

Anti-inflammatory and Analgesic Activities of *Holarrhena antidysenterica* Wall. Leaf Extract in Experimental Animal Models

P. S. Sujan Ganapathy¹ • Y. L. Ramachandra^{1*} • S. Padmalatha Rai²

¹ P. G. Department of Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta – 577 451, Karnataka, India

² Department of Biotechnology, Manipal Life Science Center, Manipal University, Manipal-576104, Karnataka, India

Corresponding author: * ylrpub@gmail.com

ABSTRACT

The methanolic extract of *Holarrhena antidysenterica* Wall. leaf was subjected to pharmacological screening using various animal models. The extract showed anti-inflammatory activity in the carrageenan-induced rat paw edema model method and analgesic activity when tested in the acetic acid-induced writhing test in Swiss albino mice and tail-flick response tests in Swiss albino rats. The plant extract, at doses of 100 and 200 mg/kg body weight, showed significant inhibition of rat paw edema at the fourth hour after carrageenan injection and peripheral as well as central analgesic activity by oral route. Therefore, the current study indicates that the methanol extract of *H. antidysenterica* leaf has significant anti-inflammatory and peripheral and central analgesic activity.

Keywords: Apocynaceae, inflammation, methanolic extract, pain

INTRODUCTION

Holarrhena antidysenterica Wall. is a dwarf tree belonging to the Apocynaceae family, commonly known as *Kutaja* in Hindi, an important plant used in indigenous systems (traditional and Ayurvedic) of medicine as a remedy for bronchitis, hematuria, spermatorrhoea, epilepsy, asthma, leprosy, eczema, diarrhea and jaundice (Bhattacharjee 2000; Guha Bakshi *et al.* 2001). The plant also has amoebicidal, anti-dysenteric, anthelmintic, antiperiodic, febrifugic and diuretic activities (Bhattacharjee 2000; Prajapati *et al.* 2004).

Various parts of *H. antidysenterica* have been reported to possess antibacterial activity (Jolly and Mechery 1996; Sujan Ganapathy *et al.* 2008). The bark has been reported to have astringent and antidiarrheal properties (Chopra *et al.* 1982). Leaves of the plant are used to cure scabies (Prajapati *et al.* 2004). However, very little scientific data is available to validate the folkloric claim. Therefore, this study was undertaken to evaluate: a) the anti-inflammatory potential of the methanolic extract of *H. antidysenterica* on carrageenan-induced rat paw edema and b) the analgesic activity using the acetic acid-induced writhing test in albino mice and tail immersion response in albino rats.

MATERIALS AND METHODS

Plant material

The leaves of *H. antidysenterica* plants were collected from Kuvempu University, Bhadra Wild Life Sanctuary, Karnataka (Southern India) in May 2006. Matured leaves from four different plants above 20 cm DBH (diameter at breast height) were sampled and used for the study. The taxonomic identification of the plant was confirmed by Dr. Y. L. Ramachandra, Department of Biotechnology, Kuvempu University, Shankaraghatta (voucher specimen number YLR204).

Extraction

Freshly collected leaves of *H. antidysenterica* were shade-dried then powdered using a mechanical grinder. 250 g of pulverized leaf were soaked in 750 ml of methanol (LR grade, Merck, India) and kept on a rotary shaker for 24 h. The extract was filtered under vacuum through a Whatman No. 1 filter paper and the process was repeated until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint colour. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). The *H. antidysenterica* extract was administered as a suspension in 2% gum acacia to the animals.

Animals

Albino Wistar rats (150-200 g) and Swiss albino mice (25-30 g) of both sexes were procured from Venkateshwara Enterprises, Bangalore. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; 60-70% relative humidity) in a 12 h light-dark cycle. The animals were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during experimental hours. All experimental protocols were approved by the institutional animal ethics committee (No. NCP/IAEC. Clear/05/2007-08).

Chemicals and drugs

The following chemicals and drugs were used: Lambda carrageenan (Sigma Aldrich, Bangalore), Diclofenac sodium (Dr. Reddy Labs, Hyderabad), acetic acid (Merck specialities Pvt. Ltd., Mumbai), aspirin (Vikash Pharma, Mumbai), Pentazocine (Pharma Impex Laboratories Pvt. Ltd, Kolkata, India), methanol (Universal Laboratories Pvt. Ltd., Mumbai).

