Original Article

ANTINOCICEPTIVE AND GASTROPROTECTIVE EFFECT OF THE CRUDE ETHANOLIC EXTRACTS OF EXCOECARIA AGALLOCHA LINN.

Nusrat SUBHAN¹, Ashraful ALAM²*, Firoj AHMED³, Israt Zahan SHAHID³

¹ Northern University, Department of Pharmacy, Dhaka, BANGLADESH
² Stamford University, Department of Pharmacy, Dhaka, BANGLADESH
³ Khulna University, Pharmacy Discipline, Phytochemical and Pharmacognosy Laboratory, Khulna, BANGLADESH

Abstract

The effect of alcoholic extracts of bark from Excoecaria agallocha Linn. (Family: Euphorbiaceae) was evaluated in experimental models of pain and ulceration. Crude extracts of Excoecaria agallocha (300 mg/kg dose) showed maximum time needed for the response against thermal stimuli (6.72±0.43 seconds) which is comparable to diclofenac sodium (8.20±0.21 seconds) in the hot plate test. Hot tail immersion test also showed similar results as in hot plate test. The bark extracts at 500 and 250 mg/kg showed significant reduction in acetic acid induced writhings in mice with a maximum effect of 53.87% reduction at 500 mg/kg dose. The effect produced by the alcoholic extract at the highest dose was comparable to that of diclofenac sodium at 100 mg/kg (70.56%). It has also been seen anti-ulcerogenic activity compared to acetylsalicylic acid, which may be due to the protective effect of the extract. The result suggest that the analgesic effect of the extract as claimed in folklore medicine, which may be mediated via both peripheral and central mechanism having gastro-protective effect.

Key Words: Excoecaria agallocha, Euphorbiaceae, hot plate test, gastric protection.

Correspondence: E-mail: sonaliagun@yahoo.com
INTRODUCTION

Scanty literature is available on the studies of biological activities of mangroves. The mangroves provide food and wide variety of traditional products and artifacts for the mangrove dwellers. Extracts and chemicals from mangroves are used mainly in folkloric medicine (e.g. bush medicine), as insecticides and pesticides and these practices continue to this day. *Excoecaria agallocha* is a small mangrove tree with acrid milky juice, alternate leaves and capsules of three cocci, found in tidal forests and swamps of the Sundarbans and other coastal areas of Bangladesh (1). Chemical investigations have revealed that the plant contains diterpenoids (2, 3) and phorbole esters acting as anti-HIV agent was isolated from the leaves and stems of *Excoecaria agallocha* collected in northwest Australia (4). Latex contains three alcohols, exocarol, agalocol and isoagalocol (1). The piscicidal constituent excoecariatoxin characterizing the daphnane diterpene ester and some related compounds has been obtained from the twigs, bark, and latex of *Excoecaria agallocha* in Japan and Thailand respectively (5). The plant is used to treat sores and stings from marine creatures. Smoke from the bark is used to treat leprosy (1). It is also used as caustic in obstinate ulcers and as purgative (1). Bark is used as purgative and emetic. Boiled oil, which found from its latex, is applied in rheumatism, leprosy and paralysis (1). The plant is being tested for modern medical uses. Modern clinical trials show that the plant may have anti-HIV, anti-cancer, anti-bacterial and anti-viral properties (6). As a part of our on-going investigation on Bangladeshi plants for phytochemical and pharmacological properties, we now report on the pharmacological investigation of the ethanolic extracts of the barks of *Excoecaria agallocha* in laboratory rodents.

EXPERIMENTAL

*Plant Material*

*Excoecaria agallocha* was collected from the Sundarbans of Karomjol, Dacope region. The time of collection of the plant parts was October 2003 at day time. The stem barks were collected from the fresh tree from the bank of the river. The plant was identified at Bangladesh National Herbarium where a voucher specimen was deposited (Accession No. -30209).

