
EVALUATION OF *MURRAYA KOEINIGII* LEAVES FOR PRELIMINARY NEUROPHARMACOLOGICAL ACTIVITY *INVITRO* AND *INVIVO* IN MICE

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ABSTRACT

The desire to capture the wisdom of traditional healing method has led to a resurgence of present study to evaluate pharmacological activity of Murrayakoeinigii leaf extract against induced Neuronal disturbances and Behavioural alterations. Identification and extraction of the Murrayakoeinigiiplant 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 Murrayakoeinigii, 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 in-vitroestimation of antioxidant activityand invivo Anxiolytic activity by General behavioral tests, Motor coordinationactivityandLocomotor activity.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: *Murrayakoeinigii, Hydroalcoholic, Neuropharmacological, Anxiolytic, Antioxidant.*

1. INTRODUCTION

Herbal medicine is the branch of science dealing with the plant based formulations usage in the alleviating the diseases. Biodiversities in natural resources like plants, animals, microbes, marine source has served the needs for human and also useful in healthcare from immemorial times. In every era of ages there are different systems of medicine but the common thing is botanical remedies used universally. The green pharmacy from the people of preliterate societies has surprised the civilized societies with compendiums of healing herbs and this herbal healing lore was passed from generation to generation by word of mouth.

India is a varietal emporium of medicinal plants and is the richest resource of medicinal plants. The traditional system of medicine like Ayurveda, Unani and Siddha used over hundreds of plant species for combating human ailments. Ayurveda is a system of ethno medicine that takes in to consideration of the physical, psychological, philosophical and ethical well being of the human and shows great importance on living in harmony with the universe. The old definitive text "CharakSamhitha", specifies on the complete treatment of diseases using herbs. The importance of medicinal plants and traditional systems of medicine in solving the health care problems of the world is increased nowadays. Most of the developing countries have adopted traditional medical practice due to its advantage over the available systems.[1]

Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion as well as for enhancement of the span and quality of life. However, there is a lack of a systematic approach to assess their safety and effectiveness. The holistic approach to health care makes herbal medicine very attractive to many people, but it also makes scientific evaluation very challenging because so many factors must be taken into account. Herbal medicines are in widespread use and although many believe herbal medicines are safe, they are often used in combination and are drawn from plant sources with their own variability in species, growing conditions, and biologically active constituents. Herbal extracts may be contaminated, adulterated, and may contain toxic compounds.[2] But, there is little data on the composition and quality of most herbal medicines not only due to lack of adequate policies or government requirements but also due to a lack of adequate or accepted research methodology for

evaluating traditional medicines.[3] In addition, there is very little research on whole herbal mixtures because the drug approval process does not accommodate undifferentiated mixtures of natural chemicals. To isolate each active ingredient from each herb would be immensely time-consuming at a high cost, making it not cost-effective for manufacturers.[4]

1.1 Herbs used for anxiety

Lavender is a herb with properties that is excellent for treating panic and anxiety. It affects the central nervous system in much the same way as some drugs without the negative side effects.

Passion flower can help in high blood pressure and when used as herbs for anxiety it can be put in tea or food. It is also an ingredient in many herbal remedies.

Ginseng has long been used for anxiety and is a natural immune booster. The Chinese have known this for a long time.

Cannabis sativa is usually smoked but can be eaten and is a great anxiety reliever. Many are aware of this and people worldwide are abusing this remedy.

Valerian is used throughout the world as a natural sedative and helps with insomnia and panic attacks. It is also a mild painkiller and is considered very safe for short term use

Kava kava is a root used for anxiety and is also well known in the treatment of sleep disorders such as insomnia.

Lemon balm is good for headaches and also for relieving stress and anxiety. It is a natural sedative and is good for easing tension. Chamomille is put into tea and has been a highly touted herb for anxiety.[5, 6, 7]

1.2 Plant introduction

MurrayaKoeinigii 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 *Murrayakoenigii* are also used as an herb in Ayurvedic medicine. They are believed to possess anti-diabetic properties.[8, 9] Some of the primary alkaloids found in the *Murrayakoenigii* leaves, stems, and seeds are: Mahanimbine, girinimbine, koenimbine, isomahanine, mahanine, Undecalactone, 2-methoxy-3-methyl-carbazole.[10]A study ongirinimbine, a carbazolealkaloid isolated from this plant, found that it inhibited the growth and induced apoptosis in human hepatocellular carcinoma, HepG2 cells *in vitro*.[11]

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.[12] 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.[13]

2. EXPERIMENTAL

2.1 Collection Of Material

The leaves of *Murrayakoeinigii*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.

