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ANTIDIABETIC EFFICACY OF MURRAYA KOEINGII IN STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN ALBINO WISTAR RATS

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ABSTRACT

The objective of the study was to evaluate the effect of Murraya Koeingii on plasma glucose, insulin and C-peptide, blood hemoglobin and glycosylated hemoglobin, glycogen and carbohydrate metabolic enzymes in experimental diabetes in albino Wistar rats. Diabetes was induced by intraperitoneal injection of streptozotocin (45mg/kg) to albino Wistar rats. Murraya Koeingii(500 mg/kg and 1000 mg/kg) dissolved in 0.2% dimethyl sulfoxide was administrated orally to diabetic rats using an intragastric tube daily for a period of 35 days. A significant increase in plasma glucose, blood glycosylated hemoglobin and hexokinase activity and a decrease in plasma insulin and C-peptide, blood hemoglobin, glycogen (liver and muscle) and the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney were observed in STZ-induced diabetic rats. Oral administration of Murraya Koeingii restored all these biochemical parameters to near normal. Our present study clearly revealed that Murraya Koeingii possesses potent antihyperglycemic effect in STZ-induced diabetic rats.

ORIGINAL RESEARCH ARTICLE

Introduction

Diabetes mellitus (DM) is characterized by hyperglycemia due to a disturbance in the group of metabolism of carbohydrates, fat, and protein, resulting from defects in insulin secretion, action or both [1,2]. Currently, there are over 150 million diabetics worldwide and this number is likely to increase due to increase in sedentary lifestyle, consumption of energy-rich diet and obesity [3].In modern medicine, there is still no satisfactory effective therapy available to cure diabetes [4].

Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for use in developing and under-developed countries. Many indigenous medicinal plants have been found to be useful for the successful management of diabetes [5].

Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for the induction of diabetes mellitus in animals [6]. Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. Previously reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used [7]. STZ-induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the

resulting hyperglycemia. STZ is a pancreatic β-cell toxin that induces rapid and irreversible necrosis of β-cells 8.

Recently there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Plant sources are usually considered to be non-toxic, with fewer side effects than synthetic sources. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms.

Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species1. Murraya Koenigii is a highly values plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are Pgurjunene, P-caryophyllene, P-element and O-phellandrene. The plant is a rich source of carbazole alkaloids. Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed by Ito3 . M. koenigii is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Bark and roots are used as a stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for the cure of dysentery, diarrhea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders4,5. Several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases. M. koenigii contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, antiulcer, positive inotropic and cholesterol reducing activities. Therefore the present review summarizes the available literature till date on isolation of phytoconstituents, biological activities of the isolated compounds and pharmacological actions of extracts along with the clinical studies.

The present study was thus designed to evaluate the influence of Murraya Koeingiion biochemical parameters and the activities of carbohydrate metabolic key enzymes in normal and STZ- induced diabetic rats.

Materials and Methods

Experimental animals

Female albino Wistar rats (150-200 g) obtained from Pharmacy dept. Bhagwant University Ajmer was used in this study. They were housed in polypropylene cages (47cm x 34cm x 20cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22º C and had free access to water and food.

The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Rajasthan, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen-free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drug and chemicals

Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai. Murraya Koeingii was collected local area of Ajmer Rajasthan. Glucose, hemoglobin

and glycosylated hemoglobin kits were purchased from Agappe diagnostics, Kerala, India. Insulin and C-peptide kits were obtained from Agappe diagnostics, Kerala. All other chemicals used in the study were of analytical grade.

Collection and preparation of the extract

The leaves of Murraya koenigii were collected from Local Area of Ajmer Rajasthan & identified by the specimen stored in NBRI Lucknow, were dried under shade and pulverized. Thimbles were made out of the powdered leaves and were extracted using methanol in Soxhlet extraction apparatus, dried in a rotary vacuum evaporator. The aqueous extract was taken as a decoction. The hydroalcoholic extract was taken as a 1:1 combination of methanol and water as described earlier in Soxhlet extraction apparatus. All the extracts were stored under refrigeration after drying.

Induction of experimental diabetes

Streptozotocin was used to induce diabetes mellitus in normoglycemic female albino Wistar rats. A freshly prepared solution of STZ (45mg/kg body weight) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1ml/kg body weight to overnight fasted rats. After 48 hours of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment [16].

