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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON *MICROCEPHALA LAMELLATA* (BUNGE.) POBED METHANOLIC EXTRACT IN MICE

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ABSTRACT

Microcephala lamellata (Asteraceae) is a traditional medicinal plant of the Balochistan. Traditionally it is used for jaundice, colic pain, fever and dysentery in Childs. Present study aimed to investigate phytochemistry and Pharmacological (Analgesic and Central Nervous System depressant) activity of the *M.lamellata*crude methanolic extract of leaves and stems. Phytochemical tests were determine to detect the presence of alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids and phlabotannins. Acetic acid induced writhing and Formalin test was used to determine analgesic activity. Central Nervous System (CNS) depressant activity was carried out byopen field, cage cross, rearing, traction and force swimming tests.In phytochemical test results were positive for the presence of glycosides, saponins, tannins & phlabotannins. *M.lamellata* crude methanolic extract showed significant ($p<0.05$) analgesic activity at both 250 and 500mg/kg oral doses. The *M.lamellata* crude methanolic showed significant ($p<0.05$) CNS depressant activity inopen field, cage cross,

rearing, traction and force swimming tests.It is concluded that *M.lamellata*contain important secondary metabolites and possess significant analgesic and CNS depressant activity

Keywords: Analgesic, Balochistan, *Microcephalalamellata,* Painphuli, Sedative.

INTRODUCTION

Plants have been utilized as a source of medicine since thousands of year [1, 2, 3]. Natural sources are the only source for the search of new drugs [4]. Traditional use of natural drugs have a great importance for search of new drug [5]. Use of herbal medicines in Asia signifies an extended antiquity of human communications with the environment. Plants used in traditional medicines containing a wide range of chemical substances, that can be used for chronic treatment and Infectious diseases [6]. The continuous use of the synthetic drugs resulting in increased resistance to the antibiotics, unwanted side/adverse effects and huge cost for combination therapy resulted in developingflow in alternative approaches likeanti-inflammatory agents, antioxidants, herbal extracts, probiotics and phytoceuticals [7].

Balochistan is largest province of Pakistan and native home of many plants [8]. *Microcephala lamellate* (Bunge) Pobed (Family: Asteraceae) is one of oldest traditional medicinal plant of Balochistan. Locally it is known as Painphuli [9].It grows in Kalat, Noshki, Ziarat, Pishin and Quetta. Flowering period is April to August. Traditionally *M.lamellata* has been used to treat diverse complaints i.e. jaundice, long standing fever, colic pain and the dysentery of children [10]. Till present no data was available on phytochemistry and Pharmacological activities of plant. In this regard Phytochemical and Pharmacological (Analgesic, CNS depressant) studies of the plant were carried out. Best of our knowledge *M. lamellata* was not evaluated previouslyfor these phytochemical and pharmacological studies.

MATERIAL AND METHOD

Plant materials

Leaves and stemsof *M. lamellate* were collected from Noshki and kalat Districts of Balochistan, Pakistan. Mrs. Bushra Aziz Khan, (Chairperson Department of Pharmacognosy Faculty of Pharmacy & Health Sciences, University of Balochistan, Pakistan) identified the plant, Voucher specimen No.MA.395 was deposited in the department of Pharmacognosy.

Preparation of methanol extract

After collection, the plant was dried under shade for 15 days. After drying plant was converted into fine powder by the help of mincer. Then the crush plant material was soaked in air tight glass jar with methanol

for 7 days. Solvent was filtered and evaporated by using Rotary evaporator, Dark green semi-solid extract was obtained.

Experimental Animals

Albino mice (25- 30 grams)of either sex were used for study, were acquired from Dow University of Health Sciences Karachi, Pakistan. Animals were kept under controlled environmental conditions (12/12 light and dark). Animals were kept in typical enclosures (5 mice in each cage), fed on standard food and had easy access to water, throughout the whole research period [11].