2.2 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.[14]

2.3 Phytochemical screening

The Hydro-alcoholic extract of *MurrayaKoeinigii*was screened for the presence of various Phytochemical constituents like Carbohydrates, alkaloids, Tannins, steroids, Glycosides, Saponins and proteins and amino acids[15] and later the extracts of *MurrayaKoeinigii*is used for further evaluation.

2.4 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.

2.5 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 $23^{o}C \pm 2^{o}C$ 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).

2.6 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).[15, 16]

3. PHARMACOLOGICAL ACTIVITY

3.1 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 *MurrayaKoeinigii*(*MK*) termed as MEMK, HEMK and AQMK.

3.2 In-Vitro method of estimation of antioxidant activity

DPPH scavenging activity procedure:

DPPH radical scavenging activity was measured using the method of Cotelle et al. with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 μM in methanol) 2.8 ml of test solution, at various concentrations (5, 10, 20, 40, 80, 160 320 μg/ml) of the synthetic compound was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the following equation:

$$\%\text{DPPH radical scavenged} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100$$

IC_{50} will obtain from a plot between concentration of test compounds and % scavenging. Ascorbic acid is used as standard for comparison.

3.3 In vivo methods

3.3.1 General behavioral tests:

Swiss albino mice were divided into five groups (6 in each group). The first three groups were treated with *MK*and*NN*leaves extract at different doses (50, 100 and 200mg/kg p.o), the fourth group was treated with control vehicle 2% tween 80 (10ml/kg p.o) and the fifth group received Diazepam (DZP, 2mg/kg p.o.) which served as standard drug. The activities were recorded at 30 min intervals in the first hour and at hourly intervals for the next 4 hrs for the following parameters.

Spontaneous activity, awareness and alertness were evaluated by placing a mouse in a bell jar. By this property of mice Sound responses was evaluated. Touch responses was noted when the animal was touched with a forceps (or) pencil at various parts (i.e. on the side of the neck, on the abdomen and on the groin).Pain response was graded when a small artery clamp was attached to the base of tail.

3.3.2 Motor coordination activity:

It was recorded in five groups of (n=6) to the integrity of motor co-ordination was assessed with a rota-rod apparatus at a rotating speed of 8 rpm, by counting the number of falls from the rod in 3 min after 30, 60 and 120 min of treatments. The animals were placed on a rotating bar (2.5 cm diameter). Unaclimatised mice were able to remain on the rod for 3 or more min in two successive trials was selected for testing. 5 Groups of six mice in each were treated with NnG extract at doses 50mg/kg, 100mg/kg and 200mg/kg p.o. and 2% tween 80(10 ml/kg p.o.), Diazepam (2mg/kg i.p).

3.3.3 Locomotor activity:

The locomotor activity was measured using an act photometer (IM CORP; Ambala, India).

The movement the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. Each mouse was placed individually in the actophtometer for 10 min and basal activity score was obtained. Subsequently, the animals were divided into groups, each consisting of six animals. *MK*and*NN*leaves (50, 100, and 200mg/kg), 2% Tween 80, diazepam (2mg/kg, i.p) was administered and after 30 min the mice were placed again in the actophotometer for recording the

activity scoring. The locomotor activity count was expressed in terms of total photo beam counts/ 10 min per group.[18]

4. RESULTS:

4.1 Percentage of Yield

The yield after the extraction of the plant leaves for *MurrayaKoeinigii*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.

4.2 Phytochemical Screening Methods

In identification of Alkaloids, Phytosterols, tannins,had showed positive results for *MurrayaKoeinigii*, 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	-	-	-

4.3 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.

4.4 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.

4.5*Effect of MurrayaKoeinigii*leaves extracts on DDPH radical scavenging activity

The test compounds have been reported to show high scavenging activity against the DPPH free radical generating system. The antiradical activity of test compound and ascorbic acid against DPPH was shown in Table and the IC_{50} values were found to increased with respectively concentrations with that of reference standard, ascorbic acid.

The results clearly indicate the free radical scavenging activity of test compound in vitro and this activity comparable with that of standard drug ascorbic acid.

