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EVALUATION OF MURRAYA KOEINIGII LEAVES FOR NEUROPHARMACOLOGICAL ACTIVITY IN MICE

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Abstract:
Researchers are developing drugs to treat many different neurological disorders even though plant based formulations usage is common thing in botanical remedies used universally. The present study is to evaluate pharmacological activity of Murraya koeinigii leaf extract against induced Neuronal disturbances and behavior alterations. Identification and extraction of the Murraya koeinigii plant has been done using aqueous and alcoholic solvents. Preliminary phytochemical studies were performed for chemical components of extracts. The Methanolic, Hydroalcoholic and Aqueous extracts of Murraya koeinigii, had been evaluated for the neuropharmacological action. The dose is determined by toxicity test and optimum safe concentration was 100mg/kg, 200 mg/kg body weight for the extract. Neuropharmacological profile is illustrated by Anxiolytic activity, Antistress, Antiepileptic, Depression induction by tail suspension, forced swim test, compulsive gnawing, Isoniazid induced epilepsy and Restrained stress. The effective doses are MEMK, HEMK, (200mg/kg) and AQMK(100mg/kg) has shown the moderate response. Hydroalcoholic extract is found to be more effective among all the three extracts in different concentrations. Hydroalcohlic fraction showed the presence of more percentage of Alkaloids and glycosides, had showed better neuropharmacololgical behaviour which can be inferred that the content in extracts is responsible for the activity.

Keywords: *Murraya koeinigii, Hydroalcoholic, Neuropharmacological, Anxiolytic, Antistress, Antiepileptic, Depression.*

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INTRODUCTION:

Herbal medicines are an essential and growing part of the International Pharmacopeia. The resurgence of interest in plant remedies has been spurred on by various factors like the effectiveness of plant medicine, direct source for therapeutic agents, it is easily affordable by people, these form the taxonomic markers for discovery of new compounds, these are of renewable source, the high cost; adverse effects; dissatisfaction with the results of most modern drugs and improvement in the quality safety and efficacy of herbal medicine with the development of science and technology. Antioxidants are playing a vital role in the treatment of diseases which are readily available in most of plants [1].

There is abundance in exemplifying the medicinal herbs like *Rauwolfia serpentina* for hypertension, *Catharanthus rosesus* for leukemia, *Papaver somniferum* for arthritis and insomnia *Digitalis* leaves for heart therapy [2]. Catechin is aflavonoid isolated from *Uncaria gambir* showed to reduce the hepatotoxicity. Glycirrizhin a component licorice root (*Glycirrhiza glabra*) has been used in chronic hepatitis and Silymarin a flavonoid found in milk thistle (*Silybum Marianum*) has showed hepatoprotective effects [3]. Quinine, theophylline, morphine, vincristine, cyclosporine are the drugs forming the corner stones of modern pharmaceutical care and they are all natural products. Herbal drugs

are marketed in various forms which are available in classical forms like capsules, lotions, syrups, ointments, creams, granules, etc..,

a. Plant introduction

Murraya Koeinigii belongs to the family of Rutaceac. It is a small tree, growing 4–6 m (13–20 feet) tall, with a trunk up to 40 cm (16 in) diameter. The aromatic leaves are pinnate, with 11-21 leaflets, each leaflet 2–4 cm (0.79–1.57 in) long and 1–2 cm (0.39–0.79 in) broad. The plant produces small white flowers which can self-pollinate to produce small shiny-black berries containing a single, large viable seed. Though the berry pulp is edible with a sweet but medicinal flavor in general, neither the pulp nor seed are used for culinary purposes.

The leaves are highly valued as seasoning in southern and west-coast Indian cooking, and Sri Lankan cooking, especially in curries, usually fried along with the chopped onion in the first stage of the preparation. They are also used to make thoran, vada, rasam and kadhi. In their fresh form, they have a short shelf life and do not keep well in the refrigerator. They are also available dried, though the aroma is largely inferior.

The leaves of *Murraya koenigii* are also used as an herb in Ayurvedic medicine. They are believed to possess anti-diabetic properties [4,5]. Some of the primary alkaloids found in the *Murraya koenigii* leaves, stems, and seeds are: Mahanimbine, girinimbine, koenimbine, isomahanine, mahanine, Undecalactone, 2-methoxy-3-methyl-carbazole [6]. A study on girinimbine, a carbazolealkaloid isolated from this plant, found that it inhibited the growth and induced apoptosis in human hepatocellular carcinoma, HepG2 cells *in vitro* [7].