Experimental design

A total of 36 rats were used in the present investigation. The animals were randomly divided into 6 groups of 6 rats in each group.

Group	1:	Normal control rats
Group	2:	Normal rats + Murraya Koeingii(500 mg/kg)
Group	3:	Normal rats + Murraya Koeingii(1000 mg/kg)
Group	4:	Diabetic control rats
Group	5:	Diabetic + Murraya Koeingii(500 mg/kg)
Group	6:	Diabetic + Murraya Koeingii(1000 mg/kg)

Murraya Koeingiiwas dissolved in 0.2% dimethyl sulfoxide and administrated to rats orally using an intragastric tube daily for a period of 35 days.

Sample collection

At the end of the treatment period, all rats were fasted for 12 hours and sacrificed by cervical decapitation. The blood was collected into heparinized tubes and plasma was separated by centrifugation and used for biochemical analysis. Liver and kidney were dissected out, washed in ice-cold physiological saline, patted dry and weighed. The tissues were then homogenized in the 0.1M Tris-HCl buffer, pH 7.4. The homogenate was used for the estimation of carbohydrate metabolic enzymes.

Statistical analysis

Results were expressed as mean ± SD for six rats in each experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one-way analysis of variance (ANOVA) and group means were compared with Duncan's Multiple Range Test (DMRT). P values < 0.05 were considered as significant.

Results

Effect of Murraya Koeingii on plasma glucose, insulin, and C-Peptide

Table 1 shows the effect of Murraya Koeingiion the levels of plasma glucose, insulin, and C-peptide in normal and STZ-induced diabetic rats. Rats induced with STZ, showed a significant ($p<0.05$) increase in the level of plasma glucose and decrease in the

levels of plasma insulin and C-peptide as compared to normal rats. Oral administration of Murraya Koeingiifor a period of 35 days significantly ($p<0.05$) decreased the level of plasma glucose and increased the levels of plasma insulin and C-peptide in STZ-induced diabetic rats.

Effect of Murraya Koeingiion hemoglobin and glycosylated hemoglobin

The levels of hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats are presented in Table 2. The diabetic rats showed a significant ($p<0.05$) decrease in the level of hemoglobin and a significant ($p<0.05$) increase in the level of glycosylated hemoglobin when compared to normal rats. Oral administration of Murraya Koeingiiin STZ-induced diabetic rats reversed the changes in the levels of hemoglobin and glycosylated hemoglobin to near normal.

Effect of Murraya Koeingii on liver and muscle glycogen

The effect of Murraya Koeingii on liver and muscle glycogen content of normal and STZ-induced diabetic rats are depicted in Table 3. A significant ($p<0.05$) reduction in liver and muscle glycogen was observed in STZ-induced diabetic rats as compared to normal rats. Treatment with Murraya Koeingiisignificantly ($p<0.05$) increased the concentration of liver and muscle glycogen when compared with untreated diabetic rats.

Effect of Murraya Koeingii on carbohydrate metabolic enzymes

Table 4 and 5 illustrate the effect of Murraya Koeingiion carbohydrate metabolic enzymes in liver and kidney of normal and STZ-induced diabetic rats. The activity of hexokinase was significantly ($p<0.05$) decreased in liver, whereas the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase were significantly ($p<0.05$) increased in the liver and kidney of diabetic rats when compared with normal rats.

Oral administration of Murraya Koeingii significantly ($p<0.05$) increased the activity of hexokinase in the liver and decreased the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney of STZ-induced diabetic rats when compared with diabetic controls.

Table 1: Effect of Murraya Koeingii on the levels of hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats

Groups	Hemoglobin (g/dl)	Glycosylated hemoglobin (mg/g Hb)
Normal control	16.62 ± 0.5^{a}	7.74 ± 0.30^{a}
Normal + Murraya Koeingii(500mg/kg)	16.72 ± 0.9^{a}	7.73 ± 0.25^{a}
Normal + Murraya Koeingii(1000mg/kg)	16.66 ± 0.5^{a}	7.78 ± 0.2^{a}
Diabetic control	8.42 ± 0.03^{b}	12.66 ± 0.35^{b}
Diabetic + Murraya Koeingii(500mg/kg)	12.51 ± 0.64^{c}	10.53 ± 0.29^{c}
Diabetic + Murraya Koeingii(1000mg/kg)	14.61 ± 0.12^{d}	8.80 ± 0.5^{d}

Each value is mean±S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other ($P<0.05$, DMRT).

