Screening of Neuropharmacological Activities of *Calotropis gigantea* roots

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ABSTRACT

The present study has been envisaged to investigate *Calotropis gigantea* (L.) Dryand (Milkweed; family – Asclepiadaceae) for neuropharmacological activities. The powdered plant material was extracted in a Soxhlet apparatus successively using solvents in order of increasing polarity viz., petroleum ether (60-80ºC), chloroform and methanol. Ethyl acetate fraction (EAF) was prepared by partitioning methanol extract with ethyl acetate. A single dose of 2 g/kg of methanol and chloroform extract was administered orally to observe toxicity in mice. The methanol extract (200 or 400 mg/kg, p.o.) and EAF (25 or 50 mg/kg, p.o.) were evaluated for antianxiety, anticonvulsant, antidepressant, sedative, antistress and analgesic activities in mice using well established models viz., elevated plus maze, maximal electroshock test, despair swim test, thiopentone sodium induced sleeping assay, cold swim test and tail immersion test respectively. The methanol extract and EAF of *C. gigantea* roots exhibited statistically significant antianxiety, anticonvulsant, antidepressant, sedative, antistress and analgesic activities with respect to control, but antianxiety, anticonvulsant and sedative activities were statistically not equivalent to the standard drug. Acute toxicity studies of chloroform extract showed 100% mortality whereas methanol extract showed no sign of lethality in mice. Preliminary phytochemical screening of methanol extract and EAF showed presence of flavonoids as major class of phytoconstituents. As flavonoids play a pivotal role in treating brain disorders, it is suggested that these constituents may be responsible for CNS activities. It is finally concluded that the present studies scientifically validated traditional claims of *C. gigantea* for neuropharmacological activities.

Keywords: Antianxiety; antidepressant; analgesic; *Calotropis gigantea*; sedative

INTRODUCTION

The pressures of modern life, depredations of famine, and pestilences and stressful situations have enormously increased the magnitude and burden of mental disorder [1]. It has been estimated that more than 650 million people worldwide suffer from common mental disorder such as anxiety and...
Serious neurological and behavioural disorders make up 13% of the global disease burden surpassing both cardiovascular diseases and cancer [3]. The Ministry of Health and Family Welfare estimated that 6-7% of India’s population suffers from a mental disorder [4]. Psychotropic agents such as barbiturates, benzodiazepines, serotonin reuptake inhibitors and calcium-channel blockers are prescribed in treating neurological disorders but regular use of synthetic drugs results in deterioration of cognitive functioning, addiction, physical dependence and tolerance [5]. Thus, researchers, are now exploring natural resources to find out more efficacious and safer drugs. Indian flora and fauna is so vast that the Indian scientists are investigating plants, based on their use in traditional system of medicine, for the treatment of neurological disorders.

*Calotropis gigantea* (L.) Dryand (Milkweed; family – Asclepiadaceae) is one of such plants which has long tradition of use in the treatment of neuropharmacological disorders [6]. The plant is distributed throughout India, mostly in Andaman Island up to 900 m altitude in hills. Scientifically, *C. gigantea* has been reported to exhibit anxiolytic, anticonvulsant and analgesic activities [7]. But crude alcoholic extracts of *C. gigantea* have been employed in these pharmacological studies. Therefore, systematic research is needed on the plant to validate its traditional claims especially for CNS activities. Thus, it was planned to: (a) prepare different extracts successsively of plant material; (b) screen extracts for neuropharmacological activities; and (c) fractionate bioactive extract by ethyl acetate and screen fraction for various neuropharmacological activities.

**MATERIALS AND METHODS**

**Collection and Identification of Plant Material**

*Calotropis gigantea* roots were procured from Himalaya Herbs Store, Madhav Nagar, Saharanpur, (Uttar Pradesh), India in September, 2012. Identity of plant was confirmed by Dr. Sunita Garg, Chief Scientist and Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi (Reference no. NISCAIR/RHMD/Consult/2013/2242/23, dated 21/05/2013).

**Animals**

Laca mice (either sex) of body weight 20-25 g purchased from the Central Research Institute, Kasauli, India were used for pharmacological and acute toxicological studies. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Punjabi University, Patiala before carrying out animal studies (107/99/CPCSEA/2013-52, dated 18/10/2013). The animals were acclimatized to laboratory conditions daily for 1 h for continuous seven days before the start of experiment. All the experiments were carried out from 9 AM to 12 PM as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals. Groups of six animals were used in all sets of experiments. The animals were overnight fasted before use. The doses were administered orally with the help of an oral cannula fitted on a tuberculin syringe.