Fresh leaves, dried leaf powder, and essential oil are widely used for flavouring soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations. The essential oil is also utilized by soap and cosmetic aromatherapy industry [8]. Curry leaves are boiled with coconut oil till they are reduced to blanked residue which is then used as an excellent hair tonic for retaining natural hair tone and stimulating hair growth. It is traditionally used as a whole or in parts as antiemetics, antidiarrheal, febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [9].

EXPERIMENTAL:

Collection Of Material

The leaves of *Murraya koeinigii* were collected from local region and were authenticated by taxonomists of Sri Venkateswara University, Tirupathi, A.P., India.

The voucher specimen (MK-E1) of the plant has been kept in the laboratory for future reference.

Extraction Method

The dried and powdered plants were defatted with petroleum ether (60-80°C) and the following extracts were prepared.

Aqueous extract by decoction method

Methanolic extract by Soxhlet extraction method

Hydroalcoholic extract (70% ethanol) by Soxhlet extraction method

(i) ***Decoction method:***

The powdered leaves were extracted with water at at 80°C and extraction was continued for 1.5 hour. The extract so obtained was dried at 50°C in hot air oven and completely dried extract was used for further studies.

(ii) ***Soxhlet extraction:***

Continuous hot extraction was used for the extraction of dried powdered leaves. The dried, powdered leaves were extracted with the help of Soxhlet apparatus using different solvents, starting with petroleum ether (60-80°C) followed by methanol. Hydroalcoholic extract was prepared by extracting with 50% methanol water/70% ethanol water mixture. Per batch, 100 g. of powder was wrapped in filter paper and placed in the Soxhlet apparatus with the respective solvent for extraction. The extract so obtained was concentrated by solvent recovery, dried completely using hot air

oven (50°C) and the powdered drug was stored in a desiccator. The extracts so obtained were dissolved in suitable vehicle for carrying out further experiments [10].

Phytochemical screening

The Hydro-alcoholic extract of *Murraya Koeinigii* was screened for the presence of various Phytochemical constituents like Carbohydrates, alkaloids, Tannins, steroids, Glycosides, Saponins and proteins and amino acids [11], and later the extracts of *Murraya Koeinigii* is used for further evaluation.

Materials

Chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Ranbaxy Laboratories Ltd. India), Phenobarbitone sodium (Rhone-Poulenc India Limited, India), Diethyl ether and all other chemicals of highest available purity were obtained from Merck, Mumbai, India. Diazepam 2mg (Natco pharma Ltd) was used as the standard sedative, and anxiolytic drug, Tween-80 used as vehicle Piracetam 500mg (Micro labs), Scopolamine hydrochloride(Sigma Aldrich Bangalore), flouxetine and Glass Distilled water.

Experimental Animals

Adult male albino Wister rats weighing 140-200g (4-8 weeks) were used for the study acquired from MAHAVEER ENTERPRISES. They were housed in polypropylene cages and were maintained at room temperature of 23^{o}C ± 2^{o}C and relative humidity 50%. They were maintained in 12hr: 12hr light: dark cycle throughout the period of acclimatization and experimental study. All the study protocols were

reviewed and approved by Institutional Animal Ethical Committee (IAEC).

Acute Toxicity Study

The Acute Toxicity Studies was performed using female rats as per OECD Guideline No. 423 (2000) (short term toxicity). The median lethal dose of the pet-ether alcohol and aqueous were determined by orally administering the extracts in increasing dose levels of 0.1,0.2,0.5, 1, 1.5 and 2 g/kg body weight to healthy adult albino rats of either sex. The animals will be observed continuously for 2 hrs under the following profiles:

I. Behavioural profile: Alertness, restlessness, irritability and fearfulness.

II. Neurological profile: Spontaneous activity, reactivity, touches response, pain response and gait.

III. Autonomic profile: Defecation and urination. After a period of 24 h they will be observed for any lethality or death (% of mortality) [12,13].

Pharmacological Activity

a. Animal grouping and treatment

For the following activities the animals divided into eleven groups, each group containing six animals except general behavioral study.

Group I for control,

Group II for standard,

Group III, IV and V for methanolic extract ME (50,100 and 200mg/kg),

Group VI, VII and VIII for hydroalcoholic extract HE (50,100 and200 mg/kg),

Group IX, X and XI for aqueous extract AQ (50,100 and 200 mg/kg) respectively.

The extracts of *Murraya Koeinigii* (*MK*) termed as MEMK, HEMK and AQMK.

