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Anxiolytic activity of *Murraya koenigii* leaf extract in Mice

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Abstract

The objective of the present study was to evaluate the anxiolytic activity of *Murraya koenigii (MK)* leaf extract as well as its interaction with conventional anxiolytic drugs using elevated plus maze, Open field behaviour test and Light Dark model in mice. Albino mice were treated with MK (100, 250 and 500 mg/kg, po), Diazepam as positive control (1 mg/kg, po). Effects were observed on (a) time spent on (b) number of entries into (c) number of stretch attend postures (d) number of head dips in arms of elevated plus maze and on duration of immobility in tail suspension test. Results showed that MK significantly ($P<0.05$) and dose dependently increased proportionate time spent on and number of entries into open arms while decreased number of stretch attend postures and head dips in closed arms. Dose dependent and significant ($P<0.05$) anti-immobility effect was found in mice treated with MK. Combination of MK (75 mg/kg, po) with imipramine (5 mg/ kg, po) or fluoxetine (5 mg/kg, po) also produced significant ($P<0.05$) anxiolytic activity. It is concluded that MK possess potential anxiolytic and antidepressant activities and it enhances the anxiolytic and antidepressant activities of imipramine and fluoxetine.

Key words: *Murraya koenigii (MK)*, Anxiolytic activity, elevated plus maze test, Open field behaviour test, Light Dark model.

Introduction

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat[1]. Anxiety effects one-eighth population worldwide and has become an important research area in the field of psychopharmacology [2]. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction, anticholinergic, antihistaminic effects (TCA's) have limited their use in patients [3]. Due to this many pharmaceutical companies are conducting studies to find an alternative medicine or plant-derived medications with more specific anxiolytic effects [4]. Aqueous extract of the leaves of Murraya koenigii (M. koenigii) possesses alexeteric, antihelmintic, analgesic, dysentry, purgative and blood disorders. Also they are reported to be useful in inflammation healing of wounds, injuries, antioxidative activity[5,6]. Plants can be a potential source of anxiolytic drugs. However, the potential of plants as anxiolytic agents has not been fully investigated. the anxiolytic aaivity of a water extract of mature leaves of *Murraya koenigii* (Family Rutaceae; known as karapincha in Sinhala and Karuvembu in Tamil), using rats and the shock-induced suppression of drinking test [7]. Recently the search for novel pharmacotherapy from medicinal plants for psychiatric illness has progressed significantly and thus revealed pharmacological effectiveness of different plant species in a variety of animal models [8]. The leaves, bark and the root of the plant are used in indigenous medicine as a tonic, stomachic, stimulant and carminative. An infusion of the roasted leaves is used to stop vomiting. The green tender leaves are eaten raw for the cure of dysentery. A decoction of the leaves is sometimes given with bitters as a febrifuge and the leaves have been claimed to be used with mint in the form of "chutney" to check vomiting. It has also been used as an anti-periodic and many a time the powdered dry leaf, mixed with honey and juice of betel nut, is recommended in the Ayurvedic system of medicine [9]. The leaves and roots of MK are bitter, acrid, cooling, anthelmintic, analgesic, it cures piles, allays heat of the body, reduces inflammation and itching [10]. Despite the widespread uses of the plant, no

scientific work is reported in literature regarding the effect of MK leaves against anxiety studies on Mice therefore, present study was undertaken to evaluate anxiolytic effects of aqueous extract of *Murraya koenigii (MK)* leaves using elevated plus maze test and tail suspension test in mice. Studies were also conducted to find its interaction with conventional anxiolytic drugs in order to elucidate its role in modulation of central monoamines.

Materials and methods

Dried powdered leaves of Murraya.

Procedure for Extraction of *Murraya koenigii (MK) leaf*

The dried and powdered leaves of MK were defatted with petroleum ether (60-80°C) and the following extracts were prepared:

i. Aqueous extract by decoction method
ii. Methanolic extract by Soxhlet extraction method
iii. Hydroalcoholic extract (70% ethanol) by Soxhlet extraction method

i. Aqueous extract by decoction method:

This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume [14.]

ii. Methanolic extract by Soxhlet extraction method:

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. Required amount of plant material is extracted with methanol. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled [15].

iii. Hydroalcoholic extract (70% ethanol) by Soxhlet extraction method:

The required leaf extract is obtained using soxhlation using 70% ethanol[15.]