**Preparation of Extracts and Fraction**

Methanol (S.D. Fine Chemicals, Mumbai, India), chloroform, ethyl acetate (E Merck, Delhi, India) and petroleum ether (60-80°C) (RFCL Ltd., New Delhi, India), of LR grade, were used for preparation of crude extracts and fraction of *C. gigantea* roots. *C. gigantea* roots were rinsed with normal saline to remove dirt, dried under sunlight and powdered in a grinder. Dried and powdered plant material (5 kg) was extracted in a Soxhlet apparatus successively using solvents in increasing order of polarity *viz.*, petroleum ether, chloroform and methanol. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator (BUCHI, Switzerland).
The methanol extract (20 g) of plant material was suspended uniformly in water, placed in a round bottom flask and partitioned with ethyl acetate by heating at 50°C for 30 min along with continuous stirring. This procedure of partitioning with ethyl acetate was repeated for ten times. All the separated layers of ethyl acetate were pooled and concentrated under reduced pressure to get ethyl acetate fraction (EAF; 3.05 g). Dried extracts and fraction were stored in a vacuum desiccator. The methanol extract and EAF were subjected to preliminary phytochemical screening using standard procedures [8].

**Vehicle and Standard Drugs**
Distilled water + Tween 80 (2%) was used as vehicle for preparing various test doses in such a concentration as to administer a volume ranging 0.2 to 0.25 ml to the mice. Diazepam (1 mg/kg, i.p.), diazepam (2 mg/kg, i.p.), thioptene sodium (80 mg/kg, i.p.), phenytoin sodium injection (20 mg/kg, i.p.), imipramine (15 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) were used as standard antistress, anxiolytic, sedative, anticonvulsant, antidepressant and analgesic drugs respectively.

**Acute Toxicity Studies**
Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-423 [9]. After oral administration of the chloroform and methanol extracts of *C. gigantea* roots, animals were observed individually for behavioural profile (alertness, restlessness, irritability and fearfulness), neurological profile (spontaneous activity, reactivity, touch response and pain response) and autonomic profile (defecation and urination) for at least once during the first 30 min and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. A total of six mice were used and each received a single oral dose of 2000 mg/kg/p.o. (limit test). Animals were kept overnight fasting prior to drug administration and food was withheld for further 3-4 h.

**Experimental Design**
Animals were divided into six (I-VI) groups.
- Group I - Control group received vehicle (0.25 ml, p.o).
- Group II - Standard group received respective standard drug.
- Group III - Test group received methanol extract (200 mg/kg, p.o).
- Group IV - Test group received methanol extract (400 mg/kg, p.o).
- Group V - Test group received EAF (25 mg/kg, p.o).
- Group VI - Test group received EAF (50 mg/kg, p.o).

**Antianxiety Activity**
The elevated plus-maze apparatus (EPM) consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour in animals [10]. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms, 45 min after the treatments. During the 5 min experiment, the number of entries into the open arms and average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in arms) were recorded in mice.

**Anticonvulsant Activity**
Maximal electroconvulsive shock (MES) was applied through ear-clip electrodes of electroconvulsometer (Rolex, Patiala, India) to induce tonic hind limb extension in mice [11]. The maximal electroshock stimulus used for mice was 50 mA for 0.2 sec. Mice were exposed to current, 45 min after the treatments. Time spent by the mice in tonic
extensor phase of convulsions and percentage protection of animals were recorded.

**Antidepressant Activity**
Mice were forced to swim, after 1 h of administration of test substances, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the level of 15 cm, and maintained at 25 ± 2°C [11]. Mice were allowed to swim for 6 min. During this test period, the total duration of immobility (floating in the water in a slightly hunched but upright position, its nose above the surface) was noted.

**Sedative Activity**
After 30 min of administration of vehicle and test extracts, thiopentone sodium (80 mg/kg, i.p.) was administered to induce sleep in mice [12]. The time taken for onset of duration of sleep was noted for all the animals. After induction of sleep, mice were placed in the inverted position. When sedation was over, the mice came to normal posture, and their sleep time was noted. This interval between loss and recovery of righting reflex was recorded as the duration of sleep. The time interval between injection of thiopentone sodium and start of sleep/loss of righting reflex was recorded as latency time.

**Antistress Activity**
Mice were forced to swim, after 1 h of administration of test drugs, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the level of 15 cm, and maintained at 10 ± 2°C [11]. Mice were allowed to swim for 6 min. During this test period, the total duration of immobility was noted.

