Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 3 (3): 190-202, 2008
ISSN 1816-496X

Ultrastructural and Biochemical Abnormalities in the Liver of Streptozotocin-Diabetic Rats: Protective Effects of *Murraya koenigii*

P. Arulselvan and S. Subramanian
Department of Biochemistry and Molecular Biology, University of Madras,
Guindy Campus, Chennai-600 025, Tamil Nadu, India

Abstract: The objective of the present study is to evaluate the antioxidant potential of ethanolic extract of *Murraya koenigii* leaf on enzymatic, non enzymatic antioxidants and ultrastructural changes in liver of streptozotocin (STZ) induced diabetic rats. Effect of oral administration of *M. koenigii* leaves extract (200 mg kg^{-1} body weight) on the levels of blood glucose, plasma insulin, glycosylated hemoglobin, Thiobarbituric Acid Reactive Substances (TBARS), hydroperoxides, enzymatic and non-enzymatic antioxidants were estimated in STZ induced diabetic rats. Ultrastructural changes in the liver were also examined. Glibenclamide was used as a standard drug. The elevated levels of blood glucose, glycosylated hemoglobin, TBARS, hydroperoxides and decreased level of insulin observed in diabetic rats were significantly altered after treatment with the *M. koenigii*. The altered enzymatic and non-enzymatic antioxidants in the liver of streptozotocin induced diabetic rats, were restored to near normal levels by treatment with the *M. koenigii* leaves extract. Ultrastructure analysis of the liver of diabetic rat revealed a reduction in the Rough Endoplasmic Reticulum (RER) and swelling of mitochondria in the hepatocytes and these abnormalities were restored to near normal morphology by the treatment of rats with *M. koenigii* leaf extract. Our results suggested that the ethanolic extract of *M. koenigii* possess potent antioxidant properties which may be due to the presence of biologically active ingredients such as carbazole alkaloids, glycosides, triterpenoids and phenolic compounds. Thus the hepatoprotective and antidiabetic properties of *M. koenigii* leaves were probably of its antioxidant property.

Key words: Antioxidants, diabetes mellitus, *Murraya koenigii*, free radicals and streptozotocin

INTRODUCTION

Modern science recognizes that life is based on a complex and finely tuned network of reduction-oxidation (redox) reactions that are under homeostatic control. Cells or organisms are constantly subjected to factors that can alter this redox balance, often resulting in overt generation of free-radicals (oxidative stress) (Nedelco *et al*., 2004).

Diabetes mellitus is one of the oldest disorders known to mankind. It has been presumed that diabetes results from inherent stresses in modern lifestyle and the rising incidence of diabetes is becoming a significant public health problem (Pickup and Williams, 1997). Diabetes mellitus has been shown to be a state of increased free radicals formation. Oxidative stress may increase in diabetes owing to a higher production of Reactive Oxygen Species (ROS) such as superoxide radical, hydroxide radical, hydrogen peroxide and/or deficiency in antioxidant defense systems (Baynes and Thorpe, 1999).

Corresponding Author: Dr. S. Subramanian, Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai- 600 025, Tamil Nadu, India
Tel/Fax: 91-44-22300488

In modern medicine, the beneficial effects of glycemic control are well documented. The preventing activity of present day drugs against progressive nature of diabetes and its complications was modest and not always effective. Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short self-life, requirement of constant refrigeration and in the event of excess dosage-fetal hypoglycemia limits its usage (Kasiviswanath *et al*., 2005). The doubts about the efficacy and safety of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes (Reaven *et al*., 1983). Nature has been a potential source of variety of plants with diversified medicinal values and herbs were used for the treatment of various ailments for thousands of years and plant based drugs continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (Grover and Vats, 2001). Herbal medicines have been employed for the treatment of diabetic patients and are currently accepted as an alternative therapy for diabetes treatment. More than 1200 plants have been described in the scientific and popular literature of their hypoglycemic agents. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Wang and Ng, 1999).

In the present study, *Murraya koenigii* (L) Spreng (Rutaceae) was chosen since it is one of the most widely acclaimed remedies for the treatment of diabetes. *M. koenigii* leaves are used as flavouring, condiment and folk medicine for the treatment of various metabolic and infectious diseases (Chakraborty *et al*., 1965). The leaves, bark and the roots are used intensively in indigenous medicine from ancient time, as a tonic for stomachache, stimulant and carminative (Kong *et al*., 1986). *M. koenigii* leaves were popularly known as curry leaves in India. Phytochemical screening of *M. koenigii* revealed the presence of some vitamins, carbazole alkaloids, triterpenoids, phenolic compounds and mineral contents such as iron, calcium, zinc and vanadium etc (Arulselvan *et al*., 2006; Narendhirakannan *et al*., 2005). In addition, carbozole alkaloids present in *M. koenigii* were reported to have antioxidant activities (Tachibana *et al*., 2001).

