ratio was 8:1. A voucher specimen has been deposited in the Department of Biochemistry, Sri Krishnadevaraya University, Anantapur, under number DSKCM-09. The extract was stored at 0–4 °C and dissolved in water just before use.

2.3. *Animals*

Male Wistar rats weighing 160–190 g (8 to 9 weeks old) were procured from Sri Venkateswara Enterprises (Bangalore, India), acclimatized for 7 days in our animal house (Regd. no. 470/01/a/ CPCSEA) before dietary manipulation. Animals were housed two per cage in an air-conditioned room (22 ± 2 °C) with 12 h light/dark cycle and had free access to standard pellet diet and water. All the procedures were performed in accordance with the Institutional Animal Ethics Committee.

2.4. *Rat feed*

The fructose diet and standard pellet diet were procured from the National Centre for Animal Science, National Institute of Nutrition (Hyderabad, India). Fructose diet contains 66% fructose, 15% protein, 8% fat, 4% cellulose, and 3.5% of each mineral and vitamin mix.

2.5. *Experimental design*

Animals were randomly assigned into four groups of eight each: Control rats fed with standard pellet diet (C); control rats treated with CM (C + CM); high-fructose diet-fed rats (F); and fructose-fed rats treated with CM (F + CM). F + CM and C + CM animals received CM suspension (200 mg/kg body weight) in 2 mL of 5% Tween 80 daily for 60 days through orogastric tube, whereas 2 mL of 5% Tween 80 was administered to C and F group rats daily for 60 days. Based on preliminary experiment on dose-dependent antihyperglycemic effect of CM, a dose less than 200 mg/kg bw was expected to be an effective dose in rats (Ramesh et al., 2011). At the end of experimental period of 60 days, animals were sacrificed by cervical decapitation.

Pancreas, heart, liver and kidney were collected.

2.6. *Assessment of lipid peroxidation, protein oxidation and GSH in pancreas and heart*

The concentration of lipid peroxidation intermediates [Thiobarbituric acid reactive substances (TBARS)] in different experimental groups were measured by the method of Utley et al. (1967), using 10% pancreas and heart homogenate in 0.15 M KCl and expressed as nmoles of malondialdehyde. The extent of protein oxidation was measured by the method of Levine et al. (1990) and reduced glutathione (GSH) levels by the method of Ellman's (1959) in pancreas and heart of different experimental groups. Protein content in the pancreas and heart homogenate were measured by the method of Lowry et al. (1951) based on the principle that tyrosine and tryptophan present in the proteins react with Folin–Ciocalteu reagent in the presence of alkaline copper to give colored complex with a maximum absorbance at 750 nm.

2.7. *Enzyme assays*

Ten percent pancreas and heart homogenate were prepared in ice-cold 0.15 M KCl, centrifuged at 12,000 rpm for 45 min in Sigma Laboratory centrifuges 3K 18 models, rotor no. 12150. The clear supernatant thus obtained was used for the assay of superoxide dismutase (SOD; E.C.1.15.1.10) Soon and Tan (2002), catalase (CAT; E.C.1.11.1.6) Beers and Sizer, (1952), glutathione peroxidase (GPx; E.C.1.15.1.9) Rotruck et al. (1973), glutathione-S-transferase (GST; E.C.2.5.1.14) Habig et al. (1974) and glutathione reductase (GR; E.C.1.8.1.7) Pinto and Bartley (1969).

