An investigation on anti-inflammatory and anti-nociceptive effect of *Shorea tumbuggaia* Roxb. traditionally used medicinal plants

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ABSTRACT

*Shorea tumbuggaia* Roxb belonging to the family Dipterocarpaceae, is a globally threatened medicinal tree taxa is valued for its timber and pharmaceutical properties. Present work is focused on screen secondary metabolites; anti-inflammatory and anti-nociceptive effects on the leaves extract of *Shorea tumbuggaia* Roxb were examined. Acute toxicity studies were performed as per OECD guide (line no.420 & 423) and method of CPCSEA for the methanolic and hydro alcoholic extracts and it was concluded that both the extract showed no toxicity effect upto 3.5gm/kg body weight. The anti-inflammatory activity was performed by using formalin-induced sub plantar region of right hind paw edema model and anti-nociceptive activity was performed by hot plate and acetic acid induced writhing method. Diclofenac sodium (DS) was used as a positive control; significant reduction in the inflammation and pain were observed which indicate that the *Shorea tumbuggaia* Roxb leaves extract possess anti-inflammatory and anti-nociceptive. Phytochemical studies indicate that flavonoids, anthocyanins, tannins, saponins and phenols were the major component in the methanolic extract. Although existence of anti-inflammatory and anti-nociceptive activity, suggest a NSAID-like mechanism. Hence further investigation on the separation and isolation of active principle will lead to a potent anti-inflammatory and anti-nociceptive agent.

Key words: *Shorea tumbuggaia* Roxb, secondary metabolitesanti-nociceptive and anti-inflammatory.

INTRODUCTION

The practice of traditional medicine is extensive using natural products for curing common to chronic element globally; these plants still represent anenomoususcradle of natural antioxidants that might serve as leads for the development of novel drugs. Numerous anti-inflammatory, neuroprotective, digestive, hepatoprotective, and antinecrotic medicines have lately been exposed to have an antioxidant and/or radical scavenging mechanism as part of their activity [1-3]. The mechanism of inflammation injury is ascribed, in part, to proclamation of reactive oxygen species from triggered neutrophils and macrophages. This over production pointers to tissue injury by destructive macromolecules and lipid peroxidation of tissues[4,5]. Reactive oxygen species proliferate inflammation by excitingproclamation of cytokines such as interleukin-1, tumor necrosis factor, and interferon, which excite recruitment of surplusneutrophils and macrophages. Thus free radicals are vitalmediators that incite or sustain
inflammatory processes and, subsequently, their nullification by antioxidants and radicals scavengers can diminish inflammation [6, 7]. Most clinically imperative remedies belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Nevertheless these have potent activity; long-term management is obligatory for treatment of chronic disease. Many activities have been reported for the most of the phenolic compound from plants they act as anti-oxidant, anti-inflammatory, anti-viral and anti-carcinogenic agents [8].

Shorea tumbuggaia Roxb is tree taxa, IUCN Red List of Threatened Species, belonging to the family Dipterocarpaceae; S. tumbuggaia Roxb with economic and medicinal values. The genus Shorea (family Dipterocarpaceae) is native to Southeast Asia, from northern India to Malaysia, Indonesia and the Philippines. It is a tropical genus with 196 species of mainly rainforest trees, out of which 148 species are currently listed in the IUCN Red List; majority of them are listed as critically endangered [9]. Many species are economically important timber trees. Leaf juice is used as ear-pain drops for children. The stem barks having anti-ulcer activity. Stem bark is good source for secondary metabolites maximum total phenolic content has been reported in stem bark after flower buds of Shorea. The traditional usages of Shorea tumbuggaia as folklore medicine, the plant parts are administered to counteract heavy sweating. The gum is used in indigenous medicine as an external stimulant and a substitute for arbutus [10,11]. The present investigation was to investigate the anti-inflammatory and anti-nociceptive activity of leaf extract of Shorea tumbuggaia Roxb traditionally used medicinal plants.