*Preparation of ethanol extracts*

Dried ground barks (200 gm) were extracted with 80% of ethanol in a Soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to have gummy concentrate of reddish black color (yield appx. 7.5%).

*Phytochemical screening*

Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorffs reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulphuric acid.

*Animals*

Swiss albino mice (20-30 g) of either sex were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65 %, r.t. 23.0±2.0 °C and 12 h light: dark cycle). The animals were fed with standard diet and water ad
*libitum*. The University Animal Research Ethical Committee approved the experimental protocol.

**Antinociceptive Activity**

**Hot Plate Test**

Albino mice were placed in aluminum hot plate kept at a temperature of 55 ± 0.5 °C for a maximum time of 10 second (7). Reaction time was recorded when animals licked their fore, hind paws and jumped at before and at 0, 15, 30 and 45 min followed by oral administration of crude extract (100, 200 and 300 mg/kg). Diclofenac sodium 100 mg/kg was used as a reference drug.

**Tail immersion test**

Mice were treated with diclofenac sodium (100 mg/kg) and three doses of the crude extract (100, 200 and 300 mg/kg). Antinociceptive effect of the test substances was determined by the tail immersion test method described by Sewell and Spencer (8). One to two centimeter of the tail of mice was immersed in warm water kept constant at 50°C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30 and 60 min after the administration of drugs. The maximum reaction time was fixed at 10 seconds.

**Acetic Acid-Induced Writhing Test.**

Antinociceptive response of *Excoecaria agallocha* extracts (200 and 400 mg/kg) was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) induced by 1% acetic acid solution (1mL: 100 mL) in mice (9). Number of writhes per animal was counted during 30 min test period, beginning 3 min after the injection of acetic acid. Diclofenac sodium 100 mg/kg was used as a reference drug.

**Studies on acetylsalicylic acid-induced ulceration**

The adult mice used in this study were fasted for 18 h with water given *ad libitum*. The animals were separated into five groups (5 mice per group). The first group received normal saline (30 ml/kg i.p.), the second group received Cimetidine (100 mg/kg i.p), and the remaining two groups received various doses of the extract (200- 400 mg/kg p.o.). 30 min later, an aqueous suspension of acetylsalicylic (ASA) was administered to each rat at a dose of 200 mg /kg p.o. Mice were then put in restraining cages for 4 h (10). At the end of the 4 h period, mice were sacrificed and the stomach removed and opened along the greater curvature. The stomach was rinsed under a stream of water and pinned flat on a cork board. The stomach was coded to avoid observer bias and examined with a microscope (X10 magnification). The scoring of the severity of ulceration was as described by Asuzu and Onu (1990) and Akah *et al.*: (11, 12)

- < 1 mm (pin point) = 1
- 1-2 mm = 2
- > 2 mm = 3
- > 3 mm = 4

The mean ulcer index was determined by dividing the total ulcer indices in a group by the total number of animals in that group. The percentage severity of ulceration was determined by dividing the scores of ulcers of each group by the total number of scores in the control group and the result multiplied by 100 (13, 14).
Statistical Analysis

Data was analyzed by one way ANOVA using SPSS-12 for Windows, further subjected to Dunnett t test in post hoc and expressed as statistical mean ± standard error of mean. Differences between means were regarded significant at P<0.05.

RESULTS

Phytochemical screening of the extracts indicated the presence of alkaloids sponine, gum and tannins in the ethanolic extracts (Table 1)

