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GLYCEMIC EFFECT OF FREEZE DRIED *MURRAYA KOENIGII* - AN EVIDENCE BASED STUDY

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ABSTRACT

The scientific evaluation of hypoglycemic and antidiabetic activity of freeze dried leaves of *Murraya koenigii* was carried out in normal and Streptozotocin-induced sub- and mild- diabetic rats. A single oral administration of variable doses of 100, 200, 300 and 400 mg kg^{-1} lowered Blood Glucose Level in normal and diabetic rats. A fall of 41.5% in normal, 34.3% in sub-diabetic and 37.9% in mild-diabetic rats was observed with the dose of 300 mg kg^{-1} after 6 h of oral administration. Same dose also improved glucose tolerance as indicative from the fall of 38.7% in normal, 54.3% in sub-diabetic and 51.7% in mild-diabetic rats in Glucose Tolerance Test after 3 h. The findings of glycemic and phytochemical studies suggest that the identified phytochemicals might be responsible for its glycemic effect and hence, the freeze dried powder may be prescribed as adjunct to dietary therapy and drug treatment for managing diabetes mellitus.

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KEYWORDS

Diabetes mellitus, *Murraya koenigii*, Freeze dried leaf powder and Streptozotocin.

INTRODUCTION

Diabetes currently is a major health problem for the people of the world. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post-prandial blood sugar levels. Approximately 215 million people worldwide suffer from Diabetes Mellitus and 90-95% of them from the Type 2 diabetes. In view of rapid increase in diabetic cases, WHO (World Health Organization) and American Diabetes Association have reduced the figure of Blood Glucose Level (BGL) from 140 mg dl^{-1} for the risk of Diabetes [1,2].

The synthetic oral hypoglycemic agents can produce serious side effects [3,4]. Natural remedies from medicinal plants are considered to be the cost effective and safe alternative treatments for various diseases. Medicinal herbs have consistently been considered a leading source of pharmaceuticals, employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality [5]. Recently many plants have been explored and claimed to be useful for the treatment of Diabetes Mellitus by our earlier and present Research Groups [6-13].

People are looking beyond conventional medical treatments towards Complementary or Alternative Medicine (CAM) for new ways to treat

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this disease, stay healthy, feel better and live longer without any side effects or toxicity. In the past, there have been many ethno-botanical medicinal plants, which have been used in traditional medicines for their antidiabetic properties without any scientific support and pharmacological evidences [14]. Natural medicines can be integrated into conventional treatment by exploring and evaluating chemopreventive potential of identified medicinal plants with demonstrated biological activity.

Murraya koenigii (L.) Spreng (family: Rutaceae) is commonly known as 'Curry Leaf Tree' in English and 'Curry Patta' in Hindi and is widely and regularly used as a spice and condiment in India and other tropical countries. The aqueous extract of *Murraya koenigii* leaves had already been evaluated by our research group [15] for its hypoglycemic activity in normal and alloxan-induced diabetic rabbits. This plant is promising as it is widely and regularly used as a spice for food flavouring and as such, it appears to be without any side effects and toxicity. *Murraya koenigii* leaves mixed with fat separated butter has been reported for the treatment of amoebiasis, diabetes and hepatitis [16-18]. Its hypoglycemic effect has also been evaluated in diabetic dogs [19].

The present study therefore, carries immense global importance in order to develop alternative drug therapy with minimal side effects and toxicity and maximum efficacy. Thus our aim of developing modern-new-better-oral drugs for Diabetes management from plant sources is of high relevance as it may one day make insulin injection an unpleasant memory. Hence, the practical and scientific utility of the present study cannot be denied.

MATERIALS AND METHODS

(i) Plant collection and identification

Fresh leaves of *Murraya koenigii* (5 Kg) were collected locally from Allahabad and authenticated by Dr. S. L. Bondya, Taxonomist, Botanical Survey of India, Allahabad, India. A voucher specimen has been submitted in this regard. The leaves were freeze dried at -40°C to get a powder. This powder was dissolved in distilled water and used for preliminary phytochemical screening based on chemical tests and thin layer chromatographic profile and evaluation of its glycemic effect.

