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Phyto-chemical Characterisation and Evaluation of europharmacological Activities of *Mallotus Philippensis* (Lamk) Muell. Arg.

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Abstract

The Central nervous system activity of Mallotus phillipensis was assessed in rats taking into consideration the effect on the spontaneous and forced motor activity. The leaves of the plant collected locally were extracted using methanol and water. The phytochemical analysis of various extracts was done using standard techniques. The central nervous system activity of the extract at 250 mg/kg body weight orally was assessed based on the effect on general behavioral pattern and the effect on spontaneous and forced motor activity using digital photoactameter, Rota rod apparatus respectively and compared with diazepam @ 1 mg/kg. The acute oral toxicity of the extract was assessed in rats at limit dose of 2000 mg/kg. Phytochemical analysis revealed the presence of phenolics, alkaloids, glycosides, tannins, terpenes and flavonoids in the extract. The extract in general reduced the spontaneous activity, alertness and awareness, but the effect was not as pronounced as that of diazepam. The forced motor and spontaneous motor activities were depressed better than diazepam and aqueous extract. From the study it could be concluded that the methanolic extract of M. philippensis at 250 mg/kg B.W. was safe depressant of the CNS.

Keywords- Mallotus philippensis, Spontaneous motor activity, Forced motor activity, Actaphotometer, Rota-rod apparatus.

I.INTRODUCTION

Neurological diseases can be some of the most devastating and dangerous clinical problems seen in humans and animals. According to World Health Report, about 450 million people suffer from a mental or behavioural disorder. This amounts to 12.3 % of the global burden of disease, and predicted to rise up to 15 % by 2020. Fifty million people are suffering from epilepsy worldwide. Eighty percent of epilepsy patients are living in the developing countries, where, three-fourths of the patients are not receiving adequate treatment [1]. Most of the central nervous system acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamine increase the activity. In other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity. [2].

Several ethno-medicinal plants have been documented for hypnotic and anticonvulsant properties, these ethno-medicinal plants could serve as sources of effective medication that may be more readily accessible and inexpensive, would thus be helpful in improving the present status [3]. The

identification of reliable targets for improved pharmacological treatment in psychiatry and neurology is particularly complex and challenging. Traditional medicine involves the use of herbal medicine, animal parts and minerals. Herbal medicines contain an active ingredient, aerial or underground parts of plants as their petal or seeds materials or combinations thereof, whether in the crude state or as plant preparations. Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs.

Mallotus (family: Euphorbiaceae) is a large genus of trees and shrubs distributed chiefly in the tropical and subtropical regions of the Old World with around 20 species in India. *Mallotus philippensis* Muell. (Commonly called Kamala, Kampillaka, Kunkumadamara, Kapila, and Shendri). *Mallotus philippensis* has been widely used as traditional medicine and very part of this plant possesses its specific medicinal properties, used mainly in Ayurveda to fight against intestinal worms in domestic and grazing animals when administered with jaggery. Ethno medically used for anti-filarial, anti-bacterial, anti-inflammatory, and immune-regulatory activities and also used as purgative, anthelmintic, vulnerary, detergent, maturant, carminative and alexiteric [4]. But literature is scarce on the central nervous system (CNS) activity of the plant. Hence the present study has been undertaken to evaluate the CNS activity of methanolic extract of leaves of *M. philippensis* in albino Wistar rats.

II. MATERIALS AND METHODS

1.1. Plant Material.

The leaves of *M. philippensis* were collected from different parts of the district of Wayanad, identified and authenticated by a Botanist at M. S. Swaminathan Research Foundation, Kalpetta. It was then dried under shade and pulverized. The pulverized plant leaves were extracted using methanol in Soxhlet extraction apparatus, dried using a rotary vacuum evaporator and stored under refrigeration till further use. Aqueous extract was taken as a decoction.

1.2. Phytochemical characterization.

The extract was analyzed qualitatively for various phytochemical constituents as per standard procedures.

2.2.1. Test for Detection of Steroids

Salkowski test

About 5mg of the extract was dissolved in 3ml of chloroform and then shaken with about 3ml concentrated sulphuric acid. If a red colour develops, indicates the presence of steroids.