Table 1: Phytochemical screening of *Excoecaria agallocha* ethanolic extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids</th>
<th>Gum</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract of <em>Excoecaria</em></td>
<td>++</td>
<td>+++</td>
<td>--</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><em>agallocha</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Effect of *Excoecaria agallocha* ethanolic extracts on Hot plate test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o)</th>
<th>0 min (Latency)</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% aq. tween 80)</td>
<td>10ml/kg</td>
<td>1.78±0.24</td>
<td>2.50±0.17</td>
<td>2.90±0.33</td>
<td>3.08±0.40</td>
</tr>
<tr>
<td>Diclofenac-Na</td>
<td>100</td>
<td>1.98±0.18</td>
<td>5.76±0.50</td>
<td>6.72±0.36</td>
<td>8.20±0.21</td>
</tr>
<tr>
<td><em>Excoecaria agallocha</em> extract</td>
<td>200</td>
<td>2.04±0.14</td>
<td>4.74±0.25</td>
<td>4.70±0.21</td>
<td>5.82±0.48</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.94±0.20</td>
<td>5.14±0.29</td>
<td>6.16±0.29</td>
<td>6.72±0.43</td>
</tr>
</tbody>
</table>

ANOVA F test - (0.001) (0.001) (0.001)
Beginning 30 min after oral administration of test agents (or 15 min after Diclofenac-Na.), the nociceptive response was measured every 15 min over a 45-min period. Each datum represents the mean latency of nociceptive responses (sec) ± S.E.M. (standard error of mean); One way ANOVA using SPSS-12 for Windows, Significant at 0.05 level compared to control, * Tukey HSD; a Dunnett t test.

Hot Plate Test

Three doses of extracts of *Excoecaria agallocha* increased the reaction time in a dose-dependent manner to the thermal stimulus which was summarized in Table- 2. The highest nociceptive inhibition of thermal stimulus was exhibited at a higher dose 300 mg/kg of crude extract which has maximum time needed for the response against thermal stimuli (6.72±0.43 seconds) which is comparable to diclofenac sodium (8.20±0.21 seconds) and found statistically significant.

Tail immersion test
Three doses of extracts of *Excoecaria agallocha* increased the reaction time in a dose-dependent manner to the thermal stimulus as seen in the hot plate test which was summarized in Table-3. The highest nociceptive inhibition of thermal stimulus was exhibited at a higher dose 300 mg/kg of crude extract (8.12±0.30 seconds), which is comparable to diclofenac sodium (8.12±0.25 seconds) and was statistically significant at a level p<0.05.

**Table 3:** Effect of *Excoecaria agallocha* ethanolic extracts on Tail immersion test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o)</th>
<th>Response Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% aq. tween 80)</td>
<td>10ml/kg</td>
<td>2.20±0.42</td>
</tr>
<tr>
<td>Diclofenac-Na</td>
<td>100</td>
<td>4.20±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Excoecaria agallocha</em></td>
<td>100</td>
<td>2.40±0.45</td>
</tr>
<tr>
<td>extract</td>
<td>200</td>
<td>4.40±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>4.20±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ANOVA F test (0.001) (0.001)  
Beginning 30 min after oral administration of test agents (or 15 min after Diclofenac-Na), the nociceptive response was measured every 30 min over a 60-min period. Each datum represents the statistical mean latency of nociceptive responses (sec) ± S.E.M. One way ANOVA using SPSS-12 for Windows, Significant at 0.05 level compared to control, * Tukey HSD; a Dunnett t test.

**Acetic Acid-Induced Writhing Test**

Dose dependent antinoceptive effect was also noted with the extract at the tested dose levels in acetic acid-induced writhing test (Table- 4). Maximum percentage of inhibition of writhing response exhibited 53.87% by the extract at 500 mg/kg while the same at 250 mg/kg showed 38.13 % reduction in acetic acid induced writhing response. It was very much comparable to that of standard diclofenac sodium (100 mg/kg) caused 70.56% pain inhibition.