**Analgesic Activity**
Groups of mice were subjected to noxious stimulus (radiant heat) by placing 5 cm of the tail in a 500 ml beaker containing 450 ml water maintained at 55 ± 2°C before and after treatment with test drugs [11]. The tail withdrawal from the heat (flicking response) was taken as end point. A cut off period 15 sec was observed to prevent damage to the tail. Three basal reaction times for each mouse at a gap of 5 min were taken to confirm normal behaviour of the mice. The reaction time at 30 min, 1 h, 2 h and 3 h were recorded after the treatment. The percentage maximum possible effect (% MPE) is calculated from the formula as given below:

\[
\text{% MPE} = \frac{\text{Reaction time – basal time}}{\text{Cut off time – basal time}} \times 100
\]

**Statistical Analysis**
The results have been expressed as mean ± standard deviation (SD). The test drugs were compared with standard drug and control by one way analysis of variance (ANOVA) followed by Student Newman Keul’s test [13].

**RESULTS**

**Acute Toxicity Studies**
Acute toxicity studies showed 100% mortality in mice after oral administration of 2 g/kg dose of chloroform extract of *C. gigantea* roots, whereas methanol extract did not show lethality and toxic reactions in mice observed until the end of the study period. The methanol extract is said to be “unclassified” under the toxicity scale. Even after acute administration of 200 mg/kg dose (1/10th of the dose used for acute toxicity study) of chloroform extract, 100% mortality in mice was observed. Therefore, chloroform extract was not subjected for further pharmacological studies. In the light of these results, only methanol extract of *C. gigantea* roots was selected for detailed pharmacological investigations.

**Phytochemical Screening**
Percentage yields of chloroform and methanol extracts *C. gigantea* roots were found to be 4.40 and 12.80% w/w, respectively. The methanol extract
of *C. gigantea* roots was subjected to standard phytochemical screening procedures in order to ascertain various classes of phytoconstituents present therein. The results of phytochemical screening showed presence of flavonoids as one of the major classes of phytoconstituents. Thus, ethyl acetate fraction was separated from methanol extract using standard procedure. Preliminary phytochemical screening of EAF showed presence of flavonoids whereas remaining methanol extract tested negative for flavonoids, indicating that all the flavonoids have been taken up by EAF.

**Antianxiety Activity**

The mean number of entries and average time spent in open arms of EPM apparatus after administration of methanol extract (200 or 400 mg/kg, *p.o.*), EAF (25 or 50 mg/kg, *p.o.*), diazepam (2 mg/kg, *i.p.*) and vehicle, *p.o.* in mice have been shown in Table 1. The methanol extract and EAF exhibited significant (*P < 0.001*) antianxiety activity with respect to control at tested doses. The activity shown by test drugs was not equivalent to the standard drug as none of the doses of methanol extract and EAF could increase significantly (*P < 0.001*) number of entries and mean time spent in open arms in comparison to the standard drug. A slight decrease in anxiolytic activity at higher dose (400 mg/kg) of methanol extract was observed. This observation suggests mild sedative activity at higher dose of methanol extract.

**Table 1: Antianxiety activity of ME and EAF of *C. gigantea* roots using EPM model**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean number of entries in open arms Mean ± S.D.</th>
<th>Mean time spent in open arms (sec) Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>Vehicle</td>
<td>2.33 ± 0.81a</td>
<td>4.00 ± 0.60a</td>
</tr>
<tr>
<td>2.</td>
<td>Diazepam</td>
<td>2</td>
<td>9.83 ± 1.72*</td>
<td>12.89 ± 1.78*</td>
</tr>
<tr>
<td>3.</td>
<td>ME</td>
<td>200</td>
<td>6.15 ± 1.31a</td>
<td>9.56 ± 1.23a</td>
</tr>
<tr>
<td>4.</td>
<td>EAF</td>
<td>25</td>
<td>5.65 ± 1.42a</td>
<td>8.56 ± 1.49a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>7.88 ± 1.60a</td>
<td>9.47 ± 1.23a</td>
</tr>
</tbody>
</table>

n=6; The data is expressed as Mean ± S.D.; *P<0.05* vs Control; *aP<0.05* vs Standard; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction, EPM = elevated plus maze

