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Antinociceptive and Anti-inflammatory Activities of *Alstonia scholaris* Linn. R.br.,

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ABSTRACT - *Alstonia scholaris* linn. (apocynaceae) is traditionally used as an antinociceptive and anti-inflammatory agent. The objective of this study was to investigate experimentally the possible antinociceptive and anti-inflammatory properties of *alstonia scholaris*. The effect of ethanolic extract of leaves of *alstonia scholaris* (EEAS) was evaluated in experimental models of pain and inflammation. The leaf extract at 200 and 400 mg/kg showed significant decrease in acetic acid induced writhings in mice with a maximum of 65.76 % at 400 mg/kg. in hot plate method, the percentage of pain inhibition was found to be 73.90 % and 79.56 % with 200, 400 mg/kg of EEAS respectively. There was a significant ($p<0.001$) inhibition in carrageenan induced paw edema with EEAS 200 and 400 mg/kg. The anti-inflammatory effects observed with the extract were comparable to that of standard. There was a significant antiulcerogenic property. The present study indicates that the ethanolic extract of *alstonia scholaris* exhibit significant antinociceptive, anti-inflammatory and antiulcerogenic activities.
KEY WORDS: Antinociceptive, Anti-Inflammatory, *Alstonia Scholaris*, Antiulcerogenic

INTRODUCTION

Alstonia scholaris is an antimalarial (1) drug used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The plant *Alstonia scholaris* Linn. R.Br., belongs to the family Apocynaceae and is native of India. It grows throughout India, in deciduous and evergreen forests, also in plains (2).

The bark is bitter, astringent, acrid, thermogenic, digestive, laxative, anthelmintic, febrifuge, antipyretic, depurative, galactogogue, stomachic, cardiotonic and tonic (2). It is useful in fevers, malarial fevers, abdominal disorders, dyspepsia, leprosy, skin diseases, pruritus, tumours, chronic and foul ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia and debility (2, 3). Juice of the leaves and tincture of the bark acts in certain cases as a powerful galactogogue. The drug is also used in cases of snake-bite (2).

The methanolic extract of this plant was found to exhibit pronounced antiplasmodial activity (4). The plant is reported to have anti-mutagenic effect (5). The bark extract of *Alstonia scholaris* has immunostimulating effect. The aqueous extract at low dose induced the cellular immune response while at high dose inhibited the delayed type of hypersensitivity reaction (6). Echitamine chloride, an indole alkaloid, extracted from the bark of *Alstonia scholaris* has got highly promising anticancer (7,8) effect against sarcoma – 180. The plant has hepatoprotective activity on liver injury induced by CCl_4, β-D-galactosamine, acetaminophen and ethanol (9).

In folklore medicine, milky juice of the plant is applied on wounds, ulcers and rheumatic pains; mixed with oil and dropped into ear, it relieves ear ache (2). Since, the plant is reported to relieve rheumatic pains in folklore medicine (2), it was decided to study the antinociceptive, anti-inflammatory activities of leaf extract of *Alstonia scholaris*. Most of the anti-inflammatory drugs are ulcerogenic. From this viewpoint, in the present study, antiulcerogenic property of *Alstonia scholaris* is also recorded.

MATERIALS AND METHODS

Plant Material and Extraction

The leaves of *Alstonia scholaris* (Family: Apocynaceae) were collected in the month of April May 2006 from hills of Savanthwadi, Maharashtra, India. The plant

material was taxonomically identified by the Botanical Survey of India (BSI), Pune and the voucher specimen AS-1 was retained in herbarium of BSI, Pune for future reference. The dried powdered leaves (500 g) were defatted using petroleum ether and subjected to subsequent extraction in a Soxhlet apparatus by using chloroform and ethanol. The solvents were removed from the respective extracts under reduced pressure to obtain a semisolid mass and vacuum dried to yield solid residues (5.24 % w/w chloroform extract and 6.22 % w/w ethanolic extract). This ethanolic extract of *Alstonia scholaris* is named as EEAS. On preliminary phytochemical screening, EEAS showed positive for the presence of alkaloids, tannins, saponins, glycosides, triterpenoids and flavonoids.