Leiberman Burchardt test

About 5mg of the extract was dissolved in 3ml of chloroform. Then five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it through the sides. A reddish ring if obtain at the junction of two layers, indicates the presence of steroids.

2.2.2. Tests for Detection of Alkaloids

About 0.5g of the extract was mixed with 5ml ammonia and then extracted with equal volume of chloroform solution. To this, 5ml dilute hydrochloric acid was added. The acid layer obtained was used for chemical tests for the alkaloids

Mayer's test

To 1ml of the acid layer, few drops of Mayer's reagent (Potassium mercuric iodide) was added. If a creamy white precipitate is formed, indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (Solution of Iodine in Potassium iodide) was added to 1ml of the acid layer. If there is presence of reddish brown coloured precipitate, the presence of alkaloids is indicated.

Hager's test

To 1ml of the acid layer, few drops of Hager's reagent (Saturated solution of picric acid) was mixed. A yellow precipitate is formed, if the alkaloids are present.

Dragendroff's test

Few drops of Dragendroff's reagent (Solution of Potassium and Bismuth iodide) was mixed with 1ml of the acid layer. Presence of alkaloids is indicated, if a reddish brown precipitate is seen.

2.2.3. Test for Detection of Tannins

Ferric chloride test

Two milligram of the extract was mixed with 3ml of one percent ferric chloride solution. If blue, green or brownish green colour is obtained, it indicates the presence of tannins.

Gelatin test:-

About 0.5g of the extract was mixed with few drops of one percent solution of gelatin containing 10 percent sodium chloride. If white precipitate is formed, indicates the presence of tannins.

2.2.4. Test for Detection of Flavonoids

Ferric chloride test

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), few drops of neutral ferric chloride solution was mixed. If development of green colour occur, indicates the presence of flavonoids.

Lead acetate test

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), few drops of neutral 10 percent lead acetate was mixed. If yellow precipitate appears, the presence of flavonoids is indicated.

2.2.5 Test for Presence of Glycosides

Sodium hydroxide reagent

Dissolved a small amount of the extract (about 5mg) in 1 ml water and added 5-6 drops of sodium hydroxide solution. A yellow colour if obtain, indicates the presence of glycosides.

Benedict's test

To about 1ml of the extract (0.5g extract in 1ml water), 5ml of Benedict's reagent was added. The mixture was boiled for two minutes. Development of brown to red colour if occur, indicates the presence of glycosides.

2.2.6. Test for Presence of Phenolic Compounds

About 5mg of the extract was dissolved in 1ml of water and five drops of 10 percent ferric chloride solution was added to it. Development of dark brown colour occur, if the phenolic compounds are present.

2.2.7. Test for Detection of Diterpenes

About 5mg of the extract was mixed with 3ml of copper acetate solution. If there is development of green colour, the presence of diterpenes is indicated.

2.2.8. Test for the Presence of Triterpenes

Salkowski test

About 3mg of the extract was dissolved in 3ml chloroform and then it was shaken with concentrated sulphuric acid. If lower layer turn to yellow on standing, indicates the presence of triterpenes.

Lieberman Burchardt test

Few drops of acetic acid and 1ml concentrated sulphuric acid was added to 3ml chloroform solution of the extract (about 3mg extract in 3ml chloroform). Deep red ring at the junction of two layers if appear, indicates the presence of triterpenes.

2.2.9. Test for Presence of Saponins

Foam test

A small amount of the extract (about 5mg) was shaken with 3ml of water. If the foam produced persists for 10 minutes, the presence of saponins is confirmed.

1.3. Animals.

Twenty four number of Wistar rats of either sex were used for experiment. They were maintained under standard environmental conditions and provided with feed and water adlibitum.

2.4 Chemicals.

Diazepam (Ranbaxy laboratories Ltd. Bengaluru) @ 1 mg/kg orally
Methanol (Emplura®, Mercks specialities pvt. Ltd. Mumbai)
Distilled water (Milli-Q® system from Millipore, Bedford, MA, USA).

