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The efficacy of aqueous methanolic extract of *Murraya koenigi* (L.) Spreng in alloxan induced diabetic albino rats

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ABSTRACT

Murraya koenigi (L.) Spreng (Rutaceae), is an indigenous medicinally important herb of Indian origin, has been used for centuries in the Ayurvedic System of Medicine. The present investigation was carried out to evaluate the antidiabetic effect of solvent extract of leaf of M.koenigi (L.) in alloxan induced diabetic albino rats. The leaves, bark and the roots of the plant are used in indigenous medicine as tonic, stomachic, stimulant and carminative. An infusion of the roasted leaves is used to prevent vomiting. The present study throws light on the further understanding and bioactivity of M.koenigii (L.) Spreng. In comparison with the efficacy of other crude extracts, aqueous methanolic extract display promising activity and significantly decreased the elevated blood glucose level, cholesterol, LDL, triglycerides, phospholipids, VLDL in comparison with standard control (glibenclamide - a standard drug used to treat Diabetes mellitus).

Key words: Antidiabetic activity, Alloxan, blood glucose, Lipid profile, *Murraya koenigi* (L) Spreng.

INTRODUCTION

Diabetes mellitus (type I, II) is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanidines and sulfonyl ureas are presently available to reduce hyperglycemia and effective for diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems [11].

Recently the medicinal values of important plant extracts have been screened by many scientists in the field of diabetic research [5]. The pharmacognosy and mechanism of action of these herbals used has not been defined. Many traditional plants used for treatments for diabetes are also used. But most of the evidence for their beneficial effects is anecdotal [2].

Murraya koenigi (L.) Spreng is widely distributed in India and commonly known as ‘curry patta’, belongs to the family Rutaceae. The plant leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. This plant is known to be the richest source of carbazole alkaloids. It has been reported by authors that carbazole alkaloids present in *M.koenigi* (L.) Spreng and display various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities [20]. It was further proved by authors, [4] isolated

mukonine, a carbazole alkaloids from the stem bark of *M. koenigi* (L.) Spreng. The green tender leaves are eaten raw for the cure of dysentery. The juice of the root is taken to relieve pain associated with kidney ailments [19].

The present studies throw light on the efficacy of crude extracts of *Murraya koenigii* (L.) Spreng in alloxan induced diabetic rats. Leaves of *M. koenigii* (L.) Spreng were used as source for bioactive molecules in the present study to clarify their effect in the treatment of alloxan induced diabetes, on blood glucose and possible effects on pancreatic in rat model. A comparison was made with the Glibenclamide (GBC), a standard drug used in treating diabetes mellitus.

MATERIALS AND METHODS

Animals

Male albino rats of wistar strain weighing about 150 – 200 g obtained from the Periyar Maniammai pharmaceutical college of Trichy were used for the study. They were fed a standard rat pellet diet (Sai Durga feeds, Bangalore) and water was provided *adlibitum* and maintained under standard laboratory conditions (Temperature 24-28°C, relative humidity 60 - 70%). Animals described as fasted were deprived of food for 16 hours but had free access to water.

Induction of Diabetics

Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate (S.D Fine Chem. Ltd., Mumbai, India), in sterile saline [15]. After 72 hours of alloxan injection, the diabetic rats (glucose level > 250 mg/dl) were separated and used for the study [13].

Plant material and Preparation of Plant Extract

The plant materials of *M. koenigi* (L.) Spreng were collected from Thanjavur, Tamil nadu, India and identified with the help of flora of Madras Presidency (Gamble, 1928). The authentication of the plant was confirmed by Dr. Narasimhan, Taxonomist, Department of Botany, Madras Christian College, Chennai. The voucher specimen was deposited at the Entomology Research Institute (ERI) herbarium collections, (LC/ERI/Herb.208) Loyola College.

The fresh leaf and stem of plant was rinsed with water to remove sand and dried in an incubator at room temperature for one month. It was pulverized to reduce the surface area using pulverizer machine, the leaf powder (10 kg) and stem powder (12 kg) was kept in air-tight cellophane bags until it's used.