Table 2: Effect of Murraya Koeingiion liver and muscle glycogen content in normal and STZ-induced diabetic rats

Groups	Liver glycogen (mg/g tissue)	Muscle glycogen (mg/g tissue)
Normal control	53.43 ± 1.32[a]	8.613 ± 0.99[a]
Normal + Murraya Koeingii (500 mg/kg)	53.75 ± 4.68[a]	8.566 ± 0.97[a]
Normal + Murraya Koeingii (1000mg/kg)	53.28 ± 4.65[a]	8.650 ± 1.09[a]
Diabetic control	30.25 ± 3.12[b]	3.848 ± 0.51[b]
Diabetic + Murraya Koeingii (500 mg/kg)	43.50± 2.10[c]	6.208 ± 0.58[c]
Diabetic + Murraya Koeingii (1000mg/kg)	48.65 ± 2.28[d]	7.580 ± 0.24[d]

Each value is mean±S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 3: Effect of Murraya Koeingiion plasma glucose, insulin and C- Peptide in normal and STZ-induced diabetic rats

Groups	Glucose (mg/dl)	Insulin(µU/ml)	C-Peptide(ng/ml)
Normal control	83.35 ± 4.4[a]	16.61 ± 0.60[a]	5.89 ± 1.61[a]
Normal + Murraya Koeingii(500mg/kg)	83.45 ± 5.1[a]	16.21 ± 1.20[a]	6.23 ± 1.54[a]
Normal + Murraya Koeingii(1000mg/kg)	82.24 ± 4.73[a]	16.04 ± 0.66[a]	5.04 ± 1.20[a]
Diabetic control	278.33 ± 6.40[b]	8.20 ± 0.50[b]	0.56 ± 0.08[b]
Diabetic + Murraya Koeingii(500mg/kg)	153.00 ± 5.70[c]	13.11 ± 1.40[c]	1.64 ± 0.58[c]
Diabetic + Murraya Koeingii(1000mg/kg)	111.66 ± 4.7[d]	14.37 ± 1.30[d]	2.73 ± 0.62[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 4: Effect of Murraya Koeingiion the activity of hexokinase in liver of normal and STZ-induced

STZ-induced diabetic rats Groups	Hexokinase(Unit[A]/h/mg protein)
Normal control	0.836 ± 0.07[a]
Normal + Murraya Koeingii(500mg/kg)	0.846 ± 0.16[a]
Normal + Murraya Koeingii(1000mg/kg)	0.848 ± 0.19[a]
Diabetic control	0.238 ± 0.01[b]
Diabetic + Murraya Koeingii(500mg/kg)	0.659 ± 0.05[c]
Diabetic + Murraya Koeingii(1000mg/kg)	0.759 ± 0.06[d] A - µmoles of glucose phosphorylated

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table: 5 Effect of Murraya Koeingii on the activities of gluconeogenic enzymes in liver and kidney of normal and STZ-induced diabetic rats

Groups	Glu-cose-6-phosphatase (Unit[B]/min/mg protein)		Fructose-1,6-bisphosphatase (Unit[C]/h/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal control	0.162 ± 0.01[a]	0.163 ± 0.03[a]	0.425 ± 0.06[a]	0.574 ± 0.16[a]
Normal + Murraya Koeingii (500mg/kg)	0.154 ± 0.02[a]	0.155 ± 0.02[a]	0.415 ± 0.05[a]	0.449 ± 0.06[a]
Normal + Murraya Koeingii (1000mg/kg)	0.166 ± 0.01[a]	0.143 ± 0.01[a]	0.126 ± 0.06[a]	0.401 ± 0.06[a]
Diabetic control	0.363 ± 0.03[b]	0.363 ± 0.02[b]	0.909 ± 0.09[b]	2.609 ± 0.56[b]
Diabetic + Murraya Koeingii (500mg/kg)	0.276 ± 0.02[c]	0.260 ± 0.01[c]	0.790 ± 0.05[c]	1.861 ± 0.05[c]
Diabetic + Murraya Koeingii (1000mg/kg)	0.223 ± 0.04[d]	0.200 ± 0.02[d]	0.569 ± 0.04[d]	1.390 ± 0.03[d]

Murraya Koeingii
B- µmoles of inorganic phosphorous liberated C- µmoles of inorganic phosphorous liberated
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Discussion

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to an absolute or relative deficiency of circulating insulin levels. In the present study, diabetic rats exhibited a significant increase in plasma glucose level. This result is inconsistent with other studies in rats [21].