2.8. *Evaluation of aspartate transaminase (AST) and alanine transaminase (ALT)*

The frozen liver and kidney tissues were thawed, and 10% tissue homogenate was prepared in ice cold 0.1 M Tris–HCl buffer, pH 7.4 and centrifuged at 12,000 rpm for 45 min. Transaminases of cytosolic fraction were assayed. Pyruvate gives a brown colored compound with 2, 4-dinitrophenyl hydrazine (DNPH) which is measured calorimetrically at 520 nm (Reitman and Frankel, 1957). To 1.0 mL of GOT buffered substrate (0.19 M of DL aspartic acid, 0.02 M of α-ketoglutarate in 0.1 M disodium and monopotassium phosphate buffer, pH 7.4) or GPT buffered substrate (0.202 M of L-alanine and 0.02 M of α-ketoglutarate in 0.1 M disodium and monopotassium phosphate buffer, pH 7.4), 0.2 mL of enzyme source was added and incubated at 37 °C for 60 min. The reaction was arrested by the addition of 1.0 mL of 1 mM 2, 4-DNPH in 1.1 N HCl. After 20 min, 10 mL of 0.4 N NaOH was added and left at room temperature for another 10 min. A series of pyruvate standards (10–50 μg) were also treated in a similar manner. The reddish brown color developed was read at 520 nm against the reagent blank. The enzyme activities are expressed as μg of pyruvate liberated/min/mg protein.

2.9. *Statistical analysis*

The results were expressed as means ± SEM. Data were analyzed for significant difference using Duncan's Multiple Range (DMR) test ($P < 0.05$) (Duncan, 1955).

3. Results

3.1. *Pancreas and heart oxidative stress markers and enzymatic antioxidants*

Table 1, Figs 1, 2 and 3 summarize the levels of MDA, GSH, protein carbonyl groups and activities of enzymatic antioxidants

Table 1 – Effect of *C. mukul* on pancreas and heart oxidative stress markers and enzymatic antioxidants.

Parameters	Tissues	C	C + CM	F	F + CM
SOD	Pancreas	27.83 ± 2.04[a]	30.41 ± 0.784[a]	18.69 ± 5.29[b]	19.93 ± 2.07[a]
Units/mg protein	Heart	30.84 ± 3.29[a]	32.08 ± 2.73[a]	22.14 ± 1.76[b]	29.02 ± 0.91[a]
CAT	Pancreas	15.87 ± 0.62[a]	17.10 ± 0.41[a]	11.23 ± 0.50[b]	16.14 ± 0.66[a]
µmol of H2O2 consumed/min/mg protein	Heart	26.39 ± 0.97[a]	26.56 ± 1.63[a]	20.91 ± 0.61[b]	25.71 ± 1.20[a]
GST	Pancreas	0.042 ± .001[a]	0.046 ± .001[a]	0.037 ± .002[b]	0.041 ± .001[a]
mmol of CDNB-GSH conjugate formed/min/mg protein	Heart	0.0498 ± .0026[a]	0.05 ± .0021[a]	0.039 ± .0016[b]	0.05 ± .0017[a]
GPx	Pancreas	4.98 ± 0.29[a]	5.01 ± 0.10[a]	4.02 ± 0.15[b]	5.04 ± 0.15[a]
µg of GSH consumed/min/mg protein	Heart	6.02 ± 0.25[a]	6.13 ± 0.19[a]	5.10 ± 0.11[b]	6.13 ± 0.23[a]
GR	Pancreas	22.86 ± 0.61[a]	23.60 ± 0.93[a]	19.40 ± 0.89[b]	23.63 ± 1.01[a]
µmol of NADPH oxidized/min/mg protein	Heart	27.00 ± 0.40[a]	26.92 ± 0.64[a]	22.88 ± 0.52[b]	26.78 ± 0.72[a]

Values are mean ± S.E. (n = 8 animals). Values not sharing common superscript letters differ significantly at $P < 0.05$ (D.M.R. test).