Acute toxicity study

A group of 3 Swiss albino mice weighing 22-25 g selected by random sampling technique were used in the study. The resulting ex-

Table 1 Effect of methanolic leaf extract of *H. antidysenterica* on carrageenan-induced rat paw edema.

| Group (n)-Treatment | Dose mg/kg | Mean paw volume (ml) ± SEM | | | |
|---------------------------|------------|----------------------------|---------------|---------------|---------------|
| | | 60 min | 120 min | 180 min | 240 min |
| Control | 0.5 ml | 0.68 ± 0.01 | 0.71 ± 0.02 | 0.76 ± 0.02 | 0.73 ± 0.01 |
| Diclofenac sodium | 10 | 0.53 ± 0.01** | 0.58 ± 0.01** | 0.54 ± 0.01** | 0.40 ± 0.02** |
| <i>H. antidysenterica</i> | 100 | 0.62 ± 0.01 | 0.66 ± 0.01 | 0.62 ± 0.01** | 0.54 ± 0.01** |
| <i>H. antidysenterica</i> | 200 | 0.6 ± 0.01** | 0.63 ± 0.01 | 0.60 ± 0.01** | 0.44 ± 0.02** |

n = 6 animals in each group. * $P < 0.05$; ** $P < 0.001$ compared to control

tracts were subjected to acute oral toxicity studies as per revised OECD Organization of Economic Co-operation and Development guidelines (OECD No. 423) (acute class method (Ecobichon 1997)). The animals were fasted overnight, provided only water after which extract was administered to the groups orally by gastric intubation. These extracts were devoid of any toxicity up to 5000 mg/kg body weight in albino mice for a single oral dose monitored for 14 days. If mortality was observed in 2 or 3 animals among 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hrs. The optimum conditions for experiments were decided on the basis of pilot experiments carried out using three animals per group. For further experiments, a group of at least six animals was used for individual treatment. Based on exploratory studies, anti-inflammatory and analgesic activity was investigated using the methanol extract only.

Anti-inflammatory activity: Carrageenan-induced rat paw edema

The rats were divided into 4 groups of 6 animals each (Table 1) and initial paw volume was measured at 0 hr. Further paw edema was induced by injecting 0.1 ml of 1% carrageenan in physiological saline into the subplantar tissue of the left hind paw of each rat (Winter *et al.* 1962). The two test groups were treated orally with extract (100 and 200 mg/kg body weight (bw)), the control group received normal saline (0.5 ml/kg bw) orally and standard group animals received Diclofenac sodium (10 mg/kg bw) by intraperitoneal injection 30 min prior to carrageenan administration. Paw volume was measured at an interval of 1 hr up to 4 hrs by mercury displacement method using a plethysmograph. The percentage inhibition of edema in the test drug-treated group (Table 2) was calculated by using the formula:

$$\% \text{ Inhibition} = 1 - (Vt/Vc) \times 100$$

where Vt = edema volume in the test drug treated animals; Vc = edema volume in the control group animals.

Analgesic activity: Acetic acid-induced writhing test

The prescreened animals were divided into groups as shown in Table 3. Aspirin at 30 mg/kg bw, suspended in 2% gum acacia was used as the standard drug. The two test groups were treated orally with extract (100 and 200 mg/kg bw), while the control group received normal saline (0.5 ml/kg bw). Writhing was induced 30 min later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water (Ghosh 1984). The number of writhes was counted for 20 min immediately after the acetic acid injection. The percentage protection was calculated by using the formula:

$$\% \text{ Inhibition} = 1 - (Vt/Vc) \times 100$$

where Vt = No. of writhing in the drug treated animals; Vc = No. of writhing in the control group.

Tail-flick method

The prescreened animals were divided into groups as shown in Table 4. Pentazocine 10 mg/kg bw (i.p.), served as the standard (Kumar *et al.* 2001; Vedhanayaki *et al.* 2003). The two test groups were treated orally with extract (100 and 200 mg/kg bw), while the control group received normal saline (0.5 ml/kg bw). Every 15 min, animals were held firmly to immerse the tail in a water bath maintained at constant temperature ($55 \pm 0.5^\circ\text{C}$). The time required for the typical reaction, a violent jerk of the tail, was recorded to assess response to the noxious stimulus (Turner 1965).

Statistical analysis

The data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple pair-wise comparison tests to assess the statistical significance. $P < 0.05$ was considered as statistically significant when compared to normal control group, using software ezANOVA ver. 0.98.