The increased glucose level might be due to the fact that STZ causes a notable

reduction in insulin release by the destruction of pancreatic β-cells. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in STZ-induced diabetic rats [22]. We have observed a significant decrease in glucose level in Murraya Koeingii treated diabetic rats when compared with non-treated diabetic rats. The possible mechanism of hypoglycemic action may be through potentiation of pancreatic secretion of insulin from beta cells of islets or due to enhanced transport of blood glucose to the peripheral tissue [23].

Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both insulin and C-peptide levels has been reported to be a valuable index of insulin secretion rather than insulin alone [23]. C-peptide and insulin levels were significantly decreased in STZ-induced diabetic rats due to the destruction of β-cells of pancreas thereby inhibiting insulin release.

Oral administration of Murraya Koeingii significantly increased the levels of plasma insulin and C-Peptide in STZ-induced diabetic rats when compared with diabetic control rats. Flavonoids stimulate the secretion of insulin from β-cells of the pancreas. In hyperglycemic animals, it is possible that Murraya Koeingii may act by potentiation of pancreatic secretion or increasing glucose uptake.

The decreased level of total hemoglobin observed in diabetic rats might be due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting blood glucose level [24]. Measurement of glycosylated hemoglobin remains the standard biochemical marker for the assessment of glycemic control in patients with diabetes [25].

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin [26]. Oral administration of Murraya Koeingiito STZ-induced diabetic rats reduced the formation of glycosylated hemoglobin by virtue of its normoglycemic activity. Since the level of glycosylated hemoglobin has been shown to provide an index of blood glucose concentration [27], the decreased level of glycosylated hemoglobin and the increased level of hemoglobin in treated diabetic rats showed the antihyperglycemic activity of Murraya Koeingii.

Liver and muscle glycogen content were significantly reduced in STZ- induced diabetic rats. Glycogen is the primary intracellular storage form of glucose and its levels in various tissues are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ causes selective destruction of β- cells of the pancreas resulting in a marked decrease in insulin levels, it is rational that glycogen levels in tissues decrease as they depend on insulin for the influx of glucose [28]. In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms of type 2 diabetes that result in hyperglycemia [29].

Glycogen synthesis in the rat liver and skeletal muscle is impaired in diabetes [30]. Also, hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in the diabetic state [28, 31]. Oral administration of Murraya Koeingii to STZ-induced diabetic rats significantly increased the liver and muscle glycogen content by stimulating the remnant β-cells to release insulin.

In experimental diabetes, enzymes of glucose metabolism are markedly altered. In the current study, diabetic rats showed a significant decrease in the activity of hepatic glucokinase and increase in the activities of glucose-6-phosphatase and fructose-1, 6- bisphosphatase in the liver and kidney. Insulin influences the

intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes.

One such enzyme is hexokinase that catalyzes the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis [32]. In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose [33]. Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin [34]. This impairment results in a marked reduction in the rate of glucose oxidation *via* glycolysis, which ultimately leads to hyperglycemia. Oral administration of Murraya Koeingiito STZ-induced diabetic rats resulted in a significant reversal in the activity of hexokinase, thereby increased the oxidation of glucose.

Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyzes the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis [35]. Fructose-1, 6-bisphosphatase is one of the key enzymes of gluconeogenic pathway. Hepatic glucose production is raised in diabetic state and is associated with the impaired suppression of the gluconeogenic enzyme fructose 1, 6-bisphosphatase. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes [36]. Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6- bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxykinase [37].

Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate-metabolizing enzymes and leads to diabetes [38]. Diabetic rats treated with Murraya Koeingiishowed significant decrease in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in the liver and kidney. Murraya Koeingiimay primarily is modulating and regulating the gluconeogenic enzymes through regulation of cAMP or inhibition of gluconeogenesis.

In conclusion, the results of the present study indicated that Murraya Koeingiihas a beneficial effect on normalizing glucose level and carbohydrate metabolic enzymes in STZ-induced diabetic rats. This suggests the efficacy of Murraya Koeingiiin the maintenance of glucose homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

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