The present study was carried out to investigate the analgesic activity of leaf extract of Olea dioica (Roxb) belonging to the family Oleaceae. Acute toxicity was determined by administering single oral dose of 2000mg/kg b.w to Swiss albino mice. The results showed no toxicity in terms of general behavior change and mortality and LD50 was found to be more than 2,000 mg/kg. Analgesic effect of extract was evaluated in Swiss Albino mice by Tail flick method. The animals were administered with 100, 200 and 300 mg extract/kg b.w and the positive control used for the study was Pentazocine (5mg/kg). The extract and Pentazocine showed significant analgesic activity as compared to control. The extract increased pain threshold indicating that the extract exhibit analgesic activity.

INTRODUCTION

Olea dioica Roxb. belongs to the family Oleaceae. It is a medium sized evergreen tree found commonly in evergreen and semi-evergreen forests of South India particularly throughout Western Ghats. The bark is brownish in color and rough. Leaves are simple, opposite, elliptic lanceolate with serrate margin. The leaf apex is acute. Inflorescence axillary divaricate panicles, flowers are polygamousdioecious and creamy white in color. Flowering occurs in February-April. Fruit is fleshy drupe, ellipsoidal with one seed and black when ripe (Gowda, 2004). The plant is traditionally used in some parts of India. In South-West Maharashtra, the ash of fruit is mixed with roots of Hemidesmus indicus and used for skin diseases. Bark and fruit paste is applied in rheumatism; decoction is used to wash old wounds and given in fever (Pullaiah, 2006). The tribes in the Parambikulum wildlife sanctuary, Kerala, India use ripe fruits traditionally (Yesodharan and Sujana, 2007).

The leaf of O. dioica is experimentally shown to exhibit some bioactivities. The leaf extracts have shown to display potent antioxidant activity in vitro (Poonrima et al., 2012).The leaf extract was shown to exhibit antimicrobial and cytotoxic activity (Kekuda et al., 2014).The leaf extract was shown to exhibit inhibitory inhibitory efficacy against clinical isolates of urinary tract infections and dental carries (Viveket et al., 2014a).The leaf extract was found to inhibit the mycelial growth of Colletotrichum capsici (Kambar et al., 2014) and Sclerotium rolfsii (Vivek et al., 2014b). The literature survey revealed that the analgesic effect of O. dioica is not yet carried out. Hence, the present study was undertaken to investigate analgesic activity of leaf extract of O. dioica.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The collection and identification of plant material and extraction protocol was as described in our previous study (Kekuda et al., 2014).

Animals

Swiss Albino mice of either sex (weighing 25-30g) were used. The mice were kept in standard environmental conditions (12 hours light/dark cycle, temperature 25±0.2°C). The animals had free access to standard food and water ad libitum and were fasted 12 hours prior to use. Water was supplied during fasting. The study was conducted as per the protocol, relevant standard operating procedures of the testing facility, committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines and Institutional animal ethics committee guidelines.

Acute Toxicity Testing (Limit test at 2000 mg/kg)

Acute toxicity studies were carried out using acute toxic class limit test dose guidelines 425 of Organization for Economic Co-operation and Development (OECD). 10 rats were randomly divided into two groups of five animals per sex. Acute toxicity of the PCC was carried out, using groups of five Swiss albino mice per sex, by administering a dose of 2000 mg/kg body weight, p.o., and the control group received normal saline. The toxicological effects
were assessed on the basis of mortality and behavioral changes, occurred during 48 hours (OECD. 2001).

A total of five mice were systematically selected out of a population of 40 mice by systematic randomization technique. The mice were fasted overnight prior to dosing of each occasion. A mouse was picked at a time, weighed and dosed with equivalent and 2000 mg/kg body weight of the methanol extract dissolved in 1 mL of 0.9 % saline used as the vehicle. Feeding was done using gastric feeding tube. Each animal was observed each time for the first 5 min after loading for signs of regurgitation and then kept in a metabolic cage. Each was watched for 0 hr, 4 hr, 8 hr, 12 hr and 24 hr after dosing, and then daily for the successive 48 h for the short term outcome and the remaining 12 days for the long term possible lethal outcome which in this case was death. Behavioral manifestations of acute oral toxicity were also noted. All observations were recorded with individual records being maintained for each mouse.

