INTRODUCTION

Litsea polyantha Juss. (Lauraceae) is a big tree found in India. It is known by the popular names of Kakuri, Pojo, Munga and Barkukuchita. Plant is abundant in hilly areas of Chhotanagpur region in state of Jharkhand. The bark of this plant has a long history of medicinal use among the traditional healers of Oraon and Munda community in Jharkhand, to treat diarrhoea and dysentery as well as the other gastrointestinal disorders. The powdered bark and root is used for pains, bruises and contusions and for fractures in animals. The bark is also used as anticonvulsant in folklore medicine.

It is reported that L. polyantha have antidiarrheal and depressant activities. The present work was focused to verify the acclaimed anticonvulsant effects of L. polyantha using different animal models.

EXPERIMENTAL

The bark and the aerial parts of L. polyantha Juss. (Lauraceae) were collected from BIT, Mesra of Ranchi District by the authors, with the help of tribal. The parts were authenticated and the voucher specimen (BIT 417; 2008-09) was preserved in the Department of Pharmaceutical Sciences, BIT, Mesra.

Preparation of extract: Shade-dried and powered bark of Litsea polyantha Juss. (passed through 44-mesh sieve) was defatted with petroleum ether for 2 h in Soxhlet apparatus. The petroleum extract was discarded and the residue was subsequently extracted with methanol for 48 h. The methanolic extract of Litsea polyantha Juss (MELP) was filtered through Whatman filter paper No. 1 and the filtrates obtained were vacuum evaporated followed by lyophilization. The yield of MELP was 11.79 % w/w.

Studies were carried out using inbreed albino mice of either sex weighing between 20 to 25 g. Animals were obtained from the animal house, Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h). Animals were allowed free access to standard pellet (Hindustan Lever, Mumbai, India) food and drinking water ad libitum. The animals were transferred to the laboratory at least 1 h before starting the experiment. The experiments were performed between 9.00 and 15.00 h. All procedures described were reviewed and approved by the Institute animal ethical committee (621/02/ac/CPCSEA).
Neurotoxicity screening: Neurotoxicity was determined using rotarod test\(^5\). Mice which were able to remain on the rotating rod, with a speed of 10 rpm for 5 min or more were selected and divided into four groups (n = 6). Group I was control, received 10 % propylene glycol at the dose of 10 mL/kg of body weight. Group II, III & IV were treated with MELP 50, 75 and 100 mg/kg, i.p. respectively. All animals were placed on the rotarod after 0.5 h of treatment and average retention time on the rod was calculated. Neurotoxicity was assessed as inability of the animal to maintain equilibrium on the rotating rod for at least 3 min (180 s) in each of the three trials.

Hole board test (exploratory behaviour): The animals were divided into 5 groups (n = 6). Group I was control, received 10 % propylene glycol at the dose of 10 mL/kg of body weight. Group II, III & IV were treated with MELP 50, 75 and 100 mg/kg, i.p. respectively; Group V received diazepam (4 mg/kg, i.p.), as a standard drug. 0.5 h after dosing, experiment was conducted by placing each animal on a wooden board and number of head dipping and time spend in head dipping during 3 min duration was recorded\(^6\).

Effect of methanolic extract of *Litsea polyantha* Juss on hypnosis test: The sedative and hypnotic effect of the extract to potentiate the barbiturate induced loss of righting reflex (sleep) was investigated\(^7\). Three different experimental groups of mice (n = 6) received the extract at doses i.e. 50, 75 and 100 mg/kg (i.p.). The animals in the control group received equal volume of vehicle (10 % PG, i.p.). After 0.5 h, all animals received i.p. injection of pentobarbital sodium (40 mg/kg). The onset and the duration of the loss of the righting reflex were noted.

Anticonvulsant activity

Maximal electroshock induced convulsions: Maximal electroshock convulsion model was used to evaluate the anticonvulsant activity of extract. Convulsions were induced in mice by delivering transauricular electroshock of 50 mA for 0.2 s by means of a convulsiometer (Research Stimulator, Model SS 44), through a pair of crocodile ear clips. Five groups of mice (n = 6) each pretreated with varying doses of MELP (50, 75 and 100 mg/kg, i.p.), vehicle 10 % propylene glycol (control group) and phenytoin 25 mg/kg (standard group) were tested after 0.5 h for maximum electroshock seizure response. Duration of tonic hind limb extension was noted in all groups\(^8\). All the MELP treated groups were compared with control in order to determine the significant anticonvulsant activity.