RESULTS

The results of the anti-inflammatory and analgesic studies are shown in Tables 1-4. In the acute inflammation model, the methanolic extract of *H. antidysenterica* in doses of 100 and 200 mg/kg produced dose-dependent inhibition of paw edema. The test and the standard drugs produced significant inhibition of paw edema as compared to the control ($P < 0.001$) at 3 and 4 h duration. The methanolic leaf extract of *H. antidysenterica* (100 and 200 mg/kg) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner. The standard drug, aspirin produced increased inhibition of writhing. These results were found to be highly significant ($P < 0.001$) in comparison with the control. The results of the tail flick model showed significant difference in the mean pre-drug reaction time between the different groups. 15 min after drug administration, reaction time increased significantly for the test and standard groups when compared to the pre-drug reaction time. The test drug produced a dose dependent increase in the reaction time at various time intervals.

Table 2 Effect of methanolic leaf extract of *H. antidysenterica* on percentage inhibition of carrageenan-induced rat paw edema.

| Group (n)-Treatment | Dose mg/kg | 60 min | 120 min | 180 min | 240 min |
|---------------------------|------------|--------|---------|---------|---------|
| Diclofenac sodium | 10 | 22.05 | 18.3 | 28.94 | 45.2 |
| <i>H. antidysenterica</i> | 100 | 8.82 | 7.04 | 18.42 | 26.02 |
| <i>H. antidysenterica</i> | 200 | 11.76 | 11.26 | 21.05 | 39.72 |

n = 6 animals in each group.

Table 3 Effect of methanol leaf extract of *H. antidysenterica* on acetic acid-induced (writhing test) pain in mice.

| Group (n)-Treatment | Dose mg/kg p.o | No of writhing (20 min) | Writhing inhibition (%) |
|---------------------------|----------------|-------------------------|-------------------------|
| Control | 0.5 ml | 79.00 ± 2.46 | --- |
| Aspirin | 30 | 26.17 ± 1.90* | 66.87 |
| <i>H. antidysenterica</i> | 100 | 54.17 ± 1.17* | 31.43 |
| <i>H. antidysenterica</i> | 200 | 43.00 ± 1.29* | 45.56 |

n = 6 number of animals in each group. Values are mean ± SEM. * $P < 0.001$ compared to control

Table 4 Analgesic activity of *H. antidysenterica* methanolic leaf extract by tail flick method in rats.

| Group (n)-Treatment | Dose mg/kg | Basal reaction time (second) after | | | |
|---------------------------|------------|------------------------------------|-------------|--------------|----------------|
| | | 0 min | 15 min | 30 min | 45 min |
| Control | 0.5ml | 3.67 ± 0.33 | 3.67 ± 0.33 | 3.67 ± 0.33 | 3.67 ± 0.33 |
| Pentazocine | 10 | 3.67 ± 0.33 | 5.83 ± 0.31 | 8.17 ± 0.70* | 10.50 ± 0.76** |
| <i>H. antidysenterica</i> | 100 | 3.50 ± 0.43 | 4.83 ± 0.48 | 6.00 ± 0.58 | 6.83 ± 0.60 |
| <i>H. antidysenterica</i> | 200 | 3.50 ± 0.22 | 5.50 ± 0.43 | 7.17 ± 0.60* | 8.33 ± 0.71** |

n = 6 number of animals in each group. Values are mean ± SEM. * $P < 0.05$; ** $P < 0.001$ compared to control

DISCUSSION

The development of carrageenan-induced edema is believed to be biphasic (Olajide *et al.* 2000). Early phase of acute inflammation is due to release of histamine and serotonin stores in the cells and late response are due to stimulating effect on the synthesis of prostaglandins (Crunkhorn and Meacock 1971). The increase in paw volume following carrageenan administration in the control- and standard-treated groups correspond with the findings of previous groups (Singh and Pandey 1996; Jana *et al.* 1999). Methanolic leaf extract of *H. antidysenterica* showed anti-inflammatory activity throughout a 4-hr observation period. Hence it is possible that the anti-inflammatory effect of leaf extracts of *H. antidysenterica* is due to its effect on synthesis of prostaglandins. However slow absorption from gastrointestinal tract or other factors which affect bioavailability, could not be ruled out and require further studies to know the exact mechanism of anti-inflammatory activity of *H. antidysenterica*.

The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ribeiro *et al.* 2000), acid sensing ion channels (Voilley 2004) and the prostaglandin pathways (Vogel and Vogel 1997). The significant antinociceptive activity of methanolic leaf extract of *H. antidysenterica* might be due to the presence of analgesic principles acting with the prostaglandin pathways. In the tail flick method of analgesic activity assay, the extract increased the stress tolerance capacity of the animals and hence also indicated the possible involvement of a higher center (Whittle 1964). The number of writhing movements during a 20 min observation in the control group was (79.00 ± 2.46) which corresponds with the other (Effraim *et al.* 1998; Hajare *et al.* 2000). However, the analgesic activity of methanolic leaf extract of *H. antidysenterica* was found to be more significant on the acetic acid-induced model ($P < 0.001$) than the tail flick ($P < 0.05$) model and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

Although methanolic leaf extract of *H. antidysenterica* exhibited significant anti-inflammatory and analgesic activities, exact mechanisms underlying the observed pharmacological effects can only be elucidated after isolation of active constituents using a wide range of experimental models.