(ii) Experimental Animals and Induction of Diabetes

Normal albino Wistar rats of same age group and body weight (100-150 g) were selected for the experiments. Rats obtained from National Institute of Communicable Disease (NICD), New Delhi, India, were housed in polypropylene cages in standard conditions of temperature of 23±5°C and relative humidity of 55±5% with a 12 h each of dark and light cycles. Rats were fed pellet diet (Golden feed, Varanasi, India) and water *ad libitum*. The Institutional Animal Ethical Committee in accordance with "Principles of Laboratory Animal Care" has approved the study.

In order to assess the hypoglycemic and antidiabetic activity of the freeze dried powder on sub- and mild-diabetic rats, diabetes was induced by a single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (55 mg kg^{-1}) in 0.1M citrate buffer (pH 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, depending upon their Blood Glucose Levels (BGLs) based on Fasting Blood Level (FBG) and Glucose Tolerance Test (GTT) Studies, the above normal rats were divided into two groups:

- Sub-diabetic with normal FBG 80-120 mg dl^{-1} and high GTT > 210 mg dl^{-1}.
- Mild-diabetic with high FBG 120-250 mg dl^{-1} and high GTT > 320 mg dl^{-1}.

(iii) Experimental Design

BGL was estimated by Glucose Oxidase Method [20] using standard kit of Bayer Diagnostics India Limited. Initial screening of the freeze dried

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powder for identifying the most effective dose was done with a range of variable doses of 100, 200, 300 and 400 mg kg^{-1} in normal, sub-diabetic as well as mild-diabetic rats by conducting FBG and GTT studies.

(a) Assessment of hypoglycemic activity in normal rats - FBG & GTT Studies

Thirty six normal rats were fasted overnight and six groups of six rats each were made to use in the experiment. Group I served as untreated control and received vehicle (i.e. distilled water only) whereas Groups II, III, IV and V received graded doses of 100, 200, 300 and 400 mg kg^{-1} respectively, of freeze dried powder suspended in distilled water. Rats of Group VI received 2.5 mg kg^{-1} of a known antidiabetic drug, Glipizide as reference drug. FBG was taken initially by collecting blood from the tail vein before administering the above doses and then again blood samples were collected from the tail vein at 2, 4, 6 and 8 h after administering the above graded doses.

After one week, the above rats were once again fasted overnight for GTT studies. FBG assessment and dose administration were conducted as mentioned in FBG studies. Thereafter, the rats were orally administered with 2 g kg^{-1} of glucose. BGL was then assessed after 90 minutes and this was considered as 0 h value for GTT. Glucose tolerance of all the rats was subsequently studied upto 3 h at regular intervals of 1 h each. Collection of blood samples for above GTT studies in normal rats was carried out as similar to their FBG studies.

(b)Assessment of antidiabetic activity in sub- and mild-diabetic rats – FBG and GTT Studies

Six groups of six rats each of overnight fasted rats were made separately for both sub- diabetic as well as mild-diabetic rats. Their FBG and GTT studies were carried out similarly as in case of normal rats.

(c) Preliminary Phytochemical Screening

Initial phytochemical screening of the freeze dried powder of *M. koenigii* leaves was carried out and polyphenols were identified by Folin-Ciocalteu method [21]. 200 mg freeze dried powder was dissolved in 1 ml ethanol and Folin-Ciocalteu reagent was added drop by drop. The change in colour from pink to blue indicated the presence of polyphenols. Further investigation revealed the presence of flavonoids by standard colour test [22,23]. Addition of a few drops of dilute sodium hydroxide to 1 ml of the extract produced intense yellow colour, which becomes colourless on addition of a few drops of dilute acid (2N HCl), indicating thereby the presence of flavonoids. Though, polyphenols have already been reported in *M. koenigii* but flavonoids have been identified for the first time in *M. koenigii* extract.