Phytochemical tests

Test for Alkaloids

1ml solution of samples was taken and Mayer's reagent (few drops) were added. Off -white or palecolor precipitate confirms the alkaloids presence [12].

Test for Saponins

1gm extract was added in distilled water (10 ml) and shacked continuously for 2 minutes. Froth formation for 1 cm (persistence for 30 minutes) indicates the presence of saponins [13].

Test for Tannins

5 ml extract solution was taken and ferric chloride (few drops) was added. Formation of intense purple, green, black or blue color confirms the presence of tannins.

Lead acetate test: to 5 ml of extract few drops of the lead acetate 10% were added.Precipitation indicates the tannin presence [13].

Test for cardiac glycoside

M. lamellate extract (2ml) and lead acetate (3 drops) were mixed and filtered. Filtrate was stirred with five (5) mL of the chloroform by using separating funnel. The layer of chloroform was evaporated. Remaining material was dissolved in a glacial acetic acid and ferric chloride few drops were added and treated with concentrated H_2SO_4(1ml). At the edge brown ring formation presented characteristic of a deoxy-sugar cardenolides. Beneath the brown ring a violet color ring may appear, a ring of greenish colormay formed slowly through thethin layer [14].

Test for Flavonoids

Methanolic extract 1 ml, concentrated HCl (few drops) and Mg wereadded. Presence of pink or magenta-red color specifies the occurrence of flavonoids [15].

Test for phlobatannins

Plant powder was mixed with distill water in a test tube, then shacked. Then 1% aqueous hydrochloric acid was further added and sample was boiled with the help of hot plate stirrer. Development of red colored precipitate established a positive result [16].

Test for terpenoids

Methanolic extract (5 ml), chloroform (2ml), con H_2SO_4 (3ml) were mixed, reddish brown color formation at the interface, shows the terpenoids presence [17].

Analgesic activity

Acetic acid induced writhing test.

The analgesic action of the plant was assessed by using acetic acid induced writhing test in mice [11]. In this test, writhes were produced by intra-peritoneal administration of 0.6% acetic acid solution 30 minutes after to the administration of the saline treated (control group), *M. lamellata*250 & 500 mg/kg treated group and Diclofenic sodium 50mg/kg treated group. Number of writhes were counted for 30 minutes instantly after acetic acid administration. A decrease in the number of writhing as compared with Control was taken as indication for the existence of analgesic activity in the plant extract.

Formalin test

The formalin test was performed in mice. 20 µl of 1% formalin in distilled water was injected subcutaneously into the dorsal hind paw of the mouse using a microsyringe (26-gauge needle), after 30 minutes of administration of saline (control group), *M. lamellata* 250 & 500 mg/kg oral dose and Diclofenic sodium 50mg/kg orally. The mouse was then put back into the chamber and the observation period started. The time spent by the animal on licking the injected paw or leg was recorded.The first period (early phase) was recorded O-5 minutes after the injection of formalin and the second period (late phase) was recorded 15-30 min after the injection [8,18].

CNS depressant activities

The methanolic extract was assessed for its CNS depressant activities by using open field, traction test, cage cross method, and rearing test, force swimming test. The animals were distributed into 4 following groups (5 mice in each group).

Group 1 control (saline 5ml/kg)

Group 2 *M. lamellata*crude extract 250mg/kg

Group 3 *M. lamellata* crude extract 250mg/kg

Group 4 standard drug (Diazepam) 2mg/kg

Open field test

Open field test area is made of plastic walls and floor divided into 25 square of equal area. It is used to estimate motor activity of animals. Mice were taken out of their cage and located in the apparatus individually. Numbers of quadranglesoverlappedby the micewith 4legs was calculated for 10 minutes [19, 22].

Cage cross test

A cage with rectangular shape was employed in this test. Animals were locatedin cage and number of activities r

(cage crossing) was counted for ten (10) minutes. This test is important for the motor activity of experimental animals [21].