**Analgesic Activity of Leaf Extract**

We employed Tail flick method in order to determine analgesic activity of leaf extract (Rizwani et al., 2012). Animals of control group, standard group and test groups received normal saline (2 ml/kg. b.w), Pentazocine (5 mg/kg b.w) and extract (100, 200 and 300 mg/kg b.w) respectively. The distal portion of tail of mice of control and treated groups (n = 6) was immersed in water bath maintained at 50±0.5°C. The time taken to withdraw tail from hot water was noted and considered as reaction time. The tail flick latency was determined at intervals of 60, 120 and 180 minutes after administration of standard and extract.

**RESULTS**

**Acute Toxicity Testing (Limit test at 2000 mg/kg)**

The results of the acute toxicological studies showed that the administration of methanolic extract by oral route at doses up to 2000 mg/kg didn’t produce any deaths in experimental animals and data represented in Table 1. Animals were observed for behavioural signs of toxicity include Motor activity, Tremors, Clonic convulsions, Tonic convulsions, Straub’s phenomenon, Catatonia, Sedation, Diarrhea, Lacrimation, Salivation, Writhing and irritation and there is no such behavioural signs of toxicity were recorded during 48 hours. Which was the no observed adverse effect level (NOAEL) (Alexeef et al., 2002). LD50 of phytotoxic combination was found to be more than 2,000 mg/kg/p.o. Body weight, water and food intake were not affected during 12 days of observation.

<p>| Table 1: Sequence and results of limit dose test of PCC |</p>
<table>
<thead>
<tr>
<th>Test Sequence</th>
<th>Test Dose (mg/kg)</th>
<th>Short Term Result(48 hr)</th>
<th>Long Term Result(12 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2000</td>
<td>Survived</td>
<td>Survived</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>Survived</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>2000</td>
<td>Survived</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>Survived</td>
<td>Survived</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>Survived</td>
<td>Survived</td>
</tr>
</tbody>
</table>

**Analgesic activity of leaf extract of O. dioica**

It was demonstrated that oral administration of leaf extract of O. dioica (100, 200 and 300 mg/kg) significantly prolonged the latency time to the heat stimulus (Table 2). Throughout 180 minutes observation, the mice pretreated with saline did not show a significant effect on the latency period of the tail-flick response. The extract exhibited dose dependent analgesic effect. Leaf extract at a dose of 100 and 300 mg/kg showed significant (P<0.001) analgesic activity when compared 200 mg/kg at 120th min after administration. The maximum possible analgesia remained elevated above the basal levels throughout the observation period. An increase in the tail flick latency was observed till 120 minutes and it later decreased in case of extract treated groups. As expected, Pentazocine (5 mg/kg) significantly increased the latency time to the noiceptive response compared with control group.

<p>| Table 2: Analgesic activity of leaf extract of O. dioica |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reaction Time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>Control</td>
<td>9.60±0.23</td>
</tr>
<tr>
<td>Pentazocine (5mg/kg)</td>
<td>9.93±1.33</td>
</tr>
<tr>
<td>Extract (100mg/kg)</td>
<td>8.67±0.89</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>7.81±0.22</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>9.61±0.56</td>
</tr>
</tbody>
</table>

Results expressed in mean ± SEM (n=6); Significance level *p<0.5, **p<0.01, ***p<0.001

**DISCUSSION**

It is well known that pain cannot be directly monitored in animals and it can only be studied by examining the responses of the animal to certain stimuli. Pain is defined as an unpleasant sensory and emotional experience associated with potential tissue injury. The action of chemical mediators such as prostaglandins, leucotrienes, peptides, acetylcholine, cytokines, nitric oxide and serotonin produced or released during tissue damage or exogenous irritants such as formalin and acetic acid are able to stimulate nociceptors, induce pain and are responsible for multiplicity of events that occur during pain transmission, in both peripheral and central nervous
CONCLUSION

In the present study, the leaf extract of *O. dioica* was found to exhibit analgesic effect. The tail flick test is considered selective to examine compounds acting through opioid receptor and the extract increased pain threshold indicating that it may act via centrally mediated analgesic mechanism (Kaushik et al., 2012). The analgesic effect of leaf of *O. dioica* can be ascribed to the presence of bioactive secondary metabolites. Further studies are warranted to isolate the active anti-nociceptive components from the plant, which may yield safe and effective agents to be used against pain.

REFERENCES