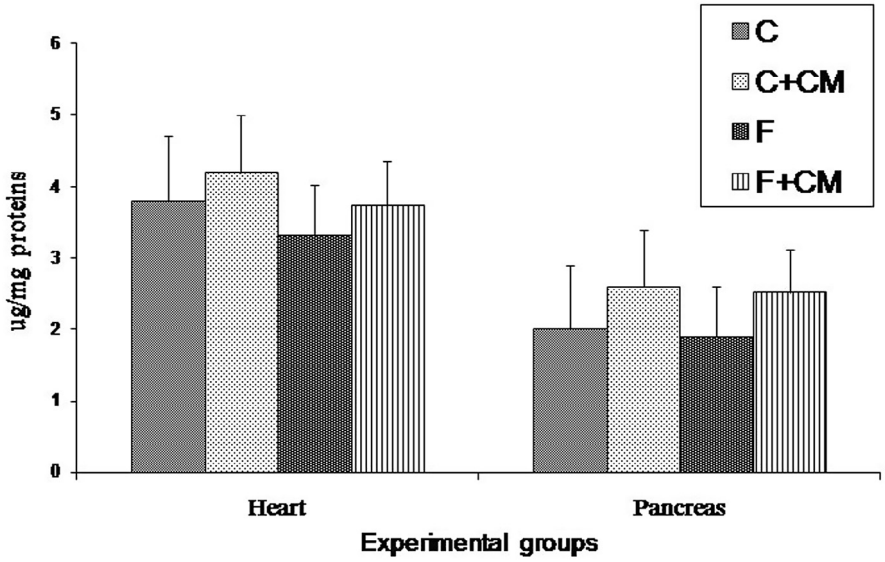


Fig. 1 – Effect of *C. mukul* treatment on pancreas and heart Glutathione in fructose fed insulin resistant rats.

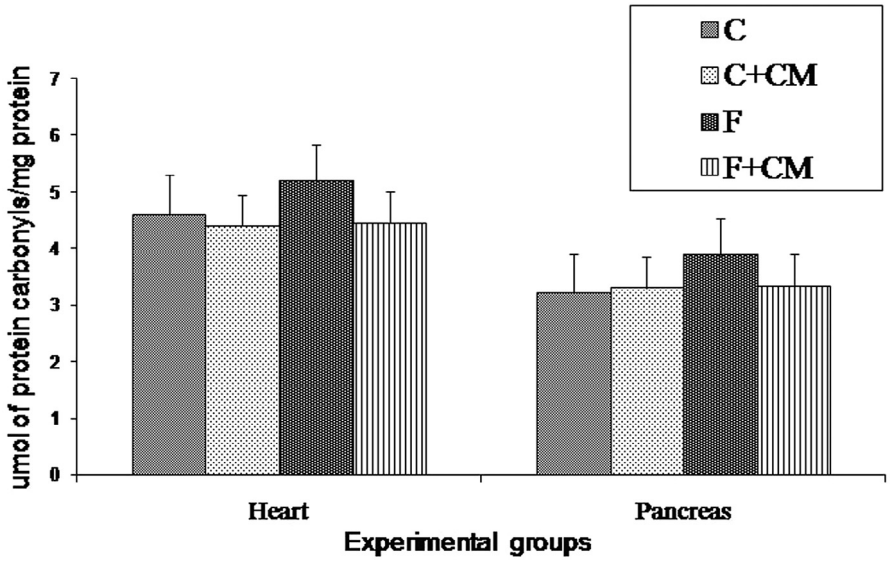


Fig. 2 – Effect of *C. mukul* treatment on pancreas and heart protein oxidation in fructose fed insulin resistant rats.

