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Research article

Neuropharmacological evaluation of *Murraya koenigii* in diabetic rodents

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ABSTRACT

The plant Murraya koenigii is used in folklore medicine to treat various diseases including diabetes mellitus, GIT and CNS disorders. In this study, the potentials of the ethanolic leaf extract on CNS complications (neuropathy) in diabetic rodents was evaluated using alloxan induced diabetes in Swiss albino mice. The extract was prepared with 70% v/v of ethanol and the alloxan induced diabetic mice were treated with the extract for 7 days after which their fasting blood glucose and the locomotor activity by Actophotometer and motor co-ordination by rota-rod were analysed. The 50, 100 and 200mg/kg of the extract significantly reduced the blood glucose, significantly increase the locomotor activity (score) and fall off time. It was concluded that the ethanolic leaf extract of Murraya koenigii possesses the dose dependent protective effect on CNS complications induced by diabetes.formulation.

Keywords: Neuropharmacology, Alloxan, Diabetes Mellitus, Murraya koenigii.

INTRODUCTION

Diabetes is a heterogeneous disorder characterized by two interrelated metabolic defects: insulin resistance coupled with impaired insulin secretion by β-cells in the pancreas. Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. Some people with nerve damage have no symptoms. Others may have symptoms such as pain, tingling, or numbness, loss of feeling, in the hands, arms, feet, and legs. Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs. About 60 to 70 percent of people with diabetes have some form of neuropathy. People with diabetes can develop nerve problems at any time, but risk rises with age and longer duration of diabetes. Murraya koenigii is a small aromatic tree with dark grey bark and closely rowded spreading dark green foliage. They are highly aromatic. All parts of the plant, especially the leaves are rich in carbazole alkaloids. The roots, bark and leaves are bitter, acrid, astringent, cooling, aromatic, demulcent, depurative, anthelmintic, febrifuge, stomachic, appetising, carminative, anti-inflammatory and antiseptic. Aerial part is spasmolytic and anti-protozoal. Root is antiprotozoal, CVS active and has effect on nictitating membrane, leaf is hypoglycaemic [1].

MATERIAL AND METHODS

Collection and Authentication of Plant the leaf was collected in the month of January from the Local market of Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia Ul Hasan (Professor), Safia College Bhopal and the specimen voucher no. assigned was 444/Bot/safia/13 [2].

Drying and Size Reduction of Plant Material

The leaves were dried under shade. It was pulverized to coarse powder. The coarse powder was passed through sieve No.20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of crude extract (by Soxhlet extraction method)

Pharmacological Activity

Animal care and handling

The experiment was carried out on Swiss albino mice of 4 months, of both sexes, weighing between 25 to 30 gm. They were provided from Truba Institute of Pharmacy, Bhopal, (M.P.). The

animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water ad libitum during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1196/a/08/CPCSEA).

Locomotor activity

The spontaneous locomotor activity was assessed with the help of photoactometer (Techno, India). Each animal was observed for a period of 10 min in a square closed field arena (30x30x30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a 6 digits resettable counter [3].

Muscle relaxant activity

The muscle relaxant activity was assessed with the help of Rota-rod apparatus (Techno, India). Turn on the instrument and select an appropriate speed (20rpm). Each animal was placed one by one on rotating rod and note down the fall of time.

Induction of diabetes

Diabetes mellitus was induced mice by a single i.p. injection of alloxan monohydrate (120 mg/kg body weight) in normal saline (0.9% NaCl). Hyperglycemia was confirmed by fasting blood glucose level measurement by glucometer on the 3rd day after the alloxan injection. Mice with consistent hyperglycemia on 3rd day (fasting blood glucose levels > 135mg/dl) was considered diabetic and was used for further studies (8).

Experimental Design

In the experiment, a total of 24 mice were used. The mice were divided into 6 groups comprising of 4 animals in each group as follows:

Group I: Normal control

Group II: Negative control mice received Alloxan 120g/kg, i.p. for inducing diabetes. Group III: Mice received Glibenclamide (2.5mg/kg, p.o.) for 7 days and Alloxan 120g/kg, i.p. on 1st day.

Group IV Mice received ethanolic Extract of Murraya koenigii, (50mg/kg p.o.) once daily for 7 days and Alloxan 120g/kg, i.p. on 1st day.