(a) Cold percolation method:

The leaf coarse powder is further taken for sequential solvent extraction in different solvents (Hexane, petroleum ether, ethyl acetate, chloroform, aqueous methanol, acetone and water). 2kg of leaf powder is taken into 10 litre three necked round bottom flak and about 7L of solvent was added to it, the mixture was agitated by mechanical overhead agitator at room temperature, 50 RPM for 48 hours. The extract was separated using fine muslin cloth and then filtered under vacuum, filtrate was taken in 10 litre single neck round bottom flask and solvent is removed in room temperature by rotary evaporator, a Greenish brown mass was obtained and it was finally dried at low temperature under reduced pressure in a high vacuum pump. A crude residue (75g) was obtained giving a yield of 3.7%. It was further dried under high vacuum condition to remove trace amount of solvent. The crude extract was kept in cold room (5^{o}C) under nitrogen atmosphere.

(b) Hot extraction method:

The leaf coarse powder (2kg) is taken into 10 litre three necked round bottom flak and about 7L of solvent was added to it, the mixture was agitated by mechanical overhead agitator at 45^{o}C by water bath for 32hours. The extract was separated using fine muslin cloth and then filtered under vacuum, filtrate was taken in 10 litre single neck round bottom flask and solvent was removed by rotary evaporator and further dried under high vacuum condition to remove trace amount of solvent. The crude extract was kept in cold room (5^{o}C) under nitrogen atmosphere.

In comparison with two methods, better yields were obtained with cold percolation method over hot extraction method.

Experimental grouping of animals

Rats were divided into the following groups.

Group I Consisted of 6 rats which served as normal control and were given only distilled water daily.

Group II Consisted of 6 Alloxan induced diabetic rats and served as diabetic control and were given distilled water only.
Group III Consisted of 6 Alloxan induced diabetic rats and was treated orally with aqueous methanolic extract of *M. koenigi* (L.) leaves at the dose of 100 mg/kg body weight daily for 30days, once a day.
Group IV Consisted of 6 Alloxan induced diabetic rats and were treated orally with aqueous methanol extract of *M. koenigi* (L.) leaves at the dose of 200 mg/kg body weight daily for 30days, once a day.
Group V Consisted of 6 Alloxan induced diabetic rats and was given glibenclamide at the dose of 5mg/kg body weight daily for 30days, once a day.

Collection of blood samples
Blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2000rpm for 20 minutes. The serum collected was used for biochemical estimations.

Biochemical parameters
Blood glucose was estimated by GOD-POD method (Trinder, 1969) using a commercial kit (Span Diagnostics,India). TC, TG and HDL were analyzed by kits (Roche Diagnostics, GmbH, D-68298 Mannheim, Germany) on Hitachi auto analyzer. LDL and VLDL (Carroll *et al*., 1956) were evaluated.

Histological Analysis
Pancreases were dissected from all the groups of rats were fixed in paraffin and histological preparations were made 5µ thick sections were cut and stained with haemato xylene and eosin. Finally the photomicrographs of histological studies were taken.

Statistical analysis
The data were analyzed using Student's t – test statistical methods and the results were considered, statistically significant at $P<0.001$.All the values expressed as Mean ± SEM.

RESULTS

Blood Glucose
Procedure and Methodology of the experiment was exactly followed by reported procedure [21].The efficacy of aqueous methanolic extract of *M. koenigii* (L.) Spreng towards diabetic activity were evaluated by oral administration of the extract to the alloxan induced diabetic rats (100 and 200 mg/kg body weight) for 30 days showed significant ($P< 0.001$) reduction in glucose. The effect of Methanolic extract on serum glucose level in alloxan induced diabetic rats presented in Table -1. In comparison with other extracts, aqueous methanolic extract shows promising activity.

Lipid Profile
The efficacy of *M.* koenigi (L.) Spreng extract on lipid profile in alloxan induced diabetic rats showed in table-2. Serum TG, serum LDL, serum VLDL levels were decreased significantly by Glibenclamide ($P< 0.001$) and *M. koenigi* leaves extract ($P< .001$) compared with diabetic control.

Histological analysis
Histologically the islets of langerhans in pancreas of control showed that normal acini and normal cellular population (fig 1a). Extensive damage to the islets of langerhans in alloxan induced diabetic rats (fig 1b) and Glibancalamide revealed that the restoration of normal cellular population in islets of langerhans with hyperplasia (fig1e) leaves extract of *M. koenigi* (L.) (100 &200 mg/kg) showed that the partial restoration of normal Cellular population and enlarged size of beta cells (fig1c and 1d).