**Anticonvulsant Activity**

The decreased duration of MES-induced tonic extension phase in the mice and percentage protection of animals were noted after administration of methanol extract (200 or 400 mg/kg, *p.o.*), EAF (25 or 50 mg/kg, *p.o.*), phenytoin (20 mg/kg, *i.p.*) and vehicle, *p.o.*, as shown in Table 2. The methanol extract and EAF exhibited significant (*P < 0.001*) anticonvulsant activity at all doses with respect to control, but the activity was not statistically equivalent (*P < 0.001*) to the standard drug, phenytoin. The standard drug completely abolished duration of tonic extension in mice and protected all animals from MES-induced convulsions, where as methanol extract and EAF reduced duration of tonic extension to 11.11 and 12.81 sec, respectively, with respect to control (22.33 sec).
Table 2: Anticonvulsant activity of ME and EAF of C. gigantea roots using MES test

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean duration of extensor phase (sec)</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>vehicle</td>
<td>22.33 ± 3.5*</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Phenytoin</td>
<td>20</td>
<td>0*</td>
<td>100</td>
</tr>
<tr>
<td>3.</td>
<td>ME</td>
<td>200</td>
<td>15.70 ± 1.25*</td>
<td>33.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>11.11 ± 1.58*</td>
<td>66.66</td>
</tr>
<tr>
<td>4.</td>
<td>EAF</td>
<td>25</td>
<td>12.81 ± 2.87*</td>
<td>83.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>12.96 ±1.91*</td>
<td>83.33</td>
</tr>
</tbody>
</table>

n=6; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; *P<0.05 vs Standard; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction, MES = maximal electroshock

Antidepressant Activity
Figure 1 shows time spent by mice in immobile state after treatment with methanol extract (200 or 400 mg/kg, p.o.), EAF (25 or 50 mg/kg, p.o.), imipramine (15 mg/kg, i.p.) and vehicle, p.o. The methanol extract exhibited significant (P < 0.001) antidepressant activity with respect to control but the activity was not equivalent (P < 0.001) to the standard drug. At higher dose of EAF, i.e., 50 mg/kg, significant (P < 0.001) antidepressant activity was observed with respect to control and the activity was also found to be statistically equivalent (P = 0.257) to the standard drug. The mice treated with 25 mg/kg dose of EAF showed significant (P < 0.001) reduction in their immobility time than the control group of mice treated with vehicle. But the antidepressant activity shown by mice treated with EAF (25 mg/kg) was significantly (P < 0.001) less than the mice treated with standard drug, imipramine.

Sedative Activity
The duration of sleep after administration of methanol extract (200 or 400 mg/kg, p.o.), EAF (25 or 50 mg/kg, p.o.), thiopentone sodium (80 mg/kg, i.p.) and vehicle, p.o, has been shown in Figure 2. It is evident from Figure 2 that the control group did not show sedative activity as vehicle could not potentiate duration of sleep in mice treated with thiopentone sodium. The sedative activity was, thus, confirmed by comparing control group with test groups. The methanol extract significantly (P < 0.001) increased duration of sleep to 96.16 and 105.66 min at a dose of 200 and 400 mg/kg, respectively, with respect to control (59.83 min), whereas EAF significantly (P < 0.001) increased duration of sleep to 93 and 113.5 min at the doses of 25 and 50 mg/kg, respectively.

Antistress Activity
Figure 3 shows the mean immobility time of the mice after treatment with methanol extract (200 or 400 mg/kg, p.o.), EAF (25 or 50 mg/kg, p.o.), diazepam (1 mg/kg) and vehicle, p.o. The methanol extract significantly (P < 0.001) reduced mean time spent by mice in immobile state in comparison to control but not statistically equivalent (P < 0.001) to standard drug, as it could not achieve therapeutic level when compared to standard drug. EAF exhibited antistress activity, which was not only statistically significant (P < 0.001) with respect to control but also statistically equivalent (P = 0.091 for 25 mg/kg; P = 0.592 for 50 mg/kg) to the standard drug.
Fig. 1: Antidepressant activity of ME and EAF of *C. gigantea* roots using despair swim test. 
n=6; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; *P<0.05 vs Standard; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction.

Fig. 2: Sedative activity of ME and EAF of *C. gigantea* roots using thiopentone sodium induced sleeping assay. 
n=6; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction.
Fig. 3: Antistress activity of ME and EAF of C. gigantea roots using cold swimming test.
n=6; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; **P<0.05 vs Standard; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction

Analgesic Activity
The tail withdrawal from the heat (flicking response) recorded in the mice after administration of methanol extract (200 or 400 mg/kg, p.o.), EAF (25 or 50 mg/kg, p.o.), morphine (5 mg/kg, p.o.) and vehicle, p.o., has been shown in Table 3. The methanol extract and EAF exhibited significant (P < 0.001) analgesic activity at all the tested dose with respect to control group. The analgesic activity was not found to be statistically equivalent (P < 0.001) to standard drug.