2.5 Assessment of the CNS activity.

2.5.1 General behavioural profile.

Evaluation of general behavioral profile was performed by using twenty four adult Wistar rats were divided into four groups comprising of six animals each. The first group was considered as negative control and was administered distilled water orally. The group II, III and IV animals were orally administered with diazepam @ dose rate of 1 mg/kg and the extract at the dose of 250 mg/kg respectively. The animals were kept under observation for behavioral changes if any, at 30 minutes interval in the first hour and at one hour intervals for next 4 hours. [7].

2.5.2 Awareness, alertness and spontaneous activity.

The awareness and alertness were recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls. The normal behavior at resting position was scored as 0. Similarly, minimum activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness (++++) were also recorded. The spontaneous activity of rats was recorded by placing the animals in a bell jar. In general the rats usually displayed a moderate degree of inquisitive behavior. Less or moderate activity was scored as (++) and strong activity as (+++). A slight or little motion was scored as (+) while it was scored as (–) if the animal slept. Excessive or very strong inquisitive activity like constant walking or running was scored as (++++). A similar test was performed with the same scoring, when the animal were removed from the jar and placed on a table [8, 9].

2.5.3 Touch, pain and sound responses.

The touch response was recorded by touching the rat with a pencil or forceps at various parts of the body (i.e. on the side of the neck, abdomen and groin). The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. The rats normally utter no sound, so that vocalization may indicate noxious stimulus [8, 9].

2.5.4 Forced motor activity/Muscle relaxant activity.

The effect of extract on muscle relaxant activity was studied by using Medicraft Rota-rod apparatus. Albino Wistar rats were placed on a horizontal steel rod (32 mm diameter) rotating at the speed of 25 rpm. The rats capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into three groups (n=6). Groups I, II, III and IV were orally administered with vehicle, diazepam (1mg/kg) and the extracts at 250 mg/kg respectively. Each group of animals was then placed on the rod at an interval of 30, 60, 90 and 120 min. The animals that failed more than once to remain on the rotating rod for 3 min were considered as positive for muscle relaxation. The average of duration that the animal remained in the Rota rod for three successive times was recorded. [8, 9].

2.5.5 Spontaneous motor activity.

The loco-motor activity was recorded with a digital photoactameter. The animals were randomly divided into 4 groups and each rat was individually placed in digital photoactameter for 3 min to score the basal reading. All the animals were treated with vehicle, standard drug diazepam (1mg/kg) and extract dose of 250 mg/kg orally. After 30 min they were again placed individually into digital

photoactameter to score loco motor activity. Mean changes in the loco motor activity was calculated for each group. [5,6,8].

2.6 Assessment of the acute oral toxicity (LD50).

The acute oral toxicity study was carried out as per guidelines set by Organization for Economic Cooperation and Development (OECD-420).

III. RESULTS

3.1 Phytochemical analysis.

The results of phytochemical analysis are shown in table 1. The presence of phenolics, alkaloids, glycosides, tannins, terpenes and flavonoids in the extracts is implicated in their CNS activity.

Table 1. Phytochemical constituents of extracts of M. philippensis (Lamk.) Muel1.Arg.

Constituents	***M. philippensis leaf***	
	Aqueous	Methanolic
Phenolics	+	+
Alkaloids	-	+
Steroids	-	-
Glycosides	+	+
Tannins	+	+
Terpenes	-	+
Saponins	+	-
Flavonoids	+	+

3.2. Assessment of the CNS activity.

3.2.1 General behavior, awareness, alertness and spontaneous activity.

The effect of extracts on general behavior pattern of rats is presented in table 2. The control drug diazepam inhibited the alertness, pain response as well as spontaneous activity when compared to the untreated animals. The methanolic extract strongly reduced the spontaneous activity but the effect on other parameters were moderate when compared with group II. There was no pronounced CNS activity in the animals of group IV.

Table 2: Effect of the MEMP on the General Behavior Pattern in albino Wistar rats.

Behavior	Group I Control (5 ml/kg)	Group II Diazepam (1 mg/kg)	Group III *M. philippensis* methanolic extract 250 mg/kg	Group IV *M. philippensis* aqueous extract 250 mg/kg
Spontaneous activity	+++	+	+	+++
Alertness	+++	-	+	+++
Awareness	+++	+	++	+++
Sound response	+++	+	++	+++
Touch response	+++	+	+	++
Pain response	+++	+	++	+++

3.2.2 Forced motor activity/Muscle relaxant activity and spontaneous motor activity.

The results of the study indicated that the methanolic extract reduced spontaneous motor activity in rats better than the control drug diazepam and aqueous extract. The activity persisted even up to 120 minutes indicating potent depression of the CNS. There was no pronounced CNS activity in the animals of group IV.