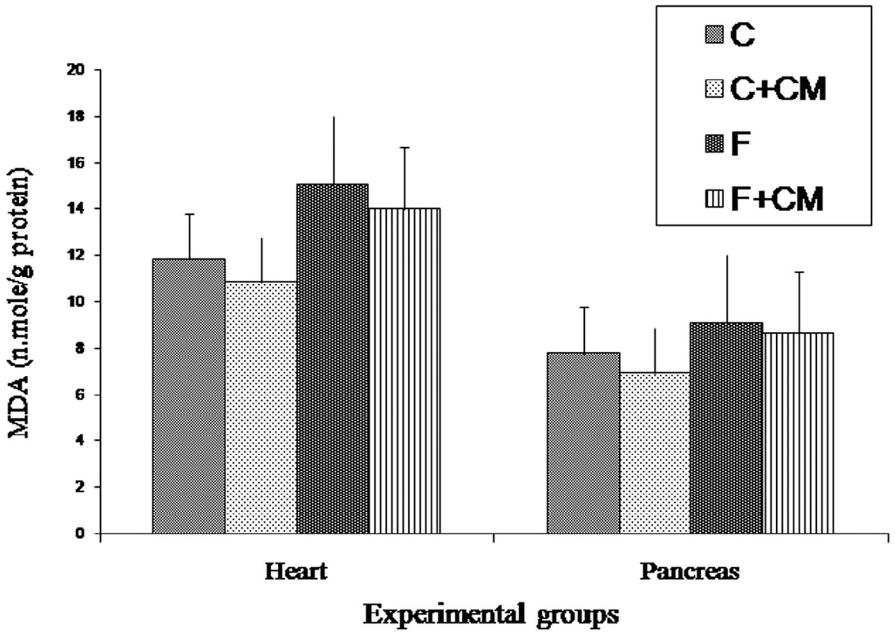


Fig. 3 – Effect of *C. mukul* treatment on pancreas and heart lipid peroxidation oxidation in fructose fed insulin resistant rats.

SOD, CAT, GST, GPx and GR in the pancreas and heart of control and experimental animals. Group-F showed significantly higher levels of TBARS (16.0, 26.5%) and protein carbonyl groups (19.0, 12.3%) as compared to group-C rats. Group-F + CM showed significantly lower TBARS (5.0, 7.0%) and protein carbonyl groups (14.2, 14.0%) when compared with group-F but still significantly higher than group-C (29% and 9%, respectively). Group-F showed depleted pancreas and heart GSH levels (4.0, 14.6%) as compared to control, but treatment with *C. mukul* limited the depletion to only 28.5, 12.6%. Group-C + CM showed 30.0, 7.9% increase in GSH levels when compared to group-C rats. The activities of enzymatic antioxidants SOD, CAT, GST, GPx and GR were significantly lower (32.8, 28.2%; 29.2, 20.7%; 12.0, 20.4%; 19.3, 17.0% and 15.0, 15.5% respectively) in group-F rats than in group-C rats. In group-F + CM, the activities were significantly higher (60.0, 31.0%; 43.7, 23.0%; 11.0, 28.2%; 25.3, 19.6% and 21.6, 17.6% respectively) as compared to group-F.

3.2. *Biochemical assay of AST, ALT activities from hepatic and renal tissues*

Fructose fed insulin resistant rats resulted in an elevation of AST and ALT activities in liver and kidney at significant level when compared to control. After the treatment of *C. mukul* gum resin ethanolic extract, both parameters in liver as well as in kidney were in the levels of control group although the ethanolic extract was able to protect these parameters partially (Table 2).

4. Discussion

The oxidative stress and resultant tissue damage are hallmark of chronic diseases and cell death, and diabetes is not an exception. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), in all tissues from glucose autooxidation and protein glycation (George and Mary, 2004). In addition, hyperinsulinemia in insulin resistance condition and enhanced FFA observed in diabetes and insulin resistance condition are also sources of free radicals (Evans et al., 2003). Diabetes mellitus and insulin resistance are postulated to be a state of increased free radical activity. Oxidative stress is currently suggested as the mechanism underlying diabetes and diabetic complications. Normalizing ROS generation not only reversed these changes, but also prevented the long-term complications of diabetes (Nishikawa et al., 2000).

The direct measurement of free radicals, particularly *in vivo*, is extremely difficult.

Usually, the products of radical damage in the cell – viz., lipids, proteins and DNA are considered good markers of

Table 2 – Effect of *C. mukul* treatment on tissue transaminases in fructose fed insulin resistant rats.

