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Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits

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Abstract

In past there have been many medicinal plants, which have been used in traditional medicines for their antidiabetic properties without any scientific support and pharmacological evidence. The aqueous extract of *Murraya koenigii* leaves has been taken to evaluate the 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 flavoring and as such it appears to be without any side effects and toxicity. Adequate characterization of hypoglycemic activity of aqueous extract has not been yet done, as no such reports are available in the literature though the activity is reported. The scientific evaluation of its hypoglycemic activity was, therefore, explored and also compared with the effect of a standard hypoglycemic drug, tolbutamide. A single oral administration of variable dose levels (200, 300 and 400 mg/kg) of aqueous extract led to lowering of blood glucose level in normal as well as in diabetic rabbits. The maximum fall of 14.68% in normal and 27.96% in mild diabetic was observed after 4 h of oral administration of 300 mg/kg. The same dose also showed a marked improvement in glucose tolerance of 46.25% in sub-diabetic (AR) and 38.5% in mild diabetic rabbits in glucose tolerance test after 2 h. The findings from this study suggest that the aqueous extract of these leaves may be prescribed as adjunct to dietary therapy and drug treatment for controlling diabetes mellitus.

Keywords: *Murraya koenigii*; Leaves; Diabetes mellitus; Aqueous extract; Alloxan

1. 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 characterised by elevation of both fasting and post-paradinal blood sugar levels. The synthetic oral hypoglycemic agents can produce serious side effects (Akhtar and Iqbal, 1991; Holman and Turner, 1991). In addition, they are not considered to be safe for use during pregnancy (Larner, 1985). Furthermore, after the recommendation made by WHO on diabetes mellitus investigation on hypoglycemic agents from medicinal plants have become more important (WHO, 1980).

Murraya koenigii (L.) Spreng (family: Rutaceae) is commonly known as 'curry patta' in Hindi and is widely and regularly used as a spice and condiment in India and other tropical countries. *Murraya koenigii* leaves mixed with fat separated butter fat is used for the treatment of amoebiasis, diabetes and hepatitis in Ayurveda (Pillai, 1958; Bose, 1985; Satyavati et al., 1987). The aqueous extract of the leaves of *Murraya koenigii* produced hypoglycemia in normal and alloxan diabetic dogs (Narayan and Sastry, 1975). Oral feeding of *Murraya koenigii* leaves diet for 60 days to normal rats showed hypoglycemic effect associated with increase in the concentration of hepatic glycogen (Khan et al., 1995). Dietary supplement with curry leaves has been shown to increase lecithin cholesterol acyl transferase activity (Khan et

Abbreviations: AR, alloxan recovered; BGL, blood glucose level; FBG, fasting blood glucose; GTT, glucose tolerance test; MD, mild diabetic; *M. koenigii*, *Murraya koenigii*; S.D., standard deviation; UGC, University Grant Commission; WHO, World Health Organisation

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al., 1996) S. Yadav has recently reported that feeding different doses of *Murraya koenigii* leaves to diabetic rats play role in control of mild diabetes but in case of moderate, severe and type I diabetes this agent alone is not likely to be useful (Yadav et al., 2002).

Several Carbazole alkaloids (Joshi et al., 1970; Narasimhan et al., 1975; Narasimhan and Kelkar, 1976; Chakraborty et al., 1978; Wang et al., 2003), Copolin-α-glucoside and free glucose (Guptha and Nigam, 1971) have been reported to be present in *Murraya koenigii*. The essential oils of *Murraya koenigii* possess antibacterial and antifungal activity (Goutam and Purohit, 1974). The present study was undertaken to study the hypoglycemic effect of aqueous extract of *Murraya koenigii* leaves in normal and alloxan induced diabetic rabbits.

2. Material and method

2.1. Plant material

Fresh leaves of *Murraya koenigii* (5 kg) were collected locally and got identified by Botanical survey of India (Allahabad Branch). The leaves were shade dried and were crushed to moderately coarse powder.

2.2. Preparation of extract

The powder was extracted with distilled water using soxhelt at boiling temperature (100 °C) up to 10 h. A dark brown colour extract is obtained. This dark brown extract was cooled and filtered to remove the residue. The extract was concentrated on rotavapour under reduced pressure and then lyophilized to get a powder weighing about 7.5 g.

2.3. Experimental animals

Male Albino rabbits weighing between 1.00 and 1.5 kg were used and were maintained on commercial diet (Hindustan Lever Ltd., Mumbai) and water ad libitum. They were acclimated to the laboratory conditions before carrying out any experimental work.