Fig 2. Effect of the MEMP on spontaneous motor activity (digital photoactameter) in albino Wistar rats (n= 6).

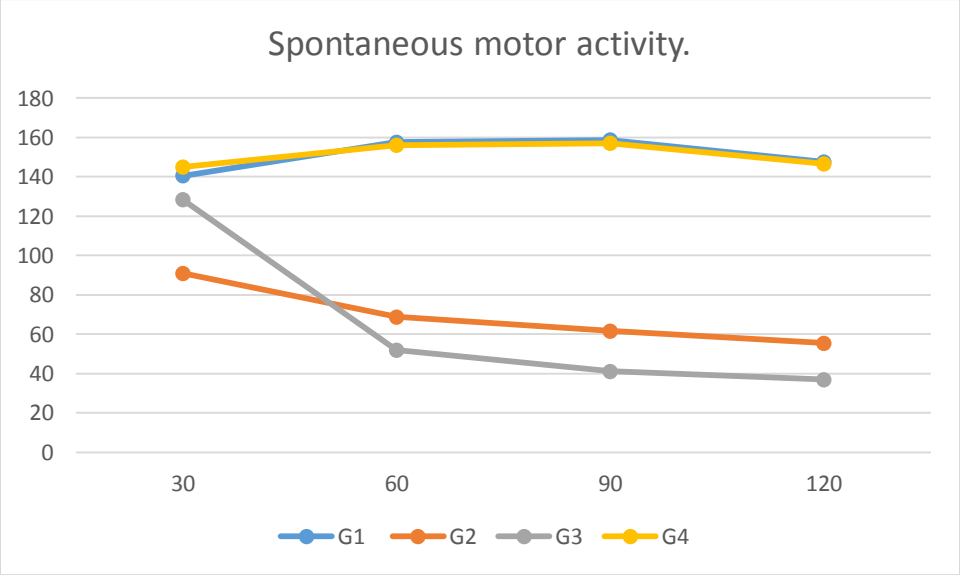
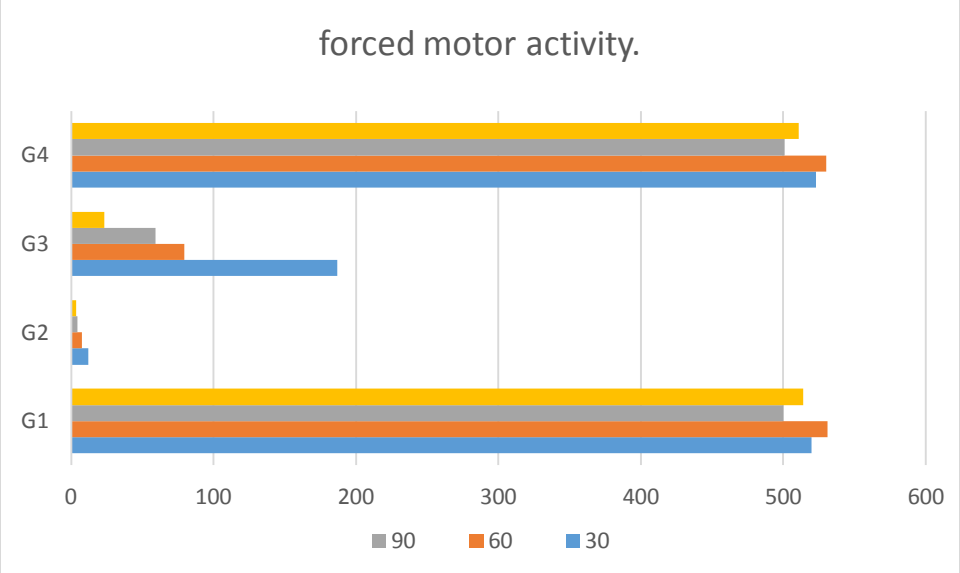


Fig 3. Effect of the MEMP on forced motor activity (Medicraft Rota-rod Apparatus) in experimental animal model (n= 6).