Since, a number of plants have been reported to possess polyphenols and flavonoids responsible for anti-diabetic activity in experimental diabetes [24,25] therefore, from the outcome of preliminary phytochemical screening, it can be conclusively stated that the glycemic potential of *M. koenigii* freeze dried leaf powder may be due to the presence of polyphenols or more precisely, flavonoids. Moreover, polyphenol-enriched *Murraya koenigii* has also been found to possess antioxidant activities, indirectly responsible for diabetes management [26]. The presence of polyphenolic flavonoids was further confirmed by TLC (Thin Layer Chromatography), using silica gel G plates; solvent system AcOH: EtOAc: Hex: CH_2O_2 (1:3:5:1), of two different spots of the same freeze dried powder (Samples 1 and 2). Both these spots turned yellow and blue individually on treatment with 10% ethanolic $FeCl_3$ and Folin-Ciocalteu reagent at Rf 0.6 and 0.57 respectively.

(d) Statistical analysis

Data was expressed as ±S.D. Two-way analysis of variance (ANOVA) was performed using Graph Pad Prism 4.00 for Windows (Graph Pad Software, San Diego CA, USA). The values were considered significant when $P<0.001$.

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RESULTS

(i) Hypoglycemic effect of Freeze Dried M. koenigii Leaf Powder in normal healthy rats

Table 1 describes the hypoglycemic effect of a single oral administration of graded doses of freeze dried leaf powder of *M. koenigii* on FBG of normal rats. Rats treated with the dose of 300 mg kg^{-1} showed a maximum fall of 41.5% in BGL after 6 h of oral administration, whereas fall of 30.5%, 37.1% and 40.8% was observed with the doses of 100, 200 and 400 mg kg^{-1}, respectively.

Table 2 depicts the hypoglycemic effect of a single oral administration of variable doses of 100, 200, 300 and 400 mg kg^{-1} of freeze dried leaf powder of *M. koenigii* during GTT of normal rats. The dose of 300 mg kg^{-1} produced a maximum fall of 38.7% in BGL of rats after 3 h of glucose administration whereas fall of 31.2%, 34.8% and 37.6% was observed in BGL after 3 h of glucose administration from the doses of 100, 200 and 400 mg kg^{-1}, respectively.

The results clearly indicate that the effectiveness of the freeze dried leaf powder is dose dependent upto a certain dose only, after that it does not increase even with increase in dose. The dose which brings the effectiveness to its optimal level, is defined as the most effective dose (300 mg kg^{-1}). Such a phenomenon of less hypoglycemic response at higher dose is not uncommon with indigenous plants.

Table 1

Effect of graded doses of freeze dried leaf powder of M. koenigii on BGL during FBG of normoglycemic rats (mean ± S.D.)

Blood Glucose Levels (mg dl^{-1})						
		Pretreatment	Post treatment (h)			
Experimental Groups	Treatment (mg kg^{-1}) bw	FBG	2 h	4 h	6 h	8 h
Group I	Control	77.6±2.9	77.4±1.2	78.3±0.9	77.3±2.1	77.2±2.1
Group II	100 mg kg^{-1}	75.3±1.5	72.5±1.9	64.5±1.7	52.3±1.3	57.2±1.7
Group III	200 mg kg^{-1}	77.5±1.2	73.2±1.3	60.5±0.8	48.7±1.4	56.6±1.7
Group IV	300 mg kg^{-1}	76.3±2.8	72.7±1.5	55.8±0.7*	44.6±3.0*	54.5±3.3
Group V	400 mg kg^{-1}	75.6±1.7	67.5±1.5	52.4±4.3	44.7±0.8	56.8±1.5
Group VI	Glipizide	74.7±0.9	68.6±1.9	53.5±5.3	45.4±1.2	55.2±0.8

*P <0.05 as compared with control

Table 2

Effect of variable doses of freeze dried leaf powder of M. koenigii on BGL during GTT of normoglycemic rats (mean ± S.D.)