DISCUSSION

The treatment in the Indian ancient pharmacopoeia mentioned specific treatments for the two types including dietary modifications, medicinal plant remedies and minerals [12]. Moreover, the researches conducted over last several decades has shown plant [2] and plant based therapies have a potential to control and treat diabetes [8] and its complications. Role of Indian medicinal plants as antidiabetic has also been reviewed by [7]. For testing, Alloxan, causes a massive distraction of β-cells of the islets of langerhans resulting in reduced synthesis and release of insulin

[9] and is widely used. It is well established that sulphonyl ureas produce hypoglycemia by increasing the secretion of insulin from pancreas and those compounds are active in mild alloxan induced diabetes whereas they are inactivate in intense alloxan diabetics [18].

Diabetes is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of disease increase so does the need for the more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of the diabetes [1] but only a few have been scientifically evaluated. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an over exertion on the endocrine system [10], which leads to the deterioration of endocrine control. Continuing to fight hyperglycemia, the efficacy of these therapeutic agents is compromised in several ways. Individual agents act only on part of the pathogenic process and only to a partial extent. This may be the reason that even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing.

The most significant findings of the present study is that the Methanol leaves extract of *M. koenigi* (L.) dose of 200 mg/kg body weight for 30 days have shown beneficial effect not only on blood glucose but also on TGL, LDL, HDL,VLDL, cholesterol level and tissue of pancreas in alloxan induced diabetic rats. Results obtained from the present study are very much promising and comparable with glibenclamide, a standard drug used to treat diabetes mellitus. *M. koenigi* (L.) leaves extract have the potential to renewal of β cells in diabetics and it has been studied in several animal models. The total β cells mass reflects the balance between the renewal and loss of cells. It also suggested that regeneration of islet β cells following destruction by alloxan may be the primary cause of the recovery of alloxan induced guinea pigs from the effects of the drugs. In alloxan induced diabetics, epicatchin [4] and *Vinca rosea* extract [6] have also shown to act by β cells regeneration. Similar effect in streptozotocin treated diabetic animals were reported by pancreas tonic [14], ephedrine [17] and *Gymnema sylvestre* leaf extract [16].

In our study, damaged in pancreas in alloxan treated diabetic control (Fig 1b), and regeneration of β cells by glibenclamide (Fig 1e) were observed. A comparable regeneration was also shown by Methanolic extract of *M. koenigi* (L.) (100 &200 mg/kg) (Fig1c and 1d) photo micrographical dated in our studies confine healing of pancreas by *M. koenigi* (L.) leaves extracts as a plausible mechanism of their anti diabetic activity

Table-1: The effect of *M. koenigi* (L.) leaves extract on serum glucose level in control and experimental rats

S. No	Groups	Treatment(Days)(Mean± SE, n=6)		
		1	15	30
1	Control(Normal saline)	91.6±7.4	91.8±8.5	92.0±7.9
2	Diabetic control(Alloxan)	218.2±15.4	220.6±16.9	215.4±17.2
3	*M. koenigi* (L.) leaves extract (100 mg/kg)	216.5±17.3	185.4±15.6*	115.7±10.2*
4	*M. koenigi* (L.) leaves extract (200 mg/kg)	209.7±17.8	152.8±12.6*	102.6±8.7*
5	Glibenclamide (Standard drug) (5mg/kg)	207.6±14.8	109.4±7.9*	96.8±6.5*

*n=6 data expressed as mean ±SE; *P<0.001 vs contro l by students't' test*

Table-2: The effect of *M. koenigi* (L.) leaves extract on lipid profile in Control and experimental rats

Treatment	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl	Total C (Cholestrol)
Control(Normal saline)	76.7±5.7	48.2±2.5	15.34±1.14	23.0±2.88	86.54±6.52
Diabetic control(Alloxan)	121.15±10.3	82.47±2.7	24.23±2.06	189.9±12.64	246.6±17.4
M. koenigi (L.) leaves extract (100 mg/kg)	107.3±8.5	37.23±2.7	21.46±1.7	116.71**±10.2	175.4±14.6
M. koenigi (L.) leaves extract (200 mg/kg)	99.45±7.6	40.58±3.4	19.89±1.52	87.93±5.68	148.4±10.6
Glibenclamide (Standard drug) (5mg/kg)	113.58±6.7	39.82*±2.79	22.71±1.34	95.77**±2.66	158.3**±6.79