Several biological activities of *M. koenigii* have been reported for its anti-hypercholesterolemic (Khan *et al*., 1996a), anti-inflammatory (Ramsewak *et al*., 1999), anti-trichomonal (Adebajo *et al*., 2006) as well as its efficacy against colon carcinogenesis (Khan *et al*., 1996b). Isolated bioactive carbazole alkaloids from fresh curry leaf such as mahanimbine, murrayanol and mahanine, have been reported to show anti-microbial properties (Ramsewak *et al*., 1999). Prolonged administration of *M. koenigii* leaves extract to experimental rats did not cause any adverse effect on Food Efficiency Ratio (FER), red blood cell count (RBC) and white blood cell count (WBC) (Khan *et al*., 1995). Recently, we have also reported the non-toxic and anti-diabetic properties of *M. koenigii* leaf extract on streptozotocin induced diabetes (Arulselvan *et al*., 2006). The effect of *M. koenigii* leaves extract on carbohydrate metabolizing enzymes in liver of STZ-diabetic rats was also reported (Narendhirakannan *et al*., 2006). More recently, we have also investigated the protective nature of *M. koenigii* leaf extract on the ultrastructural modifications in the pancreas of STZ-induced diabetes in rats (Arulselvan and Subramanian, 2007).

The liver is an organ of central metabolic importance and is known to undergo oxygen free radicals mediated injury in diabetes mellitus (Liang, 2002). The aim of the present study is to delineate alterations in the liver of diabetic rats using indicative ultrastructural and biochemical parameters. The results demonstrate that *M. koenigii* extract prevents the morphological and ultrastructural abnormalities of liver and improve the antioxidant status. The efficacy was comparable with glibenclamide, a known antidiabetic agent.

MATERIALS AND METHODS

Chemicals

Streptozotocin was procured from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade.

Plant Material and Preparation of Extract

Fresh, mature *M. koenigii* leaves (Rutaceae) were collected from a plant in Attur, Tamil Nadu, India, after identification and authentication at the herbarium of Botany Department, University of Madras by Dr. V. Kaviyarasan, Centre for Advanced Studies in Botany, University of Madras, where a voucher specimen (685) was deposited. The leaves were dried at room temperature, powdered and stored at 5°C until when needed. A 100 g of the powder was defatted with petroleum ether (60-80°C) overnight and re-extracted with 95% ethanol using soxhlet apparatus. Ethanol was evaporated in-vacuo using a rotary evaporator to give a 5.4% w/w yield.

Animals

Adult male albino rats of Wistar strain weighing approximately 150-180 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. They were acclimatized to animal house conditions, fed with commercial pelleted rat chow (Hindustan Lever Pvt. Ltd., Bangalore, India) and had free access to water. All the animal experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC 01/013/06).

Induction of Experimental Diabetes

The rats were fasted overnight and diabetes was induced in rats with a single intraperitoneal injection of a freshly prepared solution of streptozotocin (55 mg kg^{-1}) in 0.1 M cold citrate buffer of pH 4.5 (Rakieten *et al*., 1963). Rats were supplied with 5% glucose solution for 48 h after STZ injection in order to prevent severe hypoglycemia. After one week of allowing for the development and aggravation of diabetes, the rats with moderate diabetes having persistent glycosuria and hyperglycemia (Blood glucose range of above 250 mg dL^{-1}) were used for the experiments. The treatment was started on the 8th day after STZ injection and this was considered as 1st day of treatment. The rats were grouped into four, comprising of six rats in each group as follows.

Group I : Control rats receiving 0.1 M cold citrate buffer (pH 4.5)
Group II : Diabetic rats (control)
Group III : Diabetic rats given *M. koenigii* leaf extract (200 mg kg^{-1} b.w/day) in aqueous solution orally for 30 days.
Group IV : Diabetic rats given glibenclamide (0.6 mg kg^{-1} b.w/day) in aqueous solution orally for 30 days (Vijayakumar *et al*., 2006).

At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected in tubes containing EDTA for the estimation of glucose by *O*-toluidine method (Sasaki *et al*., 1972) and glycosylated hemoglobin (Nayak and Pattabiraman, 1981). The plasma was separated and used for the assay of insulin using RIA kit (for rats) supplied by Linco Research, Inc., USA.

The liver tissue was excised, rinsed in ice-cold saline and then homogenized in Tris-HCl buffer of pH 7.4 using a Teflon homogenizer. The liver homogenate were then centrifuged in a cooling centrifuge at 5000 x g to remove the debris and the supernatant was used for the analysis of biochemical parameters. The tissue homogenate was placed at -20°C until further use.

Assay of Pathophysiological Enzymes in Liver

The activities of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) were assayed by the method of King (1965a,b).

Determination of Lipid Peroxidation and Non-enzymatic Antioxidants

Lipid peroxidation (TBARS) in tissue homogenate was estimated using thiobarbituric acid reactive substances by the method of Okhawa *et al.* (1979) and hydroperoxides were estimated spectrophotometrically by the method of Jiang *et al.* (1991). Non-enzymatic antioxidants, Vitamin E and Vitamin C were measured according to the methods of Desai (1984), Omaye *et al.* (1979) and Sedlak and Lindsay (1968) (GSH), respectively.

Assay of Antioxidant Enzymes

The activity of Superoxide Dismutase (SOD) and Catalase (CAT) were assayed by the methods of Misra and Fridovich (1972) and Takahara *et al.* (1960), respectively. The activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed according to the methods of Rotruck *et al.* (1973) and Habig *et al.* (1974), respectively. Protein content in tissue homogenate was measured by the method of Lowry *et al.* (1951).

Transmission Electron Microscope

A portion of liver (about 1 mm^3) from control and experimental groups of rats were fixed in 3% glutaraldehyde and then post-fixed in osmium tetroxide and embedded in araldite (epoxy resin). One micro section was cut and then stained with toluidine blue. Suitable area for ultrastructural study was chosen after examining one-micron section (60-90 nm) and cut on an LKBUM4 ultramicrotome using a diamond knife and sections were mounted on a copper grid and stained with uranyl acetate and Reynolds lead citrate (Kalender *et al.*, 2004). The grids were examined under a Philips EM201C transmission electron microscope.