Glucose Tolerance Test (mg dl^{-1})						
		Pretreatment	Post treatment (h)			
Experimental Groups	Treatment (mg kg^{-1}) b.w.	FBG	0 h	1 h	2 h	3 h
Group I	Control	75.4±1.2	74.5±1.6	75.1±1.7	74.4±1.5	76.2±0.7
Group II	100 mg kg^{-1}	76.6±1.3	73.5±1.2	76.4±1.5	73.5±1.4	52.7±4.0
Group III	200 mg kg^{-1}	78.8±0.8	73.5±4.4	78.5±1.2	72.4±1.2	51.3±0.7

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Group IV	300 mg kg^{-1}	74.6±2.9	68.2±2.2	74.0±1.7	54.6±1.6*	45.7±1.8*
Group V	400 mg kg^{-1}	76.4±1.6	69.4±0.1	75.8±2.4	57.5±2.6	47.6±2.7
Group VI	Glipizide	75.3±2.9	71.2±0.5	75.2±0.9	55.8±0.8	46.8±2.4

*P <0.05 as compared with control

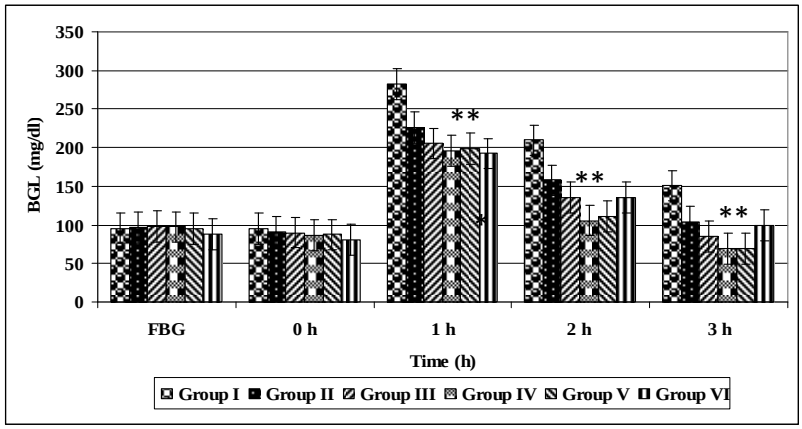
(ii) Antidiabetic effect of Freeze Dried M. koenigii Leaf Powder in sub- & mild-diabetic rats

Graph 1 depicts the antidiabetic effect of a single oral administration of variable doses of 100, 200, 300 and 400 mg kg^{-1} of freeze dried leaf powder of *M. koenigii* during GTT of sub-diabetic rats. The dose of 300 mg kg^{-1} produced a maximum fall of 54.3% in BGL of rats after 3 h of glucose administration whereas fall of 31.1%, 43.8 % and 53.9% was observed in BGL from the doses of 100, 200 and 400 mg kg^{-1}, respectively.

Graph 2 depicts the antidiabetic effect of a single oral administration of variable doses of 100, 200, 300 and 400 mg kg^{-1} of freeze dried leaf powder of *M. koenigii* during GTT of mild-diabetic rats. The dose of 300 mg kg^{-1} produced a maximum fall of 51.7% in BGL of rats after 3 h of glucose administration whereas, fall of 41.8%, 46.4% and 49.8% was observed in BGL from the doses of 100, 200 and 400 mg kg^{-1}, respectively.

Graph 1

Effect of variable doses of freeze dried powder of M. koenigii leaves on BGL during GTT of sub-diabetic rats (mean ± S.D.)



**p <0.01 as compared with control

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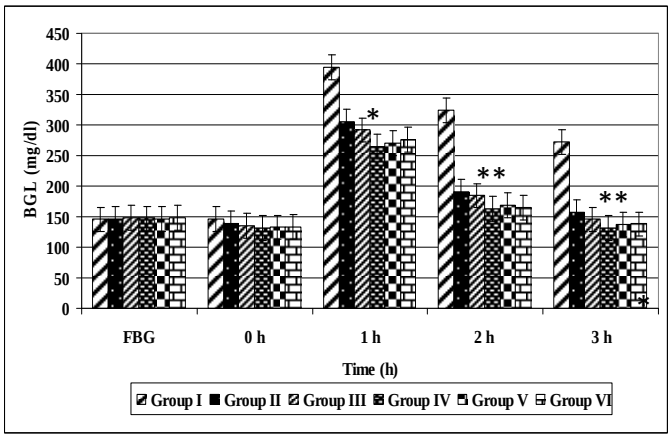
Graph 2

Effect of variable doses of freeze dried powder of M. koenigii leaves on BGL during GTT of mild-diabetic rats (mean ± S.D.)