Traction test

In this test the mice were trained on traction equipment to check its learning power and ability to cross it with balance. The observation was to define the time taken by the mice to travel an iron rod of one-meter length. Then the readings of control group of animals and the drug treated group of animal are compared. Any increase or decrease in the activity of animals indicates the stimulant or sedative activity of drug on animals [22].

Rearing test

This test is also a behaviorial trial test. A beaker (glass), one liter capacity was used. The upward movementsand attempt to erect body in the beaker was observed. Observation period was [23] monutes.

Forced swimming test

This test describes the CNS activity of the crude extract in mice.Cylindrical apparatus containing clean water, temperature 25^{o}C were utilized in this experiment. Mice were placed and allowed to swim for 6 minutes. After placing the mice in the water the mice suddenly starts moving its front and hind paws. The mobility time of mouse was observed with the help of a stop watch [8, 24].

Statistical analysis

Results were presented as Mean ± Standard error of the mean. The significance of difference between means was determined by t-test and the results were considered as significant at $p<0.05$. $P<0.001$ was taken to be the level of highly significance [8].

RESULTS

Phytochemical test

The results of phytochemical analysis of the plant were positive for the presence of glycosides, saponins, tannins and phlobatannins (table 1).

Analgesic Activity

Writhing test

Results shows that in saline treated (control group) the number of writhes after administration of acetic acid in mice was58.6$\pm$ 3.62. While in *M. lamellata*250mg/kg crude extract treated group numbers of the writhes were 26.4$\pm$1.5. In*M. lamellata*500mg/kg of crude extract numbers of the writhes were20$\pm$1.26 and with standard drug (Diazepam) treated group the activity was 17$\pm$1.41. (Table 2).

Formalin Test

1st phase

Results shows that in saline treated (control group) the number of licking was 16.8±0.86 and time spent on licking was 26.6±2.32 seconds. While in 250mg/kg *M.*

lamellata crude extract treated group numbers licking was 33 ±1.58 and time spent on this was 45.2±2.54 seconds. In 500mg/kg of crude extract numbers of licking was 42.8±0.73 and time spent was 40.4±2.44 seconds and with standard drug (Diclofenicsodium) treated group the number of licking was 41.4±3.24 and time spent was 37.8±1.16 seconds (table 3).

2nd phase

Results shows that in saline treated (control group) numbers of the licking were 12.4±1.33 and time spent on this was 25±3.71 seconds. While in 250mg/kg *M. lamellata* crude extract treated group numbers the activity was 25±3.71 and time spent on this was 13±3.32 seconds. In 500mg/kg of crude extract numbers the activity were 14±2.47and time spent was 17.8±2.77 secondsand with standard drug (Diclofenic sodium) treated group the activity was 17.2±2.04 and time spent was 12.6±1.63seconds (table 4).

CNS depressant Activity

Open Field activity

Results shows that in saline treated control group) the open field activities were 190±1.52, while in 250mg/kg *M. lamellata* crude extract treated group numbers of the activities were 158±1.98. In 500mg/kg of crude extract was 72.08± 1.16, and with standard drug (Diazepam) treated group the activity was 60.06 ± 0.51 (table 5).

Cage cross activity

Results shows that in saline treated (control group) the cage cross activity was 40.6±3.51

While in 250mg/kg *M. lamellata* crude extract treated group numbers the activity was 33.8±2.62. In 500mg/kg of crude extract was 27.6±1.89 and with standard drug (Diazepam) treated group the activity was 22±0.7(Table No 5).

Traction activity

Results shows that in saline treated (control group) the traction test (time taken by mice to cross the steel rod) was 14.6±2.29 seconds. While in 250mg/kg *M. lameletta* crude extract treated group traction time was 38.4±1.36 seconds. In 500mg/kg of crude extract traction time was 34.4±3.73 seconds and with standard drug (Diazepam) treated group the traction time was 49.2± 2.58 seconds (Table 5).