Statistical Analysis

All the grouped data were statistically evaluated with SPSS/10.00 software (SPSS, Chicago, IL, USA). Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test. $P<0.05$ was considered to be statistically significant. All the results were expressed as Mean±SD for six rats in each group.

RESULTS

Levels of Blood Glucose, Glycosylated Hemoglobin and Insulin in Control and Experimental Groups of Rats

Table 1 shows the change in blood glucose, glycosylated hemoglobin and insulin levels of control and experimental groups of rats. These biochemical variables were significantly altered in STZ-induced diabetic rats when compared to control rats. The effect of oral administration of *M. koenigii* extract and glibenclamide to diabetic rats, were found to be similar to that of control rats.

Table 1: Levels of blood glucose, plasma insulin and glycosylated hemoglobin in control and experimental groups of rats

Groups	Blood glucose (mg dL^{-1})	Plasma insulin (μU mL^{-1})	Glycosylated hemoglobin (%Hb)
Control	86.15±5.85	16.81±0.94	6.8±0.24
Diabetic control	295.35±22.15*	4.63±0.29*	14.2±0.75*
Diabetic+*Murraya koenigii*	91.62±5.95**	14.90±0.72**	7.6±0.41**
Diabetic+Glibenclamide	94.61±6.71**	13.25±0.68**	8.0±0.29**

Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *$p<0.05$ and **$p<0.05$.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **_M. koenigii_ and glibenclamide treated groups of rats were compared with diabetic control

Table 2: Activities of AST, ALT and ALP in serum of control and experimental groups of rats

Groups	AST	ALT	ALP
Control	81.52±5.46	18.27±0.95	69.24±5.26
Diabetic control	118.05±8.26*	43.07±2.75*	126.32±8.46*
Diabetic+*Murraya koenigii*	85.64±5.82**	19.13±0.82**	72.19±4.62**
Diabetic+Glibenclamide	86.35±6.21**	20.07±1.00**	73.94±5.24**

The enzyme activities expressed as: AST and ALT-μmoles of pyruvate/h/mg of protein
ALP-μmoles of phenol liberated/min/mg of protein
Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *p<0.05 and **p<0.05.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **M. koenigii and glibenclamide treated groups of rats were compared with diabetic control.

Table 3: Activities of AST, ALT and ALP in liver of control and experimental groups of rats

Groups	AST	ALT	ALP
Control	10.52±0.43	15.27±0.64	0.16±0.03
Diabetic control	7.05±0.41*	10.07±0.58*	0.09±0.01*
Diabetic+*Murraya koenigii*	10.04±0.35**	14.73±0.56**	0.15±0.02**
Diabetic+Glibenclamide	9.35±0.31**	14.28±0.59**	0.14±0.02**

The enzyme activities expressed as: AST and ALT-moles of pyruvate/min/mg of protein
ALP-μmol of phenol liberated/min/mg of protein
Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *p<0.05 and **p<0.05.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **M. koenigii and glibenclamide treated groups of rats were compared with diabetic control.

Table 4: Levels of TBARS and hydroperoxides in liver of control and experimental groups of rats

Groups	TBARS (mM 100 g^{-1} of tissue)	Hydroperoxides (mM 100 g^{-1} of tissue)
Control	0.83±0.04	67.04±3.95
Diabetic control	1.80±0.09*	95.38±5.10*
Diabetic+*Murraya koenigii*	1.03±0.05**	71.60±4.00**
Diabetic+Glibenclamide	1.12±0.06**	76.34±4.50**

Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *p<0.05 and **p<0.05.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **M. koenigii and glibenclamide treated groups of rats were compared with diabetic control

Activities of AST, ALT and ALP in Control and Experimental Groups of Rats

The activities of AST, ALT and ALP enzymes were found to increase significantly in serum with a concomitant decrease in liver of diabetic rats when compared with the control group. Administration of *M. koenigii* leaves extract normalizes the activities of AST, ALT and ALP to near normal when compared with control rats (Table 2 and 3).

Levels of TBARS and Hydroperoxides

STZ-induced diabetes results in a significant increase in the level of TBARS and hydroperoxides in diabetic rats. Administration of *M. koenigii* leaves extract for 30 days to diabetic rats resulted in a significant decrease in the levels of TBARS and hydroperoxides to near normal level (Table 4).

Vitamin C, Vitamin E and Glutathione Levels in Liver of Control and Experimental Groups of Rats

A significant decrease in the levels of vitamin C, GSH and concomitant increase in the level of vitamin E was observed in the liver of STZ-diabetic rats. Oral administration of *M. koenigii* leaves extract increase the levels to normal (Table 5).