In vivo methods

Forced Swim Test (FST):

Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25^{o}C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the above table [14].

Tail Suspension Test (TST):

The total duration of immobility induced by tail suspension was measured. Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm. from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug [14]

Compulsive Gnawing:

Mice were given amitriptyline (5 or 10 mg/kg I.P.) or imipramine (20 or 40 mg/kg i.P.).Fifteen minutes later apomorphine was injected subcutaneously (10 mg/kg). The animals were placed in cages, two mice in each cage, for one hour. A cage consists of a 30 cm high box, 12x25 cm, without bottom and lid. The cages were placed on corrugated paper. If compulsion to gnaw occurred, the mice would begin to bite the paper within 5-10 min. The gnawing intensity was estimated, Five groups, each consisting of two mice, were used at each dose level.The gnaw-compulsion syndrome was studied in mice pretreated with a-methyl-L-tyrosine (a-MT, 50 or 100 mg/kg i.P. 4 hr before test). Both doses of a-MT significantly reduced the gnawing intensities. (-)-DOPA (200 mg/kg i.P. 1 hr before testing) completely reactivated the mice pretreated with a-MT.

Furthermore it was shown that pretreatment with sodium diethyl dithiocarbamate (three doses of 500 mg/kg i.P., 18, 6 and 3 hr before testing) hardly affected the gnawing intensities, although the animals were markedly sedated [15].

Isoniazid Induced Epilepsy:

INH is administered Intra Peritoneal to the animals and seizure induction is observed in a span of 2 minutes. INH is thought to cause seizures by interfering with g-aminobutyric acid syntheses [16]. Specifically, INH inhibits glutamic acid decarboxylase7 by inhibiting pyridoxal 5 phosphate, a co-factor for glutamic acid decarboxylase enzyme. The consequent reduction in GABA level increases the susceptibility to seizures. Thus, neurologic effects of isoniazid are specifically countered by administration of pyridoxine [17].

Restraint stress (RS) model:

After 18 h fasting (food deprivation) of rats, one stress session consisting of a 2.5 h immobilization period inside the cylindrical steel tube (7cm diameter, 17.5 cm long, with holes for ventilation) at room temperature was performed during the early phase of the light cycle (07:00 to 09:30 h) and after 1 h the animals were sacrificed [18].

RESULTS:

Percentage of Yield

The yield after the extraction of the plant leaves for *Murraya Koeinigii* with methanol, hydro alcohol, aqueous showed more yield in methanol (ME), Hydro alcohol (HE) than Aqueous (AQE), summarized in Table no. 1

Table.1 Percentage yield of the extracts

S. No	Extract/ Fraction	% of Yield
1.	MEMK	43.12
2.	HEMK	45.58
3.	AQMK	16.40

Yield of extracts calculated with respect to the raw material used and for fractions was with respect to the corresponding alcoholic extract used for fractionation.

Phytochemical Screening Methods

In identification of Alkaloids, Phytosterols, tannins, had showed positive results for *Murraya Koeinigii*, for methanolic and hydroalcoholic extracts and aqueous extract had showed positive results for Alkaloids, Carbohydrates, Glycoside tannins, saponins but negative results for phytosterols. The aqueous extract has showed identification of Alkaloids, Carbohydrates and Glycoside components which may be due to its solubility.

Table 2: Phytochemical screening of the extracts

Test/Reagent Used	HE(70% Ethanol)	ME	AQE
Alkaloids			
Mayer's test	+	+	+
Dragendroff's test	+	+	+
Hager's test	+	+	+
Wagner's test	+	+	+
Carbohydrates and Glycosides			
Molisch's test	-	-	+
Fehling's Test	-	-	+
Phytosterols			
Liebermann's Burchard's test	+	+	-
Fixed Oils and Fats			
Saponification test	-	-	-
Saponins			
Foam test	-	-	+
Phenolic Compounds and Tannins			
Ferric chloride test	+	+	+
Proteins and Amino Acids			
Biuret test	-	-	-

Acute Toxicity Study

The oral acute toxicity study in mice was performed as per the OECD guidelines **(No 423)** to evaluate the undesirable effects or toxicity of MK leaf extracts. Swiss Albino rats either Male or Female of weight 140-180 g are used for the test.

Mortality, and Signs and Symptoms of Toxicity:

Leaf extracts were found to be safe till a dose of 5000 mg/kg since no mortality and abnormal toxicity was observed at this dose. Animals receiving the mentioned doses did not produce any significant changes in behavioural pattern and failed to elicit any clinical abnormality.