Animals

Healthy male Albino Wistar rats weighing between 150–200 g, Albino mice (20 – 25 g) of either sex were maintained in our animal house facility under standard animal house conditions and used for anti-inflammatory and anti-nociceptive activities respectively. CPCSEA guidelines were adhered to during the maintenance and experiment. Experimental protocol was submitted to Institutional Animal Ethics Committee and approval was taken

Acute Toxicity studies

Acute toxicity study was carried out for EEAS following OECD guidelines (10). Overnight fasted, healthy Wistar Albino rats (n=3) were administered orally the EEAS in the dose of 2000 mg/kg body weight and observed continuously for 4 h. No visible change was observed in any test animal and all animals survived beyond 24.

Anti-Nociceptive Activity

Hot Plate Method (11)

The prescreened Swiss Albino mice (reaction time: 2-4 sec) were divided into four groups of six animals each as following: control, standard (Tramadol 5 mg/kg), EEAS 200 mg/kg and EEAS 400 mg/kg. The delay in reaction time (hind paw licking/jumping response) of animals when placed on hot plate maintained at 55 ± 0.1° C (Eddy's analgesiometer, INCO) was recorded at 0, 30 min, 1, 2, 3 h and tabulated. A cut-off reaction time was fixed at 15 sec to avoid damage to the paws.

Acetic acid induced writhing (12)

Swiss Albino mice were assigned into four groups - control, standard (Aspirin 100 mg/kg), EEAS 200 mg/kg and EEAS 400 mg/kg. Writhing was induced after 30 min by intraperitoneal injection of 0.1 ml of 0.6 % acetic acid. The number of writhes was counted for 30 min immediately after acetic acid injection in all animals. Percentage protection was calculated for all groups.

Anti-inflammatory activity

EEAS was evaluated for anti-inflammatory activity by carageenan induced rat paw oedema method (13, 14). Male albino Wistar rats (150–200 g) were randomly distributed into 4 groups of 6 animals each. First group served as a control, second group served as the standard (received diclofenac sodium 10 mg/kg, *po*), while the third and fourth groups received 200 mg/kg, 400 mg/kg body weight of EEAS respectively. After 1 h 0.1 ml of 1 % w/v suspension of carageenan was injected into the sub plantar region of left hind paw to all the four groups. The paw volumes were measured using plethysmometer every hour till 6 h after carageenan injection, and mean increase in paw volumes were noted.

Anti-Ulcerogenic activity (15)

Animals of four groups of six rats in each were fasted for 16 h. Control, diclofenac sodium (10 mg/kg), EEAS 200 mg/kg, 400 mg/kg were orally administered. Animals were sacrificed 4 h after the administration of the drugs, 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. In the second model (16), the above said procedure was followed after administering the respective drugs orally for 7 days.

Statistical analysis

The difference in the paw volume at different time intervals and ulcer scores were analysed for statistical significance by performing one-way ANOVA followed by Newmans-Keul multiple comparison test. $p < 0.05$ implies significance.

RESULTS

Hot plate Method

There was a significant ($p < 0.01$) increase in the basal reaction time on treatment with EEAS throughout the study (Fig 1). EEAS was found to increase the basal reaction time in a dose-dependent manner. The highest nociception inhibition of thermal stimulus was exhibited at 3 h (79.56 %) with 400 mg/kg, which was comparable to the standard (80.02 %).

Acetic acid induced Writhing

Dose dependent antinociceptive effect was noted with EEAS at the tested dose levels (Fig 2). Maximum percentage inhibition of writhing response exhibited by the EEAS was at 400 mg/kg (65.76 %) while 200 mg/kg showed 44.30 % reduction in acetic acid induced

Table 1: Anti-inflammatory effect of EEAS on carrageenan induced paw edema

Group	Paw volume					
	1 h	2 h	3 h	4 h	5 h	6 h
Control	7.33 ± 0.2108	7.66 ± 0.2108	7.66 ± 0.2108	7.83 ± 0.166	7.66 ± 0.2108	7.33 ± 0.2108
Standard	4.16 ± 0.16***	4.16 ± 0.16***	4.16 ± 0.16***	4.5 ± 0.2236***	6.33 ± 0.2108***	6.50 ± 0.2236
EEAS 200 mg/kg	7.33 ± 0.2108	7.50 ± 0.2236	6.84 ± 0.166**	5.66 ± 0.2108***	5.166 ± 0.166***	6.0 0± 0.36**
EEAS 400 mg/kg	7.50 ± 0.2236	7.50 ± 0.2236	6.5 ± 0.2236**	4.5 ± 0.2236***	4.16 ± 0.16***	5.66 ± 0.2108***