Table 5: Levels of vitamin C, vitamin E and reduced glutathione in liver of control and experimental groups of rats

Groups	Vitamin C ($\mu g\ mg^{-1}$ of protein)	Vitamin E ($\mu g\ mg^{-1}$ of protein)	GSH (mg $100\ g^{-1}$ of tissue)
Control	1.38±0.04	0.83±0.02	48.53±2.71
Diabetic control	0.72±0.02*	1.52±0.04*	26.94±1.29*
Diabetic+*Murraya koenigii*	1.26±0.05**	1.06±0.03**	47.31±2.50**
Diabetic+Glibenclamide	1.17±0.04**	1.14±0.03**	45.82±2.61**

Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *p<0.05 and **p<0.05.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **_M. koenigii_ and glibenclamide treated groups of rats were compared with diabetic control

Table 6: Activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in liver of control and experimental groups of rats

Groups	SOD	CAT	GPx	GST
Control	9.85±0.45	82.64±4.87	9.62±0.39	7.15±0.27
Diabetic control	4.20±0.17*	41.38±2.35*	4.94±0.21*	3.17±0.11*
Diabetic+*Murraya koenigii*	8.93±0.38**	79.01±4.97**	8.72±0.38**	6.74±0.26**
Diabetic+Glibenclamide	8.31±0.35**	71.45±4.14**	7.94±0.30**	6.31±0.25**

The enzyme activities expressed as: 50% of inhibition of epinephrine autoxidation/min for SOD; µmoles of hydrogen peroxide decomposed/min/mg of protein for Catalase; µmoles of glutathione oxidized/min/mg of protein for GPx; Units/min/mg of protein for GST.
Values are given as mean±SD for groups of six rats in each.
Values are statistically significant at *p<0.05 and **p<0.05.
Statistical significance determined by ANOVA was compared within the groups as follows:
*Diabetic control rats were compared with control rats; **_M. koenigii_ and glibenclamide treated groups of rats were compared with diabetic control

Enzymatic Antioxidants

In STZ-diabetic rats, the activities of these enzymes were found to be significantly decreased when compared to that of the normal control rats. Administration of *M. koenigii* leaves to diabetic rats restored the activities to near normal control levels (Table 6).

Transmission Electron Microscopic Studies

Figure 1(a-e) depicts the transmission electron microscope examinations of liver in control and experimental groups of rats. At the electron microscope level, the liver of control rats had a normal cytological appearance (Fig. 1a). Both Fig. 1b and 1c depicts the alterations in the liver tissue of STZ-diabetic rats, showing major alterations in the structural integrity of intracellular organelles, including mitochondria. Administration of *M. koenigii* leaf extract and glibenclamide to STZ-diabetic rats restored the liver ultrastructural changes (Fig. 1d and 1e).

DISCUSSION

Oxidative stress has been defined as a disturbance in the balance between the production of Reactive Oxygen Species (ROS) and antioxidant defense system, which can lead to tissue injury. The levels of reactive oxygen species are regulated by a variety of cellular defenses mechanisms consisting of enzymatic and non-enzymatic antioxidant systems. Antioxidant level in the tissues is an important factor for sensitivity of individual tissue to oxidative stress (Baynes, 1991). It has been suggested that oxidative stress can play an important role in tissue damage associated with diabetes and complications (Baynes and Thorpe, 1999).

Blood glucose is an index for the diagnosis of diabetes mellitus. In the present study oral administration of *M. koenigii* leaves decreased the levels of blood glucose and increased levels of insulin in STZ-diabetic rats. Antidiabetic effect of medicinal plant extract is generally dependent upon the degree of β-cell destruction (Grover *et al*., 2000). *M. koenigii* leaf extract may bring about its

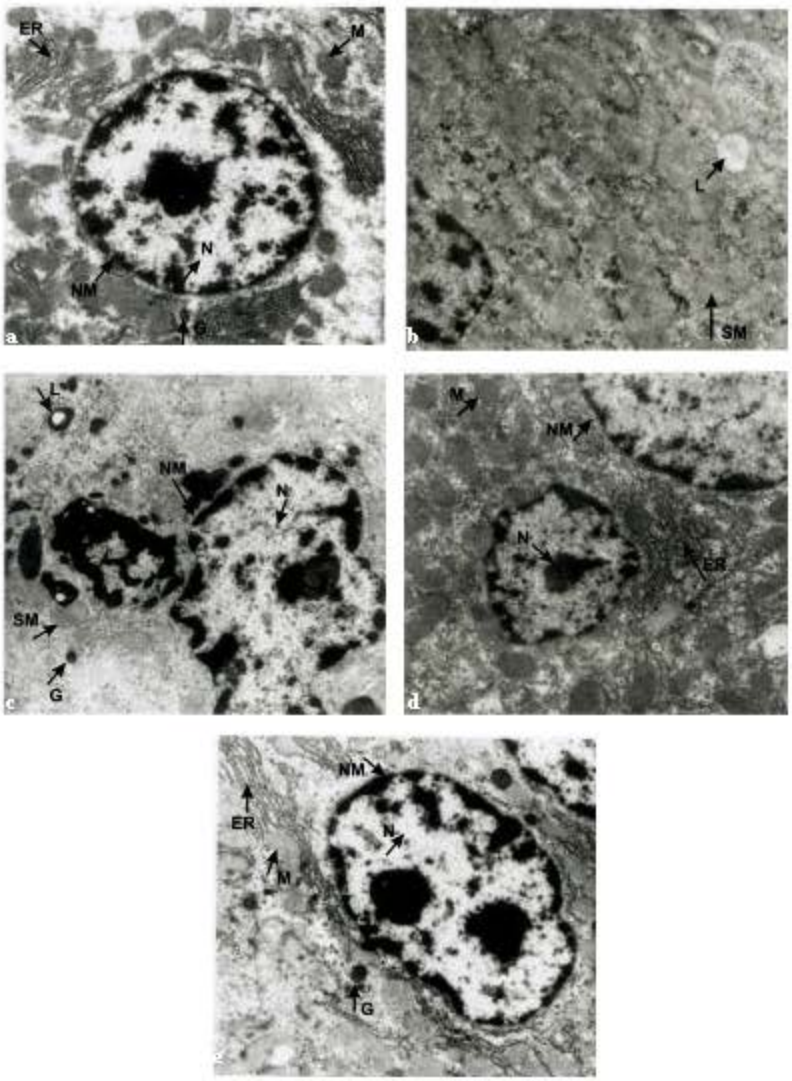

Fig. 1: Electron microscopic observations of liver in control and experimental groups of rats