The results of the study indicated that the methanolic extract reduced forced motor activity in albino Wistar rats of Group III from 60th min and reduced considerably at 120th min as compared to that of standard drug diazepam which reduced the loco motor activity immediately after 30th min. There was no pronounced CNS activity in the animals of group IV.

3.3 Acute oral toxicity.

No mortality was detected in animals treated with the extract. Also no untoward clinical signs were noticed in any of the animals treated with the extract during the entire period of observation.

IV. DISCUSSION

The plant for the study is selected based on their use in folklore and traditional medicine of the district of Wayanad, Kerala for the treatment of stomach ailments and associated pain of unknown origin. Ethno medically used for anti-filarial, anti-bacterial, anti-inflammatory, and immune-regulatory activities and also used as purgative, anthelmintic, vulnerary, detergent, maturant, carminative and alexiteric [9]. Most of the drugs used to treat CNS disorders affect the quality of life of the patient. 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 [10] and are acclaimed to have a lower side-effect profile than conventional drugs. The spontaneous and forced motor activity models are widely used to screen new muscle relaxant drugs. These tests are quite sensitive and relatively specific to all major classes of CNS acting drugs including tricyclics, serotonin specific reuptake inhibitors, monoamine oxidase inhibitors and atypical [11].

Methanolic extract of *M. philippiensis* leaves decreases the CNS activity in experimental animals at dose 250 mg/kg compared with standard drug diazepam at the dose 1 mg/kg. The active principles of many drugs found in plants are secondary metabolites and are responsible for several biological activity

in man and animals. This study suggests that methanolic extract possess considerable neuropharmacological activity attributed due to the presence of active phytoconstituents like alkaloids, glycosides, flavonoids, tannins and saponins. However, only a few reports are attributed to this plant and its different parts and there is a large scope for investigation. [12]. The change in behaviour occurs when the animal is treated by a drug which increases serotonin, norepinephrine and dopamine levels in the nerve terminals. An increase in all the three neurotransmitters could be effected by inhibition of monoamine oxidase (MAO) activity in the brain. Tannic acid being a non-selective inhibitor of monoamine oxidase causes an increase in the levels of mono aminergic neurotransmitters in the brain [13]. As the plant *M. philippiensis* contains tannin, the antidepressant activity may be due to MAO inhibition, thereby increasing norepinephrine and dopamine levels in the brain. Locomotor activity and muscle coordination are an index of alertness and muscle relaxation. Reduction indicates that it may possess a sedative and skeletal muscle relaxant effect. Decrease in motor activity and muscle relaxation is an indication of CNS depressant property.

At a dose of 250 mg/kg, the methanolic extract exhibits a mild sedation and muscle relaxation which may be due to the presence of flavanoids in *M. philippiensis* and their interaction with the Benzodiazepine site of GABA-A Receptors [14]. It is as well establisged fact that γ-aminobutyric acid (GABA) the principal inhibitory neurotransimitter in the CNS, activates two types of receptor, the ionotropic $GABA_A$ receptor and the metabotropic (G protein-coupled) $GABA_B$ receptor. The $GABA_A$ receptor is a pentameric protein whose activation by agonists opens an associated chloride ion channel, leading to an increase in chloride ion influx that results in membrane hyperpolarization. [15]. Apart from this, it is of interest to note that several established antidepressants decrease locomotor activity. So the sedative effect was not significant when compared with antidepressant activity.

The results revealed that the methanolic extract at 250 mg/kg orally caused a significant reduction in the general behavior profile, spontaneous motor activity (digital photoactameter), forced motor activity (Rota rod test). The extracts did not possess any toxicity in rats. We believe that *M. philippensis* has the potential to be used as an adjuvant in the treatment of locomotor disorders and related ailments. Further research is required to gain closer insights into the exact mechanism of its action. Hence it could be concluded that the extract exhibit notable neuropharmacological effects in tested animal model and the methanolic extract of *M. philippensis* can form a lead for the synthesis of a novel herbal anti-depressant and muscle relaxant agent.

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