Diabetes mellitus was induced by administering intravenous injection of alloxan monohydrate (80 mg/kg body weight, Sigma chemicals, USA) (Shukla et al., 1995) dissolved in citrate buffer (pH 4.00) to the overnight fasted rabbits through their marginal ear vein. Fasting blood glucose (FBG) level was checked regularly up to stable hyperglycemia after 3 weeks of alloxan administration. The blood glucose level (BGL) was determined by glucose oxidase method. Depending on their glucose levels the animals were divided in to different groups. The rabbits having FBG value between 120 and 250 mg/dl were considered as Mild diabetic (MD) group. In rabbits whose FBG value increased initially but returned to normal or slightly elevated FBG value (120 mg/dl or below) but showed abnormal Glucose Tolerance Test (GTT) were designated as alloxan recovered (AR) sub-diabetic group.

Rabbits that did not show any increase in FBG levels even initially after alloxan injection were considered as totally resistant and were excluded from studies. Hypoglycemic activity was assessed by lowering of BGL in normal and MD rabbits and improvement in glucose tolerance in GTT in AR and MD rabbits.

2.4. Experimental design

2.4.1. Effect of Murraya koenigii extract on blood glucose level of normal healthy rabbits

In overnight, fasted rabbits FBG was checked and then they were divided into four groups of six rabbits each. Group I served as control and received vehicle (Tween 80 in distilled water) only. Groups II, III and IV were given aqueous leaves extract suspended in distilled water using Tween 80 orally at doses 200, 300 and 400 mk/kg body weight, respectively. Blood samples were collected from the marginal ear vein at 2, 4 and 6 h after giving the extract.

2.4.2. Effect of Murraya koenigii on blood glucose level of diabetic rabbits

After checking the FBG in overnight fasted mild diabetic rabbits, they were divided into four groups of six rabbits each. Control rabbits (group I) were given vehicle (Tween 80 in distilled water) only while other groups II, III and IV received aqueous leaves extract suspended in distilled water using Tween 80 orally at doses 200, 300 and 400 mg/kg, respectively. The effect of extract on BGL was studied upto 6 h, at 2 h interval.

2.4.3. Effect of Murraya koenigii extract on glucose tolerance in diabetic rabbits

The hypoglycemic effect of aqueous extract of *Murraya koenigii* leaves in mild and subdiabetic rabbits was also assessed by improvement of glucose tolerance. The rabbits were divided into several groups. Control received vehicle (Tween 80 in distilled water) only, whereas variable doses of 200 and 300 mg/kg of leaf extract and drug Tolbutamide were administered to other groups. The rabbits of all the groups were given glucose (2 g/kg), 90 min after the extract and drug administration. Blood samples were collected just prior to glucose administration (0 h) and 1, 2 and 3 h after glucose loading and blood glucose levels were measured by glucose oxidase method.

2.5. Statstical analysis

All the group data were statistically evaluated using student's *t*-test, expressed as the mean ± S.D. from six rabbits in each group. *P*-value of 0.05 or less was considered to be significant.

Table 1
Hypoglycemic effect of graded doses of aqueous extract of *M. koenigii* leaves in normal rabbits (mean ± S.D.)

Experimental groups	Treatment and dose	Blood glucose level (mg%)			
		0 h	2 h	4 h	6 h
I	Control	86.3 ± 7.2	85.5 ± 7.7	84.6 ± 8.5	82.6 ± 8.2
II	+Leaf extract 200 mg/kg	79.2 ± 5.9	76.8 ± 3.8	72.6 ± 4.2**	73.0 ± 5.8
III	+Leaf extract 300 mg/kg	82.4 ± 3.7	77.3 ± 4.2	70.3 ± 3.1*	71.8 ± 3.6
IV	+Leaf extract 400 mg/kg	79.6 ± 4.8	75.7 ± 4.1	72.2 ± 5.1**	73.6 ± 4.9

* $P < 0.001$ compared with initial value.
** $P < 0.05$ with compared to initial value.

3. Results

3.1. Assessment of hypoglycemic activity in normal healthy rabbits

Table 1, depicts hypoglycemic effect of a single oral administration at doses 200, 300 and 400 mg/kg of aqueous extract of *Murraya koenigii* leaves in normal healthy rabbits. Rabbits treated with 300 mg/kg showed a significant fall of 14.68% fall in BGL after 4 h of oral administration, whereas fall of 8.3% and 9.3% was observed in BGL levels at doses 200 and 400 mg/kg was, respectively, after 4 h of oral administration.

3.2. Assessment of hypoglycemic activity in diabetic rabbits

Table 2 depicts hypoglycemic effect of a single oral administration at doses 200, 300 and 400 mg/kg of aqueous extract of *Murraya koenigii* in mild diabetic rabbits. The most effective dose was found to be 300 mg/kg as it produces significant fall of 27.96% in FBG, after 4 h of oral administration. Fall of BGL levels for doses 200 and 400 mg/kg was found to be 11.86% and 14.5%, respectively, after 4 h of oral administration. However, after 6 h BGL rises slightly as compare to that of 4 h.