1a: Electromicrographs of hepatocytes of the control rats. Expansion in the Nucleus (N), Nuclear membrane (NM), mitochondria (M), Endoplasmic reticulum (ER) and Glycogen (G) Magnification: X x15000

1b: Electromicrographs of hepatocytes of the diabetic rats. Swelling in mitochondria (SM) and lipid droplets (L) Magnification: X x15000.

1c: Electron micrographs of STZ-diabetic rats. Swelling in mitochondria (SM), damaged Nuclear Membrane (NM), Lipid droplets (L), Nucleus (N) and Glycogen (G) Magnification: X x15000.

1d: Electromicrographs of hepatocytes of the diabetic rats given *M. koenigii* extract. Normal Nuclease (N), Nuclear membrane (NM), Endoplasmic reticulum (ER), Normal mitochondria (M) and increased level of glycogen (G) Magnification: X x15000.

1e: Electron micrographs of STZ-diabetic rat given glibenclamide. Normal Nuclease (N), Nuclear membrane (NM), Endoplasmic reticulum (ER) and normal structure of mitochondria (M) Magnification: X x15000

antidiabetic effect through insulin secretion from the remnant β-cells and from regenerated β-cells (Arulselvan and Subramanian, 2007). This was clearly evident by the increased level of insulin in diabetic rats treated with *M. koenigii* extract.

Glycosylated hemoglobin or HbA_{1C} has been commonly measured to monitor the glycemic control mechanism in patients with diabetes mellitus. The increased blood glucose present in diabetic conditions reacts with hemoglobin to form HbA_{1C} (Koenig *et al*., 1976). The amount of increase in glycosylated hemoglobin is directly proportional to excess glucose present in blood (Al Yassin and Ibrahim, 1981). Administration of *M. koenigii* extract to diabetic rats decreased the level of HbA_{1C} by virtue of its hypoglycemic activity. This normalization of HbA_{1C} indicates anti-diabetic activity of *M. koenigii* leaf extract.

The measurement of enzyme activities of transaminases (AST and ALT) and Alkaline Phosphatase (ALP) is of clinical and toxicological importance as changes in their activities is often used as a tool of tissue damage by toxicants or in disease conditions. The altered activities of serum and liver AST, ALT and ALP indicate that, diabetes may induce hepatic dysfunction, which is resulting from necrosis of the liver (Larcan *et al*., 1979). The increased activities of AST, ALT and ALP in the serum and decreased activities of these enzymes in the liver may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which may probably be an indication of the hepatotoxic effect of STZ (El-Demerdash *et al*., 2005). However, treatment of STZ-diabetic rats with *M. koenigii* and glibenclamide for 30 days restored the activities of these liver enzymes to their control levels, by restoring the liver damage induced by STZ.

Lipid peroxidation of unsaturated fatty acids is commonly used as an index of increased oxidative stress and subsequent cytotoxicity (Anwer *et al*., 2007). Previous studies indicated that lipid peroxidation increases in naturally (Nishigaki *et al*., 1981) as well as in chemically induced diabetes (Higuchi, 1982). The elevated level of lipid peroxidation is due to enhanced production of reactive oxygen species (superoxide radicals, hydrogen peroxide and hydroxyl radicals). The products of lipid peroxidation (lipid radical and lipid peroxide) are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and cancer (Acworth *et al*., 1997). In our study, there was significant increase in liver TBARS content in diabetic rats compared with control rats. *M. koenigii* and glibenclamide treated rats showed a significant reduction in TBARS, probably indicating a decrease rate of lipid peroxidation. These findings suggest that the *M. koenigii* extract possesses antioxidant effect which can protect the liver from lipid peroxidation induced by STZ.

Hydroperoxides are molecules with high toxic potential and capable of destroying enzymes and cell membranes (Wang *et al*., 1996). The increased level of hydroperoxides in liver may be due to decrease in the activities of antioxidant enzymes, which is a favorable factor for uncontrolled generation of free radicals and subsequent generation of lipid hydroperoxides (Matkovics *et al*., 1998). *M. koenigii* leaf extract, administered orally, decreased significantly hydroperoxides produced in the liver of STZ-diabetic rats. These observations contemplate the antioxidant and anti-lipid peroxidant nature of *M. koenigii* leaf extract.

Non-enzymatic antioxidants such as vitamin C, vitamin E and GSH play an excellent role in preventing the cells from oxidative threats. GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the antioxidant defense processes in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reactions (Gueeri, 1995). The decreased level of GSH in the liver in diabetes represents its increased utilization due to oxidative stress (Anuradha and Selvam, 1993). This may be due to the attempt by the hepatocytes to counteract the increased formation of lipid peroxides. In our study, decreased level of GSH in STZ-diabetic rats was reverted to near normal after administration of *M. koenigii* leaf extract. This indicates that the *M. koenigii* extract probably increase synthesis of GSH as well causing reduction in oxidative stress.

