Analgesic, Anti-inflammatory and Anti-ulcerogenic Activities of Fractions from Alstonia scholaris

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Abstract: Background: The analgesic and anti-inflammatory effect of ethanolic extract of leaves of Alstonia scholaris Linn. (Apocynaceae) has been reported earlier. The objective of this study was to investigate analgesic and anti-inflammatory effect of the fraction which is responsible for the activity.

Materials and Methods: The Dichloromethane (DCM) and Ethylacetate Fractions (EA) from ethanolic extract of leaves of Alstonia scholaris Linn. were subjected to analgesic, anti-inflammatory activity using hot plate, acetic acid induced writhing and carrageenan induced inflammatory models. The ulcerogenicity of the fractions was also tested using pylorus ligation model. Results: There was a significant (p<0.01) decrease in the writhing and decrease (p<0.01) in the paw volume in carrageenan induced inflammation with DCM fraction, whereas there was no effect in basal reaction time in hot plate method. There was a significant (p<0.01) decrease in the number of writhes with EA whereas there was no effect in basal reaction time and paw volume with EA fraction. Conclusion: The results obtained indicate that DCM has peripheral analgesic activity, anti-inflammatory activity and lack ulcerogenicity, whereas EA did not possess analgesic and anti-inflammatory activities. This confirms that the DCM fraction consist of components responsible for the analgesic and anti-inflammatory activities of Alstonia scholaris Linn.

Keywords: Alstonia scholaris, analgesic, anti-inflammatory, anti-ulcerogenic.

INTRODUCTION

Alstonia scholaris Linn. R. Br. (AS) belongs to family Apocynaceae and is native of India. It grows wild throughout in deciduous, evergreen forests and even in plains. Bark of AS possess spectrum of pharmacological activity, ranging from bitter, astringent, thermogenic, laxative, antipyretic, anthelmintic to galactoogogue and cardiotonic properties, therefore used in fever, malarial fever, abdominal disorder, dyspepsia, leprosy, skin diseases, asthma, bronchitis, cardiopathy etc. (Nadkarni, 1976; Kirtikar and Basu, 2002). An antimalarial Ayurvedic preparation, Ayush-64, containing AS is marketed (Vrisha et al., 2003). Folklore use includes application of milky juice of leaves on wounds, ulcers and for rheumatic pain, as well as a mixed form with oil which is applied for earache (Nadkarni, 1976).

The leaves of AS were reported to contain several alkaloids such as scholaricine, 19, 20-dihydrocondlyocarpine alkaloid, 19, 20-Z-Vallesamine, alstonamine, rhazimamine, 19-epischolaricine, N,N'-methylscholaricine, N,N'-methylburnamine, vallesamine N oxide, narelne ethyl ether, 5-epi-narelne ethyl ether and scholarine-N″-oxide, picrinine, angustilobine B acid, losbanine (6, 7-seco-6-nor-angustilobine B), tubotaiwine, its oxide, 6, 7-seco-angustilobine B, Lagunamine (19-hydroxytubotaiwine), manilamine, 5-methoxystreitamine, 19, 20 E-alstothesolarine and 19, 20 Z-alstothesolarine, alschomine, isoalschomine, netuline (Arulmozhi et al., 2007a). The leaves also contain non-alkaloid constituents like ursonic acid, cycloexadecanol, a-amyrin acetate, b-amyrin 3-palmitate, 20 (20)-lupen-3-ol, 20 (29)-lupen-3-palmitate, b-sitosterol, squalene, a-tocopherol, a-tocopherol quinone, bis (2-ethylhexyl) phthalate, dibutyl phthalate, 1-hydoxy-3, 5-dimethoxyxanthone, 7, 3, 4-trimethoxy-5-hydroxyflavone and 3, 5, 7, 4-tetrahydroxyflavone-3-O-β-D glucoside, alstonic acid A and alstonic acid B. Eight flavonoids namely, kaempferol, quercetin, isoherbertin, kaempferol-3-O-β-D-galactopyranoside, quercetin-3-O-β-D-galactopyranoside, isohamnetin-3-O-β-D-galactopyranoside, kaempferol-3-O-β-D-xylopyranosyl-(2′-1)-O-β-D-galactopyranoside, quercetin-3-O-β-D-xylopyranosyl-(2′-1)-O-β-D-galactopyranoside were also reported to be present in the leaves of AS (Hui et al., 2009).

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Extracts of AS is reported to possess several pharmacological activities of interest that include antiplasmodial activity (Keawpradub et al., 1999), antimutagenic activity (Lim-Sylviaco et al., 1990), immunostimulatory effect (Iwo et al., 2000), hepatoprotective activity (Lin et al., 1996), anticancer activity (Saraswathi et al., 1998, 1999), antidiabetic and antihyperlipidemic activities (Arulmozhi et al., 2010).