Rearing activity

Results shows that in saline treated (control group) the rearing activity was 57.2± 3.68, while in 250mg/kg *M. lamellata* crude extract treated group numbers the activity was 16.8±1.46. In 500mg/kg of crude extract was 11±0.7 and with standard drug (Diazepam) treated group the activity was 12.8±3.55 (Table 5).

Force swimming Test

Mobility time

Results shows that in saline treated (control group) the mobility time was 3.31± 0.007 minutes. While in 250mg/kg *M. lamellata* crude extract treated group mobility time was 3.15±0.01 minutes.In *M. lamellate* 500 mg/kg crude extract treated group mobility time was 2.55 ±0.005 minutes and with standard drug (Diazepam) treated group the mobility was 1.55±0.004 minutes (table 6).

Immobility time

Results shows that in saline treated (control group) the immobility time was 2.29 ± 0.007 minutes. While in 250mg/kg *M. lamellata* crude extract treated group immobility was 2.51±0.003 minutes. In 500mg/kg of *M. lamellata*crude extract *M. lamellata* was 3.4±0.005 minutes and with standard drug (Diazepam) treated group the immobility time was 4.4±0.004 minutes (table 6).

DISCUSSION

Nature has blessed a wide range of plant based active chemical substance that probablypromote the health and thesepoly constituents increases action of each other [26]. The phytochemical screening of *M. lamellata*crude extract of leaves and stems showed that the plantcontains glycoside, tannins, phlobatannins and saponins. These chemical constituents are known to have medicinal activity as well as having physiological activity [26].

M. lamellata crude extract showed significant ($p<0.05$) results in acetic acid induced writhing and formalin test. To evaluate the analgesic potential of medicinal agents, acetic acid induced writhing test is used. Pain is elicited through a localized inflammatory response which is the result of arachidonic acid release from tissue phospholipids catalyzed by cyclooxygenase and afterwards production of prostaglandins, PGE_2 and $PGE_2\alpha$ [27]. The test determined a mark reduction in writhing reflux. It was observed that the analgesic effect of the drug at the dose of 250& 500mg/kg was comparable with the Non-Steroidal Anti-inflammatory standard drug i.e. Diclofenac Sodium (Table 4).

To determine the mechanism of action and site of the analgesic effect of the drug,formalin model was used. Inflammatory (15-30 min) and neurogenic (0-5 min) pain are used for representation of biphasic model [28]. The inhibition of late phase occurs with the use of centrally acting narcotics [29]. It is obvious that the suppression of inflammatory and neurogenic pain suggests presence of active analgesic principle in *M. lamellata* crude extract may acting centrally and peripherally.Therefore, acute and chronic

pain may be managed using the *M. lamellata* crude extract.

M. lamellata crude extract showed significant ($p<0.05$) CNS depressant effect in Open field, cage crossing, traction, rearing and forced swimming test and results were comparable with standard drug diazepam. In phytochemical studies results were positive for the presence of Saponins, tannins, glycosides and phlobatannins, previous studies reveals that saponins, tannin, and flavonoid containing medicinal plants are utilized for management of various CNS disorders [30]. So, the CNS depressant activity may be due to these active constituents.One of the major inhibitory neurotransmitters in our brain is GABA (Gamma-aminobutyric acid). In this regard it has been suggested by earlier investigations that neuroactive phytoconstituents are ligands for the GABA receptors [31]. Therefore, these substances may act as benzodiazepine (GABA agonist) like molecules. GABA has antianxiety properties and inhibits the death of cortical neurons in vitro by inhibiting generation of ROS and amyloid beta induced glutamate release. GABA improves functions of neurons and protects critical brain regions involved with cognition after cerebral damage by ischemia. Activities of *M. lamellata* crude extract are linked with protection of neuronal abnormalities like antidepressant, anti-anxiety [30].

CONCLUSION

M. lamellata crude methanolic extract possess strong analgesic and CNS depressant activity. However further are required to isolate and characterize the chemical constituents responsible for pharmacologic effects.

