



International Journal of **Biological Chemistry**

ISSN 1819-155X



Academic
Journals Inc.

www.academicjournals.com

Effect of *Murraya koenigii* Leaf Extract on Carbohydrate Metabolism Studied in Streptozotocin Induced Diabetic Rats

P. Arulselvan and S. Subramanian

Department of Biochemistry and Molecular Biology, University of Madras,
Guindy Campus, Chennai-600 025, Tamil Nadu, India

Abstract: In the present study, antihyperglycemic effect of oral administration of ethanolic extract of *M. koenigii* leaves (200 mg/kg/day) for 30 days on carbohydrate metabolizing enzymes in the liver and kidney of streptozotocin induced diabetic rats was evaluated. Glibenclamide was used as a standard reference drug. Significant increase in blood glucose and glycosylated hemoglobin and a concomitant decrease in the levels of insulin and liver glycogen were observed in diabetic rats. Increased activities of lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-diphosphatase and glycogen phosphorylase and decreased activities of hexokinase, pyruvate kinase and glycogen synthase were observed in diabetic rats. These alterations were restored to near normal in the liver and kidney after treatment with *M. koenigii* leaf extract indicating that the therapeutic role of *M. koenigii* leaves in moderate streptozotocin induced diabetes.

Key words: *Murraya koenigii*, streptozotocin, diabetes, carbohydrate metabolic enzymes

INTRODUCTION

Diabetes mellitus is a heterogeneous broad spectrum disease often characterized by disturbed carbohydrate and lipid metabolism (Vallance-Owen, 1975). It is the most prevalent chronic disease in the world affecting nearly 25% of the population. From literature review, it has been revealed that 15-20% of diabetic patients are suffering from insulin dependent diabetes mellitus (IDDM) or type-I (Urger and Foster, 1998). Diabetes mellitus is a disorder due to the abnormality in carbohydrate metabolism and is mainly linked with low blood insulin level (Type I) or insensitivity of target organs to Insulin (Type II). Diabetes has been shown to depress the activities of glycolytic and pentose phosphate pathway enzymes while promoting gluconeogenic and lipolytic activities (Weber *et al.*, 1966; Storey and Bailey, 1978; Nathan, 1994).

Hence, the treatment for diabetes mellitus is based on administration of insulin and oral hypoglycemic agents. The oral antihyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects, leading to increasing demand for herbal products with antidiabetic activity having minimum side effects (Vetrichelvan *et al.*, 2002; Mansour *et al.*, 2002). Throughout the world, approximately 80% of the population are almost dependent on traditional medicines. These traditional medicines are invariably from plant sources that do not from the constituents of our normal diet. In recent decades, several health beneficial physiological effects of species have been evidenced based on experimental animals as well as on clinical trails (Srinivasan, 2005). The number of many traditional plants used for treatments of diabetes has increased (Gray and Flatt, 1997). However, few traditional antidiabetic plants have received proper scientific screening. The world health organization has recommended that this area warrants further evaluation (WHO, 1980).

Murraya koenigii (L.) spreng (*Rutaceae*) is one of the most widely acclaimed remedies for the treatment of diabetes mellitus, hence its choice in this present study. The plant is well known in India and commonly referred to as Curry Leaf Tree. The leaves are widely used as a condiment. Various

parts of the plant have been used in traditional or folk medicine for the treatment of headache, stomachache, influenza, rheumatism, traumatic injury, insect and snake bites and as an antidiarrheal and astringent (Kong *et al.*, 1986). Recent studies revealed strong antioxidative activity for carbazoles of *M. koenigii* (Tachibana *et al.*, 2001), reduction of total serum cholesterol content (Khan *et al.*, 1996a). Extracts and carbazoles have been reported to have antimicrobial activity (Nutan *et al.*, 1998), anti-trichomonal (Adebajo *et al.*, 2006) and anti-inflammatory (Ramsewak *et al.*, 1999), activities. Ethanolic extract significantly reduced the blood glucose of STZ-induced diabetic rats (Arulselvan *et al.*, 2006). Its efficacy against colon carcinogenesis was also proved (Khan *et al.*, 1996b).

However, the present study is aimed at investigating the effect of the leaf extract on carbohydrate metabolic enzymes in the liver and kidney of streptozotocin-induced diabetic rats, comparing with that of a standard hypoglycemic drug, glibenclamide.