**Table 4:** Effect of *Excoecaria agallocha* ethanolic extract on acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>Route of administration</th>
<th>Writhings**</th>
<th>% of writhing</th>
<th>% inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% aq. tween 80)</td>
<td>10ml/kg</td>
<td>p.o.</td>
<td>20.72±0.81</td>
<td>100</td>
<td>--</td>
</tr>
<tr>
<td>Diclofenac-Na</td>
<td>100</td>
<td>i.p.</td>
<td>6.10±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.44</td>
<td>70.56%</td>
</tr>
<tr>
<td><em>Excoecaria agallocha</em></td>
<td>200</td>
<td>p.o.</td>
<td>12.82±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.87</td>
<td>38.13%</td>
</tr>
<tr>
<td>extract</td>
<td>400</td>
<td>p.o.</td>
<td>9.56±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.13</td>
<td>53.87%</td>
</tr>
</tbody>
</table>

ANOVA F test (0.001)  
<sup>a</sup> Administered 45 min before 0.7% acetic acid administration (10 ml/kg, i.p.).  
<sup>b</sup> Counted for 15 min, starting 5 min after acetic acid administration; % inhibition of writhing was calculated as % inhibition=(1-writhing of test/writhing of control) ×100.  
One way ANOVA using SPSS-12 for Windows, Significant at 0.05 level compared to control, Dunnett t test; values are statistical mean ±S.E.M (N=5).
Table 5: Gastro protective effect of the ethanolic extract of *Excoecaria agallocha*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (mg/kg i.p)</th>
<th>Mean ulcer score</th>
<th>Ulcer index</th>
<th>% of ulcer Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline + acetylsalicylic</td>
<td>14.20±0.66</td>
<td>2.84</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>Cimetidine (100 mg/kg i.p) + acetylsalicylic acid</td>
<td>4.40±0.51**</td>
<td>0.88</td>
<td>69.01</td>
</tr>
<tr>
<td>Group III</td>
<td><em>E. agallocha</em> extract (200 mg/Kg) + acetylsalicylic acid</td>
<td>6.60±0.40**</td>
<td>1.32</td>
<td>53.52</td>
</tr>
<tr>
<td>Group IV</td>
<td><em>E. agallocha</em> extract (400 mg/Kg) + acetylsalicylic acid</td>
<td>4.80±0.37**</td>
<td>0.96</td>
<td>66.20</td>
</tr>
</tbody>
</table>

ANOVA F test (0.001)

** One way ANOVA using SPSS-12 for Windows, Significant at 0.05 level compared to control, Dunnett t test; values are statistical mean ±S.E.M (N=5).

Studies on acetylsalicylic acid-induced ulceration

The aqueous extract of *Excoecaria agallocha* stem bark (200-400 mg/kg i.p.) significantly (P < 0.05) inhibited ulceration induced by acetylsalicylic acid (200 mg/kg p.o.) dose dependently in rats. The results obtained compared with Cimetidine (100 mg/kg i.p.; Table 5).

DISCUSSION

The study of Premnathan *et al.* (15, 16) revealed that the mangroves were found highly effective for antiviral activity as compared to seaweeds and sea grasses. Kokpal *et al.* also reported the bioactive compounds from mangrove plants such as insecticidal activity (17). Earlier chemical investigations have revealed that the plant contains diterpenoids (2, 3) and phorbole esters (4). All the methods for investigating analgesic effects of the crude ethanolic extract of *Excoecaria agallocha* were selected such that both centrally and peripherally mediated effects were investigated. Pain and inflammation are associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases (18, 19, 20). A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation. As preliminary phytochemical results indicated, it could be suggested that the antinociceptive and anti-inflammatory effects of the extracts may be due to their content of alkaloids (21, 22), diterpinoids (23), saponins (24) and few reports on the role of tannins in antinociceptive and anti-inflammatory activities (25).