Vitamin C is an important non-enzymatic antioxidant in *in vivo* and *in vitro*. Its major roles, are to act as free radicals scavenger and prevention of oxidative damage under all types of oxidative stress (Prakasam *et al*., 2005). In our study, the level of vitamin C is decreased due to increased utilization of ascorbic acid as an antioxidant defense against to a decrease in the GSH level, since GSH is required for the recycling of ascorbic acid (Hunt, 1996). Treatment with *M. koenigii* and glibenclamide brought back the levels of Vitamin C to near normal level which could be due to natural source of ascorbic acid in the plant extract as it helps to increase the level of vitamin C in diabetic rats.

It is well known that vitamin E, a lipid-soluble and antioxidant vitamin protects unsaturated fatty acids, a main component of cell membranes, from the attack of oxygen derived free radicals. Unlike vitamin C, contradictory results have been reported concerning Vitamin E concentrations in diabetes. The discrepancies among reports seem to be explained on the ground that vitamin E concentration depends on the experimental conditions such as duration and stage of diabetes (Sun *et al*., 1999). Increased level of vitamin E in liver has been reported in diabetic conditions (Sukalski *et al*., 1981). The elevation of tissue tocopherol may be partly explained by the fact that plasma Vitamin E may be increased by mobilization with lipids from the liver resulting from hyperlipidemia accompanied by diabetes (Sun *et al*., 1999). In our study, elevated level of Vitamin E in the liver of diabetic rats are partly due to mobilization of Vitamin E from adipose tissue to the liver or due to impairment in utilization and storage mechanism of Vitamin E by the tissues of diabetic rats. Our results corroborate these observations which may be facilitated by the *M. koenigii* leaf extract.

Streptozotocin-induced hyperglycemia induces generation of O_2 and hydroxyl radicals (OH), which induces various injuries in organs and plays an important role in several clinical disorders. Endogenous antioxidant enzymes such as SOD, CAT are responsible the detoxification of deleterious oxygen radicals (Del Maestro, 1980). Key metabolic steps are the superoxide dismutase (SOD) catalysis of the dismutation of superoxide to hydrogen peroxide and oxygen and the conversion of H_2O_2 into water and oxygen by catalase (CAT). The decreased activities of SOD, CAT in the liver of diabetic rats have been reported (Ramachandran *et al*., 2004). The reduction activities of SOD and CAT in the liver during STZ-induced diabetes may be due to increased production of free radicals as well as decreased the level of antioxidant defensive nature. However oral administration of *M. koenigii* leaf extract reversed the activities of these enzymatic antioxidants. This suggests direct or indirect antioxidant nature of the *M. koenigii* leaf extract, which could be due to the free radical scavenging of carbazoles alkaloids (Tachibana *et al*., 2003) present in the *M. koenigii* leaf acting as a strong free radical scavenger, thereby improving the antioxidant nature in STZ-diabetic rats.

Glutathione-S-Transferase (GST) is a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional groups. They play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds (Ji *et al*., 1992). Glutathione peroxidase (GPx), an enzyme with selenium and GST, catalyses the reduction of toxic compounds hydrogen peroxide to non-toxic compounds (Illing *et al*., 1991). The significant decrease in the activities of GPx and GST were observed in STZ-diabetic rats. The altered activities of the antioxidant enzymes in diabetic rats treated with *M. koenigii* indicate antioxidant replenishment potential of *M. koenigii*. These results suggest that treated rats with *M. koenigii* leaf extract significantly modulates the oxidative stress induced by diabetes and is likely to play a major role in preventing the pathogenesis of diabetic complications and oxidative damage.

The electron microscopic observations demonstrated the degeneration of the liver cells, decreased level of glycogen granules, increased level of lipid droplets and also reduction in the rough ER and swelling of mitochondria in liver of diabetic rats. These results agree with previous reports (Thakran *et al*., 2004). Oral administration of *M. koenigii* leaf extract prevented STZ-induced diabetic dysfunction and inhibited the generation of free radicals in the damaged hepatic tissue. These observations concluded the cytoprotective role of *M. koenigii* leaf extract in the liver STZ-diabetic rats.

CONCLUSION

Excess production of active oxygen radicals causes oxygen stress in cell membrane and DNA, which consequently induces toxic effects and diseases. Self defense system against these oxidative damages is facilitated by antioxidants. Many new antioxidants have been isolated and identified from herbs and spices. The daily intake of these foods might be one of the most promising sources against major diseases leading to a healthier life. It is strongly expected that biochemical evaluation of antioxidants in edible plants may lead to chemoprevention of lipid peroxidation, inflammation, cancer, diabetes and aging in human beings. Oral administration of ethanolic extract of *M. koenigii* leaves attenuate these problems in the STZ-induced diabetes in rats as a consequence of its potential antioxidant properties. This could be due to the presence of biologically active ingredients such as alkaloids, glycosides, triterpenes and phenolics in the leaf extract (Tachibana *et al*., 2003; Arulselvan *et al*., 2006). Further studies are in progress to isolate and characterized the active components such as carbazole alkaloids and phenolic compounds in *M. koenigii* and their role in controlling diabetes and its related complications.

REFERENCES

Acworth, I.N., D.R. McCabe and T. Maher, 1997. The Analysis of Free Radicals, Their Reaction Products and Antioxidants. In: Oxidants, Antioxidants and Free Radicals, Baskin, S.I. and H. Salem (Eds.). Taylor and Francis, Washington DC, Ch.2.