MATERIALS AND METHODS

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 by Dr. V. Kaviyaran, Centre for Advanced Studies in Botany, University of Madras and a voucher specimen (685) was deposited at the herbarium of Botany Department, University of Madras. 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

Male albino rats of Wistar strain (160-180 g) obtained from Tamil Nadu Veterinary and Animals Sciences University, Chennai, India, acclimatized to animal house conditions. They were fed with commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. All the rat 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).

Experimental

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 experiment. 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: Normal rats given 0.1 M cold citrate buffer (pH 4.5): Normal glycaemic negative control group.
- Group II: Diabetic rats given 0.1 M cold citrate buffer (pH 4.5): Diabetic negative control.
- Group III: Diabetic rats given *M. koenigii* leaf extract (200 mg/kg/day) in aqueous solution orally for 30 days.
- Group IV: Diabetic rats given glibenclamide (0.6 mg/kg/day) in aqueous solution orally for 30 days.

At the end of the experimental period of 37 days and after an overnight fasting, the rats were anaesthetized and sacrificed by cervical dislocation and blood was collected using EDTA as anticoagulant for the estimation of glucose level (Sasaki *et al.*, 1972), glycosylated haemoglobin were estimated according to methods of Nayak and Pattabiraman (Nayak and Pattabiraman, 1981). The plasma was separated and used for the assay of insulin using RIA assay kit (for rats) supplied by Linco Research Inc., USA.

For enzymes and glycogen assay, a portion of the liver and kidney tissues were dissected out, washed with ice-cold saline immediately and the tissues were homogenized in 0.1 M Tris HCl buffer (pH 7.4). The homogenate was centrifuged at 10000 rpm to remove the debris and the supernatant was used as the enzyme source for the assays of hexokinase (Brandstrup *et al.*, 1957), glucose-6-phosphatase (Koiide and Oda, 1959), fructose 1,6-diphosphatase (Gancedo and Gancedo, 1971), lactate dehydrogenase (King, 1959), glycogen synthase (Leloir and Goldenberg, 1979), glycogen phosphorylase (Camblath *et al.*, 1963), glucose-6-phosphate dehydrogenase (Koiide and Oda, 1959) and pyruvate kinase (Pogson and Denton, 1967). Another portion of wet liver tissue was used for the estimation of glycogen content (Camblath *et al.*, 1963).

Statistical Analysis

All the grouped data were statistically evaluated with SPSS/10.00 software. Hypothesis testing methods included one-way analysis of variance followed by Least Significant Difference (LSD) test. $p < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean \pm S.D for six rats in each group.

RESULTS

Levels of Blood Glucose, Glycosylated Hemoglobin and Insulin of Normal and Streptozotocin-diabetic Groups of Rats

There were significant increases in the levels of blood glucose, glycosylated hemoglobin and a concomitant decrease in the level of insulin in STZ-induced diabetic rats. The oral administration of the leaf extract and glibenclamide tended to bring these values back to those of the normal (control) rats (Table 1).

Activities of Glycolytic Enzymes in Normal and Streptozotocin-diabetic Groups of Rats

The significant decreases in the activities of hexokinase, pyruvate kinase and increase in lactate dehydrogenase in livers of STZ-diabetic rats over those of normal were reversed by the extract and glibenclamide as shown in Table 2. Similarly, the increased activities of these enzymes in the kidneys of the diabetic rats were significantly lowered by the extract and glibenclamide to values close to those of the normoglycaemic rats (Table 3).

Activities of Gluconeogenic and Glycogen Metabolic Enzymes in Normal and Streptozotocin-diabetic Groups of Rats

Significantly increased activities of hepatic glucose-6-phosphatase, fructose 1,6-diphosphatase and glycogen phosphorylase as well as concomitant decreases in the levels of glycogen and activities of glucose-6-phosphate dehydrogenase and glycogen synthase were observed in diabetic rats. Administration of the leaf extract, similar to that of glibenclamide, tended to revert the levels of glycogen and activities in the diabetic rats to those of normal rats (Table 4 and 5). The activities of the gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1, 6-diphosphatase and glucose-6-phosphate dehydrogenase that were significantly increased in the kidneys of the diabetic rats were brought back to values of the normal rats after treatment with the extract and glibenclamide (Fig. 1).