The crude extracts of *Excoecaria agallocha* demonstrated significant anti-nociceptive activity at three different dose levels in various animal models of pain. Acetic acid-induced writhing response elucidated peripheral activity, while the hot plate tests, hot tail flick test investigated both peripheral and central activity (26, 27). Nociceptive reaction towards thermal stimuli in hot plate test and tail immersion in hot water test using mice is a well-validated model for the detection of opiate analgesic as well as several types of analgesic drugs from spinal origin (8, 28). Nociceptive pain inhibition was noticed highest in both the test at 45 minutes after administration of the extracts and the response time is increased from 1.94 seconds to 6.72 seconds in hot plate test at dose 300mg/kg while it was also increased from 4.20 seconds to 8.12 seconds in tail flick test at the same dose level. Other doses used in this study also increases the latent period significantly with the time being in both tests. Acetic acid-induced writhing test has been used as a model of chemonociceptive induced pain, which increases PGE$_2$ and PGF$_{2a}$.
The crude ethanolic extract of *Excoecaria agallocha* showed significant reduction of abdominal contraction in mice. Local peritoneal receptors were postulated to be partly involved in the abdominal constriction (writhing) response (9, 28). The method has been associated with prostanoids in general, i.e. increased levels of PGE₂ and PGF₂α in peritoneal fluids (9) as well as lipoxygenase products by some researchers (28). The extract might inhibit the synthesis and/or release these endogenous substances. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is commonly employed in the treatment and/or management of rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis (29, 30) and for its anti-inflammatory and analgesic effects (31). Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (32, 33, 34). The drug also affects polymorphonuclear leukocytes function in vitro, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation and neutral protease production (35, 36). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (36, 37, 38). In the present study, the reduction of the antinociceptive process obtained within the first hour is probably related to reduction in the release of preformed inflammatory agents, rather than to a reduced synthesis of the inflammatory mediators by inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators). Thus the anti-nociceptive activity shown by crude extracts of *Excoecaria agallocha* in hot plate, hot tail-flick and acetic acid induced writhing test indicate that alcoholic extracts of the plant might possess centrally and peripherally mediated antinociceptive properties.

Gastric mucosal layers play a role of a barrier that limits an exposure of the gastric mucosal cells to numerous injurious luminal agents and irritants of exogenous and endogenous origin. Mucosal surface epithelium is a subject of attack by physical, chemical or microbiological agents acting from the gastric lumen, which are involved in multiple pathologies, such as gastritis, peptic ulcer or gastric cancer. Pretreatment with different substances could effectively prevent gastric mucosa from the development of erosions and ulcerations. This action, called gastro- or cytoprotection is not related to the inhibition of gastric acid secretion and known to account for gastro-protection by various irritants and ulcerogens (39). The dose dependent reduction in acetylsalicylic acid induced ulceration by the aqueous extract of *Excoecaria agallocha* probably suggests the presence of some active ingredients i.e. antioxidants which act through scavenging the free radical generated incase of ulceration or one or more ulcer protecting mechanisms. Since, terpenes were associated to antiulcerogenic activity in other plants (40). Some triterpenes are known as anti-ulcer drugs and their action has been suggested to be due to the reduction of mucosal prostaglandins metabolism, cytoprotective action and reduction of gastric vascular permeability (41). The results obtained were comparable to that of cimetidine which has been reported to have ulcer healing mechanism involving competitive inhibition of H₂ receptors (Table 5, Figure 1) (42,43).
Figure 1: Typical photomicrograph of the ulcer induced by acetylsalicylic acid. A- Untreated control group. B- Cimetidine (100 mg/kg i.p) + acetylsalicylic acid, C- *Excoecaria agallocha* extract (200 mg/Kg) + acetylsalicylic acid, D- *Excoecaria agallocha* extract (400 mg/Kg) + acetylsalicylic acid. (Axistar Plus with cannon A 610/620 photo documentation system, Magnification× 10)

CONCLUSION

The ability of the extracts to suppress abdominal writhes, increase pain threshold latency, suppression of the acetylsalicylic acid induced inflammation confirms the analgesic and anti-ulcerative properties of the extract. These findings justify traditional use of this plant in the treatment of pain and other inflammatory conditions and validate its claim of being used for the said purpose in folklore medicine. It can be concluded that alcoholic extracts of *Excoecaria agallocha* possesses analgesic properties, which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms which may be of potential benefit for the management of pain where ulceration is a problem with NSAID therapy. Further research should be necessary for elucidating the active principle as well as toxicological studies.
REFERENCES


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