MATERIALS AND METHODS

Plant material
Leaves of Shorea tumbuggaia Roxb were collected from Tirumala hills, Chittoor District Andhra Pradesh, India during September 2011 and the plant was identified and authenticated by Dr. Madavashetty, Department of Botany, S.V.University, Tirupathi. The materials were washed thoroughly and shade dried.

Extraction and phytochemical screening of plant material:
The leaves of Shorea tumbuggaia Roxb after shade drying were pulverized by a power-driven grinder and the powder were passed through sieve (40-mesh), to get a fine powder. The powder material (2kg), were subjected for successive extraction with increase in the polarity of the solvents n-hexane, ethyl acetate, methanol, and hydro-alcohol at 60-70°C by Soxhlet extractor. Each solvent were extract with the material until the solvent used shows colourless in the syphon; and the solvents were collected separately and the material were air dried at room temperature and the same materials were used for extraction with other solvents; this step was repeated for all the solvent according to their polarity. All the extracted solvents were concentrated under reduced pressure using Heidolph rotavapor evaporator. These extracts were subjected for phytochemical screening by employing standard phytochemical tests [12].

Experimental animals
Experimental animals Healthy Swiss albino mice (25–30g) and adult Wistar rats (150–180g) housed and maintained (23 ± 4°C, relative humidity (60–70%) in the animal house facility on a standard diet with water ad libitum, were acclimatized for two weeks before the experimentations. All animal experiments were carried out in accordance with the approval CPCSEA APPROVED REG. NO. 1230/a/08/CPCSEA, and guidelines of the Institutional Animal Ethics Committee IAEC I.D. NO: - IAE/SKIPS/2012/MAY08/I.

Acute toxicity studies
The acute oral toxicity (p.o) at 0–2000 mg/kg study was performed for methanolic and hydro-alcoholic extract in adult swiss albino mice of both sexes. This method was carried out according to OECD guide (line no 423) [13], by adopting fixed dose method. Six animals per treatment group at different dose range 100, 300,1000, 2000 mg/kg respectively (n=6) and observed at 30 mints time interval for 4h and 6,12,18 and 24h and then daily for next 14 days to record symptom softoxicity and death [14]. No acute toxic effects(agility, muscular tonus, tremors, convulsions, and problem in breathing, body weight, urination, and water or food intake) or mortality was observed following treatment, so the procedure was repeated up to 3.5gm/kg p.o. None of the treated groups displayed any significant change of behavior as compared with the untreated controls.
Anti-inflammatory activity:
Formalin Induced Paw Oedema-Experimental Procedure:
Wistar rats (150–180g) of eight groups were housed as groups of 6, fasted overnight prior to experiment and during the experiment have free access to water. Group A was served as toxicant control treated with 2% Tween 80 solution, and toxicant Formalin; group B with diclofenac (30 mg/kg p.o.) that served as standard. The rats of groups C to E (received methanolic extract), F to H (received hydro-alcoholic extract) ranging from 100, 200, and 400 mg/Kg (medium and high dose p.o) respectively. All the rats were administered with 0.1ml of 2.5% of Formalin into sub plantar region of right hind paw of rats 1 h after administration of vehicle, Diclofenac and extracts. Immediately thereafter the oedema volumes of the injected paws were measured plethysmographically at prefixed time intervals. For comparison purpose, the volume of oedema was measured at prefixed time intervals[15, 16]. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume:    

\[
\text{Percentage inhibition} = \left( \frac{C_t - C_o}{C_t - C_o} \right) \times 100 
\]

Where \(C_t\) = Thickness of paw after carrageenan injection and \(C_o\) = Thickness of paw before carrageenan injection.

Anti-nociceptive activity:
Hot plate method:
Experimental animals (Swiss albino mice (25–30g)), of either sex were randomly selected and divided into eight groups designated as group-(I to VIII) consisting of six mice in each group for control, positive control and test sample (methanolic and hydro-alcoholic) leaf extract of *Shorea tmbuggaia Roxb* respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water, 10ml/kg, p.o.), positive control (Diclofenac sodium 30mg/kg, p.o.) and the test sample (ethanolic and hydro-alcoholic extract of 100, 200, and 400 mg/kg, p.o.) respectively. The animals were positioned on Eddy’s hot plate kept at a temperature of 55±0.5°C. A cut off period of 15 seconds was observed to avoid damage to the paw; Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples [17-20].The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time, \(I\%\), was derived, using the formula [21]

\[
I\% = \left( \frac{I_t - I_o}{I_o} \right) \times 100
\]

Where \(I_t\) = reaction time at time, \(t\), and \(I_o\) = reaction time at time zero (0).

