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By Krishna Prasad. D, S. N.Sri Harsha, D. Yashwanth kumar & K. S. S. N. Neelima
PACIFIC University, India

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Evaluation of Anti Depressant Activity of Murraya Koenigii Leaf Extract in Mice

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Abstract- Murraya koenigii Spreng (Rutaceae), a medicinally important herb of Indian origin, has been Used for centuries in the Ayurvedic System of Medicine. Aqueous extract of the leaves of Murraya 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. In folklore practice, the decoction of M. koenigii leaves has been reported to be useful in diarrhoea. In the present study the effect of hydroalcoholic extract of seeds was evaluated for antidepressant activity in mice. The models selected for the study were tail suspension test and despair swimming test. The extract at the doses of 100mg/kg, 250mg/kg and 500mg/kg significantly reduced the duration of immobility of mice in tail suspension test and despair swimming test as compared to the untreated group. Further, it potentiated the amphetamine induced stereotypic behaviour and enhanced the compulsive gnawing behaviour in mice. In conclusion, our results suggest that Murraya koenigii exerts antidepressant-like effects comparable to those of imipramine in experimental animal models.

Keywords: murraya koenigii (mk), anti depressant activity, aqueous extract, mice.

I. Introduction

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a depressive episode in their lifetime with suicide being one of the most common outcome of depression (WHO 1998, Stahl SM, et.al., 1998, Richelson E, et.al., 2001). Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects (Tripathi KD 2008, Hardman JG, et.al., 2007). Furthermore, it is difficult to predict which patient will respond to any given treatment. It has been reported in earlier studies that only two out of three patients responds to any given antidepressant treatment, and of these, one would probably have responded to placebo alone (Walker R, et.al., 1999). Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon (Sarandol A, et.al., 2007, Ibrahim E, et.al., 2007). Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Tripathi KD, et.al., 2008, Sembulingam K, et.al., 1997). On one hand these agents have a less adverse.

Effect profile, and on the other hand they have been shown to be comparable in efficacy to their synthetic counterparts. As various parts of Murraya koenigii L.are used for the treatments of various diseases so this plant has its own identity identity in medicinal, pharmaceutical and chemical sciences, due to anti-oxidant, anti-microbial antidiarrhea, antidibetic ,antinflammatory, hepatoprotective, hypercholestero-lemic, antiviral , diuretic, carminative properties how promising results in the treatment of diarrhoea. 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 (Kirtikar KR, et.al., 2008, Swaroop VR, et.al., 2011).

Along with the above mentioned properties Murraya also posses the anti dipreseent activity. so, the present study is designed to evaluate the anti depressant activity in mice.

II. Materials and Methods

Dried powdered leaves of Murraya.

a) 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:

1. Aqueous extract by decoction method
2. Methanolic extract by Soxhlet extraction method
3. 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

Authors α σ ρ ω : Pacific College of Pharmacy, PACIFIC University, Udaipur – Rajasthan. e-mail: yashwanth.pharma@gmail.com

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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% ethanol15.

The Etractive yield of Murraya leaf is mentioned in Table No: 1.

b) Phytochemical Evaluation of Murraya Leaf

The qualitative Phytochemical Screening of Various Extracts Obtained from Murraya koenigii Leaves are in Table No: 2

Conclusion: Phytochemical investigations revealed presence of phytosterols in pet ether extract, carbohydrates, glycosides, proteins, amino acids in aqueous extract and alkaloids and phenolic compounds in hydro-alcoholic and methanolic extract.

c) Evaluation of Anti depressant activity

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) - SIP/CPCSEA/IAEC/2012/I/09.

d) Extract and standard drug

The hydroalcoholic extract was formulated as suspension using 0.1% Sodium carboxymethyl cellulose (CMC). Imipramine (Cipla Ltd, Mumbai, India) was used as reference drug. The extract was adjusted to give a fixed volume of 10 ml/ kg orally in doses of 100mg/kg, 250mg/kg and 500mg/kg. Imipramine (Depsonil 25 mg Sarabhai Piramal Pharma Ltd, Vadodara): at a dose of 25 mg/kg was used as a positive standard. Reserpine and apomorphine was purchased from Boehringer Ingelheim BI. D-Amphetamine (Dexamphetamine IP) was purchased from Smith Kline and French, India.