The Etractive yield of *Murraya leaf* is mentioned in table No:1

Table No: 1

Plant Name	Extractive Yield Values (% w/v)		
	Aqueous Extract	Methanolic Extract	Hydro alcoholic extract
Murraya koeinigii	30.3	35.1	32.8

Selection of Animals:

Healthy Swiss albino mice (20-30 g) of either sex were used for the studies. Each experimental group consisted of at least six animals. The animals were housed for a minimum of five days prior to the pharmacological experiments, with free access to standard rodent pellet diet (Lipton India Ltd) and tap water, and maintained on a 12/12 h light-dark cycle. All experiments were conducted in accordance with international Animal Ethics Committee guidelines. The experimental protocols were approved by the institutional animal ethics committee (IAEC).

Elevated plus maze test (EPM)

The elevated plus maze test is a rapid and selective technique (22) for detecting anxiolytic drug effects under identical conditions. For more sensitive measures of effects of new anxiolytic compounds, risk assessment behaviors (behavior related to anxiety/fear) such as stretch attend postures and head dips were also measured in addition to measure of time spent on and number of entries into arms in the EPM (23). The plus maze was in the shape of a cross or plus with two closed arms each with roof open measuring 30×5×20 cm, extending from a central region (5×5 cm) running along amnorth-south axis and two open arms each measuring 30×5 cm running east-west. The wooden apparatus was elevated to a height of 50 cm from the floor in a dimly illuminated room. Mice were placed individually in the central area of the maze with open access to any arm. The amount of time spent on and number of entries in both open and closed arms whereas numbers of stretch attend postures and head dips in closed arms were measured manually during the 5 min test period. An arm entry was defined as all four feet in the arm. Stretch attend posture was defined as mice stretching forward and then retracting to original position from closed (protected) or open (unprotected) arms. Head dipping is defined as mice protruding the head over the edge of closed or open arms down towards floor. The apparatus was cleaned after each mouse was tested to remove any residue or odour. For the purpose of analysis, open arm stay was quantified as the amount of time that the mouse spent in open arm relative to the total amount of time spent in open and closed arm (open/total×100) and open arm entries were quantified as the number of entries in open arm relative to the total number of entries in open and closed arm (open/total× 100).

Open Field Behavior Test: (Kulkarni *et al* 1999)

This behavioral model is based on the induction of anxiety state such as ambulation or freezing by exposing the animals to a highly novel field environment. Anxiolytics increase the exploratory behavior and diminish freezing time. The open field area is a circular arena (diameter 48cm) made of thermocol like material. It has four radial arms

projecting from the center (a small circular region) and each arm has slots of equal area to monitor the animal's exploratory behavior. The model is placed at a height to induce anxiety state in mice.

Light Dark Model (Vogel *et al* 1997 43):

In Light Dark Model, exploration of mice/rats is inhibited by bright illumination, which is highly aversive for rodents. The animals are placed on brightly lit side of a two-compartment chamber and number of crossings between light and dark sites is recorded. Anxiolytics produce dose dependent increase in crossings, whereas non-anxiolytics do not have this facilitatory effect. Furthermore, relative potency of anxiolytics in increasing exploratory behavior in two-compartment chamber agrees well with potency found in clinical trials. One-third of cage (40 x 60 cm) is darkened with a cover and separated with a wall from otherwise brightly illuminated area. A round hole (Diameter 13 cm) allows the animal to pass from illuminated to darkened Compartment.

D r u g s:

0.1% Na CMC, Thiopentone sodium (Sun Pharmaceutical Industries Baroda), Diazepam (Ranbaxy) were purchased and used in the study.

V e h i c l e

All the drugs and extract used in the study were suspended in 2% gum acacia and were administered orally to the respective animals 1 h prior to start of experiment. The volume for oral administration was 10 ml/kg.

Experimental design

Different groups of mice were used to explore antianxiety activity. To ensure consistency of experience prior to the test session, animals were brought to the testing room 1 h prior to the start of behaviour testing. Test room lighting, temperature and noise level were kept constant for all mice used in the study. Mice were divided into various groups according to the treatments they received. Each group consisted of 6 animals. Individual mice were subjected to test 1 h after drug administration. Mice were tested in EPM only once in order to avoid influence of repeated experience on anxiolytic activity of drugs.

Drug treatment

Mice were treated with aqueous extract of MK at the dose of 100, 250, 500 mg/kg for dose dependent effect against EPM induced anxiety-like behaviour. Doses of MK were selected on the basis of earlier studies conducted using aqueous extract of MK for evaluation of its analgesic activity (27). To compare the effects of test drug with standard anxiolytic drugs mice were treated with diazepam (1 mg/kg). To assess influence of MK on anxiolytic activity of standard drugs, mice were treated with diazepam (1 mg/ kg) alone and in combination with MK (100 mg/kg). Mice treated with vehicle (2% gum acacia) at the dose of 10 ml/kg, served as control group.

Statistical analysis

Results are represented as Mean±SEM. All the data were analyzed using one-way ANOVA followed by Tukey multiple comparison tests. P values <0.05 were considered as statistically significant.