3.3. Assessment of hypoglycemic activity by GTT in diabetic rabbits

Table 3 depicts hypoglycemic effect of a single oral administration at doses 200 and 300 mg/kg of aqueous extract of *Murraya koenigii* leaves and at a dose of 300 mg/kg of tolbutamide in AR and MD rabbits. The dose of 300 mg/kg produced a maximum fall of 46.25% in AR and 38.5% in MD rabbits in BGL after 2 h of glucose administration. The same dose of 300 mg/kg of tolbutamide produced maximum fall of 45.1% and 39.5%, respectively, in AR and MD rabbits,

Table 2
Hypoglycemic effect of graded doses of aqueous extract of *M. koenigii* leaves in diabetic rabbits (mean ± S.D.)

Experimental groups	Treatment and doses	Blood glucose level (mg%)			
		0 h	2 h	4 h	6 h
I	Control	235.2 ± 8.2	240.1 ± 8.4	236.0 ± 7.9	237.6 ± 8.9
II	Leaf extract 200 mg/kg	232.3 ± 7.7	218.6 ± 7.9	202.8 ± 8.5*	212.8 ± 7.5*
III	Leaf extract 300 mg/kg	240.3 ± 9.1	210.6 ± 10.4	173.1 ± 11.2*	184.2 ± 8.8*
IV	Leaf extract 400 mg/kg	238.2 ± 8.3	221.8 ± 7.7	203.6 ± 8.1*	210.4 ± 7.7*

* $P < 0.001$ compared with initial value.

Table 3
Effect of oral administration of the graded dose of the aqueous extract on the glucose tolerance in AR and MD rabbits (mean ± S.D.)

Experimental groups	Dose (mg/kg)	Blood glucose level (mg%)			
		0 h	1 h	2 h	3 h
Subdiabetic					
Control		97.4 ± 5.8	278.0 ± 5.6	196.5 ± 5.2	115.2 ± 4.8
Tolbutamide	300	95.7 ± 4.2	160.2 ± 5.4*	107.8 ± 6.1*	97.2 ± 5.1
Aqueous extract	200	98.2 ± 4.3	235.4 ± 5.4*	168.4 ± 6.1*	103.8 ± 4.9
	300	91.2 ± 5.3	168.2 ± 6.5*	105.6 ± 5.2*	99.2 ± 5.6
Mild diabetic					
Control		181.6 ± 8.8	397.2 ± 5.6	322 ± 10.2	216 ± 9.2
Tolbutamide	300	176.2 ± 9.6	252.6 ± 10.6**	194.8 ± 9.2*	183.2 ± 8.8
Aqueous extract	200	174.6 ± 8.2	316.6 ± 10.2*	259.2 ± 9.8**	198.4 ± 9.2
	300	177.4 ± 9.8	261.6 ± 9.2*	197.8 ± 10.2**	189.6 ± 9.5

* $P < 0.001$ compared with control.
** $P < 0.01$ compared with control.

after 2 h of glucose administration. Thus, the fall produced by aqueous extract of *Murraya koenigii* and tolbutamide at dose 300 mg/kg is almost similar.

4. Discussion

The knowledge regarding diabetes existed since Brahmic period and treatment of diabetes has been mentioned in Sushruta Samhita (Dhanukar and Thatte, 1989). In this ancient text, two form of diabetes were described; one genetically based and other as a result of dietary indiscretion (Dhanukar and Thatte, 1989). 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 (Iyer and Mani, 1990; Vats et al., 2002).

The conclusions derived from results revealed a defined role of the water extract of *Murraya koenigii* leaves in suppressing blood glucose level in normal and diabetic rabbits. The aqueous extract in all the three doses produced significant hypoglycemic effect after 4 h administration. It is also clear that the hypoglycemic effect of variable dose of extract had began +2 h and was maximum at +4 h in all the groups. As far as most effective dose is concern it has been found to be 300 mg/kg in all the groups. This dose has almost same effect as of synthetic drug tolbutamide.

The effect was dose dependent upto 300 mg equivalent of extract. However, the response decreased at 400 mg/kg dose. Such a phenomenon of less hypoglycemic response at higher dose is not uncommon with indigenous plants and has been observed with *Vinca rosea* (Chattopadhyay et al., 1991), *Cinnamomum tamala* (Sharma et al., 1996) and *Aegle marmelose* (Rao et al., 1995).

Murraya koenigii, thus seems to be a promising plant with respect to its hypoglycemic effect and may be prescribed as adjunct to dietary therapy and drug treatment for controlling diabetes mellitus. Further comprehensive pharmacological investigation is in progress to elucidate the exact mechanism of action of this extract.

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