e) Despair Swimming Test

Mice were made to swim individually in a polypropylene vessel (45×40×30 cm) with a water level of 20 cm. This ensured that the mouse's feet did not touch the floor of the vessel and that it could not climb out of it. Each mouse was allowed to swim for 10 min. Thereafter, during the next 10 min, the periods of total immobility, characterized by complete cessation of swimming with the head floating just above water level, was noted. This immobility period, after the initial frenzied attempts to escape, is postulated to represent behavioural despair as an experimental model of endogenous depression (S.K. Bhattacharya et al., 2003).

f) Tail Suspension Test

The method described by N.N. Jain et al. (2003) was used. Mice were divided in groups of six each. They were suspended by tying a thread to their tail from a height of 50 cm above the table top. Duration of immobility was recorded for 10 min. Mice were considered immobile only when they hung passively and remain motionless. Mice were treated with vehicle, MP (100, 250 and 500 mg/kg p.o) 60 min before the test and imipramine (25 mg/kg p.o) 30 min before the test.

g) Enhancement of Amphetamine -Induced Excitation

Imipramine like antidepressant drugs, enhance and prolong the behavioral effects of amphetamine (Turner, 1971). Groups of 6 male mice were treated orally with test drugs and were then challenged 90 minutes later with an i.p. dose of 3mg/kg of d-Amphetamine. Each animal was then scored every 30 minutes for 5 hr post amphetamine treatment according to the following scale: 0 = No activity, 1 = normal activity, 2 = increased motor activity, 3 = stereotyped head searching, 4 = continuous licking. The change of the cumulative total activity score for drug treated mice was calculated by comparison to a control group.

h) Compulsive Gnawing in Mice

Male mice with a body weight between 18 and 20 gm were injected with 10mg/kg apomorphine S.C, 30 minutes, prior to apomorphine injection the animals were treated with the test drug or the vehicle. Immediately after apomorphine injection, 6 mice were placed into a cage with wired lid. The bottom of the cage was covered with corrugated paper, the corrugation facing upwards. The mice started biting into paper causing fine holes or tearing the paper. The number of bites into the corrugated paper was evaluated by placing template upon paper. The template had 10 rectangle windows divided into 10 areas of the same size. In a total of 100 areas the number of bites was checked. In this way percentage of damaged paper was calculated. Percent gnawing of the test compound was compared with that of standard antidepressant drug imipramine, considering its value as 100% (Turner, 1971).

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i) Statistical analysis

The data was analyzed using Prism Graph Pad software and showed as mean±S.D. Comparison between control and drug treated groups were made by one-way analysis of variance (ANOVA) followed by Dunett's test, P values of less than 0.05 were considered to be significant.

III. Results

a) Despair Swimming Test

MP at the doses of 250 and 500mg/kg significantly reduced the immobility time as compared to the untreated animals ($P<0.05$). However, MP 100mg/kg did not show significant reduction in immobility time. The reduction in immobility time was comparable to imipramine 25mg/kg. The observations are given in Graph 1.

b) Tail Suspension Test

The total duration of immobility in vehicle-treated mice was 76.16 ± 6.8 s. Oral administration of MP (100, 250 and 500 mg/kg) significantly reduced the duration of immobility ($P<0.05$). Imipramine (25 mg/kg p.o) also reduced the duration of immobility. The observations are given in Graph 2.

c) Enhancement of Amphetamine -Induced Excitation

MP at the doses of 100, 250 and 500 mg/kg significantly increased amphetamine induced behaviour as evident by the average cumulative scores in mice, as compared to the vehicle treated animals($P<0.05$). The observations are given in Graph 3.

d) Compulsive Gnawing in Mice

MP at the doses of 500 mg/kg significantly increased the average number of bites induced by apomorphine administration as compared to untreated mice ($P<0.05$). The observations are given in Graph 4.

IV. Discussion and Conclusion

Depression is one of the most prevalent and disabling neuropsychiatric disease. The available antidepressant drugs are safe and effective but half of the patient exhibit partial, refractory or intolerant responses to treatment, thus emphasizing the need to discover new antidepressants. Murraya koenigii (MK) is reported to be an aphrodisiac and tonic. Therefore, the present work evaluated the effect of MK in mice models of depression.

The despair swimming test (DST) is the tool most widely used for assessing preclinical antidepressant activity. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the DST, while neuroleptics and anxiolytics produce different effects (Porsolt et al., 1979). It is suggested that mice or rats, forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behavior reflects a state of despair, which can be reduced by antidepressants, which are therapeutically effective in human depression. In our study MK at the doses of 100, 250 and 500mg/kg significantly reduced the immobility time of mice in FST. Further, the MK extract was evaluated in tail suspension test. The "tail suspension test" has been described by Jain et al. (2003) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair, which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail. Oral administration of MK extracts (100, 250 and 500 mg/kg) significantly reduced the duration of immobility ($P<0.05$). The results from the FST and tail suspension test prove the potential antidepressant action of MK hydroalcoholic extract.