Table 1: Levels of blood glucose, plasma insulin and glycosylated haemoglobin in normal and streptozotocin-diabetic groups of rats

Groups	Blood glucose (mg dL ⁻¹)	Plasma insulin (μU mL ⁻¹)	Glycosylated haemoglobin(%Hb)
Normoglycaemic (normal) rats	89.02±6.05	14.72±0.75	6.2±0.25
Diabetic control	287.92±21.59*	4.36±0.15*	13.4±0.61*
Diabetic+ <i>Murraya koenigii</i>	97.64±6.73 [#]	12.68±0.63 [#]	7.5±0.33 [#]
Diabetic+Glibenclamide	105.72±7.18 [#]	11.24±0.42 [#]	8.1±0.34 [#]

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 normal rats; [#]Experimental groups of rats were compared with diabetic control

Table 2: Activities of hexokinase, pyruvate kinase and lactate dehydrogenase in livers of normal and streptozotocin-diabetic groups of rats

Groups	Hexokinase	Pyruvate kinase	Lactate dehydrogenase
Normoglycaemic (normal) rats	261.51±16.99	9.84±0.46	243.89±16.82
Diabetic control	127.86±9.07*	3.92±0.20*	459.34±33.99*
Diabetic+ <i>Murraya koenigii</i>	252.63±17.93 [#]	8.46±0.45 [#]	246.39±17.74 [#]
Diabetic+Glibenclamide	246.72±18.25 [#]	8.27±0.46 [#]	275.89±20.13 [#]

The enzyme activities expressed as: Hexokinase-μ moles glucose-6-phosphate formed/h/mg of protein. Pyruvate kinase (μ moles of pyruvate formed/min/mg of protein). Lactate dehydrogenase-μ moles pyruvate formed/h/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 normal rats; [#]Experimental groups of rats were compared with diabetic control

Table 3: Activities of hexokinase, pyruvate kinase and lactate dehydrogenase in kidneys of normal and streptozotocin-diabetic groups of rats

Groups	Hexokinase	Pyruvate kinase	Lactate dehydrogenase
Normoglycaemic (normal) rats	145.73±10.34	9.14±0.49	485.61±35.93
Diabetic control	292.16±21.03*	20.64±1.01*	700.63±49.74*
Diabetic+ <i>Murraya koenigii</i>	158.08±11.06 [#]	10.33±0.54 [#]	517.24±37.75 [#]
Diabetic+Glibenclamide	165.72±12.09 [#]	10.96±0.58 [#]	525.68±36.27 [#]

The enzyme activities expressed as: Hexokinase-μ moles glucose-6-phosphate formed/h/mg of protein. Pyruvate kinase (μ moles of pyruvate formed/min/mg of protein). Lactate dehydrogenase-μ moles of pyruvate formed/h/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 normal rats; [#]Experimental groups of rats were compared with diabetic control

Table 4: Activities of Glucose-6-phosphatase, fructose 1, 6-di phosphatase and glucose-6 phosphate dehydrogenase in livers of normal and streptozotocin-diabetic groups of rats

Groups	Glucose-6-phosphatase	Fructose 1,6-di phosphatase	Glucose-6-phosphate dehydrogenase
Normoglycaemic (normal) rats	1041.23±82.25	498.79±36.41	7.4±0.27
Diabetic control	1972.10±147.90*	780.14±55.38*	3.1±0.10*
Diabetic+ <i>Murraya koenigii</i>	1098.36±86.77 [#]	504.18±34.78 [#]	6.6±0.23 [#]
Diabetic+Glibenclamide	1152.21±89.87 [#]	525.36±37.30 [#]	5.9±0.21 [#]

Activities are expressed as: Glucose-6-phosphatase, fructose 1,6 di-phosphatase- μ moles of phosphate liberated/h/mg of protein; Glucose-6-phosphate dehydrogenase-μmoles of NADPH formed/min/g tissue. 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 normal rats; [#]Experimental groups of rats were compared with diabetic control

Table 5: Level of glycogen and activities of glycogen synthase and glycogen phosphorylase in livers of normal and streptozotocin-diabetic groups of rats