DISCUSSION
The methanol extract and EAF of C. gigantea roots exhibited antianxiety activity using well established model, i.e., EPM by decreasing motor activity in animals which increased due to fear of height (acrophobia) [14]. The test drugs significantly inhibited tonic extension in mice, suggesting its anticonvulsant activity against generalized tonic-clonic and cortical focal seizures [15]. When the mice were forced to swim in a restricted space, from which they cannot escape, induces characteristic behaviour of depression [16]. Test drugs significantly decreased duration of immobility, confirming their antidepressant activity. The activity of test drugs was assessed by recording mean latency time and duration of sleep. The increase in duration of sleep after treatment correspond the sedative activity in experimental mice. The test drugs exhibited significant sedative activity by increasing thiopentone sodium induced sleeping time, thus, suggested to potentiate GABA mediated postsynaptic inhibition through allosteric modification of GABA$_A$ receptors [12]. When mice are individually kept in a cold environment leads to a sharp increase in the level of adrenocorticoids. This increased level of neurotransmitter induces stress in animals [17]. The methanol extract and EAF significantly reduced mean time spent by the animals in immobile state in cold environment as compared to control group, thus, inferring its antistress activity. In the tail immersion model, the analgesic activity of the test drug in different doses
Table 3: Analgesic activity of ME and EAF of C. gigantea roots using tail immersion test

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Basal reading (sec)</th>
<th>Mean reaction time (sec)</th>
<th>% MPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.D.</td>
<td>30 min</td>
<td>1 h</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>Vehicle</td>
<td>2.20 ± 0.57</td>
<td>2.82 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Morphine</td>
<td>5</td>
<td>2.32 ± 0.03</td>
<td>10.55 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>ME</td>
<td>200</td>
<td>2.60 ± 0.14</td>
<td>6.70 ± 0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400 2.50 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>EAF</td>
<td>25</td>
<td>2.30 ± 0.42</td>
<td>6.42 ± 0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>2.00 ± 0.07</td>
<td>8.17 ± 0.48</td>
<td></td>
</tr>
</tbody>
</table>

n=6; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; **P<0.05 vs Standard; one way ANOVA followed by Student Newman Keul’s test. S.D. = standard deviation, ANOVA = analysis of variance, ME = methanol extract; EAF = ethyl acetate fraction, MPE = maximum possible effect

are assessed in terms of significant increase in pain threshold during the period of observation, and this indicates the involvement of a higher centre [18]. The methanol extract and EAF of C. gigantea significantly increased pain threshold with respect to control, thus, confirmed their analgesic activity. The suggested modes of actions for neuropharmacological activities of C. gigantea are: (a) Involvement of gamma-aminobutyric acid type A (GABA_A) receptor, the chloride ion channel complex and 5-hydroxytryptamine1A (5HT_1A) [19], and (b) Monoamine inhibitory activity (tribulin activity) [20]. Preliminary phytochemical studies showed presence of flavonoids in bioactive EAF of C. gigantea roots. The available literature reveals that a large number of flavonoids – chrysin [21], apigenin [22], linarin [23] and goodyerin [24] have been reported to exhibit varied neuropharmacological activities. In agreement to these reports, it is suggested from our results that neuropharmacological activities of C. gigantea roots are attributed to flavonoids. Though, a previous report supported role of C. gigantea roots in treating anxiety, convulsions and pain but this study employed uncharacterized crude alcoholic extract [7]. This study is too preliminary to conclude scientific validation of C. gigantea for neuropharmacological activities. The present research work reported by the authors is different from previous study as: (a) various extracts of C. gigantea roots are prepared successively for evaluation of various neuropharmacological activities such as antianxiety, anticonvulsant, antidepressant, antistress, seadive and analgesic; (b) acute toxicity of extracts is preformed; (c) fractionation of bioactive extract is done to purify
crude extract; (d) fraction is also evaluated for neuropharmacological activities and (e) specific class of phytoconstituents responsible for activities and their probable modes of action are suggested. Detailed investigations are in progress to isolate bioactive constituent(s) from bioactive EAF of \( C. \ gigantea \) roots responsible for neuropharmacological activities.

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