*Values are mean ± SEM, (n= 6). * *p< 0.01, ***p< 0.001 compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test).*

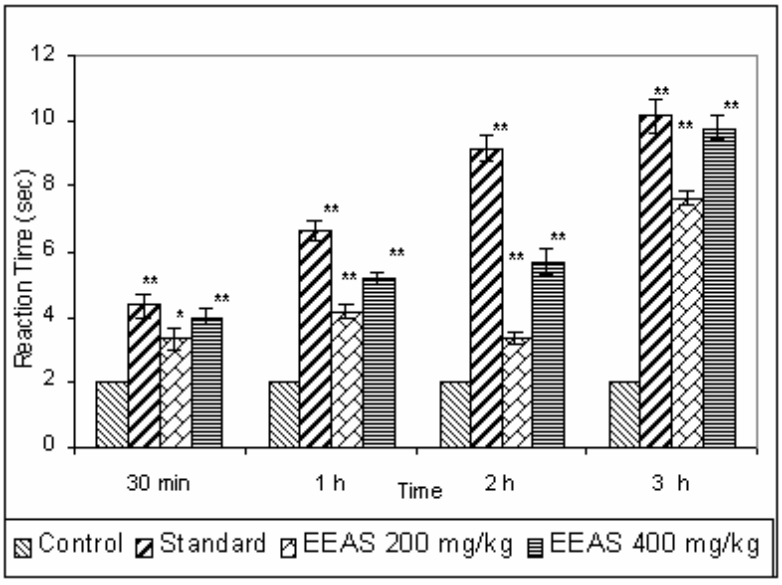
Table 2: Antiulcerogenic activity of EEAS

Parameter	Control	Diclofenac sodium (10 mg/kg)	EEAS	
			200 mg/kg	400 mg/kg
Ulcer Score (4 hrs)	0.26 ± 0.10	3.33 ± 0.34***	0.26 ± 0.12	0.38 ± 0.82
Ulcer score (7 days)	0.25 ± 0.12	3.84 ± 0.16**	0.54 ± 0.14	0.66 ± 0.22

Ulcer scores: *0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions.*

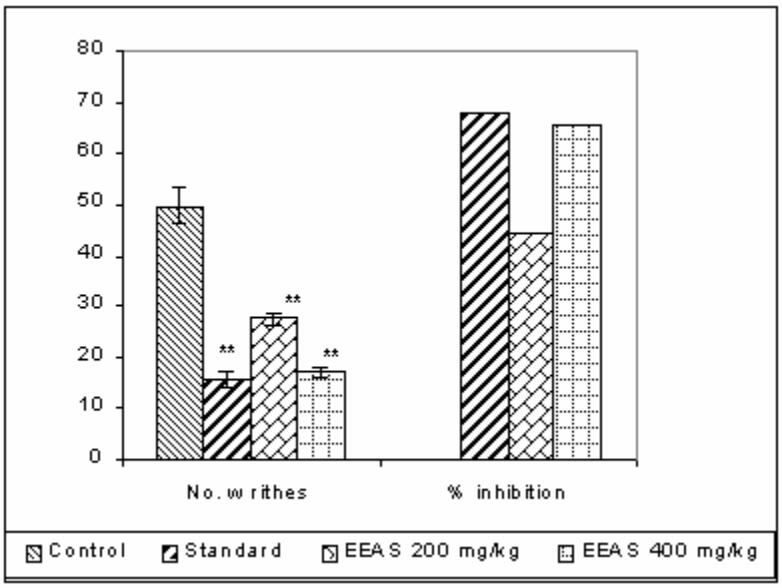
Values are mean ± SEM, (n=6). * *p <0.01, ***p< 0.001 compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test).

Figure 1: Antinociceptive effect of EEAS on thermal stimulation



**p< 0.05, * *p< 0.01, compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test).*

Figure 2: Antinociceptive effect of EEAS on acetic acid induced writhing



** *p< 0.01 compared to control group (One-way ANOVA followed by Dunnet's Multiple Comparison test).*

writhing response, which was comparable to that of standard that caused 68.78 % inhibition.