Results and Discussion

Effect of MK against EPM induced anxiety-like behaviour in mice. Results of the present study showed that Aqueous extract of MK at the dose of 75 mg/kg did not show significant change (P>0.05) in mean time spent on and in mean number of entries into open arms as well as in mean number of protected stretch attend postures and head dips as compared to control (Table II). This suggests that MK at the dose of 250 mg/kg is sub effective than MK at the dose of 150 and 300 mg/kg(Table III & IV respectively).

Discussion:

Elevated plus maze is well established paradigm has a long and successful history

in assessing .anxiety-like behaviour in rodents. The model is based on naturalaversion of rodents for open spaces (afraid possibly of falling off). Rodents tend to avoid the open areas, especially when they are brightly lit, favouring darker, and more enclosed spaces. Inconsistent results with anxiolytic compounds and a desire for more targeted therapeutic treatments suggest that scoring additional, ethologically relevant behavioural indicators (e.g. stretch attend postures and head dips) may provide more sensitive measures of the effects of new anxiolytic compounds. This ethological approach overcome locomotor confounds and has increased the value of plus maze as important tool to study anxiolytic activity. Present study showed that MK attenuated anxiety parameters in the elevated plus maze test. MK administration at the dose (250 mg/kg) significantly and dose dependency increased open arm activity by increasing time spent on and number of entries into open arms while decreased risk assessment behaviour by decreasing number of stretch attend posture and head dips. Anxiolytic activities of MK on Open field behaviour at the dose (250mg/kg) significantly (300 mg/kg) shows maximum time spent was recorded & in Dark and Light model at the dose (500mg/kg) shows number of segments crossed with four paws recorded were comparable to standard drugs diazepam.

In conclusion the aqueous leaf extract of *Murraya koenigii (MK)* showed significant anxiolytic activity. Hence *Murraya koenigii (MK)* may be served as a potential resource for natural psychotherapeutic agent against stress related disorder such as anxiety.

Graphs &Tables:
Elevated Plus Maze Model: (Bogdanski *et al* 1956)
Effect of MK hydroalcoholic extract on plus maze test
Pretreatment with MK did not improve entries in the open arm and time spent in the open arm.
Graph and table showing effect of MK on number of entries in open arm
Graph 1

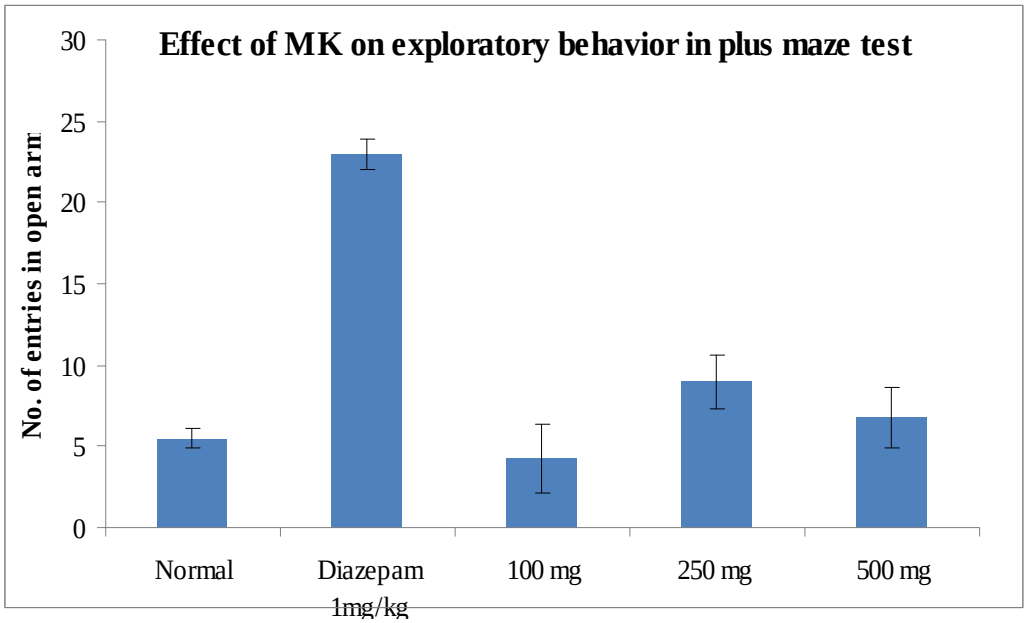

Table No: II

	Normal	**Diazepam 1mg/kg**	**100 mg**	**250 mg**	**500 mg**
No. of entries in open arm	5.5	23**	4.21	8.97	6.78
S.D	0.60	0.90	2.10	1.70	1.90