Groups	Glycogen (mg of glucose/g of liver tissue)	Glycogen synthase (μmol of UDP formed /h mg protein)	Glycogen phosphorylase (μmol of phosphate liberated/h/mg protein)
Normoglycaemic(normal)rats	58.10±3.60	832.71±57.48	639.64±42.85
Diabetic control	22.38±1.36*	547.41±37.22*	897.42±62.81*
Diabetic+ <i>Murraya koenigii</i>	55.98±3.97 [#]	808.12±57.37 [#]	695.24±47.27 [#]
Diabetic+Glibenclamide	51.82±3.52 [#]	782.21±53.97 [#]	734.19±50.65 [#]

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 normal rats; [#]Experimental groups of rats were compared with diabetic control

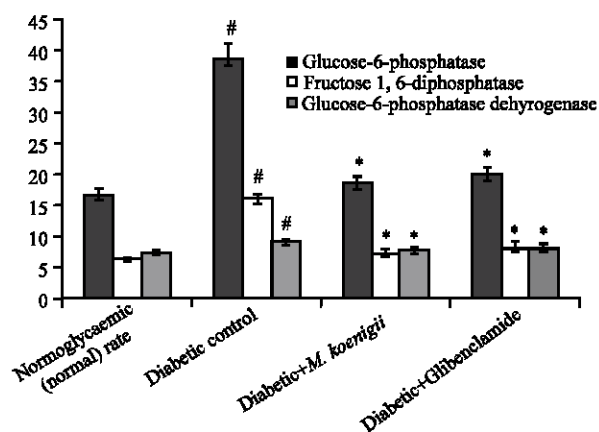


Fig. 1: Activities of glucose-6-phosphatase, fructose 1, 6-diphosphatase and glucose-6-phosphate dehydrogenase in kidneys of normal and streptozotocin-diabetic groups of rats. Activities are expressed as: Glucose-6-phosphatase, fructose 1, 6-diphosphatase- μ moles of phosphate liberated/h/mg of protein; Glucose-6-phosphate dehydrogenase- μ moles of NADPH formed/min/g tissue. Values are given as mean \pm 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 normal rats; #Experimental groups of rats were compared with diabetic control

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β -cell cytotoxicity and has been widely used to induce diabetes mellitus in experimental rats. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al.*, 2000).

Based on the histological observations made on the pancreatic tissue, the prevalence of diabetes was confirmed and the dose (200 mg kg^{-1}) was selected after preliminary behavioral and acute toxicity tests. The extract of the drug was found to be safe for biological studies as no lethality was observed at 1000 mg kg^{-1} b.w in rats. Assay of pathophysiological enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and histological observations made on vital organs revealed the non-toxic nature of the plant extract. Similar results were also reported for normal rats by Adebajo *et al.* (2006). The report that sub-chronic administration of 200 mg kg^{-1} of the extract for 14 days gave a better reduction in blood glucose and the enzymes studied (Adebajo *et al.*, 2006), agreed with our choice of same dose and duration of treatment used in this present study.

Glibenclamide is often used as a standard drug in STZ-induced moderate diabetic models to compare antidiabetic properties of a variety of compounds and its effectiveness in insulin stimulation and by extra pancreatic effects is also reported (Andrade-Cetto *et al.*, 2000). The elevated blood glucose concentration in the STZ induced diabetic rats clearly indicated persistent hyperglycemia in the rats used. However, treatment with *M. koenigii* leaf extract markedly lowered the blood glucose concentration in diabetic rats. The increase in plasma insulin stimulated by both the extract and glibenclamide suggested that the extract probably exerted its effect by stimulating insulin secretion from the remnant β -cells or from regenerated β -cells (Table 1). A number of other plants have also been reported to elicit their antihyperglycemic effects by a stimulatory effect on insulin release (Latha and Pari, 2003).

A significant increase in the level of glycosylated hemoglobin was reported in STZ-induced diabetes. The rate of glycosylation is proportional to the concentration of blood glucose. The peak of the glucose tolerance curve correlates with glycosylation and with the improvement of glycemic

control, glycosylated hemoglobin also decreases (Koenig *et al.*, 1976). The estimation of glycosylation of hemoglobin is a well accepted parameter useful in the management and prognosis of the diabetes (Alyassin and Ibrahim, 1981; Sheela and Augusti, 1992). The significant increase in the level of glycosylated haemoglobin observed in STZ-induced diabetic rats was lowered by the extract and glibenclamide (Table 1). This further demonstrated the antihyperglycaemic effect of the plant (Koenig *et al.*, 1976).