MK extract significantly increased the stereotypic behaviour of amphetamine. Further, it also enhanced the apomorphine gnawing behaviour. The compulsive gnawing in mice is induced by apomorphine is due to dopaminergic stimulation. Based on these findings, it can be postulated that the antidepressant effect of MK is possibly due to increase in the neurotransmitters level at the synaptic cleft.

In conclusion, our results suggest that Murraya koenigii leaf extract exerts antidepressant-like effects comparable to those of imipramine in experimental animal models.

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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

Table No : 2

Test/Reagent Used	Hydro alcoholic Extract (70% Ethanol)	Methanolic Extract	Aqueous Extract
Alkaloids			
Mayer's test	+	+	+
Dragendroff's test	+	+	+
Hager's test	+	+	+
Wagner's test	+	+	+
Carbohydrates and Glycosides			
Molisch's test	-	-	+
Fehling's Test	-	-	+
Barfoed's test	-	-	+
Benedicts test	-	-	+
Phytosterols			
Liebermann's Burchard's test	+	+	-
Fixed Oils and Fats			
Spot test	-	-	-
Saponification test	-	-	-
Saponins			

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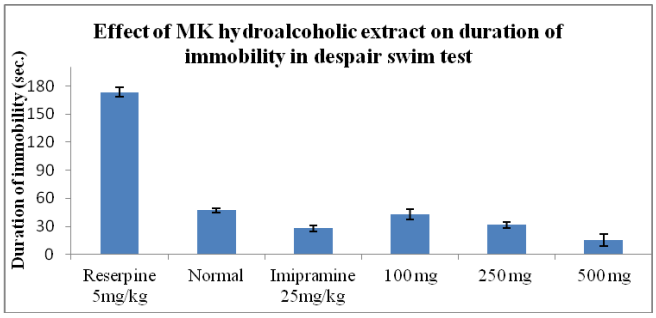
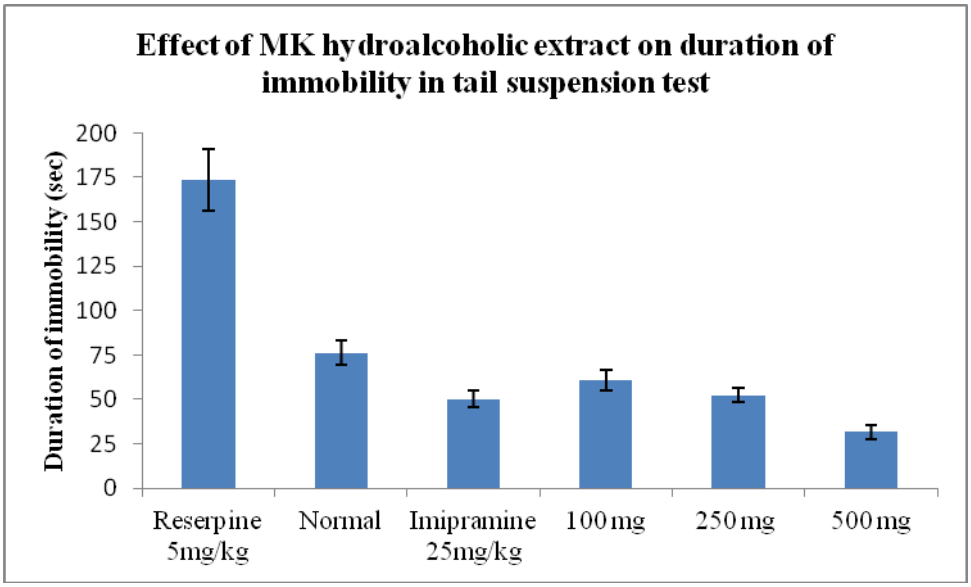
Foam test	-	-	+
Haemolysis test	-	-	+
Phenolic Compounds and Tannins			
Ferric chloride test	+	+	+
Gelatin test	+	+	+
Lead acetate test	+	+	+
Proteins and Amino Acids			
Biuret test	-	-	-
Ninhydrin test	-	+	+
Coumarins			
Fluorescence test	-	-	-

Graph No 1



Graph No 2



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