

## Antioxidant and anti-inflammatory activity of *Alstonia scholaris* R.Br. stem bark extract

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### ABSTRACT

**Background:** The plant *Alstonia scholaris* R.Br. (Labiatae) is widely cultivated throughout India. The bark of this tree is medicinally used as an astringent, tonic, anthelmintic, antiperiodic and febrifuge. **Methods:** The effect of Methanolic extract of stem bark of *Alstonia scholaris* R.Br. was investigated to evaluate the antioxidant activity by TLC method and anti-inflammatory activity by Carrageenan induced rat paw odema, dextran induced rat paw odema and cotton pellet induced granuloma. **Result:** Anti-inflammatory activity of the tested extract and its fractions was comparable with that of the standard drug Indomethacin (10 mg/kg). **Conclusion:** The present investigation for the first time, confirms the potential anti-inflammatory activity in the methanol extract of *Alstonia scholaris* stem bark. Antioxidant property of the extract was confirmed quantitatively by  $\beta$ -carotene–linoleate oxidation method by thin layer chromatography.

**Keywords:** anti-inflammatory activity, *Alstonia scholaris*, dextran.

### INTRODUCTION

The plant *Alstonia scholaris* R.Br. (Labiatae) is widely cultivated throughout India and found in Subhimalayan tract from the Jumna eastward ascending to 3000 feet. It is abundantly found in Bengal and South India. The bark of this tree is medicinally used as an astringent, tonic, anthelmintic, antiperiodic and febrifuge.<sup>[1]</sup> Recent report indicates that the plant has got bronchovasodilator activity.<sup>[2]</sup> However, there is no report on the anti-inflammatory activity of this plant though inflammation is a common occurrence in infective conditions. Therefore, the aim of the present work is to evaluate, for the first time, the anti-inflammatory activity of *Alstonia scholaris* stem bark extract.

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### MATERIALS AND METHODOLOGY

#### Plant Material

The stem bark of *Alstonia scholaris* R.Br. were collected from Udupi district, India during March, April and May, 2005. The voucher specimens have been identified and deposited in NGSIM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore, India.

#### Extraction

Coarsely powdered dry stem bark (1 kg) were successively extracted in cold with 95% methanol for 72 h at room temperature. The whole extract was filtered and the solvent was evaporated to dryness under reduced pressure at 40°C–45°C. The chemical tests indicated the presence of flavonoids.

#### Animals used

Albino Wistar rats of either sex weighing 160–180 g each were housed in standard metal cages at room temperature.

They were provided with food and water *ad libitum*. The rats were allowed a one-week acclimatization period before the experimental sessions.

### Carrageenan-induced rat paw edema

The rats were divided into eleven groups, each group consisting of six animals. Edema was induced by subplantar injection of 0.1% freshly prepared carrageenan suspension into the right hind paw of each rat. The paw volume was measured at 0 h and at 3 h after the injection of carrageenan, using a plathysmometer.<sup>[3]</sup> The methanol extract of *Alstonia scholaris* at 200 and 400 mg kg<sup>-1</sup> and its fractions at 25 and 50 mg kg<sup>-1</sup> doses was administered orally to nine groups of rats. The tenth and eleventh groups of rats received 5 ml kg<sup>-1</sup> propylene glycol orally as vehicle control or 10 mg kg<sup>-1</sup> Indomethacin as drug control respectively, for assessing comparative pharmacological significance. Drug pretreatment was given 1 h before the injection of carrageenan.

### Dextran-induced rat paw edema

The paw was induced in the right hind paw by subplantar injection of 0.1 ml of freshly prepared 1% dextran solution.<sup>[4]</sup> Paw volume was measured 30 min before and after dextran injection. The rats were treated as described above. The percentage inhibition of edema was calculated for both models.<sup>[5]</sup>

### Cotton pellet-induced granuloma

The eleven groups of rats, eight in each group was included in this study. After shaving off the fur the animals were anaesthetized. Sterile preweighed cotton pellets (10 mg) were implanted in the axilla region of each rat through a single needle incision.<sup>[6]</sup> Methanol extract of *Alstonia scholaris* stem bark at 200 and 400 mg kg<sup>-1</sup>, and its fractions at 25 and 50 mg kg<sup>-1</sup>, indomethacin at 10 mg kg<sup>-1</sup> (standard) or propylene glycol at 5 mg kg<sup>-1</sup> (control) were administered orally to the respective group of animals for seven consecutive days, from the day of cotton-pellet implantation. On the eighth day, the animals were anaesthetized again, the cotton pellets were removed surgically and made free from extraneous

tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. The increase in the dry weight of the pellets was taken as measure of granuloma formation.<sup>[7]</sup>