Values are expressed as mean ±S.E, n=6
**P<0.01 Vs control; **P<0.001 Vs control by students't' test*

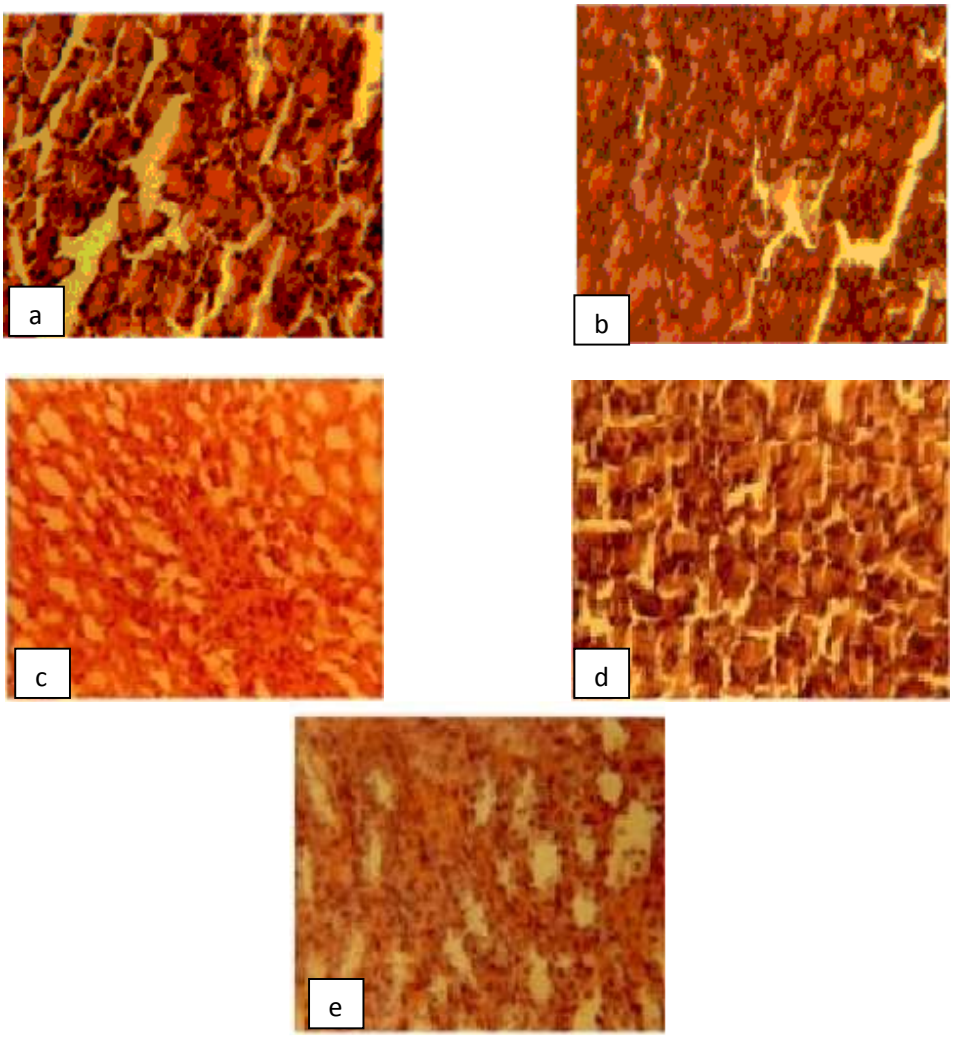

Fig1: Photomicrograph of islet of rats stained with Haemato xylene and Eosin X40.

a-Normal saline Injected Group, b-Alloxan induced Diabetic rats, c-Diabetic rats treated with M. koenigi (L.) leaves extract (100mg/kg), d-Diabetic rats treated with M. koenigi (L.) leaves extract (200mg/kg), e-Diabetic rats treated with Glibenclamide (5mg/kg).

CONCLUSION

Many plants were mentioned in Ayurvedic and Homeopathy literature for hypoglycemic treatment. The results of this study substantiate the traditional use of this drug in the treatment of diabetes. In near future the aqueous methanolic extract of *Murraya koenigii* (L.) Spreng will be a potential source of drug for management of diabetes mellitus. Further detailed studies may be carried out to identify the active principles responsible for the hypoglycemic effect and to understand the exact mechanism of action.

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