Adebajo, A.C., O.F. Ahoola, A.A. Iwalewa, A.A. Akindahunsi, N.O. Omisore, C.O. Adewunmi and T.K. Adenowo, 2006. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of *Murraya koenigii* growing in Nigeria. Phytomedicine, 13: 246-254.

Al Yassin, D. and K. Ibrahim, 1981. A minor hemoglobin fraction and the level of fasting blood glucose. J. Fac. Med. Baghdad, 23: 373-380.

Anuradha, C.V. and R. Selvam, 1993. Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan-induced diabetic rats. J. Nutr. Biochem., 4: 212-217.

Anwer, T., M. Sharma, K.K. Pillai, S.E. Haque, M.M. Alam and M.S. Zaman, 2007. Protective effect of benzafibrate on streptozotocin-induced oxidative stress and toxicity in rats. Toxicology, 229: 165-172.

Arulselvan, P., G.P. Senthilkumar, D. Sathiskumar and S. Subramanian, 2006. Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats. Pharmazie, 61: 874-877.

Arulselvan, P. and S.P. Subramanian, 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic β-cells in experimental diabetes in rats. Chem. Biol. Interact., 165: 155-164.

Baynes, J.W., 1991. Perspective in diabetes. Role of oxidative stress in development complications in diabetes. Diabetes, 40: 405-412.

Baynes, J.W. and S. Thorpe, 1999. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 48: 1-9.

Chakraborty, D.P., B.K. Barma and P.K. Bose, 1965. On the constitution of murrayanine, a carbazole derivative isolated from *Murraya koenigii* Spreng. Tetrahedron, 21: 681-685.

Del Maestro, R.F., 1980. An approach to free radicals in medicine and biology. Acta. Physiol. Scand., 492: 153-168.

Desai, I.D., 1984. Vitamin E analysis methods for animal tissues. Methods Enzymol., 105: 138-147.

El-Demerdash, F.M., M.I. Yousef and N.I. Abou El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem. Toxicol., 43: 57-63.

Grover, J.K., V. Vats and S.S. Rathi, 2000. Anti-hyperglycemic effect of *Eugenia jambolona* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J. Ethnopharmacol., 73: 461-470.

Grover, J.K. and V. Vats, 2001. Shifting paradigm from conventional to alternative medicine. An introduction on Traditional Indian Medicine. Asia Pacific Biotech. News, 5: 28-32.

Gueeri, H., 1995. Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. Adv. Exp. Med. Biol., 23: 12-14.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Higuchi, Y., 1982. Lipid peroxides and alpha-tocopherol in rat streptozotocin induced diabetes mellitus. Acta. Med. Okayama., 36: 165-175.

Hunt, J.V., 1996. Ascorbic acid and diabetes mellitus. Subcell. Biochem., 25: 369-405.

Illing, E.K.B., C.H. Gray and R.D. Lawrence, 1991. Blood glutathione and non glucose reducing substances in diabetes. Biochem. J., 48: 637.

Ji, X., P. Zhang, R.N. Armstrong and G.L. Gilliland, 1992. The three dimensional structure of a glutathione-S-transferase from the Mu gene class. Structural analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2-A resolution. Biochem., 31: 10169-10184.

Jiang, Z.Y., J.V. Hunt and S.D. Wolff, 1991. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem., 202: 384-389.

Kalender, Y., S. Kalender, A. Uzunhisarcikli, F. Ogutcu, F. Acikgoz and D. Durak, 2004. Effect of endosulfan on β-cells of Langerhans islets in rat pancreas. Toxicology, 200: 205-211.

Kasiviswanath, R., A. Ramesh and K.E. Kumar, 2005. Hypoglycemic and antihyperglycemic effect of *Gmelina asiatica* Linn. In normal and in alloxan induced diabetic rats. Biol. Pharm. Bull., 28: 729-732.

Khan, B.A., A. Abraham and S. Leelamma, 1995. Haematological and histological studies after curry leaf (*Murraya koenigii*) and mustard (*Brassica juncea*) feeding in rats. Ind. J. Med. Res., 102: 184-186.

Khan, B.A., A. Abraham and S. Leelamma, 1996a. Biochemical response in rats to the addition of curry leaf (*Murraya koenigii*) and mustard seeds (*Brassic juncea*) to the diet. Plant. Foods Hum. Nutr., 49: 295-299.

Khan, B.A., A. Abraham and S. Leelamma, 1996b. *Murraya koenigii* and *Brassica juncea*-alterations on lipid profile in 1-2 dimethyl hydrazine induced colon carcinogenesis. Invest. New Drugs, 14: 365-369.

King, J., 1965a. The Transaminases: Alanine and Asparate Transaminases. In: Practical Clinical Enzymology. Van, D. (Ed.). Nostran Co., London, pp: 363-395.

King, J., 1965b. The Hydrolases-acid and Alkaline Phosphatases. In: Practical Clinical Enzymology, Van, D. (Ed.). Nostran Co., London, pp: 199-208.

Koenig, R.L., C.M. Peterson, R.L. Jones and C. Saudek *et al*., 1976. Correlation of glucose regulation and hemoglobin A_{1C} in diabetes mellitus. New Eng. J. Med., 295: 417-420.