Anti-inflammatory activity

EEAS showed significant (p< 0.01) decrease in paw oedema after 3 h of injection of carrageenan (Table 1). However the effect was more prominent (p< 0.01) at 4, 5 and 6 h. The percentage inhibition of paw volume of EEAS 400 mg/kg was comparable to that of standard at 4 h and more prominent at 5 h and 6 h.

Antiulcerogenic activity

The groups of animals treated with EEAS 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 diclofenac sodium (Table 2). Treatment of the extracts for seven days did not show any ulceration whereas the ulcer score was significantly (p <0.01) high with diclofenac sodium treated rats (Table 2).

DISCUSSION

The thermal stimuli in hotplate test and the writhing response of the animals to an intra-peritoneal injection of noxious chemical are used to screen both peripherally and centrally acting analgesic activity. Acetic acid causes algesia by liberating endogenous substances that excite the pain nerve endings (17). From the results it is apparent that the EEAS showed a significant antinociceptive effect in hot plate test and writhing response, which are comparable to that of the standard. Studies demonstrate that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produced significant antinociceptive and anti-inflammatory activities (18, 19). There are also few reports on the role of tannins in antinociceptive and anti-inflammatory activities (20). NSAIDs can inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors (21). The mechanisms of antinociceptive action of EEAS could be due to the presence of flavonoids and mediated through central and peripheral mechanisms.

Carrageenan induced paw oedema was taken as 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 carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin (22, 23) and the delayed phase is sustained by the leucotrienes and prostaglandins (24). Flavonoids and tannins are reported to inhibit PG synthesis (25). As phytochemical tests showed presence of triterpenoids, tannins and flavonoids in EEAS, it might suppress the formation of PG or antagonize their action and exert the activity. A

strong correlation between the potency of NSAIDs as an inhibitor of prostaglandin (PG) synthesis and ulcerogenic activity has been suggested (26). Most of the NSAIDs have well-balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity. The ethanolic extract of *Alstonia scholaris* possesses a marked anti-inflammatory activity and its lack of ulcerogenic activity is suggestive that it does not act mainly by PG synthetase inhibition, (but through some selective mechanism viz. Cox-2). Further, chronic administration of the extracts did not produce ulcer which proves the safety of the extracts. This is a point of distinct advantage when considering the chronic administration.

CONCLUSION

The ethanolic extract of *Alstonia scholaris* Linn. comprises antinociceptive and anti-inflammatory activities. EEAS, whose spectrum of anti-inflammatory activity appears to be different from classical NSAIDs, with the distinct advantage of its freedom from gastric ulcerogenic effects, is likely to have therapeutic potential. It is worthwhile to isolate the bioactive principles, which are responsible for these activities, which is in process. Studies are underway to evaluate the anti-arthritic property of the extract. However, further studies are essential to elucidate the detailed mechanisms of action for antinociceptive and anti-inflammatory activities.

