Full Length Research Paper

Protective effect of *Murraya koenigii* (L) leaves extract in streptozotocin induced diabetics rats involving possible antioxidant mechanism

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Diabetes causes increased oxidative stress, which is thought to play an important role in the pathogenesis of various diabetic complications. The antioxidant actions are keys to preventing or reversing diabetes and its complications. Thus the aim of the present study was to evaluate the antioxidant potential of ethanolic *Murraya koenigii* leaves (MKL) extract on Thiobarbituric Acid Reactive Substances (TBARS) as index of lipid peroxidation and on the glycemic control in streptozotocin induced diabetic rats. Effect of oral administration of MKL (300 and 500 mg/kg) on the level of blood glucose, glycosylated haemoglobin, on TBARS and glycogen levels were estimated in streptozotocin induced diabetic rats. Glibenclamide was used as a standard drug. The elevated level of blood glucose, glycosylated haemoglobin, TBARS observed in diabetic rats were significantly altered compared to control but it not come to normal level after treatment with MKL for 15 days in diabetic rats. From the results it suggest that the ethanolic extract of *M. koenigii* possessed potent antioxidant properties which may be due to the presence of biological active ingredient such as carbazole alkaloids, glycoside, triterpenoids and phenolic compounds. Thus the antidiabetic activity of *M. koenigii* leaves was probably due to the presence of its antioxidant property.

Key words: Diabetes mellitus, *Murraya koenigii,* antioxidant.

INTRODUCTION

Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defense potential (Gumieniczek et al., 2002). Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs), and enhanced glucose flux through the polyol pathway (Oberley, 1988; Tiwari and Rao, 2002). Elevated generation of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes

and may enhance susceptibility to lipid peroxidation (Baynes, 1991). Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production (Halliwell and Gutterridge, 1994).

Diabetes mellitus is one of the oldest known to mankind. It has been presumed that diabetes results from inherent stress in modern lifestyle and the rising incidence of diabetes is becoming a significant public health problem. Many herbal plants possess both effect and have been used in traditional medicinal for the treatment of diabetes (Ajgaonkar, 1984; Wang and Ng, 1999; Sheela and Augusti, 1992).

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

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control, yet its short coming such as ineffectiveness on oral administration, short shelf-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 a 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. More than 1200 plants have been described in the scientific and popular literature of their hypoglycemic agents. Plants drugs are frequently considered to be less toxic and free side effects than synthetic ones (Wang and Ng, 1999).

In the present study, *Murraya koenigii* (L) (MKL) was chosen since it is one of the most widely acclaimed remedies for the treatment of diabetes. *M. koenigii* leaves are used as flavoring, condiment and folk medicine for the treatment of various metabolic and infectious diseases. The leaves, barks and the roots are used intensively in indigenous system of medicine from ancient time, as a tonic for stomach ache, stimulant and carminative (Anonymous, 1998; Prajapati et al., 2003). *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 (Narendhirakannan et al., 2005; Chakraborty et al., 1965; Kong et al., 1986). In addition, carbazole 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, b), anti-inflammatory (Ramsewak et al., 1999) as well as its efficacy against colon carcinogenesis (Khan et al., 1996b). Isolated carbazole alkaloids from fresh curry leaf such as mahanimbine, murrayanol and mahanine, have been reported to show anti-microbial properties (Ramsewak et al., 1999). Recently we have also reported antidiabetic activity, and some physiological parameters like weight loss and cholesterol lowering effects at prolonged administration of fruit juice of *M. koenigii* (Tembhurne and Sakarkar, 2009a, b). More recently, we observed an increase in the gastrointestinal motility by *M. koenigii* (Tembhurne and Sakarkar 2009c; 2010).

Diabetes mellitus has been shown to be a state of increased free radicals formation. Oxidative stress may increase in diabeties owing to a higher production of reactive oxygen species results into deficiency in antioxidant defense systems (Bayane and Thorpe, 1999). Thus the antioxidant actions are keys to preventing or reversing diabetes and its complications (DeFronzo, 1999). Thus the aim of the present study was to evaluate the protective antioxidant effects of ethanolic extract of *M. koenigii* leaves (MKL) on the glycemic control in streptozotocin induced diabetic animals.

MATERIALS AND METHODS

Plant

Fresh leaves of *M. koenigii* were collected from its natural habitat at Sakoli village in Nagpur region, Maharastra, India. The plant was authenticated by Dr. N. M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Chemicals

Glibenclamide as a standard (Glenmark Pharmaceutical Mumbai), Glucose estimation kit (Hi-Media Lab, Mumbai), Streptozotocin (Gift sample from Nicholas Piramal Mumbai), haemoglobin kit (Drabkins reagent), Thiobarbituric acid, Glycosylated Hb Kit. All the reagents and chemicals used in present study were of analytical grade.