The administration of leaf the extract treatment to STZ-induced diabetic rats modulated key carbohydrate metabolizing enzymes in liver and kidney, resulting in normal blood glucose homeostasis. Hexokinase, that is responsible for the first phosphorylation step of glucose in glycolysis, was significantly reduced in the liver of diabetic rats resulting in the diminished utilization of glucose and the increased amount of blood glucose (Vestergaard, 1999). Enhanced hexokinase activity in *M. koenigii* treated rats suggested activation of glycolysis leading to greater uptake of glucose from blood by the liver cells and attendant increase in the utilization of glucose for energy production (Table 2).

Pyruvate kinase is an insulin dependent enzyme and its activity is regulated at the mRNA levels (Noguchi *et al.*, 1985). The observed diminished levels of the plasma insulin and pyruvate kinase in Table 1 and 2, respectively, confirmed the diabetic states of the rats used. The increased activities of pyruvate kinase obtained with the diabetic rats fed with the extract (Table 2) might have led to enhanced glucose metabolism (utilization) in the rats.

Pozzilli *et al.* (1997) have shown increased activity of LDH in diabetes mellitus. Normal LDH activity is indicative of improved channeling of glucose (pyruvate) for mitochondrial oxidation and an abnormally high LDH activity showed a blockage in the glucose metabolism (Table 2). Hence, the reversal effects of *M. koenigii*, similar to glibenclamide, on the values of hexokinase, pyruvate kinase and LDH confirmed the ability of the plant to induce better glucose utilization in the diabetic rats.

In STZ-diabetic rats, the renal hexokinase, pyruvate kinase and LDH activities were significantly increased (Table 3) and were consistent with the raised renal glucose-6-phosphatase, fructose-1, 6-diphosphatase and glucose-6-phosphate dehydrogenase levels (Fig. 1). These results were further confirmed by the reduction in the glycogen content and glycogen synthase activity in the liver (Table 4). The extract and glibenclamide however successfully reversed this trend. Diabetic nephropathy is a serious microvascular renal complication in Type II diabetes and involves a series of metabolic events in its pathogenesis (Bolli, 1999).

Glucose-6-phosphatase catalyses the final step of glucose production in liver and kidney. Glucose-6-phosphatase and fructose-1,6-diphosphatase are regulatory enzymes of gluconeogenic pathway. The activities of both glucose-6-phosphatase and fructose-1,6-diphosphatase were increased in the liver and kidney tissues during diabetic condition resulting in a decrease of glycolytic flux. Administration of the leaf extract and glibenclamide significantly reduced the increased activities glucose-6-phosphatase and fructose 1,6-diphosphatase in these two organs during diabetic conditions (Table 4) similar to insulin treatment or pancreas transplantation (Makoff *et al.*, 1983). Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme of the Hexose Mono Phosphate (HMP) shunt pathway producing NADPH. The reversal of the changes in this enzyme activity achieved by the extract and glibenclamide in the diabetic rats, revealed improvement in the formation of NADPH, favouring lipogenesis and the use of an alternative channel to dispose excess glucose via the HMP pathway (39). These findings are similar to that of Ramachandran *et al.* (2003).

Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen (Roesler and Khandelwal, 1986). Glycogen synthase and glycogen phosphorylase are the two key regulatory enzymes that catalyze glycogen synthesis (glycogenesis) and degradation (glycogenolysis), respectively. An increased glycogen phosphorylase, a decreased glycogen synthase with the resultant decrease in the glycogen content was observed in STZ-diabetic rats. However, in the rats treated with the extract and glibenclamide, these effects were effectively reversed (Table 5). Hence, the *M. koenigii* leaf extract maintained the glucose homeostasis in STZ-induced diabetic rats additionally by altering the activities of carbohydrate metabolizing enzymes.