Effect of *Murraya Koeinigii* leaves extracts on Anti depressant Forced swim test

Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25oC. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the below table.

MEMK (200mg/kg), HEMK (200mg/kg) had illustrated the maximum response and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate response, in comparision to the standard drug.

Table 3: Effect of *Murraya Koeinigii* extracts on Anti depressant Forced swim test

S.No	Treatment	Dose	Immobility period (secs) Pre treatment	Posttreatment	
				7 Days	14Days
1	2%Tween80	10 ml/kg	150.2±9.3	144.0±11.2	146.3±9.7
2	MEMK	50mg/kg	140.3±15.2	138.6±11.5*	130.4±12.4*
3		100mg/kg	153.1±12.8	140.4±9.4*	132.5±10.6*
4		200mg/kg	157.2±10.5	138.4±11.8*#	120.3±9.6*#
5	HEMK	50 mg/kg	160.2±11.4	152.6±10.6*	148.2±20.6*
6		100mg/kg	158.0±13.2	149.2±16.5*	130.2±10.8*
7		200mg/kg	164.2±10.2	138.2±12.5*#	125.2±12.1*#
8	AQMK	50 mg/kg	156.2±13.1	140.9±10.5*	138.3±15.3*
9		100mg/kg	158.5±12.6	150.2±9.7*	142.3±13.0*
10		200mg/kg	165.4±10.3	157.2±9.6*	140.2±10.4*
11	Fluoxetine	20 mg/kg	169.1±10.1	132.3±9.3*	119.2±10.6*

Values are expressed as (Mean ± SD), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnet test

Effect of *Murraya Koeinigii* leaves extracts on Anti depressant Tail suspension test

The total duration of immobility induced by tail suspension was measured Immobility time was recorded during a 6 min period. Changes in the immobility duration were studied after administering. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

MEMK (200mg/kg), HEMK (200mg/kg) had showed the maximum response and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate response, in comparision to the standard drug.

Table 4: Effect of *Murraya Koeinigii* extracts on Anti depressant Tail suspension test

G.No	Treatment	Dose	Immobility period (secs) Pre treatment	Posttreatment 7 Days	14Days
1	2%Tween 80	10 ml/kg	154.2±10.2	149.0±10.2	146.3±10.7
2	MEMK	50mg/kg	162.3±12.5	157.5±10.5*	150.2±11.8*
3		100mg/kg	159.1±10.8	143.1±11.9*	131.2±10.5*
4		200mg/kg	160.3±15.9	138.2±10.6*#	123.1±12.4*#
5	HEMK	50 mg/kg	160.8±12.6	157.4±16.5*	149±12.3*
6		100mg/kg	152.5±12.6	141.5±12.8*	130.3±5.5*
7		200mg/kg	164.5±13.1	142.5±11.0*#	120.4±11.5*#
8	AQMK	50 mg/kg	158.4±15.2	149.5±13.1*	140.1±11.3*
9		100mg/kg	159.2±16.0	152.3±10.8*	147.2±12.0*
10		200mg/kg	158.2±10.4	146.2±9.1*	140.1±11.5*
11	Fluoxetine	20 mg/kg	163.1±10.4	122.3±10.2*	111.2±10.3*

Values are expressed as (Mean ± SD), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnet test

Effect of *Murraya Koeinigii* leaves extracts on Compulsive Gnawing

The cages were placed on corrugated paper. If compulsion to gnaw occurred, the mice would begin to bite the paper within 5-10 min. The gnawing intensity was estimated. Very high gnawing intensities were obtained with the tricyclic antidepressants. MEMK (200mg/kg), HEMK (200mg/kg) had showed the maximum Gnawing intensity and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate Gnawing intensity, in comparision to the standard drug.