Preparation of extracts of *M. koenigii* leaves

The collected leaves of *M. koenigii* (L) were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by maceration in air tight container by using ethanol as a solvent (125 g/500 ml ratio). The extract was concentrated by evaporation at room temperature and used for pharmacological studies.

Administration of extract

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using Tween 20 (0.2% v/v) as a suspending agent. The extract was administered in a dose of 300 and 500 mg/kg respectively to streptozotocin induced diabetic Wister rats. Control groups were given only 0.5% carboxymethyl cellulose with Tween 20 (0.2% v/v).

Experimental animals

Male Wister rats weighing 175 - 200 g were used. The animals were fed with standard diet (Amrut feed, Sangali, Maharastra), had free access to water under well ventilated condition of 12 h day light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) of Sudhakarrao Naik Institute of Pharmacy Pusad (729/02/a/CPCSEA).

EXPERIMENTAL DESIGN

Antidiabetic study

Preliminary hypoglycemic study

The hypoglycemic activity was performed in normal glycemic and

glucose loaded rat [Oral Glucose Tolerance Test (OGTT) model]. Male Wister rats weighing 175 - 200 g were assigning to each contain 6 animals. Four groups of animals were used for normal glycemic model and OGTT models respectively (Achyut et al., 2005; Akhtar and Iqbal, 1991; Tembhurne and Sakarkar, 2009a).

In normoglycemic, groups 1 and 2 were normal and vehicle control, groups 3 and 4 received orally 300 and 500 mg/kg of MKL respectively. While in OGTT model group 1 was vehicle control, group 2 was standard Glibenclamide (300 mg/kg p.o.) and group 3 and 4 received the extract of MKL at same dose levels.

In normoglycemic and OGTT model, initial blood was taken by retro-orbital at 0 min. After the administration of drugs further blood was taken at 30, 60 and 120 min intervals respectively. In OGTT model, glucose was given (3.0 g/kg) to all groups of animal and subsequent blood samples were taken at time interval of 30, 60 and 120 min intervals respectively.

Antidiabetic study of EMKL in streptozotocin induced diabetic model

Induction of experimental diabetes: After fasting, diabetes was induced by single intravenous (*i.v.*) injection of STZ (55 mg/kg) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight for prevention of mortality due to hypoglycemic convulsing reaction. After 13 days of STZ administration the remaining survival animals which showed blood glucose concentration more than 250 mg/dl considered as diabetics and were used for experimentation.

Antidiabetic activity in streptozotocin induced diabetic rats: 13 days after streptozotocin, control and survival diabetic rats were randomly divided in five groups, each consisting of six animals: Group one as a normal vehicle control received 0.5% sodium CMC with twin 20 (0.2%v/v). Group 2 as a diabetic control and received vehicle only. Group 3 diabetic animals received glibenclamide (10 mg/kg, p.o.) Group 4 and 5 diabetic animals received 300 and 500 mg/kg of MKL extract respectively. After 15 days of above treatments schedule animal's blood was withdrawal by retro-orbital plexus (fasted animals) for determination of glucose and glycosylated haemoglobin, triglyceride, and cholesterol (Verspohl, 2002). Liver was isolated from respective group of animals for determination of thiobarbituric acid reactive substance (Verspohl, 2002; Tembhurne et al., 2009d).

Determination of blood glucose, and glycosylated hemoglobin: Fasting blood glucose was estimated by glucose estimation kit by GOD-POD method while glycosylated hemoglobin was assayed according to reported method by using enzymatic kit (Nayak and Pattabiraman, 1981).

Determination of thiobarbituric acid reactive substance (TBARS): After sacrificing, the liver were isolated from respective groups of animals and homogenized in 0.15 M KCl respectively. Homogenates were centrifuged and supernatant was used as a source of polyunsaturated fatty acid for determination of extent of TBARS. The content of TBARS indicates as index of lipid peroxidation or oxidative stress in the form of liver damage in diabetic animals (Ohkwa et al., 1979).

Statistical analysis: All the experimental results were expressed as the mean ± standard deviation. Student unpaired t-test was used to detect further difference between groups respectively, values of p < 0.05 were considered significant.

RESULTS AND DISCUSSION

STZ-induced experimental diabetes is a valuable model for induction of diabetes. Further, the STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunctions through oxidative stress (Ozturk et al., 1996). Through different types of oral hypoglycemic agents are available along insulin for the treatment of diabeties, there is an increasing demand by patient to use the natural products with antidiabetic activity to overcome the side effects and toxicity of synthetic drugs (Jahodar, 1993). Herbal antidiabetic drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Jahodar, 1993). Thus the aim of the present work was to evaluate the antidiabetic activity of MKL in terms of its effects on glycemic status and on the oxidative stress in STZ induced diabetic rats.

