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ANTI-NOCICEPTIVE ACTIVITY OF *HYGROPHILA AURICULATA* (SCHUM) HEINE

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Abstract

Hygrophila auriculata (Schum) Heine (syn) *Asteracantha longifolia* Nees, Acanthaceae was described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha. The plant was extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, etc. The aqueous extract of aerial parts (HAA) and root (HAR) were screened for its anti-nociceptive property using both chemical and thermal methods of nociception in mice. In chemical method acetic acid writhing test and in thermal methods hot plate and tail flick tests were performed. Both the extracts at doses 100 and 200 mg/kg/p.o inhibited the abdominal constrictions induced by acetic acid and also increased the pain threshold of mice towards the thermal source in a dose dependent manner. The activity exhibited by the extracts was comparable to that of the standard drug aspirin (100 mg/kg/p.o). From the results it was concluded that both extracts exhibited anti-nociceptive activity by central and peripheral mechanism(s).

Key words: *Hygrophila auriculata*, *Asteracantha longifolia*, Anti-nociceptive, Writhing, Hot plate, Tail flick test.

Introduction

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history (Almeida *et al.*, 2001). The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success (Akah and Nwambie, 1994). Natural products in general, and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and antiinflammatory drugs.

Hygrophila auriculata (Schum) Heine (syn) *Asteracantha longifolia* Nees, Acanthaceae is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha “having eyes like the Kokila or Indian Cuckoo.” The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, urinary infections, oedema and gout. It is classified in ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of premeham (Diabetes), athisaram (Dysentry) etc., (Nadkarni, 1978, Chopra *et al.*, 1986).

The plant is known to possess antitumor (Ahmed *et al.*, 2001; Mazumdar *et al.*, 1997), hypoglycaemic (Fernando *et al.*, 1991), antibacterial (Boily and Vanpuyvelde, 1986; Vlientick *et al.*, 1995) and hepatoprotective (Anubha Singh and Handa, 1995) activities. The literature survey revealed that there are no scientific studies carried out regarding anti-nociceptive and anti-inflammatory activities on the aerial parts and roots of *Hygrophila auriculata* to substantiate their therapeutic claim. Hence in the present study the aqueous extract of aerial parts and roots were examined for its anti-nociceptive property

Materials and Methods

Collection of plant material

Fresh aerial parts and roots of *Hygrophila auriculata* were collected from Red Hills, Thiruvalluvar Taluk, Tamilnadu, Chennai, India in the month of August. The plant specimen was authenticated by Dr. S. Jayaraman, Plant Anatomy Research Center, Chennai, Tamilnadu. A voucher specimen (No: 12/2002) has been deposited at the herbarium unit of the Department of Pharmacology and Environmental Toxicology, University of Madras, Taramani, Chennai.

Preparation of plant extract

The air-dried aerial parts and roots of *Hygrophila auriculata* were made in to a coarse powder. The powdered material was macerated using distilled water for 24 hrs. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum dried. The aerial parts and root yielded a brownish residue of 20% and 24% extract, respectively with reference to dry starting material.

Animals

Wistar albino mice (20 ± 5 g) of either sex, procured from TANUVAS (Tamilnadu University of Veterinary and Animal Sciences) were used for the study. The animals were housed in large polypropylene cages in a temperature-controlled room ($22^\circ \pm 2^\circ\text{C}$) and provided with standardized pelleted feed (TANUVAS) and clean drinking water *ad libitum*. The study has got the clearance from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Anti-nociceptive activity

Three models, viz. acetic acid induced writhing response (chemical method); hot plate reaction time and tail flick assay (thermal methods) using albino mice were employed to study the anti-nociceptive effect according to the method of (Turner, 1965). The animals were divided into six groups of six animals each. Group I served as normal control and received distilled water (1 ml/kg, p.o), group II served as reference group and received aspirin (100 mg/kg/p.o), groups III-VI served as treatment groups in which groups III & IV received HAA at the doses of 100 and 200 mg/kg/p.o, respectively and groups V & VI received HAR at the doses of 100 and 200 mg/kg/p.o, respectively.