Table 5: Effect of *Murraya Koeinigii* extracts on Compulsive Gnawing

G.No	Treatment	Dose	Gnawing intensity(%)
1	2%Tween 80	10 ml/kg	0
2	MEMK	50mg/kg	16.7*
3		100mg/kg	50.0*
4		200mg/kg	83.4*#
5	HEMK	50 mg/kg	33.4*
6		100mg/kg	83.4*
7		200mg/kg	100*#
8	AQMK	50 mg/kg	16.7*
9		100mg/kg	33.4*
10		200mg/kg	50.0*
11	FLouxetine	20 mg/kg	100*

Values are expressed as (Mean ± SD), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnet test

Effect of *Murraya Koeinigii* leaves extracts on INH induced Epilepsy

INH often produces seizures that are usually refractory to anticonvulsant therapy and causes neurological side effects, including peripheral neuritis, dizziness, and insomnia. MEMK (200mg/kg), HEMK (200mg/kg) had showed the maximum against the INH iduced seizures and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate protection, in comparision to the standard drug

Table 6: Effect of *Murraya Koeinigii* extracts on INH induced Epilepsy

G.No	Treatment	Dose	Reduction in Seizure occurrence (%)
1	2%Tween80	10 ml/kg	0.0
2	MEMK	50mg/kg	16.7*
3		100mg/kg	33.4*
4		200mg/kg	66.7*
5	HEMK	50 mg/kg	33.4*
6		100mg/kg	50.0*
7		200mg/kg	83.4*#
8	AQMK	50 mg/kg	16.7*
9		100mg/kg	33.4*
10		200mg/kg	50.0*
11	Phenytoin	50 mg/kg	100.0*

Values are expressed as (Mean ± SD), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnet test

Effect of *Murraya Koeinigii* leaves extracts on restrained stress

The stress session consisting of a 2.5 h immobilization period inside the cylindrical steel tube (7cm diameter, 17.5 cm long, with holes for ventilation) at room temperature was performed during the early phase of the light cycle and after 1 h the animals were sacrificed.

The levels of corticosterone were measured and MEMK (200mg/kg), HEMK (200mg/kg) had showed the maximum response against restrained stress and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate response, in comparision to the standard drug

Table 7: Effect of *Murraya Koeinigii* extracts on Restrained stress

G.No	Treatment	Dose	Levels of corticosterone
1	2%Tween 80	10 ml/kg	100.6±6.3
2	MEMK	50mg/kg	85.3±7.5*
3		100mg/kg	72.3±9.8*
4		200mg/kg	66.3±4.5*#
5	HEMK	50 mg/kg	82.4±5.6*
6		100mg/kg	70.3±5.9*
7		200mg/kg	58±.3.7*#
8	AQMK	50 mg/kg	80.2±6.2*
9		100mg/kg	75.1±9.2*
10		200mg/kg	70.5±6.7*#
11	Melatonin	20 mg/kg	69.3±7.1

Values are expressed as (Mean ± SD), n= 6, All groups were compared with Normal control group *p<0.05 and standard group and significance shown by #p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnet test

SUMMMARY AND CONCLUSION:

The leaves of *Murraya Koeinigii were* extracted and the percentage yield of the methanolic extracts and hydro alcoholic extracts were considerable good compared to that of aqueous extract. The phytochemical study revealed that the presence of Alkaloids, Phytosterols, tannins, had showed positive results for *Murraya Koeinigii*, for methanolic and hydro alcoholic extracts and aqueous extract had showed positive results for Alkaloids, Carbohydrates, Glycoside Tannins, Saponins but negative results for phytosterols.

The leaves were found to be safe till a dose of 5000 mg/kg since no mortality and abnormality was observed at this dose. Animals receiving the mentioned doses did not produce any significant changes in behavioural pattern and failed to elicit any clinical abnormality.

According to OECD guidelines, $1/10^{th}$ of maximal safe dose can be selected for the study. Hence three doses of MK [ME, HE and AQE] were selected for the study: 50 mg/kg, 100 mg/kg and 200 mg/kg. The results indicate that the extract influences anti-anxiety response similar to that observed at 2 mg/kg of diazepam.

MEMK, HEMK and HEMK (200mg/kg) had showed the maximum protection against the INH induced seizures and extracts MEMK, HEMK (100mg/kg) had showed moderate protection, in comparision to the standard drug.

MEMK (200mg/kg), HEMK (200mg/kg) had showed the maximum Gnawing intensity and extracts MEMK (100mg/kg), HEMK (100mg/kg), AQMK (100mg/kg) had showed moderate Gnawing intensity, in comparision to the standard drug.

There are studies that indicate that ‘‘stress’’ is one of the factors leading to cognitive deficits, anxiety and peptic ulcers. Prolonged stress immobilization, extreme heat, cold and other stressors are associated with neuron cell degeneration in the hippocampal and other areas of the brain.[19]

The levels of corticosterone were measured and MEMK, HEMK (200mg/kg) had showed the maximum response against restrained stress and extracts MEMK, HEMK (100mg/kg) had showed moderate response, in comparision to the standard drug.

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