Acetic acid induced writhing method:
In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation [22]. Overnight fasted swiss albino mice were grouped into eight groups and with each group consist of six mice of both sexes. Diclofenac sodium (10mg/kg) was used as a positive control. The methanolic and hydro-alcoholic leaf extracts of *Shorea tmbuggaia Roxb*(100, 200 and 400 mg/kg) was used as test and vehicle (1.0% aqueous Tween 80 solution) as positive control, were administrated orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. The number of writhes in each treated group was compared to that of a control group. The number of writhing was recorded and the percentage protection was calculated using the following ratio: \% protection = (control mean- treated mean/control mean)×100.

RESULTS AND DISCUSSION

Current pharmacological evaluation of *Shorea tmbuggaia Roxb* methanolic(ME-STR) and hydro-alcoholic (HAE-STR), leaf extract to assess its anti-inflammatory and anti-nociceptive potential to justify its use in the traditional medicine. The phytochemical test indicates the presence of flavonoids, anthocyanins, tannins, saponins and phenols were the major component in the methanolic extract and hydro-alcoholic extracts. Acute toxicity study was performed for ME-STR and HAE-STR, mortality was not observed at the dose of 2000 mg/kg and was extended upto 3500 mg/kg (p.o) observed for 0 to 24h, 7 and 14 days. Therefore 2000 mg/kg was considered as ALD\(_{50}\) cut off dose under Globally Harmonised Classification System (GHS) category 5 (safe dose), as per OECD guideline 423 (Annexure 2d).

Anti-inflammatory:
Anti-inflammatory studies were executed by formalin induced paw oedema experimental procedure which is commonly performed for the assessment of anti-inflammatory activity for natural products. It is well known that
inhibition of formalin induced paw oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain [23,24]. The cotton-pellet-induced granuloma method [25], is limited to a measurement of sub-acute or chronic inflammation, and is time-consuming. Formalin persuaded paw oedema in rats is one of the most appropriate test methods to screen the acute inflammation and it is supposed to be a biphasic event. Among the various phytoconstituents flavonoids have satisfactory properties in the management of inflammatory conditions and that the anti-inflammatory activity is a common property of many phenolic and terpenoids [26]. Flavonoids are particularly reported for significant antioxidant, vasculo-protector, anti-hepatotoxic, anti-allergic, anti-inflammatory and anti-tumor activity [27]. The anti-inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipooxygenase and cycloxygenase activities [28]. Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. LPO level was increased during inflammation [29]. Administration of formalin produced an elevated level of LPO, which may due to the free radicals and is responsible for damaging cell membranes thereby further intensifying inflammatory damage [30]. Formalin induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as mucopolysaccharides, glycoprotein, hexosamine and hydroxyl proline, sialic acid [31]. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response. Thus formalin induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity on inflammation.

Figure 1: Anti-inflammatory activity by formalin induced paw oedema for the leaf extract of *Shorea tumbugaia* Roxb.
In the present investigation the formalin induced paw oedema was performed for leaf extracts of *Shorea tumbuggaia* Roxb methanolic (ME-STR) and hydro-alcoholic (HAE-STR) in the concentration ranging from 100, 200 and 400 mg/kg. The results were statistically calculated by using one way ANOVA, graphpad Prism 6.0 not RM, Dunnett’s multiple comparisons test version. The results was evident that the methanolic extract showed highest amount of % inhibition as increase in the dose and time after injection of toxicant formalin (0 to 3h). When compared with control and positive control standard diclofenac sodium (30 mg/kg), the percentage inhibition of ME-STR at high dose (400mg/kg) was 69.291 ± 3.341%, these values are considered to be significant with a P value less than 0.0001. HAE-STR at high dose (400mg/kg) % inhibition 48.819 ± 3.556 at 3h with a P value of 0.01 and positive control showed % inhibition 52.756 ± 1.494, at 3h with a P value less than 0.001 when compared with control group vehicle (Figure 1 and Table I) respectively.