The results of the present study demonstrated the antidiabetic activity of *M. koenigii*. The extract was found to decrease the serum blood glucose after 2 h by (12.20%) at 300 mg/kg and (22.79%) at 500 mg/kg in normoglycemic rats. The results of OGTT revealed that the percentage increase in serum glucose level was lowest at the dose of 500 mg/kg (54.32%) at 30 min which was comparable to standard glibenclamide (47.37%); while at 300 mg/kg it was found to be 68.29% compared to their 0.0 min reading. The reduction in the blood glucose level results could be due to delaying absorption of the glucose from the gastro intestinal tract by the extract. The MKL at both doses was found to decrease maximum blood glucose level after 120 min. Moreover, percentage reduction in serum glucose level was highest at the dose of 500 mg/kg (26.25%) which was comparable to standard glibenclamide (29.12%) at 120 min than 300 mg/kg (19.31%). This could be due to increase in the disappearance of glucose from circulation soon it is absorbed in the circulation by the extract. The result of both normoglycemic and OGTT concludes that at both doses (300 and 500 mg), MKL possess possible hypoglycemic activity (Table 1).

Rats treated with STZ develop almost identical diabetic states and exhibit symptoms like hyperglycemia, glucosuria, polyuria, polyphagia, polydipsia and weight loss. The ability of STZ to produce such diabetes has previously been reported in numerous studies (Verspohl, 2002). Similar result of STZ was obtained in our present study. While after single oral doses of the extract (300 and 500 mg/kg) produced significant (p < 0.05) decrease serum glucose levels at 5 h compared to 0 h on day 1 which was comparable to standard glibenclamide (Table 2). While there was no significant (p < 0.05) difference in the serum glucose level at 5 h compared to 3 h, it indicates a decline in further reduction of serum glucose levels in extract and glibenclamide treated diabetic animals. Once daily repeated oral administration of extract (300 and 500 mg/kg p.o) and glibenclamide (10 mg/kg

Table 1. Hypoglycemic effect of MKL in normoglycemic and in oral glucose tolerance test.

Treatments	0 min	30 min	60 min	120 min
		Effect in normoglycemic		
Normal	78.7 ± 3.14	77.79 ± 3.96 (-1.17)	78.34 ± 4.69 (+0.46)	77.96 ± 3.47 (-0.94)
Vehicle control	77.73 ± 3.77	76.13 ± 5.80 (-2.10)	76.75 ± 4.68 (-1.27)	76.54 ± 2.53 (-1.55)
MKL-300	75.58 ± 3.74	72.06 ± 3.20 (-4.88)	71.45 ± 2.68 (-5.78)	67.36 ± 2.95 (-12.20)
MKL-500	77.24 ± 4.42	72.3 ± 3.1 (-6.83)	68.19 ± 2.82 (-13.27)	62.9 ± 2.07 (-22.79)
		Effect in OGTT		
Vehicle control	76.31 ± 4.15	137.36 ± 5.14 (+80.0)	130.14 ± 4.65 (+70.54)	116.36 ± 6.77(+52.48)
Glibenclamide	75.23 ± 3.28	110.87 ± 4.97(+47.37)	94.35 ± 4.95 (+25.41)	85.86 ± 5.36(+14.13)
MKL-300	74.09 ± 5.02	124.69 ± 4.94(+68.29)	114.54 ± 5.13 (+54.59)	104.5 ± 4.75(+41.04)
MKL-500	74.75 ± 6.48	115.36 ± 5.56(+54.32)	105.36 ± 5.53 (+40.94)	91.37 ± 5.58(+22.23)

Data expressed as means ± s.d of absorbance (n=5). The values in parenthesis are in percentage '+' indicate percent rise and '–'indicate decline in blood glucose compare to 0 min reading; In OGTT model 3 gm/kg glucose was given after 0 min reading.

Table 2. Antidiabetic activity of *M. koenigii* in streptozotocin induced diabetic rat.