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test.
Open Field Behavior Test: (Kulkarni *et al* 1999)
Graph and table showing effect of MK on time spent in the open arm
Graph 2

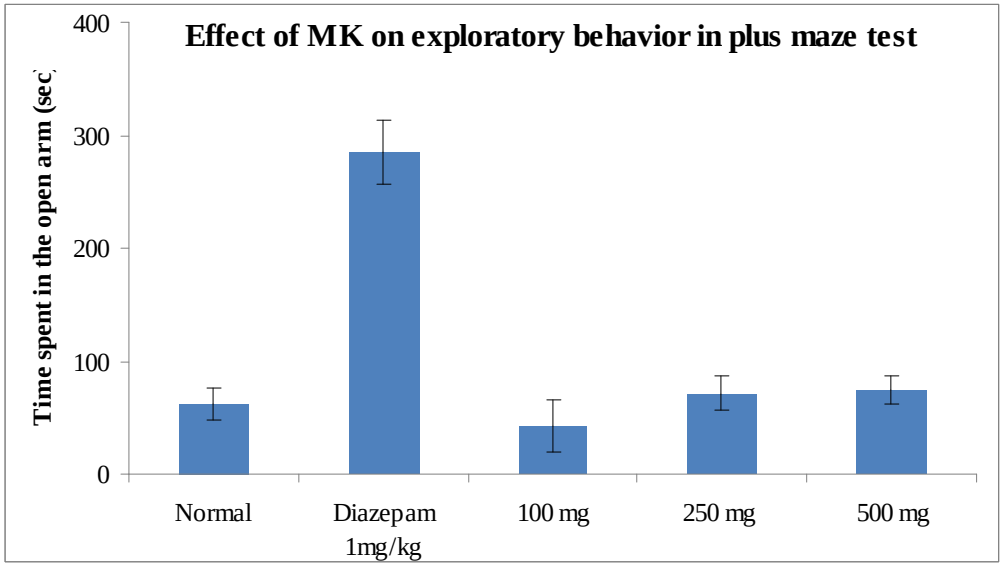

Table No: III

	Normal	Diazepam 1mg/kg	100 mg	250 mg	500 mg
Time spent in the light chamber	61.78	285.3**	42.13	71.44	74.21
S.D	14.56	27.80	23.10	15.40	12.70

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test.

Light Dark Model (Vogel *et al* 1997 **43)**

Effect of MK on light and dark model

Pretreatment with MK extract did not increase number of crossings and time spent in the light chamber as compared to normal control. Therefore MK extract does not possess anxiolytic activity.

Table and graph showing effect of MK on number of crossings in light and dark chamber**.**

Graph 3

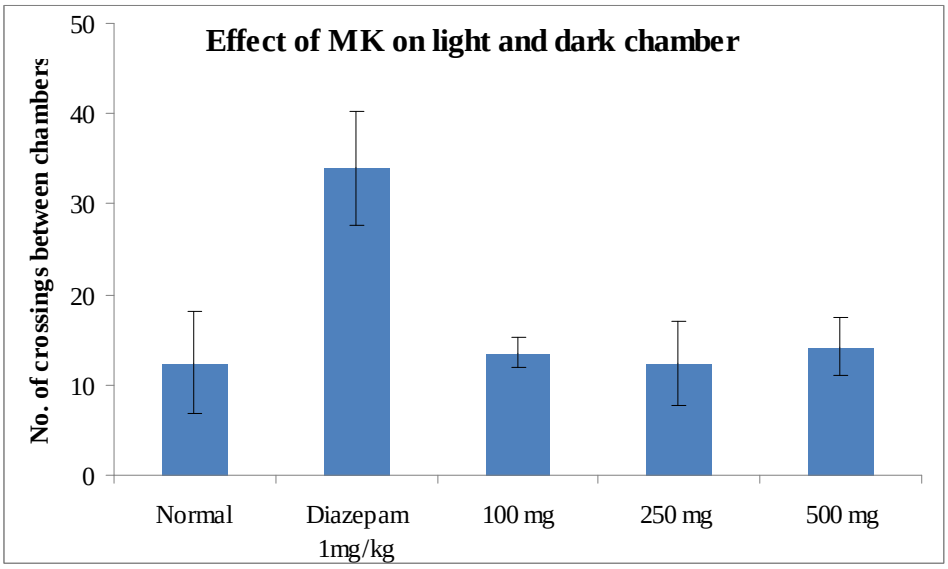

Table No: IV

	Normal	Diazepam 1mg/kg	100 mg	250 mg	500 mg
No. of crossings	12.5	34**	13.56	12.34	14.19
S.D	**5.58**	**6.28**	**1.67**	**4.67**	**3.19**

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test.

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