Table No. 1 Phytochemical Tests *M. lamellata* crude extract

Phytochemical group	Detection
Flavonoids	Not detected
Alkaloids	Not detected
Glycosides	Detected
Saponins	Detected
Tannins	Detected
Terpenoids	Not Detected
Phlabatannins	Detected

Table No. 2 Acetic acid induced writhing test of *M. lamellata* crude extract on mice

Treatment	Mean No. of writhes± SEM	% Inhibition of writhes
Control	58.6± 3.62	-
M.lamellata crude extract 250mg/kg	26.4±1.5*	54.94%
M.lamellata crude extract 500mg/kg	20±1.26*	65.87%
Diclofenac sodium 50mg/kg	17±1.41**	70.98%

All values are mean ± SEM; n=5; * = Significant results ($P<0.05$), ** = highly significant results ($P<0.01$).

Table No.3 Formalin (1st phase)test of *M. lamellata* crude extract on mice

1st phase		
Treatment	**Number of lick & bite**	**Time spent(seconds)**
Control	**16.8 ±0.86**	**26.6 ±2.32**
***M.lamellata* crude extract 250mg/kg**	**33± 1.58***	**45.2 ± 2.54**
***M.lamellata* crude extract 500mg/kg**	**42.8 ± 0.73***	**40.4 ± 2.44**
Diclofenac sodium 50mg/kg	**41.4 ± 3.24****	**37.8 ± 1.16**

All values are mean ± SEM; n=5; * = Significant results (*P*<0.05), ** = highly significant results (*P*<0.01).

Table No.4 Formalin (2nd phase) test of *M. lamellata* crude extract on mice

2nd Phase		
Treatment	**No of lick & bite**	**Time spent(seconds)**
Control	**12.4 ±1.33**	**25 ± 3.71 seconds**
***M.lamellata* crude extract 250mg/kg**	**9.2 ± 2.44***	**13 ± 3.32 seconds**
***M.lamellata* crude extract 500mg/kg**	**14 ± 2.47***	**17.8 ± 2.77 seconds**
Diclofenac sodium 50mg/kg	**17.2 ±2.04****	**12.6 ±1.63 seconds**

All values are mean ± SEM; n=5; * = Significant results (*P*<0.05), ** = highly significant results (*P*<0.01).

Table No5: CNS depressant activities of *M. lamellata* crude extract on mice

Treatments	Open field	Cage crossing	Rearing	Traction
Control	**190 ± 1.52**	**40.6±3.51**	**57.2±3.68**	**49.2±2.58**
***M.lamellata* crude extract 250mg/kg**	**158±1.98**	**33.8±2.62**	**16.8±1.46**	**38.4±1.36**
***M.lamellata* crude extract 500mg/kg**	**72.08±1.16**	**27.6±1.89**	**11±0.7**	**34.4±3.73**
Diazepam 2mg/kg	**60.06±0.51**	**22±0.7**	**12.8±3.55**	**14.6±2.29**

All values are mean ± SEM; n=5; * = Significant results (*P*<0.05), ** = highly significant results (*P*<0.01).

Table No 6: Forced Swimming test of *M. lamellata* crude extract on mice

	Mobility time Minutes (Mean)	Immobility time
***M.lamellata* crude extract 250mg/kg**	**3.31 ±0.007 minutes**	**2 .29 ± 0.007 minutes**
***M.lamellata* crude extract 500mg/kg**	**3.15 ±0.01 minutes**	**2.59 ± 0.003 minutes**
Diazepam 2mg/kg	**2 .55 ±0.005 minutes**	**3.05 ± 0.005 minutes**
***M.lamellata* crude extract 250mg/kg**	**1.55±0.004 minutes**	**4.05± 0.004 minutes**

All values are mean ± SEM; n=5; * = Significant results (*P*<0.05), ** = highly significant results (*P*<0.01).

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