The findings of our study suggests that the administration of *M. koenigii* leaf extract to diabetic rats gives good control over key glycolytic enzymes. The changes in the activities of the enzymes

effected by this extract suggest that a normal glucose metabolism, in peripheral tissues such as liver and kidney, is critical in achieving normoglycemia. Hence, the extract has anti-hyperglycaemic activity elicited by stimulation of insulin production from the pancreas and extra-pancreatic effects.

REFERENCES

- Adebajo, A.C., O.F. Ayoola, E.O. 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.
- Alyassin, D. and K. Ibrahim, 1981. A minor haemoglobin fraction and the level of fasting blood glucose. *J. Fac. Med. Baghdad*, 23: 373-380.
- Andrade-Cetto, A., H. Wiedenfeld, M.C. Revilla and I.A. Sergio, 2000. Hypoglycemic effect of *Equisetum myriochaetum* aerial parts on streptozotocin diabetic rats. *J. Ethnopharmacol.*, 72: 129-133.
- Arulselvan, P., G.P. Senthilkumar, D. Sathishkumar and S. Subramanian, 2006. Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats. *Pharmazie*, 61(In Press).
- Bolli, G.B., 1999. How to ameliorate the problem of hypoglycemia in intensive as well as nonintensive treatment of type 1 diabetes. *Diabetes. Care*, 22: B43-B52.
- Brandstrup, N., J.E. Kirk and C. Bruni, 1957. The hexokinase and phosphoglucoisomerase activities of aortic and pulmonary artery tissue in individuals of various ages. *J. Gerontol.*, 12: 166-171.
- Camblath, M., P.J. Randle, A. Parmeggiani and H.E. Morgan, 1963. Regulation of glycogenolysis in muscle. Effects of glucagon and anoxia on lactase production, glycogen content and phosphorylase activity in the perfused isolated rat heart. *J. Biol. Chem.*, 238: 1592-1597.
- Gancedo, J.M. and C. Gancedo, 1971. Fructose-1,6-diphosphatase, phosphofructokinase and glucose-6-phosphate dehydrogenase from fermenting and nonfermenting yeasts. *Arch. Mikrobiol.*, 76: 132-138.
- Gray, A.M. and P.R. Flatt, 1997. Nature's own pharmacy: The diabetes perspective. *Proc. Nutr. Soc.*, 56: 507-517.
- Khan, B.A., A. Abraham and S. Leelamma, 1996a. Biochemical response in rats to the addition of curry leaf (*Murraya koenigii*) and mustard seeds (*Brassica 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., 1959. A routine method for the estimation of lactic dehydrogenase activity. *J. Med. Lab. Technol.*, 16: 265-272.
- Koenig, R.J., C.M. Peterson and C. Kilo, 1976. Hemoglobin A₁ C as an indicator of the degree of glucose intolerance in diabetes. *Diabetes*, 25: 230-232.
- Koide, H. and T. Oda, 1959. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. *Clin. Chim. Acta*, 4: 554-561.
- Kong, Y.C., K.H. Ng, P.P. But, Q.Li, S.X. Yu, H.T. Zhang, K.F. Cheng, D.D. Soejarto, W.S. Kan and P.G. Waterman, 1986. Sources of the anti-implantation alkaloid yuehchukene in the genus *Murraya*. *J. Ethnopharmacol.*, 15: 195-200.
- Latha, M. and L. Pari, 2003. Antihyperglycemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clin. Exp. Pharmacol. Physiol.*, 30: 38-43.
- Leloir, L.F. and S.H. Goldenberg, 1979. Glycogen Synthase from Rat Liver. In: *Methods of Enzymology*. Colowick, S.P. and N.O. Kaplan (Eds.). Academic Press, New York, pp: 145-148.
- Makoff, R.A., J. Brown, Y. Mullen and W.R. Clark, 1983. Normalization of six key hepatic enzymes after fetal pancreas transplantation in diabetic rats. *Diabetes*, 32: 730-733.