### Antioxidant property by TLC method<sup>[8]</sup>

*Alstonia scholaris* stem bark extract was solubilized in methanol and subjected to TLC on 20×20 cm glass plates precoated with silica gel G. The developing solvent used was chloroform:methanol (9:1 v/v) for flavonoids and chloroform:ethyl acetate:formic acid (5:4:1 v/v) for phenolic compounds. The location of the spots was marked under UV light.  $\beta$ -carotene linoleate (a mixture of  $\beta$ -carotene in 30 ml of chloroform and 2 ml of purified Linoleic acid in 60 ml of 95% ethanol) was sprayed uniformly on the plates and exposed to daylight for about 4 hours. The background was bleached and the spots which contained the flavonoids and phenolic compound retained the yellow color which is indicative of antioxidant activity.<sup>[9]</sup>

### Statistical analysis

The results were expressed as mean + SE and the significance was evaluated by student's *t* test compared with control.<sup>[10]</sup>

## RESULTS

The results presented in Table 1 showed that the methanolic extract at 200 and 400 mg kg<sup>-1</sup>, po exhibited significant anti-inflammatory activity in all the experiment models. The methanolic extract at 400 mg kg<sup>-1</sup> exhibited maximum inhibition (64.86%) of paw edema in carrageenan-induced rat paw edema, while indomethacin showed 67.29% inhibition of edema volume after 4 h of drug treatment (Table 1). The results of the dextran-induced rat paw edema, presented in Table 2 showed that the edema suppression at 400 mg kg<sup>-1</sup> of methanolic extract was 53.08% inhibition of edema respectively whereas indomethacin produced 62.42% inhibition. In cotton pellet granuloma (chronic inflammation model) test the methanolic extract at 400 mg kg<sup>-1</sup> exhibited maximum inhibition of 54.33% (Table 3).

**Table 1 Effect of the stem bark of *Alstonia scholaris* methanol extract on Carrageenan-induced rat paw edema**

Treatment	Dose (mg/kg po)	Paw volume	% inhibition
Propylene glycol	5 ml/kg	5.35 ± 0.19	–
Indomethacin	10	1.75 ± 0.21*	67.29
Stem bark extract	200	2.54 ± 0.33*	52.53
	400	1.88 ± 0.25*	64.86

Values are mean + SE, n = 6, \*p < 0.001 compared with control, Student's *t*-test.

**Table 2 Effect of stem bark of *Alstonia scholaris* methanol extract on Dextran-induced rat paw edema**

Treatment	Dose (mg/kg po)	Paw volume	% inhibition
Propylene glycol	5 ml/kg	4.71 ± 0.19	–
Indomethacine	10	1.77 ± 0.04*	62.42
Stem bark extract	200	2.38 ± 0.55*	49.47
	400	2.21 ± 0.44*	53.08

Values are mean + SE, n = 6, \*p < 0.001 compared with control, student's t-test.

**Table 3 Effect of stem barks of *Alstonia scholaris* methanol extract on cotton-pellet granuloma in Rats**

Treatment	Dose (mg/kg po)	Paw volume	% inhibition
Propylene glycol	5 ml/kg	75.55 ± 2.70	–
Indomethacine	10	34.35 ± 1.77*	54.54
Stem bark extract	200	48.48 ± 1.95**	35.83
	400	34.23 ± 1.62*	54.69

Values are mean ± S.E. \*p < 0.05, \*\*p < 0.01 compared with control, Student's t - test's.

## DISCUSSION

The results of the three anti-inflammatory models indicated moderate to strong anti-inflammatory activity of the methanolic extract of *Alstonia scholaris* stem bark. However, the polar fractions containing a major alkaloid echitamine do not have recognizable anti-inflammatory activity. *Alstonia scholaris* is reported to contain echitamine having antineoplastic activity.<sup>[11]</sup>

*Alstonia scholaris* showed significant anti-inflammatory activity comparable to that of indomethacin against carrageenan induced acute pedel edema, dextran induced edema and cotton pellet induced granuloma. The carrageenan-induced paw edema is believed to be biphasic, of which the first phase is mediated by early release of histamine and 5 HT followed by the release of kinin in later phase.<sup>[12]</sup> On the other hand, dextran mediated inflammatory (edema) was reduced probably as a result of antihistamine effects of the extract, as dextran is known to cause inflammation through both histamine and serotonin.<sup>[13]</sup> The extract also exhibited significant anti-inflammatory effect in cotton pellet granuloma test. This reflected its efficacy to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. However, the exact mechanism of anti-inflammatory action of *Alstonia scholaris* stem bark extract and its fractions required further studies.

The present investigation for the first time, confirms that there is potential anti-inflammatory activity in the methanol extract of *Alstonia scholaris* stem bark. Antioxidant property of the extract was confirmed quantitatively by

$\beta$ -carotene–linoleate oxidation method by thin layer chromatography. Many flavonoids are reported from the stem bark of *Alstonia scholaris*.<sup>[14]</sup> The anti-inflammatory property of the extract may be due to the flavonoids present in the extract.<sup>[15]</sup>

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