Previous studies on the plant has illustrated the antinociceptive, anti-inflammatory (Arulmozhi et al., 2007b; Shang et al., 2010) and antiarthritic activities (Arulmozhi et al., 2011) of leaves of AS. Based on the above perspective, the present study was designed to investigate the most promising fraction responsible for the analgesic, anti-inflammatory property of AS. The main adverse effect and limitation of synthetic analgesic, anti-inflammatory compounds is its ulcerogenic property. Hence, we investigated the ulcerogenicity of the promising fraction.

**MATERIALS AND METHODS**

**Materials:** The leaves of AS were collected in the month of April-May 2008 from hills of Sawantwadi, Maharashtra, India. The plant material was taxonomically identified by the Botany Survey of India (BSI), Pune and the voucher specimen AS-1 was retained in herbarium of BSI, Pune for future reference. Dichlofenac sodium was procured from Shreeji Pharma International, India and carrageenan was procured from Sigma Aldrich, USA. All other chemicals used were of analytical grade and obtained from Merck, USA.

**Animals:** Swiss Albino mice of either sex (18-25 g) and male Albino Wistar rats (150-180 g) were used for the study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee (Reg. No. 626/02/a/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

**Extraction:** The dried powder of the leaf (500 g) was subjected to extraction in a Soxhlet apparatus using ethanol. The solvent was removed from the extract under reduced pressure. This extract was partitioned with petroleum ether (60-80°C), dichloromethane, ethylacetate and n-butanol in a separating funnel.

**Acute toxicity studies:** Acute toxicity study was carried out for Dichloromethane (DCM) and Ethyl Acetate (EA) fractions from ethanolic extract of leaves of AS following OECD (Organisation for economic co-operation and development) guidelines OECD 423 (OECD Guidelines 2001). Overnight fasted healthy female Wistar Albino rats (n = 3) were administered orally the DCM or EA (in 2% Tween 80) in the dose of 5 mg kg⁻¹ body weight. The animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total period of 14 days. The animals were observed for the signs of toxicity which include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The acute toxicity test was repeated with doses of 50, 300 mg kg⁻¹ bodyweight.

**Hot plate method:** This method was performed as given by Vogel (2008).

**Acetic acid induced writhing:** This method was performed as given by Eddy and Leimbach (1953)

Percentage protection was calculated following the formula:

\[
\text{% Protection} = \frac{(\text{No. of writhes in control} - \text{No. of writhes in test})}{\text{No. of writhes in control}} \times 100
\]

**Anti-inflammatory activity in carrageenan induced rat paw edema:** Male Albino Wistar rats (150-180 g) were randomly distributed into four groups of six animals each as follows: Group 1 served as control which received 2% w/v Tween 80, p.o., group 2 served as standard and was administered with indomethacin 30 mg kg⁻¹, p.o. while group 3 and group 4 were test groups which received DCM 30 mg kg⁻¹, p.o. and EA 30 mg kg⁻¹, p.o., respectively.

DCM and EA were evaluated for anti-inflammatory activity by carrageenan induced rat paw edema method (Winter et al., 1962; Turner, 1965). After 1 h of oral administration, 0.1 mL of 1% w/v suspension of carrageenan was injected into the subplantar region of left hind paw to all the four groups. The paw volumes were measured using plethysmometer (UGO Basile, Italy) every hour till 6 h after carrageenan injection and mean increase in paw volumes were noted.

**Anti-Ulcerogenic activity in pyloric ligation model:** Male albino Wistar rats were divided into four groups of six animals each as follows: Group 1 served as control which received 2% w/v Tween 80, p.o., group 2 was administered with indomethacin 30 mg kg⁻¹, p.o. while group 3 and group 4 were test groups which received DCM 30 mg kg⁻¹, p.o. and EA 30 mg kg⁻¹, p.o., respectively.
The animals were fasted for 16 h and the respective treatment was orally administered. The pyloric end was ligated and the animals were sacrificed at 4 h after ligation. The stomachs were removed and cut along the lesser curvature and the gastric mucosa were washed with normal saline and scored according to the scale. The following scale was used: 0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions (Cashin et al., 1977). In the second model (Santos et al., 2004), the above said procedure was followed after administering the respective treatment orally for 7 days.

**Statistical analysis:** Experimental results were expressed as Mean±SEM of six animals. Analysis of variance was performed by ANOVA followed by Dunnet’s multiple comparison test. P values less than 0.05 were regarded as significant.

**RESULTS**

**Fractionation:** The yields of various fractions were as follows: Petroleum Ether Fraction (PE): 2.10% w/w, Dichloromethane fraction (DCM) 1.20% w/w, Ethyl Acetate Fraction (EA) 0.30% w/w and n-butanol fraction (BUT) 2.62% w/w.

**Acute toxicity studies:** As suggested by OECD guidelines, the test animals were observed individually, after dosing at least once during the first 30 min, periodically during the first 24 h with special attention during first 4 h. The test animals did not exhibit any visible change and survived beyond recommended duration of observation with 300 mg kg⁻¹. Hence DCM and EA were safe up to 300 mg kg⁻¹.

**Hot plate method:** There was no significant change in the basal reaction time on treatment with EA or DCM throughout the study Fig. 1. However, the standard pentazocine produced a significant (p<0.01) increase in the basal reaction time.