Table 3*In-Vitro* method of antioxidant activity for *MurrayaKoeinigii*leaves extracts

Conc(µg/ml)	Percentage inhibition of DPPH radical (IC_{50})			
	MEMK	HEMK	AQMK	Std
5	18.2±0.81	18.8±0.71	15.5±0.51	47.6±0.48
10	26.7±0.51	25.8+0.24	22.6±0.82	56.15±0.65
20	34.2±1.15	33.5±1.52	31.8±0.98	65.6±0.48

40	39.3±0.47	38.4±0.54	35.4±0.32	70±1.33
80	44.6±0.77	43.5±0.62	40.8±0.49	77.8±0.82
160	49.2±0.68*	48.2±0.91*	45.3±0.47	84.9±1.1*
320	53.5±0.90*	52.4±0.12*	50.2±0.78	89.1±0.51*

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

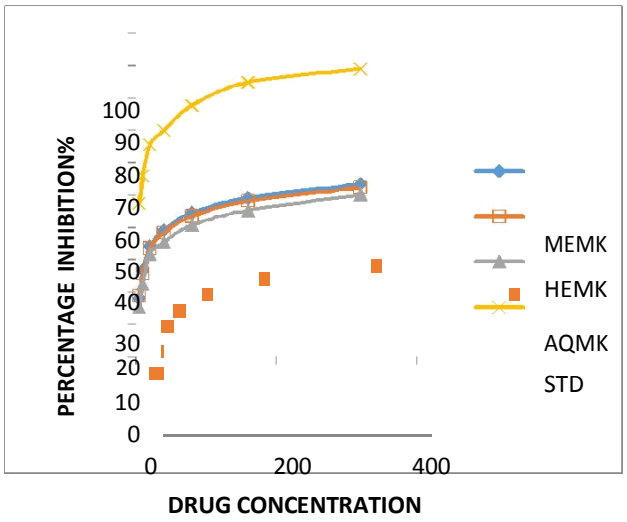

Fig: 1. In vitro concentration dependent percentage inhibition of DPPH radical by test compounds and ascorbic acid

4.6 Effect of *MurrayaKoeinigii*leaves extracts on General behaviour

Spontaneous activity, awareness and alertness were evaluated by placing a mouse in a bell jar. It usually shows a moderate degree of inquisitive behaviour. Touch responses was noted when the animal was touched with a forceps, Pain response was graded when a small artery clamp was attached to the base of tail. MEMK (200mg/kg), HEMK(200mg/kg) had showed the maximum response of behaviour and MEMK(100mg/kg), HEMK(100mg/kg), AQMK(100mg/kg) had depicted moderate responses.

Table 4. *In-Vivo* method of General behaviour activity for *MurrayaKoeinigii*leaves extracts

S.No	Treatment	Dose	spontaneousity	Alertness	Awareness	Touch pain, response
1	20% Tween 80	10Mg/Kg	-	-	-	-
2	MEMK	50Mg/Kg	++	++	++	++
		100Mg/Kg	+++	+++	+++	+++
		200Mg/Kg	++++	++++	++++	++++
3	HEMK	50Mg/Kg	++	++	++	++
		100Mg/Kg	+++	+++	+++	+++
		200Mg/Kg	++++	++++	++++	++++
4	AQMK	50Mg/Kg	-	-	-	-
		100Mg/Kg	++	++	++	++
		200Mg/Kg	+++	+++	+++	+++
9	Diazepam	2Mg/Kg	++++	++++	++++	++++

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

4.7 Effect of *MurrayaKoeinigii*leaves extracts on Motor coordination activity

The integrity of motor co-ordination was assessed with a rota-rod apparatus at a rotating speed of 8 rpm, by counting the number of falls from the rod. The difference in the fall off time from the rotating rod between the control and the treated rats (Diazepam/extract) was taken as an index of muscle relaxation. MEMK (200mg/kg), HEMK(200mg/kg) had illustrated the maximum response of motor coordination.

Table 5 Effect of *MurrayaKoeinigii*leaves extracts on Motor coordination activity

G.No	Treatment	Dose	Falling time		
			30min	60min	120 min
1	2%Tween8010	ml/kg	0.0	0.0	0.0
2	MEMK	50mg/kg	0.5± 0.02*	0.70± 0.03*	0.52± 0.01*
3		100mg/kg	0.8± 0.06*	1.2± 0.1*#	0.6± 0.05*
4		200mg/kg	1.02± 0.11*#	1.8± 0.09*#	0.63± 0.02*
5	HEMK	50 mg/kg	0.45± 0.03*	0.6± 0.02*	0.4± 0.03*
6		100mg/kg	0.71± 0.02*	0.9± 0.04*	0.6± 0.02*
7		200mg/kg	1.1± 0.1*#	1.9± 0.1*#	1.2± 0.09*#
8	AQMK	50 mg/kg	0.4± 0.01*	0.6± 0.01*	0.3± 0.02*
9		100mg/kg	0.5± 0.02	0.7± 0.02	0.4± 0.02*
10		200mg/kg	0.6± 0.01*	0.7± 0.01*	0.5± 0.02*
11	Diazepam	2 mg/kg	0.36± 0.1*	0.63± 0.2*	0.25± 0.2*