Table I: Anti-Inflammatory Activity of leave extract of *Shorea tumbuggaia* Roxb.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw volume in ml mean ± SEM</th>
<th>% Inhibition mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2% Tween 80 solution</td>
<td>0.448 ± 0.008</td>
<td>0.572 ± 0.012</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Diclofenac 30 mg/kg</td>
<td>0.455 ± 0.010</td>
<td>0.397 ± 0.018</td>
</tr>
<tr>
<td>ME-STR</td>
<td>100mg/Kg</td>
<td>0.448 ± 0.012</td>
<td>0.395 ± 0.019</td>
</tr>
<tr>
<td>ME-STR</td>
<td>200mg/kg</td>
<td>0.462 ± 0.008</td>
<td>0.350 ± 0.014</td>
</tr>
<tr>
<td>ME-STR</td>
<td>400mg/kg</td>
<td>0.457 ± 0.010</td>
<td>0.332 ± 0.012</td>
</tr>
<tr>
<td>HAE-STR</td>
<td>100mg/Kg</td>
<td>0.448 ± 0.008</td>
<td>0.477 ± 0.010</td>
</tr>
<tr>
<td>HAE-STR</td>
<td>200mg/kg</td>
<td>0.452 ± 0.012</td>
<td>0.433 ± 0.014</td>
</tr>
<tr>
<td>HAE-STR</td>
<td>400mg/kg</td>
<td>0.457 ± 0.012</td>
<td>0.422 ± 0.012</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n = 6). *P< 0.0001, **P<0.001, ***P<0.01, and ****P<0.05 as compared with the control group. One-way ANOVA of Two-way ANOVA, not RM, Dunnett’s multiple comparisons test. (ME-STR – methanolic extract and HAE-STR Hydro alcoholic extract *Shorea tumbuggaia* Roxb)

Anti-nociceptive activity:

Central analgesic effect of *Shorea tumbuggaia* Roxb extracts on the hot plate test in mice:

Several anti-inflammatory, digestive, anecrotic, neuroprotective, and hepatoprotective drugs have lately been revealed to have an antioxidant and/or radical-scavenging mechanism as part of their activity [32]. Free radicals are vital mediators that aggravate or sustain inflammatory progressions and, thus, their neutralisation by antioxidants and radical scavengers can attenuate inflammation [6]. Several flavonoids isolated from medicinal plants have been discovered to possess significant anti-nociceptive (analgesic) and/ or anti-inflammatory effects. It is, therefore, possible that both the analgesic and anti-inflammatory effects observed with this extract may be attributable to its flavonoid component. The presence of flavonoids, anthocyanins, tannins, saponins and phenols were also reported in *Shorea tumbuggaia* Roxb [33]. Highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plants[34]. The analgesic and anti-inflammatory activity is a common property of many terpenoids, phenols and sterols.

The results show that the extract of *Shorea tumbuggaia* Roxb methanolic (ME-STR) and hydro-alcoholic (HAE-STR) exhibited analgesic activity in mice in the concentration of 100, 200, and 400 mg/kg produced significant anti-nociception in the hot plate using diclofenac sodium as positive control. The methanolic extract in the concentration of 400 and 200 mg/kg (**P<0.001, ***P<0.01) and hydro-alcoholic extract in the concentration of 400mg/kg (****P<0.05) showed a significant effect on central analgesic activity when compared with positive control (diclofenac sodium 30mg/kg) **P<0.001, all the groups were compared statistically by Tukey’s multiple comparison test (RM-one-way ANOVA) with control group. The % basal reaction time is tabulated in (Figure 2 and Table II) respectively.
Peripheral analgesic effect of Shorea tumbuggaia Roxb extracts on acetic acid-induced writhing test in mice:

Substances and drugs that produce strong inhibitory effects in the hot plate test can inhibit centrally induced pain and they act as strong analgesics [35, 36]. Analgesic activity in mice, by inhibiting the acid-induced writhing, which is a model of visceral pain [37]. Acetic acid-induced writhings is a highly profound and useful test for analgesic drug development. The writhing response of the mouse to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes pain by liberating endogenous substances and many others that excite pain nerve endings [38]. Inhibition of peripheral tissues by cyclooxygenase enzyme, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the effect or the synthesis and/or release of inflammatory mediators [39]. The extract of Shorea tumbuggaia
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*Roxb* methanolic (ME-STR) and hydro-alcoholic (HAE-STR) exhibited analgesic activity in mice, by inhibiting the acetic acid-induced writhing. The percentage inhibition of writhing of extract at 100, 200 and 400 mg/kg of methanolic extract (27.724, 54.841 and 82.674) and hydro alcoholic (5.678, 27.606 and 45.407) was significant when statistically (Tukery’s multiple comparison test (RM-one-way ANOVA)), compared with positive control and control group. The results of writhing are shown in (Table III and Figure 3) respectively.

**Table III:** Analgesic activity of Leaf extract of *Shorea tumbuggaia Roxb.* by acetic acid induced writhing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Number of writhing mean ± SEM</th>
<th>% Inhibition (mean n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0% aqueous Tween 80 solution</td>
<td>30.167 ± 2.639</td>
<td>----</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Diclofenac sodium 10 mg/kg</td>
<td>14.667 ± 1.366</td>
<td>50.810</td>
</tr>
<tr>
<td>HA-STR</td>
<td>100mg/kg</td>
<td>28.333 ± 1.211</td>
<td>5.678</td>
</tr>
<tr>
<td>HA-STR</td>
<td>200mg/kg</td>
<td>21.667 ± 1.211</td>
<td>27.606</td>
</tr>
<tr>
<td>HA-STR</td>
<td>400mg/kg</td>
<td>16.333 ± 1.211</td>
<td>45.407</td>
</tr>
<tr>
<td>ME-STR</td>
<td>100mg/kg</td>
<td>21.667 ± 1.366</td>
<td>27.724</td>
</tr>
<tr>
<td>ME-STR</td>
<td>200mg/kg</td>
<td>13.500 ± 1.643</td>
<td>54.841</td>
</tr>
<tr>
<td>ME-STR</td>
<td>400mg/kg</td>
<td>5.167 ± 1.169***</td>
<td>82.647**</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M., n=6; ****P < 0.0001, ***P<0.001, **P<0.01, *P<0.05 as compared with the control group, Tukery’s multiple comparison test (RM-one-way ANOVA). (ME-STR – methanolic extract and HAE-STR Hydro alcoholic extract *Shorea tumbuggaia Roxb*).

**Figure 3:** Anti-nociceptive activity of Leaf extract of *Shorea tumbuggaia Roxb.* by acetic acid induced writhing

**CONCLUSION**

Investigational report on anti-inflammatory and anti-nociceptive activity of *Shorea tumbuggaia Roxb* were studied; from the result it is evident that both methanolic extract and hydro alcoholic extracts showed nociceptive activity but the methanolic extracts show a better activity when compared with positive control diclofenac sodium.
The leaf and bark were been used for joint pains, common pains, fever and external stimuli by the traditional healers. In vitro antioxidant tests in our previous studies (data not shown) [40], have shown that methanolic and hydro-alcoholic leaf extract proven to have good antioxidant (free radical scavengers) activity and presence of total phenol and flavonoids. As such, in the present study the observation of analgesic and anti-inflammatory properties of methanolic and hydro alcoholic leaf extract of Shorea tumbuggaia Roxb may be due to the presence of phytochemical compounds and its potent antioxidant activities, they might suppress the formation of prostaglandin and bradykinins or antagonize their action. Therefore, antioxidant compounds from this plant extract could effectively avoid these processes. Hence investigation should be made on the bioactive secondary metabolites which could be possibly acting as anti-inflammatory and analgesic activity for the extracts of Shorea tumbuggaia Roxb.

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