REFERENCES

- Bhattacharjee SK (2000) *Handbook of Medicinal Plants*, Pointer Publishers, Jaipur, India, 183 pp
- Chopra RN, Chara IC, Handa KL, Kapur LD (1982) *Indigenous Drugs of India*, Academic Press, New Delhi, India, 342 pp
- Crunkhorn P, Meacock SC (1971) Mediators of the inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology* **42**, 392-402
- Ecobichon DJ (1997) *The Basis of Toxicology Testing* (2nd Edn), CRC Press, New York, pp 43-60
- Effraim KD, Osunkwo UA, Onyeyilli P, Ngulde A (1998) Preliminary investigation of possible antinociceptive activity of aqueous leaf extract *Ziziphus spina* Christi (Linn) Desf. *Indian Journal of Pharmacology* **30**, 271-272
- Ghosh MN (1984) *Evaluation of Analgesic Agents, Fundamentals of Experimental Pharmacology* (2nd Edn), Scientific Book Agency, Calcutta, India, pp 153-157
- Guha Bakshi DN, Sensarma P, Pal DC (2001) *A Lexicon of Medicinal Plants in India* (Vol II), Naya Prokash Publishers, Calcutta, India, pp 356-358
- Hajare SW, Chandra S, Tandon SK, Sarma J, Lal J, Telang AG (2000) Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. *Indian Journal of Pharmacology* **32**, 357-60
- Jana U, Chattopadhyay RN, Shaw BP (1999) Preliminary studies on anti-inflammatory activity of *Zingiber officinale* Rosc., *Vitex negundo* Linn., and *Tinospora cordifolia* (Willid) Miers in albino rats. *Indian Journal of Pharmacology* **31**, 232-233
- Jolly CI, Mechery NR (1996) Comparative pharmacognostical, physicochemical and antibacterial studies on seeds of *Holarrhena antidysenterica* Wall and *Wrightia tinctoria* R. Br. *Indian Journal of Pharmaceutical Science* **58**, 51-53
- Kumar V, Singh PN, Bhattacharya SK (2001) Antiinflammatory and analgesic activity of Indian *Hypericum perforatum* L. *Indian Journal of Experimental Biology* **39**, 339-343
- Olajide OA, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O, Okpako DT (2000) Studies on the anti-inflammatory, antipyretic and analgesic properties of the *Aistonia boonei* stem bark. *Journal of Ethnopharmacology* **71**, 179-186
- Prajapathi ND, Purohit SS, Sharma AK, Kumar T (2004) *A Handbook of Medicinal Plants*, Agrobios Publishers, Jodhpur, India, 273 pp
- Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato ABP, Poole S, Ferreira SH, Cunha FQ (2000) Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *European Journal of Pharmacology* **387**, 111-118
- Singh RK, Pandey BL (1996) Antiinflammatory activity of seed extracts of *Pongamia pinnata* in rat. *Indian Journal of Physiology and Pharmacology* **40**, 355-358
- Sujan Ganapathy PS, Sudeep HV, Padmalatha Rai S, Ramachandra YL (2008) Screening of crude extracts of *Holarrhena antidysenterica* Wall. against clinically important pathogenic strains. *Medicinal and Aromatic Plant Science and Biotechnology* **2**, 68-71
- Turner RA (1965) *Analgesics: Screening Methods in Pharmacology*, Academic Press, New York, pp 100-116
- Vedhanayaki G, Shastri GV, Kuruvilla A (2003) Analgesic activity of *Piper longum* Linn. root. *Indian Journal of Experimental Biology* **41**, 649-651
- Vogel HG, Vogel WH (1997) Pharmacological assays. In: *Drug Discovery and Evaluation*, Springer-Verlag, Germany, pp 368-370
- Voilley N (2004) Acid sensing ion channels (ASICs): New targets for the analgesics effects of non-steroid anti-inflammatory drugs (NSAIDs). *Current Drug Targets - Inflammation and Allergy* **3**, 71-79
- Whittle BA (1964) The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *British Journal of Pharmacology and Chemotherapy* **22**, 246-253
- Winter CA, Risely EA, Nuss GW (1962) Carrageenan induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine* **111**, 544-547