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

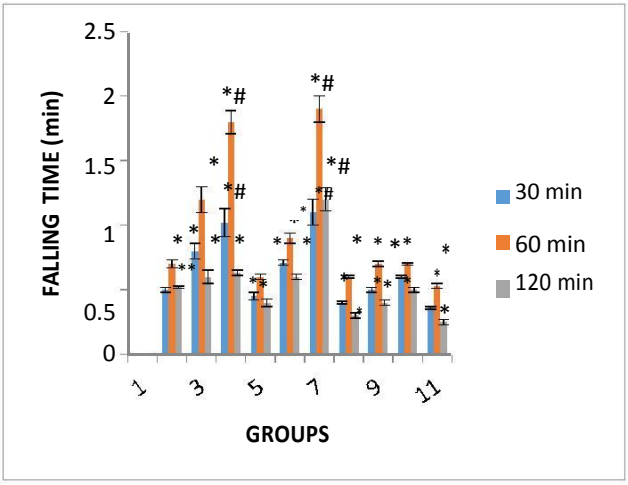

Fig: 2. *In-Vivo* method of motor coordination activity of test compounds in comparision to standard

4.8 Effect of *MurrayaKoeinigii*leaves extracts on Locomotor activity

The locomotor activity of each mouse was recorded individually for 10 min using actophotometer, which enables movement of the animal across a light beam to be recorded as a locomotion count. It demonstrates a CNS activity profile. MEMK (200mg/kg), HEMK (200mg/kg) had illustrated the maximum response of locomotory stimulating response.

The extracts MEMK (100mg/kg), HEMK(100mg/kg), AQMK(100mg/kg) had showed moderate stimulatory activity.

Table 6 Effect of *MurrayaKoeinigii*extracts on locomotor activity

G.No	Treatment	Dose	Counts/10min	
			Basal-activity	After dosing
1	2%Tween80	10 ml/kg	102.2±9.7	90.5±11.2
2	MEMK	50mg/kg	90.2±5.1	117.1±6.2*
3		100mg/kg	101.1±11.0	131±9.2*
4		200mg/kg	92.4±5.3	139.8±6.4*#
5	HEMK	50 mg/kg	100.2±4.8	121.1±5.6*
6		100mg/kg	103.5±10.4	132.4±9.8*
7		200mg/kg	97.5±5.2	143.6±8.4*#
8	AQMK	50 mg/kg	112.0±7.3	133.3±9.2*
9		100mg/kg	102.3±6.8	122.2±11.9*

10		**200mg/kg**	107.3±8.2	124.6±9.6*
11	**Diazepam**	**2 mg/kg**	125.4± 10.2	85.3± 6.8*

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

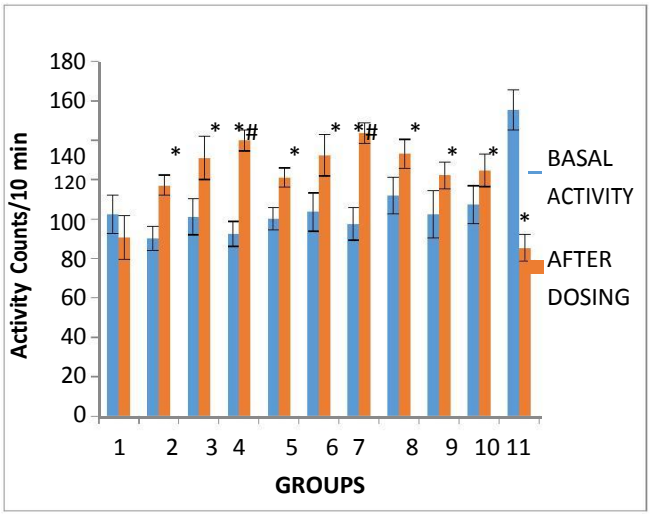

Fig: 3. *In-Vivo* method of locomotor activity using test compounds in comparision to standard

5. Summary

The plant *Murraya*used for the study was 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, hytosterols, annins, had showed positive results for *MurrayaKoeinigii*, for methanolic and hydro alcoholic extracts and aqueous extract had showed positive results for Alkaloids, Carbohydrates, Glycoside tannins,saponins but negative results for phytosterols.

According to OECD guidelines, 1/10th of maximal safe dose can be selected for the study. Hence three doses of MK [ME, HE and AQE] were selected for the study: 100 mg/kg, 250 mg/kg and 500 mg/kg.

The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.

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