Kong, Y.C., K.H. Ng, P.P. But, Q. Li, S.X. Yu, H.T. Zhang and K.F. Cheng *et al*., 1986. Sources of the anti-implantation alkaloid yuehchukene in the genus *Murraya*. J. Ethnopharmacol., 15: 195-200.

Larcan, A., H. Lambert, H. Laprevote and N. Delorme, 1979. Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients. Dia. Meta., 5: 103-112.

Liang, M.L., 2002. Effect of Tetramethylpyrazine on lipid peroxidation in streptozotocin-induced diabetic mice. Am. J. Chinese Med., 30: 601-608.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Matkovics, B., M. Kotorman, I.S. Varga, D.Q. Hai and C. Varga, 1998. Oxidative stress in experimental diabetes induced by streptozotocin. Acta. Physiol. Hung., 85: 29-38.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay of superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Narendhirakannan, R.T., S. Subramanian and M. Kandaswamy, 2005. Mineral content of some medicinal plants used in the treatment of diabetic mellitus. Biol. Trace Elem. Res., 103: 109-115.

Narendhirakannan, R.T., S. Subramanian and M. Kandaswamy, 2006. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. Clin. Exp. Pharmacol. Physiol., 33: 1150-1157.

Nayak, S.S. and T.N. Pattabiraman, 1981. A new calorimetric method for the estimation of glycosylated hemoglobin. Clin. Chem. Acta., 109: 267-274.

Nedelco, A.M., O. Marco and R.E. Michod, 2004. Sex as a response to oxidative stress: A two-fold increase in cellular reactive oxygen species activates sex genes. Proc. R. Soc. London Ser B, 271: 1591-1596.

Nishigaki, J., M. Hagihara, H. Tsunekawa, M. Maseki and K. Yagi, 1981. Lipid peroxide levels of serum lipoprotein fractions of diabetic proteins. Biochem. Med., 25: 373-378.

Okhawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric reaction. Anal. Biochem., 95: 351-358.

Omaye, S.T., J.D. Turnbull and H.E. Sauberlich, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol., 62: 3-11.

Pickup, J.C. and G. Williams, 1997. Epidemiology of Diabetes Mellitus. In: Textbook of Diabetes. 2nd Edn., Vols.1-2, Blackwell, Oxford, pp: 3.1-3.28.

Prakasam, A., S. Sethupathy and K.V. Pugalendi, 2005. Antiperoxidative and antioxidant effects of *Casearia esculenta* root extract in streptozotocin-induced diabetic rats. Yale J. Biol. Med., 78:15-23.

Rakieten, N., M.L. Rakieten and M.V. Nadkarni, 1963. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother. Rep., 29: 91-98.

Ramachandran, B., K. Ravi, V. Narayanan, M. Kandaswamy and S. Subramanian, 2004. Effect of macrocyclic binuclear oxovanadium complex on tissue defense system in streptozotocin induced diabetic rats. Clin. Chim. Acta., 345: 141-150.

Ramsewak, R.S., M.G. Nair, G.M. Strasburg, D.L. DeWitt and J.L. Nitiss, 1999. Biologically active carbazoles alkaloids from *Murraya koenigii.* J. Agric. Food Chem., 47: 444-447.

Reaven, E., D. Wright, C.E. Mondon, R. Solomon, H. Ho and G.M. Reaven, 1983. Effect of age and diet on insulin secretion and insulin action in the rat. Diabetes, 32: 175-180.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science, 179: 588-590.

Sasaki, T., S. Matsy and A. Sonae, 1972. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsh, Kagaku, 1: 346-353.

Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 293-305.

Sukalski, K.A., K.A. Piuto and J.L. Bernstein, 1981. Decreased susceptibility of liver mitochondria from diabetic rats to oxidative damage and associated increase in α-tocopherol. Free Radic. Biol. Med., 14: 57-65.

Sun, F., K. Iwaquchi, R. Shudo and Y. Nagaki *et al*., 1999. Change in tissue concentrations of lipid Hydroperoxides, vitamin C and vitamin E in rats with streptozotocin-induced diabetes. Clin. Sci., (Lond), 96: 185-190.

Tachibana, Y., H. Kikuzaki, N.H. Lajis and N. Nakatani, 2001. Antioxidative activity of carbazoles from *Murraya koenigii* leaves. J. Agric. Food Chem., 49: 5589-5594.

Tachibana, Y., H. Kikuzaki, N.H. Lajis and N. Nakatani, 2003. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* leaves. J. Agric. Food Chem., 51: 6461-6467.

Takahara, S., B.H. Hamilton, J.V. Nell, Y. Ogura and E.T. Nishimura, 1960. Hypocatalasemia, a new genetic carrier state. J. Clin. Invest., 29: 610-619.

Thakran, S., M.R. Siddiqui and N.Z. Baquer, 2004. *Trigonella foenum graecum* seed powder protects against histopathological abnormalities in tissues of diabetic rats. Mol. Cell. Biochem., 266: 151-159.

Vijayakumar, M., R. Govindarajan, G.M. Rao, Ch.V. Rao, A. Shirwaikar, S. Mehrotra and P. Pushpangadan, 2006. Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. J. Ethnopharmacol., 104: 356-361.

Wang, H.X. and T.B. Ng, 1999. Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci., 65: 2663-2677.

Wang, W., C.C. Pang, M.S. Rogers and A.M. Chang, 1996. Lipid peroxidation in cord blood at birth. Am. J. Obestet. Gynecol., 174: 62-65.