- Mansour, H.A., A.S. Newairy, M.I. Yousef and S.A. Sheweita, 2002. Biochemical study on the effects of some Egyptian herbs in alloxan induced diabetic rats. *Toxicology*, 170: 221-228.
- Nathan, D., 1994. Relationship between metabolic control and long term complications of diabetes. In: Joslin's Diabetes. Kahn, C.R. and G. Weir (Eds.), Lea and Febiger, Philadelphia, PA, pp: 620-631.
- Nayak, S.S. and T.N. Pattabiraman, 1981. A new colorimetric method for the estimation of glycosylated hemoglobin. *Clin. Chim. Acta.*, 109: 267-274.
- Noguchi, T., H. Inoue and T. Tanaka, 1985. Transcriptional and post transcriptional regulation of L-type pyruvate kinase in diabetic rat liver by insulin and dietary fructose. *J. Biol. Chem.*, 260: 14393-14397.
- Nutan, M.T.H., A. Hasnat and M.A. Rashid, 1998. Anti bacterial and cytotoxic activities of *Murraya koenigii*. *Fitoterapia*, 69: 173-175.
- Papaccio, G., F.A. Pisanti, M.V. Latronico, E. Ammendola and M. Galdieri, 2000. Multiple low dose and single high-dose treatments with streptozotocin do not generate nitric oxide. *J. Cell. Biochem.*, 77: 82-91.
- Pogson, C.I. and R.M. Denton, 1967. Effect of alloxan diabetes, starvation and refeeding on glycolytic kinase activities in rat epididymal adipose tissue. *Nature*, 216: 156-157.
- Pozzilli, A., A. Signore and R.D.G. Leslie, 1997. Infections, Immunity and Diabetes. In: International Text Book of Diabetes Mellitus. 2nd (Ed.), pp: 1231-1241.
- 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., M. Kandaswamy, V. Narayanan and S. Subramanian, 2003. Insulin mimetic effects of macrocyclic binuclear oxovanadium complexes on streptozotocin-induced experimental diabetes in rats. *Diabetes. Obes. Metab.*, 5: 455-461.
- Ramsewak, R.S., M.G. Nair, G.M. Strasburg, D.L. De Witt and J.L. Nitiss, 1999. Biologically active carbazoles alkaloids from *Murraya koenigii*. *J. Agric. Food Chem.*, 47: 444-447.
- Roesler, W.J. and R.L. Khandelwal, 1986. Quantitation of glycogen synthase and phosphorylase protein in mouse liver: Correlation between enzymatic protein and enzymatic activity. *Arch. Biochem. Biophys.*, 244: 397-407.
- 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. *Rinsho. Kagaku*, 1: 346-353.
- Sheela, C.G. and K.T. Augusti, 1992. Antidiabetic effects of S-allyl cysteine sylohexide isolated from garlic *Allium sativum* Linn. *Indian. J. Exp. Biol.*, 30: 523-526.
- Srinivasan, K., 2005. Role of species beyond food flavouring: Nutraceuticals with multiple health effects. *Food. Rev. Int.*, 21: 167-188.
- Storey, J.M. and E. Bailey, 1978. Effect of streptozotocin diabetes and insulin administration on some liver enzyme activities in the past-weaning rat. *Enzyme*, 23: 382-387.
- 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.
- Urger, R.H. and D.W. Foster, 1998. Diabetes Mellitus. In: William's Text Book of Endocrinology. Wilson, J.D., D.W. Foster, H.M. Kronenserg and P.R. Larson (Eds.), W.b. Saunders. Philadelphia, pp: 973-1060.
- Vallance-Owen, J., 1975. Diabetes its Physiological and Biochemical Basis. Lancaster: Mtp Press.
- Vestergaard, H., 1999. Studies of gene expression and activity of hexokinase, phosphofructokinase and glycogen synthase in human skeletal muscle in states of altered insulin-stimulated glucose metabolism. *Dan. Med. Bull.*, 46: 13-34.
- Vetrichelvan, T., M. Jegadeesan and B.A. Devi, 2002. Anti-diabetic activity of alcoholic extract of *Celosia argentea* Linn. seeds in rats. *Biol. Pharm. Bull.*, 25: 526-528.
- Weber, G. R.L. Singhal, N.B. Stamm, M.A. Lea and E.A. Fisher, 1966. Synchronous behavior pattern of key glycolytic enzymes: glucokinase, phosphofructokinase and pyruvate kinase. *Adv. Enzyme. Regul.*, 4: 59-81.
- World Health Organization, 1980. Second Report of the WHO Expert Committee on Diabetes Mellitus. Geneva, Technical Report Series, pp: 646: 66.