Pentylenetetrazol induced convulsions: Subcutaneous injection of pentylenetetrazol (PTZ) (80 mg/kg) was given to five groups of mice (n = 6) pretreated 30 min prior with varying doses of MELP (50, 75 and 100 mg/kg, i.p.), vehicle 10 % propylene glycol (control group) and diazepam 5 mg/kg (standard group), the latency to clonic convulsions was noted in all groups\(^9\). All the extract treated groups were compared with control in order to determine the significant anticonvulsant activity.

Modulation of anticonvulsant potency of extract by cyproheptadine in maximum electroshock induced convulsions: In order to investigate the involvement of serotonergic mechanisms for the anticonvulsant action of methanolic extract of *Litsea polyantha* Juss. (MELP), one groups of mice (n = 6) was injected with cyproheptadine hydrochloride (4 mg/kg, i.p.) 0.5 h prior to the dosing of MELP (100 mg/kg). After 0.5 h this group was tested for maximum electroshock seizures. Duration of tonic hind limb extension in this group was compared with that of MELP (100 mg/kg) per se treated group in maximum electroshock induced convulsion model.

Statistical analysis: Results are expressed as mean ± SEM. Statistical significance was determined by ANOVA, followed by Bonferroni’s Multiple Comparison Test; values with p < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Neurotoxicity was determined using rotarod test. Methanolic extract of *Litsea polyantha* Juss did not produced any motor in-coordination or loss of muscle tone in the doses administered (Table-1). There was significant reduction (p < 0.05 and p < 0.01) in number of head dips and the time spend in head dipping observed with MELP at doses 75 and 100 mg/kg, respectively. Diazepam (4 mg/kg) treated group significantly reduce (p < 0.001) number of head dips and the time spend in head dipping observed during 5 min (Table-2).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>Mean holding time (s)</th>
<th>Basal time</th>
<th>0.5 h after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG 10 %</td>
<td>10 mL/kg, i.p.</td>
<td>311.3±30.69</td>
<td>305.01±30.33</td>
<td></td>
</tr>
<tr>
<td>MELP 50</td>
<td>50 mg/kg, i.p.</td>
<td>295.3±11.20</td>
<td>302.17±11.06</td>
<td></td>
</tr>
<tr>
<td>MELP 75</td>
<td>75 mg/kg, i.p.</td>
<td>289.1±9.11</td>
<td>299.3±13.42</td>
<td></td>
</tr>
<tr>
<td>MELP 100</td>
<td>100 mg/kg, i.p.</td>
<td>301.5±11.06</td>
<td>297.8±12.57</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM (n = 6). The data were analyzed by ANOVA followed by Bonferroni’s multiple comparison test. ‘ns’ indicated statistically non-significant values from control. PG: Propylene glycol; MELP: Methanolic extract of *Litsea polyantha* Juss.

In barbiturate hypnosis test the extract showed a dose dependent decrease in sleep latency and increase in sleeping time as compared to vehicle control group. The significant
potentiation was observed at 75 mg/kg (p < 0.01) and 100 mg/kg (p < 0.001) dose of MELP (Table-3).

Anticonvulsant studies with methanolic extract of Litsea polyantha Juss. (MELP) showed a significant protection in maximum electroshock convulsion model in a dose dependent manner. There was a significant (p < 0.001) decrease in the duration of tonic hind limb extension at all the three doses of MELP (50, 75 and 100 mg/kg) in maximum electroshock model with maximum protection observed at 100 mg/kg dose, as compared to control group. Mortality was not observed in any group treated with the MELP (at all doses 50, 75 and 100 mg/kg, i.p.) in maximum electroshock model. The extract showed no protection against pentylenetetrazol induced convulsions at any dose as there was no significant (p < 0.05) change found in the latency to clonic convulsions in extract treated groups as compared to vehicle control group and complete mortality was observed in all of these groups. Whereas, diazepam treated group (standard group) showed increase in the latency to clonic convulsions as compared to vehicle control and there was no mortality in this group (Table-4).

Pretreatment with cyproheptadine (4 mg/kg, i.p.) showed inhibition of anticonvulsant effect of the extract in maximum electroshock model. As there was a significant (p < 0.001) increase in the duration of tonic hind limb extension in the group as compared to extract (100 mg/kg) per se treated group in maximum electroshock model (Table-5).

Most of conventional antiepileptic drugs are associated with many side effects such as neurotoxic effects, cognitive deficits and teratogenic effects, which decrease their clinical utility12-14. Recently, the search for novel pharmacotherapy from medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their less side effects and better tolerability15. In the present study the anticonvulsant activity of Litsea polyantha Juss. extract (MELP) has been studied owing to its ethnopharmacological uses. Almost all antiepileptic drugs show the signs of sedation, hypo or (less often) hyper-locomotion, ataxia, abnormal gait, reduced or inhibited righting reflexes and muscle relaxation in laboratory animals. These effects are commonly termed as neurotoxicity16. In laboratory neurotoxicity can be determined using rotarod, chimney and inverted screen test. In our study we have used rotarod test to determine neurotoxic effects of Litsea polyantha Juss. extract. MELP showed no neurotoxicity as there was no sedation, normal gait, no change on righting reflexes and all animals were able to maintain equilibrium on rotating rod for more than 3 min. Further treatment of Litsea polyantha Juss. extract potentiated pentobarbitone induced sleep.