Parameters	Tissues	C	C + CM	F	F + CM
ALT	Liver	1.33 ± 0.098^{a}	1.3 ± 0.0259^{a}	2.78 ± 0.095^{b}	1.35 ± 0.055^{a}
µg of pyruvate formed/min/mg protein	Kidney	0.243 ± 0.0019^{a}	0.23 ± 0.0016^{a}	0.31 ± 0.0038^{b}	0.24 ± 0.0016^{a}
AST	Liver	0.83 ± 0.0016^{a}	0.81 ± 0.0098^{a}	0.99 ± 0.031^{b}	0.85 ± 0.045^{a}
µg of pyruvate formed/min/mg protein	Kidney	0.45 ± 0.0126^{a}	0.44 ± 0.01^{a}	0.51 ± 0.009^{b}	0.46 ± 0.0115^{a}

Values are mean ± S.E. (n = 8 animals). Values not sharing common superscript letters differ significantly at $P < 0.05$ (D.M.R. test).

oxidative stress (Piconi et al., 2003). To understand the extent of oxidative stress and to assess the protective effect of *C. mukul* administration in insulin resistance conditions, oxidative markers like lipid peroxidation (LPO), protein oxidation and antioxidant status were assessed in tissues of four experimental groups. Lipid peroxidation has probably been the most extensively investigated process induced by free radicals. Lipid peroxides are derived from the oxidation of poly unsaturated fatty acids of membranes and are capable of further LPO by a free radical chain reaction (Das et al., 2002). All biomacromolecules are faced with oxidative stress including proteins. Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive ROS or indirectly by reactions with secondary byproducts of oxidative stress (Sermin et al., 2007).

However *C. mukul* treated control group, i.e., C + CM showed no deviation in the protein oxidation in pancreas and heart tissues with significantly decreased protein oxidation levels in the liver compared to C-group. From the results obtained, it is evident that increased oxidative stress as measured by the index of LPO increased in fructose fed rats which could cause impaired insulin function in insulin resistant condition (Evans et al., 2005). In the present study, among the tissues in which oxidative stress studies were conducted (pancreas and heart), pancreas and heart tissues have shown more prominent oxidative stress under insulin resistant conditions respectively.

Insulin resistance is also associated with increased LPO and free radical formation and increased formation of TBARS (thiobarbituric acid reactive substances) is associated with insulin perturbations. Previous studies strongly suggest that oxidative stress occurs in rats fed a high fructose diet (Srividhya and Anuradha, 2002). Detrimental effects of fructose are enhanced when antioxidant defenses are decreased or when free radical production is increased (Rayssiguier et al., 1993). High fructose diet has prooxidant effects (Busserolles et al., 2002).

Both enhanced oxidative damage to cellular constituents and diminished antioxidative capacity have been reported in fructose fed-rats (Busserolles et al., 2002).

Elevated plasma insulin levels reflect an insulin resistance state such that inducing higher plasma glucose levels results in increased ROS production (Paolisso and Giugliano, 1996). Earlier studies showed a direct correlation between ROS levels and fasting insulin concentration in patients with type-2 diabetes. In fructose fed rats, free radical production can be enhanced during hyperinsulinemia and hyperglycemia by mechanisms such as autoxidation of glucose, enhanced glycation, and altered polyol pathway (Paolisso and Giugliano, 1996). FFA could directly increase reactive oxygen species via peroxidation reactions and via mitochondrial production (Bakker et al., 2000). A study by Pennathur et al. (2005) showed that rats with diet-induced hyperlipidemia without hyperglycemia fail to exhibit increased protein and lipid oxidation products in the retina, whereas with linear-regression analysis Davis et al. (2002) demonstrated a significant positive correlation between plasma glucose concentration and levels of plasma TBARS, but neither plasma cholesterol nor plasma triglyceride levels correlated with plasma TBARS. However, Sies et al. (2005) observed that hyperglycemia and/or hyperlipidemia can give rise to nutritional oxidative stress under postprandial conditions. Thus the presence of elevated lipid alone can cause oxidation of proteins and lipids that can be enhanced in the association with hyperglycemia.