Group V Mice received ethanolic Extract of Murraya koenigii, (100mg/kg p.o.) once daily for 7 days and Alloxan 120g/kg, i.p. on 1st day.

Group VI Mice received ethanolic Extract of Murraya koenigii, (200mg/kg p.o.) once daily for 7 days and Alloxan 120g/kg, i.p. on 1st day.

Sample collection

Blood samples were collected by tail vein and blood glucose levels were estimated using an electronic glucometer (Gluco chek).

Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Tukey- Kramer Multiple Comparisons Test by employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at $P < 0.05$ levels [4].

RESULT OF EXPERIMENTAL DATA

Table 1: Percentage Yield of extract.

Alcoholic Extract	7.54

Table 2: Effect of ethanolic Extract of *Murraya koenigii* on locomotor activity in diabetic mice.

GROUPS	Locomotor activity (scores) in 10 min
Group I (Control)	138.43±4.22
Group II (Diabetic Control)	65.5±3.35a***
Group III Glibenclamide)	120.6±4.21a**,b***
Group IV (EE MK, 50mg/kg)	91.85±2.84a***,b*
Group V (EE MK, 100mg/kg)	109.43±1.29 a***,b***
Group VI (EE MK, 200mg/kg)	112.6±5.88 a***,b***

Values are mean ± SEM from a group of four animals. *p<0.05, **p<0.01, ***p<0.001

a- significant difference as compared to control

b- Significant difference as compared to diabetic control

Table 3: Effect of ethanolic Extract of *Murraya koenigii* on muscle relaxant activity in diabetic mice.

GROUPS	Fall of time (Sec)
Group I (Control)	150.23±4.46
Group II (Diabetic Control)	84.57±5.69a***
Group III Glibenclamide)	137.4±3.87a**,b***
Group IV (EE MK, 50mg/kg)	104.58±2.34a***, b***
Group V (EE MK, 100mg/kg)	112.54±4.76 a***, b***
Group VI (EE MK, 200mg/kg)	123.45±3.55 a***, b***

Values are mean ± SEM from a group of four animals. *p<0.05, **p<0.01, ***p<0.001

a- significant difference as compared to control

b- Significant difference as compared to diabetic control

Table 4- Effect of ethanolic Extract of *Murraya koenigii* on blood glucose level in alloxan induced diabetes in mice.

GROUPS	Blood glucose (mg/dl)	
	3rd day	7th day
Group I (Control)	77.45±2.37	83.31±2.54
Group II (Diabetic Control)	152.56±3.54	172.45±3.51a***
Group III (Glibenclamide)	146.57±3.55	120.5±1.29 a***,b***
Group IV (EE MK, 50mg/kg)	140.55±4.38	134.3±3.67 a***,b***,c**
Group V (EE MK, 100mg/kg)	147.43±5.12	129.45±3.4 a***,b***,c*
Group VI (EE MK, 200mg/kg)	152.4±2.45	126.41±2.98 a***,b***

Values are mean ± SEM from a group of four animals. *p<0.05, **p<0.01, ***p<0.001

a- significant difference as compared to control

b- Significant difference as compared to diabetic control

c- Significant difference as compared to standard

DISCUSSION AND CONCLUSION

The coarse powder of the shed dried parts of the plant was subjected to extraction by using soxhlet apparatus. The plant materials were treated with solvents for 24 hours. The plant material was extracted with alcohol. In the extract yield was obtained in alcoholic extract that was 7.54%. Neuropharmacological study was evaluated in diabetic mice through locomotor activity and motor coordination. Results of Locomotor activity reveled that ethanolic extract of Murraya koenigii produced more locomotor activity (score)

as compared to diabetic mice. Results of motor coordination test revealed that the ethanol extract also exhibited marked increase in motor coordination as compared to diabetic mice. Results shown in table 1-4. Treatment with extract at different dose 50, 100 and 200mg/kg was elicited significant inhibition of blood glucose level. The Results were compared with glibenclamide was more effective in reducing blood glucose level in normal as well as diabetic control group. On the basis of above result, the ethanolic extract of Murraya koenigii leaf produced significant blood glucose lowering potential in the animal model. On the basis of results, it may be concluded that ethanolic extract produced dose dependent protective action against neuropathy induced by diabetes [5, 6].

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