Chemical method

Acetic acid induced writhing test

Acetic acid (1% v/v) was administered intraperitoneally to all the groups at the dose of 1 ml/kg body weight 60 min after the administration of test compounds. Anti-nociception was recorded by counting the number of writhes after the injection of acetic acid for a period of 10 min. A writhe is indicated by abdominal constriction and full extension of hind limb

Thermal method**Hot plate test**

The test was performed using Eddy's hot plate maintained at a temperature of $55 \pm 1^\circ\text{C}$. The basal reaction time of all animals towards thermal heat was recorded. The animals which showed fore paw licking or jumping response within 6-8 secs were selected for the study. 60 min after the administration of test and reference compounds, the animals in all the six groups were individually exposed to the hot plate maintained at 55°C . The time taken in secs for fore paw licking or jumping was taken as reaction time. A cut off period of 15 secs is observed to avoid damage to the paws. The pain inhibition percentage (PIP) (Wu *et al.*, 2003) was calculated according to the following formula:

$$\text{Pain inhibition percentage (PIP)} = ((T_1 - T_0) / T_0) \times 100$$

T_1 is post-drug latency and T_0 is predrug latency.

Tail flick test

Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. The animals, which showed flicking response within 3-5 secs, were selected for the study. A cut off period of 15 secs is observed to avoid damage to the tail. The measurements of withdrawal time using the tail flick apparatus was conducted at 30 and 60 min after administration of drugs. PIP was calculated as above.

Statistical analysis

The data were expressed as mean \pm SEM of 6 animals. Results were analysed statistically by One-way ANOVA followed by Tukey's multiple comparison using SPSS software student's version. The difference was considered significant if $p < 0.05$.

Results and Discussion

The anti-nociceptive activity of HAA and HAR were evaluated using both chemical and thermal methods of nociception in mice. These methods are used to detect central and peripheral analgesics. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests are most sensitive to centrally acting analgesics. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE_2 and $\text{PGF}_{2\alpha}$ and their levels were increased in the peritoneal fluid of the acetic acid induced mice (Deraedt *et al.*, 1980). Thermal induced nociception indicates narcotic involvement (Besra *et al.*, 1996). Thermal nociceptive tests are more sensitive to opioid μ receptors and non-thermal tests are to opioid κ receptors (Abbott and Young, 1988, Furst *et al.*, 1988).

Both HAA and HAR significantly ($P < 0.001$) reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid in a dose-dependent manner (Figure 1). HAA exhibited a writhing inhibition percentage of 35.82% and 49.46%, respectively whereas HAR showed a writhing inhibition percentage of 33.96% and