The observations emanated in anticonvulsant studies showed that the MELP possesses anticonvulsant activity as evidenced by decrease duration of tonic hind limb extension in maximum electroshock induced convulsions. However, the extract was found to be ineffective against pentylenetetrazol induced convulsions. Carbamazepine, a clinically used anticonvulsant is effective against seizures induced by maximum electroshock but not in seizures induced by pentylenetetrazol17,18. Carbamazepine has been reported to increase serotoninergic neurotransmission as one of its proposed anticonvulsant mechanism19,20. In present studies MELP has shown similar anticonvulsant profile as that of carbamazepine, showing an evidence for the involvement of serotoninergic mechanisms for its anticonvulsant activity.

Further the role of serotonergic mechanism in our study was evidenced from the failure of Litsea polyantha Juss. extract to show anticonvulsant activity in the animals pretreated with cyproheptadine (serotonin blocker) in maximum electroshock induced convolution models.

**Conclusion**

Litsea polyantha Juss. extract increased the threshold of maximum electroshock induced convulsions with no neurotoxic effects, in a dose dependent manner. Inhibition of antiepileptic

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**TABLE-3**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>Sleep latency (min)</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG 10 %</td>
<td>10 mL/kg, i.p.</td>
<td>13.50 ± 0.62</td>
<td>63.3 ± 2.03</td>
</tr>
<tr>
<td>MELP 50</td>
<td>50 mg/kg, i.p.</td>
<td>11.50 ± 1.20</td>
<td>65.0 ± 1.21</td>
</tr>
<tr>
<td>MELP 75</td>
<td>75 mg/kg, i.p.</td>
<td>9.00 ± 0.52</td>
<td>72.8 ± 1.78</td>
</tr>
<tr>
<td>MELP 100</td>
<td>100 mg/kg, i.p.</td>
<td>7.83 ± 0.54</td>
<td>77.7 ± 1.33</td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM (n = 6). The data were analyzed by ANOVA followed by Bonferroni’s multiple comparison test. *p < 0.01, **p < 0.001 as compared with control group. PG: Propylene glycol; MELP: Methanolic extract of Litsea polyantha Juss.

**TABLE-4**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>PTZ induced convulsions latency (min)</th>
<th>Seizures latency (min)</th>
<th>MES induced convulsions tonic hind limb extension phase (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG 10 %</td>
<td>10 mL/kg, i.p.</td>
<td>4.67 ± 0.56</td>
<td>15.83 ± 0.87</td>
<td></td>
</tr>
<tr>
<td>MELP 50</td>
<td>50 mg/kg, i.p.</td>
<td>5.33 ± 0.49</td>
<td>5.17 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>MELP 75</td>
<td>75 mg/kg, i.p.</td>
<td>5.33 ± 0.80</td>
<td>3.83 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>MELP 100</td>
<td>100 mg/kg, i.p.</td>
<td>5.17 ± 0.48</td>
<td>2.83 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>5 mg/kg, i.p.</td>
<td>19.67 ± 1.48</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>25 mg/kg, i.p.</td>
<td>1.67 ± 0.33</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM (n = 6). The data were analyzed by ANOVA followed by Bonferroni’s multiple comparison test. *p < 0.001 as compared with control group. PG: Propylene glycol; MELP: Methanolic extract of Litsea polyantha Juss.

**TABLE-5**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>MES induced convulsions tonic hind limb extension phase (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELP 100</td>
<td>100 mg/kg, i.p.</td>
<td>2.83 ± 0.48</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>4 mg/kg + MELP 100</td>
<td>16.33 ± 0.67</td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM (n = 6). The data were analyzed by ANOVA followed by Bonferroni’s multiple comparison test. *p < 0.001 as compared with MELP 100. MELP: Methanolic extract of Litsea polyantha Juss.
effect of extract with cyproheptadine pretreatment showed that the extract might be mediating its effect via modulating serotonin dependent GABAergic and/or glutamatergic neurotransmission. However, further research is warranted to determine the specific mode of its anticonvulsant activity.

ACKNOWLEDGEMENTS

The authors would like to thanks Vice Chancellor, Birla Institute of Technology, Mesra, Ranchi for providing the required facilities to carry out this work.

REFERENCES