*P<0.05, **P<0.01 as compared with control

(iii) *Toxicity studies - LD_{50} Experiment*

Toxicity study was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported upto 10 and 15 times of the effective dose and there was no death reported in any of these groups. High LD_{50} indicates its high margin of safety.

(iv) Preliminary Phytochemical Analysis

The presence of polyphenols and flavonoids was identified on preliminary phytochemical analysis of *M. koenigii* freeze dried powder based on chemical tests and thin layer chromatographic profile (Figure 1).

Thin Layer Chromatographic Profile

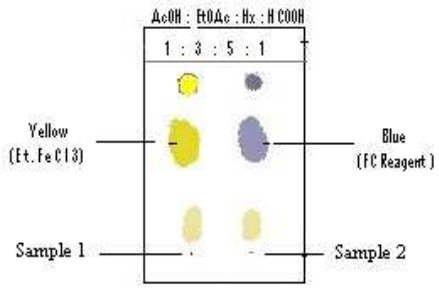


Figure 1 *Thin Layer Chromatographic Profile of two different spots of M. koenigii freeze dried powder run in the solvent system AcOH:EtOAc:Hex:CH_2O_2 (1:3:5:1)*

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DISCUSSION

The knowledge regarding diabetes existed since Brahmic period and treatment of diabetes has been mentioned in Sushruta Samhita. In this ancient text, two forms of diabetes were described; one genetically based and other as a result of dietary indiscretion [27]. Spices like Fenugreek, *Murraya koenigii*, *Brassica juncea*, etc. as a dietary constituent have been found to have beneficial effect on carbohydrate metabolism. This has been found experimentally as well as clinically [28,29]. Hyperglycemic animals have been described as a useful experimental model to study the activity of hypoglycemic agents [30].

The purpose of the present study was to develop *M. koenigii* freeze dried leaf powder as a therapy for treating Diabetes Mellitus by evaluating its potential of managing various parameters such as FBG, GTT and LD_{50} of diabetic models.

Freeze dried powder of *M. koenigii* leaves reduces the BGL in normal animals and normalizes the high BGLs of sub- and mild-diabetic rats. It also improves oral glucose tolerance in normal, sub- and mild-diabetic animals. Glipizide was used as a reference drug for positive control. It is interesting to note that the freeze dried leaf powder at a dose of 300 mg kg^{-1} was more effective than Glipizide (2.5 mg kg^{-1}).

CONCLUSION

The conclusions derived from results revealed a defined role of the freeze dried powder of *M. koenigii* leaves in managing the BGL in normal and diabetic rats. The significant hypoglycemic effect was produced with all the four doses of freeze dried powder after 6 h of administration. Though the effect on FBG of normal healthy rats had began at 2 h and was maximum at 6 h in all the groups, the effect was dose dependent upto 300 mg kg^{-1}, the dose identified as the most effective dose in normal, sub- as well as mild-diabetic rats during FBG and GTT studies. However, the response decreased at 400 mg kg^{-1} dose. Such a phenomenon of less hypoglycemic response at higher dose is not uncommon with indigenous plants and has been observed a number of times [31,32] and also reported by our research group in our previous studies [33,34]. *Murraya koenigii*, thus seems to be a promising plant with respect to its hypoglycemic and antidiabetic effect as evident from the experimental results and may be prescribed as adjunct to dietary therapy and drug treatment for controlling diabetes mellitus. Phytochemical investigation reveals the presence of polyphenols and flavonoids. Thus, the glycemic effect of *M. koenigii* freeze dried leaf powder can be very well correlated in a suggestive and meaningful manner to the presence of polyphenols and flavonoids in it.

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. Please see the experimental part for details.

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