Time Interval	Vehicle control	Diabetic	Glibenclamide	MKL-300 mg/kg	MKL-500 mg/kg
0 h	80.93 ± 3.84	308.56 ± 18.83*	302.60 ± 14.21*	304.66 ± 14.56*	307.98 ± 10.22*
1 h	80.61 ± 6.20	309.62 ± 19.08	291.038 ± 15.48	294.87 ± 16.63	294.45 ± 11.52
3 h	79.48 ± 4.35	309.99 ± 19.11	276.58 ± 17.10[a]	283.51 ± 15.43	280.73 ± 12.29[a]
5 h	80.89 ± 5.51	309.06 ± 20.20	260.42 ± 16.21[a]	273.72 ± 14.82[a]	267.65 ± 10.92[a]
5th Day	79.84 ± 4.55	310.55 ± 21.4	223.15 ± 15.43	239.65 ± 16.00	236.95 ± 9.36
10th Day	78.56 ± 4.41	312.85 ± 21.67	194.49 ± 16.08	215.77 ± 16.65	204.92 ± 9.80
15th Day	79.54 ± 3.84	316.12 ± 18.52	169.57 ± 11.98[ab]	195.94 ± 14.93[ab]	174.39 ± 12.56[ab]

Data expressed as means ± s.d; n = 5. The data are statistically ($p < 0.05$) significant (ANOVA followed by Dunnet test). *indicates significant ($p < 0.05$) induction of diabetes compare to vehicle control group at 0 hr. [a] indicates significant ($p < 0.05$) hypoglycemic effect of drugs in diabetic animals compared to 0 hr reading of respective group. [ab]indicates significant ($p < 0.05$) decline

p.o) for 15 days produced significant ($p < 0.05$) decrease in serum glucose levels on day 5, 10 and 15 which demonstrate antidiabetic activity in the extract (Table 3). The results of the present study was similar our previous finding on the fruit juice of *M. koenigii* (Tembhurne and Sakarkar, 2009a).

Diabetes is due to the excess glucose present in the blood reacting with hemoglobin to form glycosylated hemoglobin. The rate of glycation is proportional to the concentration of blood glucose (Sheela and Augusti, 1992). Glycosylated hemoglobin has been found to increase over a long period of time in diabetes (Bunn et al., 1978). There is an evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition (Gupta et al., 1997). Thus it suggested that oxidative stress can play an important role in tissue damage associated with diabeties and complications. Therefore, the measurement of glycosylated hemoglobin is supposed to be very sensitive index for glycemic control (Jain et al., 1989). In the present study, the diabetic rats had shown higher levels of glycosylated hemoglobin compared to those in normal rats indicating their poor glycemic control; while after 15 days of MKL treatment, the glycosylated hemoglobin significantly decreased as comparable to standard glibenclamide, indicating a decrease in the status of glycation.

Lipid peroxidation of unsaturated fatty acids is commonly used as an index of increased oxidative stress and subsequent cytotoxicity (Anwer et al., 2007). In the present study, elevated level of lipid peroxidation in the diabetic animals is either due to due to enhanced production of reactive oxygen species because of higher level of glycosylated haemoglobin. In present study, lipid peroxidation was measured in terms of TBARS (Ohkawa et al., 1979; Tembhurne et al., 2009d) and results indicated to significant increase in liver TBARS content in diabetic rats compared with control rats. However oral administration of MKL extract reduces the level of TBARS

Table 3. Level of glycosylated haemoglobin in STZ induced diabetic rats.

Groups	Glycosylated haemoglobin (%Hb)
Vehicle control	8.34 ± 0.731
Diabetic control	16.43 ± 0.141^{a}
MKL-300	11.098 ± 0.921^{ab}
MKL-500	9.32 ± 1.043^{ab}
Glibenclamide	11.98 ± 0987^{ab}

Values are given in mean ± SD for groups of six rats in each. Values are statistically significant at $p<0.05$ (student unpaired t test). * indicates significant ($p < 0.05$) increased in glycosylated Hb when compared with vehicle control group; whereas **indicates significant ($p < 0.05$) decreased in glycosylated Hb when compared with diabetic control group.

indicating a decrease rate of lipid peroxidation. These finding suggest that the MKL extract prevents the formation of glycosylated haemoglobin which ultimately protect from the formation of reactive oxygen species and induction of lipid peroxidation by STZ in diabetic rats. Thus based on our present finding as well as reported phytochemical literatures (Iyer and Uma, 2008; Chakrabarty et al., 1997 and Tachibana et al., 2003) demonstrated that the presence of antioxidant carbazole alkaloids of *M. koenigii* might be involved in stabilization glycemic level. Literature also suggest direct or indirect antioxidant nature of the MKL extract, which could be due to the free radical scavenging of carbazole alkaloids present in the *M. koenigii* (Tachibana et al., 2003) leaf acting as a strong free radical scavenger, thereby improving the antioxidant nature in STZ diabetic rats.

In conclusion, our study demonstrated beneficial effects of *M. koenigii* in diabetics rats. It decreases blood glucose level dose dependently and also has potential to decrease the level of TBARS by inhibiting the lipid peroxidation formation so this findings would be helpful in diabetic patient for prevention of diabetic complications related to level of oxidative stress.

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