**Acetic acid induced writhing:** There was a significant (p<0.01) decrease in the number of writhes on treatment with DCM 30 and EA 30 mg kg⁻¹ Table 1. The percentage inhibition of writhing response exhibited by the DCM was 45.06% while EA showed 28.96% decrease in acetic acid induced writhing response.

**Anti-inflammatory activity:** DCM showed significant (p<0.05) decrease in paw oedema from 3 h of injection of carrageenan Fig. 2. However, the effect was more

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**Table 1:** Effect of DCM and EA on acetic acid induced writhing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Dichlorobane sodium (10 mg kg⁻¹)</th>
<th>DCM (30 mg kg⁻¹)</th>
<th>EA (30 mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of writhes</td>
<td>48.5±2.96</td>
<td>12.3±1.26**</td>
<td>26.1±1.74**</td>
<td>33.6±5.94**</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>---</td>
<td>74.1±2.82**</td>
<td>45.6±4.92**</td>
<td>28.8±4.52**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM, n = 6. One Way ANOVA followed by Dunnet’s t test. **p<0.05, ***p<0.01 compared to Vehicle control. DCM: Dichloromethane, EA: Ethyl acetate
prominent (p<0.01) at 4, 5 and 6 h. The percentage inhibition of paw volume of DCM 30 mg kg\(^{-1}\) was comparable to that of standard at 4 h and more prominent at 5 and 6 h. There was no decrease in the paw volume on treatment with EA fraction.

**Antiuicerogenic activity:** The groups of animals treated with DCM and EA did not show ulceration in the stomach after 16 h of fasting, whereas the ulcer score was found to be significantly high (p<0.01) in rats administered Indomethacin Table 2. Treatment with the fractions for seven days did not show any ulceration whereas the ulcer score was significantly (p<0.01) high with Indomethacin treated rats.

**DISCUSSION**

Hot plate method is based on the sensitivity of the mice paw to heat at temperatures not damaging the skin which is observed as jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics whereas peripheral analgesics do not generally affect these responses (Vogel, 2008). In the present study, both DCM and EA fractions were found not to affect these jumping, withdrawal or paw licking responses which makes it evident they are not centrally acting.

In acetic acid induced writhing model, pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behaviour which is called as writhing. Acetic acid causes algesia by liberating endogenous substances that excite the pain nerve endings (Raj, 1996). In the present study, there was a significant decrease (p<0.01) in the number of wriths with DCM and EA fractions. This proves the peripheral analgesic activity of DCM and EA fractions. There are various flavonoids like rutin, quercetin, luteolin, hesperidin known to produce analgesic and anti-inflammatory activities (Galati et al., 1994; Ramesh et al., 1998). Tannins are also known to produce such effects (Ramprasath et al., 2006). The mechanism of analgesic action of DCM and EA may be due to the presence of flavonoids, as found in the preliminary phytochemical investigation. There is no change in basal reaction time with DCM or EA in hot plate method which shows that these fractions do not possess central action and their ability to significantly decrease the number of wriths suggest that their analgesic action is mediated through peripheral mechanisms.

Carrageenan induced paw edema is a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carrageenan induced edema is believed to biphasic. The early phase is attributed to the release of histamine and serotonin (Vinegar et al., 1976; Larsen and Henson, 1983) and the delayed phase is sustained by the leukotrienes and Prostaglandins (PG) (Brooks and Day, 1991). In the present study, there was a significant (p<0.05) decrease in paw volume with DCM from 3rd h onwards and was more prominent (p<0.01) at 4, 5 and 6th h. This suggests that the DCM fraction is having its action on delayed phase which is mediated by leukotrienes and prostaglandins. However, EA fraction did not show a decrease in paw volume in any of the tested hours. This indicates the lack of anti-inflammatory action of EA fraction.

There are reports which suggest that flavonoids and tannins inhibit prostaglandin synthesis (Ramprasath et al., 2006). The presence of flavonoids and tannins in the DCM fraction as evident by our preliminary phytochemical analysis, appears to inhibit prostaglandin synthesis and excerts the anti-inflammatory action. But there is always a correlation between the potency to inhibit PG synthetase and ulcerogenic activity (Boyle et al., 1982). Many of the NSAIDs have anti-inflammatory and ulcerogenic activities which are due to PG synthetase inhibitor activity. The DCM fraction has anti-inflammatory activity but its lack of ulcerogenicity even with multiple administration (7 days) suggest that the mechanism of action may not be mainly due to PG synthetase inhibition but through some selective mechanism like Cox-2. However, further studies are required to substantiate this mechanism and to pinpoint the active constituents responsible for the action.

**CONCLUSION**

The DCM fraction from ethanolic extract of *Alstonia scholaris* Linn. is proven to have analgesic and anti-inflammatory activities. The mechanism of its action is different from classical NSAIDs and its lack of ulcerogenicity proves to be advantageous in chronic administration like rheumatoid arthritis. However, further
studies are underway to isolate the compound(s) responsible for the action and to elucidate the exact mechanism of action.

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