High fructose diets may have a hypertriglyceridemic and prooxidant effect, and fructose fed rats have shown less protection from lipid peroxidation. Moreover, the susceptibility of tissues to oxidative stress may depend on alterations in lipid composition. Enhanced lipid accumulation observed in the tissues of fructose fed rats may also contribute to increase LPO in these animals. Further, increased or enhanced catabolism of fructose would result in energy depletion in cells, making them more susceptible to peroxidation (Fields et al., 1992). Besides hyperglycemia, hypertriglyceridemia and hyperinsulinemia along with lipid overload in nonadipose tissues by fructose feeding can be related to increased lipid peroxide levels found in these rats. A similar trend was also reported in Zucker diabetic fatty rats, a model of type-2 diabetes (Atkinson et al., 2003). Kelley et al. (2004) hypothesized that prooxidant stress response pathways might mediate hepatic increase VLDL secretion and delayed clearance upon fructose feeding. Fructose is a highly lipogenic nutrient. The data from the present study also revealed enhanced levels of cholesterol, TG and FFA in blood and liver of fructose fed rats.

In animal models, there is a growing recognition that cardiac dysfunction can occur early following induction of diabetes (Wichi et al., 2007). In parallel, insulin resistance was also associated with an early development of cardiac hypertrophy (Thirunavukkarasu et al., 2004). Earlier studies also indicated that cardiac hypertrophy is induced by chronic fructose feeding. Recently the role of oxidative stress in the development of atherosclerosis in the insulin resistance syndrome has been recognized. Major components of insulin resistance syndrome (IRS) (Insulin resistance, hypertension, and dyslipidemia) generate oxidative stress in response to an overproduction of superoxide anion by the activation of NADPH oxidase (Lee, 2001). Normalizing ROS generation not only reversed these changes, but also prevented the long-term complications of diabetes (Nishikawa et al., 2000).

C. mukul supplementation for 60 days to fructose fed insulin resistant rats alleviated the lipid accumulation in the skeletal muscle and heart tissues. This may depend upon its TG lowering and insulin sensitivity effects. Further, its antihyperglycemic effect could bring a favorable metabolic environment, avoiding the prooxidant conditions with reduced oxidative stress in *C. mukul* treated fructose fed rats. This protection against oxidative stress by *C. mukul* is further reflected by controlled LPO and protein oxidation in F + CM-groups. Guggulsterone has been reported to decrease the levels of LPO products in the liver membranes of treated animals (Singh et al., 1990) and also to inhibit the oxidative changes induced by Cu2+ and Fe2+ in LDL lipids and proteins in *in vitro* studies (Chander et al., 1996).

NIDDM patients (Seghrouchni et al., 2002) and fructose diet-induced insulin resistant rats (Rajasekar et al., 2005). It has recently been shown that GSH improves insulin sensitivity in insulin-resistant individuals and/or patients with type-2 diabetes (Zancan and Sola-Penna, 2005). Hence, the measurement of cellular GSH provides the information about GSH associated scavenging system against free radicals induced LPO in the metabolic disease conditions and aging.

Significant increase in the GSH content in pancreas and heart of *C. mukul* treated fructose fed rats indicates that *C. mukul* treatment activated the compensatory mechanism against the oxidative stress and cell death. Generally, antioxidant treatment can exert beneficial effects in diabetes, with preservation of *in vivo* β-cell function. Antioxidant treatment suppresses apoptosis in β-cells without changing the rate of β-cell proliferation, supporting the hypothesis that in chronic hyperglycemia, apoptosis induced by oxidative stress causes reduction in β-cell mass (Wiernsperger, 2003). The observed significant elevation of GSH content of the tissues of F + CM-rats compared with F-rats indicate that *C. mukul* might have either increased the biosynthesis of GSH or lowered the utilization of GSH due to decreased oxidative stress, or both.