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REFERENCES

1. P. Versha, B. Ghosh, B. Anroop and M. Ramanjit. Antimicrobial activity of *Alstonia scholaris* leaf extracts. *Indian drugs* **40(7):** 412-413 (2003).
2. A.K. Nadkarni. **Dr. KM Nadkarni's Indian Materia Medica**, Vol. 1, (Popular Prakashan, Bombay, 1976) pp. 80-83.
3. K.R. Kirtikar and B.D. Basu. **Indian Medicinal Plants,** Vol.1, (Lalit Mohan Basu, Allahabad, 2002) pp. 111 – 114.
4. N. Keawpradub, G.C. Kirby, J.C.P. Steele and P.J. Houghton. Antiplasmodial activity of extracts and alkaloids of three *Alstonia* species from Thailand. *Planta Medica* **65(8):** 690-694 (1999).
5. C.Y. Lim-Sylianco, A.P. Jocano and C.M. Linn. Antimutagenicity of twenty Philippine plants using the micronucleus test in mice. *Philippine Journal of Science* **117(3):** 231-235 (1990).
6. M.I. Iwo, A.A. Soemardji, D.S. Retnoningrum and U.U.M. Sukrasno. Immunostimulating effect of pule (*Alstonia scholaris* L. R.Br., Apocynaceae) bark extracts. *Clin. Hemorheol. Microcirc.* **23(2-4):** 177-183 (2000).
7. V. Saraswathi, V. Mathuram, S. Subramanian and S. Govindasamy. Modulation of the impaired drug metabolism in sarcoma-180-bearing mice by echitamine chloride. *Cancer Biochem Biophys.* **17(1-2):** 79-88 (1999).
8. V. Saraswathi, N. Ramamoorthy, S. Subramaniam, V. Mathuram, P. Gunasekaran and S. Govindasamy. Inhibition of glycolysis and respiration of sarcoma-180 cells by echitamine chloride. *Chemotherapy* **44(3):** 198-205 (1998).
9. S.C. Lin, C.C. Lin, Y.H. Lin, S. Supriyatna and S.L. Pan. The protective effect of *Alstonia scholaris R.Br.* on hepatotoxin-induced acute liver damage. *Am. J. Clin. Med.* **24(2):** 153-164 (1996).
10. "Guidance document on acute oral toxicity testing" Series on testing and assessment No. 24, 2001 Organisation for economic co-operation and development, OECD Environment, health and safety publications, Paris (www.oecd.org/ehs).
11. H.V. Gerhard and H. V. Wolfgang. **Drug Discovery and Evaluation** I Edn. (Springer-Verlag, Germany, 1997) pp. 382.
12. B. Nathan Eddy and Dorothy Leimbach. Synthetic analgesics. II Dithienybutenyl and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **107:** 385 – 393 (1953).
13. C.A. Winter, E.A. Risley and G.W. Nuss. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Bio. Med.* **111:** 545-547 (1962).
14. R.A. Turner. **Screening methods in Pharmacology** (Academic Press, New York, 1965) pp. 158.
15. C.H. Cashin, W. Dawson and E.A. Kitchen. The pharmacology of benoxaprofen (2-(4(chlorophenyl)-alpha-methyl-5-benzoxazole acetic acid), LRCL 3794, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthesis. *J. Pharm. Pharmacol.* **29(6):** 330-6 (1977).
16. L.H. Santos, C.A. Feres, F.H. Melo, M.M. Coelho, M.S. Nothenberg, S. Oga and C.A. Tagliati. Anti-inflammatory, antinociceptive and ulcerogenic activity of a zinc-diclofenac complex in rats. *Braz. J. Med. Biol. Res.* **37(8):** 1205 – 1213. (2004).

17. P.P. Raj. Pain Medicine: A Comprehensive Review, 1 edition. (Mosby-Year Book, Missouri, 1996) pp.12-23.
18. E.M. Galati, M.T. Monforte, S. Kirjavainen, A.M. Forestieri, A. Trovato and M.M. Tripodo. Biological effects of hesperidin, a citrus flavonoid: anti-inflammatory and analgesic activity. *Farmaco*. **40(11):** 709 – 712 (1994).
19. M. Ramesh Rao, Y.M. Rao, A.V. Rao, M.C. Prabhakar, C.S. Rao, N. Muralidhar and B.M. Reddy. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. *J. Ethnopharmacol*. **62(1):** 63 – 66 (1998).
20. Vanu Ramkumar Ramprasath, Palanivelu Shanthi and Panchanatham Sachdanandam. Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. nut milk extract in experimental inflammatory conditions. *Biol. Pharm. Bull*. **29 (4):** 693-700 (2006).
21. Fields HL. Analgesic Drugs. In: Day W Eds. Pain 1st ed. (USA, Mac. Graw-Hill; 272 (1987).
22. R. Vinegar, J.F. Truax and J.L. Selph. Quantitative studies of the pathway to acute carrageenan inflammation. *Fed. Proc*. **35(13):** 2447 – 56 (1976).
23. G.L. Larsen and P.M. Henson. Mediators of inflammation. *Annu. Rev. Immunol*. **1:** 335-359 (1983).
24. P.M. Brooks and R.O. Day. Nonsteroidal anti-inflammatory drugs-differences and similarities. *N. Engl. J. Med*. **324:** 1716-1725 (1991).
25. M.J. Alcaraz and M.L. Ferrandiz. Modification of arachidonic metabolism by flavonoids. *J. Ethnopharmacol*. **21(3):** 209-229 (1987).
26. E.A. Boyle, P.C. Freeman, F.R. Mangan and M.J. Thomson. New xanthone derivatives as potent anti-inflammatory agents. *J. of Pharm. Pharmacol*. **34:** 562-569 (1982).