According to Paolisso et al. (1992), intravenous infusion of GSH in Type-2 diabetic patients improved insulin secretion and glucose tolerance during oral glucose tolerance tests. Maintenance of ample concentrations of antioxidants seems to be necessary for efficient insulin action. Efficient expression of insulin receptor gene requires certain transcription factors that are activated by GSH (Araki et al., 1991). Vitamin E also has a beneficial effect on insulin action as its supplementation could restore the GSH concentration in fructose fed rats and improve the physical state of plasma membrane and insulin action in NIDDM patients (Paolisso et al., 1993).

Thus prevention of GSH depletion seen in fructose fed rats by *C. mukul* supplementation may also be responsible for the enhanced insulin sensitivity observed in F + CM-group.

Decreased activity of GPx and GST in F-rats could be directly explained by the low content of GSH found in these rats since GSH is a substrate and cofactor of GPx and GST (Domingues et al., 1998). GSH, the most important antioxidant metabolite, plays an important role in maintaining good levels of GPx activity. Flohe (1971) reported that the kinetics of GPx are of the first order in respect to GSH. Thus the decreased levels of GSH in F-rats may be one of the factors for decreased activity of GPx. GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress (Condell and Tappel, 1983). The low activity of GPx causes accumulation of H2O2 in diabetic rats. This finding could also explain the progressive decrease in SOD in later stages of the diabetes. The depletion in the activities of GST and GPx may result in the involvement of deleterious oxidative changes due to accumulation of toxic products. Thus the decreased activities of these GSH related antioxidant enzymes of D and F-rats may be responsible for elevated LPO observed in these groups of rats.

C. mukul supplementation prevented the depletion in tissue GR activity in F + CM-group by maintaining the normal levels of this enzyme in these animals. Enhanced GR activity in F + CM-groups compared to F-groups respectively reveals the protective effect of *C. mukul* against oxidative damage by keeping normal GSH levels in tissues in fructose fed conditions, which is further reflected by enhanced activities of GPx and GST in *C. mukul* treated resistance animal models.

It has been shown that fructose induced oxidative stress might modulate transcription factors that are sensitive to change in the redox state of the cell (Ramon et al., 2001). This observation indicates that the tissue specific protection of *C. mukul* eventually led to control of LPO in these tissues. The results from the present study indicate that fructose fed insulin resistant rats have increased oxidative stress and a compromised antioxidant defense system in the pancreas and heart. This increase in oxidative stress could be reverted to normal values by *C. mukul* administration. Further, *C. mukul* exerts a protective effect against LPO in liver, pancreas and heart by scavenging ROS and elevating the activities of antioxidant enzymes both in insulin deficient and insulin resistance conditions.

Many phytochemicals are reported to enhance antioxidant enzymes by inducing gene expression of these enzymes. Induction of the hepatic GSH antioxidant system by chemopreventive agents was reported in several studies (Yeh and Yen, 2006). Several phytochemicals were reported to act against the deleterious effects of oxidative stress such as anthraquinones in aloe vegetables (Malterud et al., 1993), total saponins from *Panax ginseng* (Yukozawa et al., 1996), polyphenols (Tiwari, 2001) and flavonoids from *Sideritis raeseri* (Gabrieli et al., 2005). The oleoresin of *C. mukul* is a mixture of several steroid lipids. Of these steroids, Z-guggulsterone and E-guggulsterone are the most effective (Sukh Dev, 1987). The steroid structure also contains H, CH3 and O bond, which indicate that the drug like other herbs may also quench free radicals such as hydroxyl and single oxygen due to its antioxidant effect thus causing a decrease in lipid peroxides similar to the action of probucol (Anderson et al., 1991).

Guggulsterones in the body are easily reduced to guggulsterols, which behave as powerful antioxidants. The antioxidant properties of guggulsterols could be explained by the fact that their hydroxyl groups are present at α-positions of double bonds, similar to antioxidant vitamins, and are soluble in lipids. The drug exhibits nontoxic potential, because it has no highly reactive groups in any position of the isomer structure, similar to tocopherols. The results from the present study suggest that *C. mukul* has antioxidant potential against oxidative stress along with antidiabetic and hypolipidemic potential. These results may lend further support to mount evidence to show that *C. mukul* contains compounds which, if taken in sufficient quantities, could conceivably be beneficial in attenuation and prevention of diabetes and its associated complications.

Muscle wasting, negative nitrogen balance and accelerated gluconeogenesis are among the hallmarks of uncontrolled diabetes (Buse et al., 1972). Tissue aspartate transaminase (AST) and alanine transaminase (ALT) are important enzymes that aid in making amino groups available for entry into the urea cycle. Measurement of their activity in tissues provides an indication of amino acid catabolism. Animals must metabolize proteins to amino acids, at the expense of muscle tissue, when blood sugar is low.

Higher ALT concentrations were cross-sectionally associated with obesity and wholebody and hepatic insulin resistance and prospectively associated with a decline in hepatic insulin sensitivity and the development of type-2 diabetes (Barbora et al., 2002). According to Barbora et al. (2002), high ALT is a marker of risk for type-2 diabetes and suggests a potential role of the liver in the pathogenesis of type-2 diabetes. Wijekoon et al. (2004) reported decreased concentrations of amino acids in liver and kidney of Zucker diabetic fatty rats (insulin resistance rats) which is an indication of enhanced catabolism of these amino acids under insulin resistant conditions. Liver, an

insulin dependent tissue, plays a pivotal role in glucose and lipid homeostasis and it is severely affected during diabetes. Thus risk of chronic liver disease is higher in diabetics. It is the general assumption that herbal preparations would have less side effects, but chronic consumption of large amounts and/or prolonged consumption of traditional remedies must always be taken with caution. No deviation in the activities of tissue transaminases of group-C + CM from group-C clearly indicates the non-toxic nature of *C. mukul* even in chronic treatments. Instead, the restoration of transaminase activities of liver and kidney of F + CM-rats to their respective normal levels further strengthens the protective effect of *C. mukul* against diabetes induced alterations in these tissues. The efficacy of any hepatoprotective drug is essentially dependent on its capability to either reduce harmful effects or to maintain the normal hepatic physiological mechanisms that have been unbalanced by the hepatotoxin (Sen et al., 1993). *Commiphora opobalsamum*, a related species of *C. mukul* which was a valuable medicinal agent in ancient Arabic, has been used in the treatment of diseases of liver, stomach and urinary tract. A decoction or tincture is used by local traditional healers for the treatment of chest, stomach and kidney complaints, to promote digestion; and to relieve rheumatism, scurvy and jaundice (Dymock et al., 1890). Hepatoprotective activity of an ethanolic extract of *C. opobalsamum* was demonstrated in carbon tetrachloride:liquid paraffin (1:1) induced liver toxicity and also prevented the prolongation of the barbiturate sleeping time associated with CCl4 induced liver damage in prevented mice. In the present study similar to *C. opobalsamum, C. mukul* also showed hepatoprotective and nephroprotective activity under insulin resistance conditions.

5. Conclusion

Our results show that fructose feeding to the rats results in development of oxidative stress in both pancreas and heart. This oxidative stress may play a role in pathology associated with fructose feeding such as insulin resistance. In uncontrolled diabetes, oxidative stress results from increased free radical production and depletion of antioxidants like SOD, catalase. Being a potent quencher of reactive oxygen species, *C. mukul* gum resin extract inhibited the free radical mediated lipid peroxidation, preserved the antioxidant enzymes and maintained the non-enzymic antioxidant concentrations. This ameliorative effect of *C. mukul* on tissue lipid peroxidation might also be attributed to its ability to increase glucose disposal thereby abolishing the consequences of hyperglycemia. The findings of the present study corroborate the utility of *C. mukul* as a therapeutic tool in the management of diabetic complications in which induction of oxidative stress is the major contributing mechanism.

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