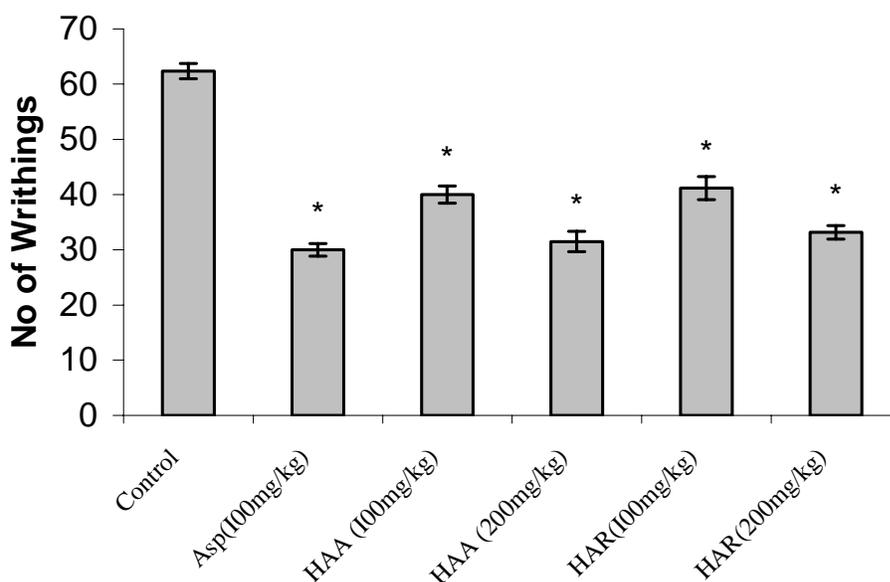


Figure 1 Effects of HAA and HAR on acetic acid induced writhing in mice. Data represent mean \pm SEM of 6 animals. * $p < 0.001$ compared to control (One way ANOVA followed by Tukey's multiple comparison test). Asp-Aspirin, HAA – aqueous extract of aerial parts, HAR- aqueous extract of root.

46.79%, respectively. The aqueous extract of the aerial parts HAA (200 mg/kg/p.o) exhibited greater activity (49.46%), which was comparable with the standard drug Aspirin (51.86%). The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the extracts exert their analgesic effect probably by inhibiting the synthesis or action of prostaglandins.

The centrally acting analgesics generally elevate the pain threshold of mice towards heat. HAA and HAR significantly ($P < 0.001$) increased the reaction time of animals towards the thermal source in a dose-dependent manner. In hot plate test HAA showed a pain inhibition percentage (PIP) of 36.5% and 67.3%, respectively whereas HAR showed a PIP of

40 and 70%, respectively (Figure 2). In tail flick test the drugs showed greater activity after 60 min of drug administration, in which HAR (200 mg/kg/p. o.) exhibited greater PIP of 77.14% (Figure 3). In thermal methods HAR exhibited a greater activity and the activity was comparable to that of the standard drug Aspirin.

From the results it could be concluded that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s).

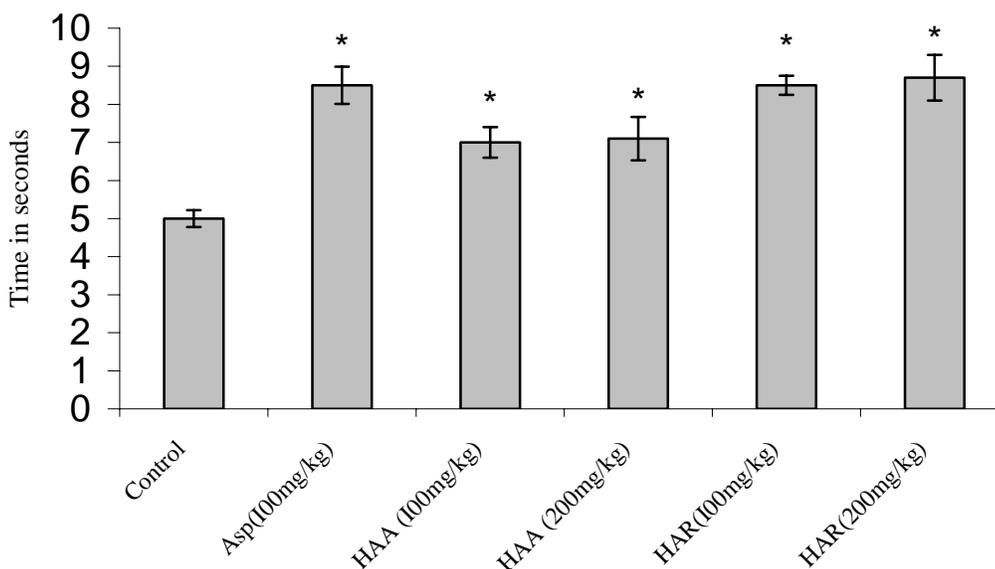


Figure 2: Effects of HAA and HAR on latency time of mice exposed to hot plate test. Data represent mean \pm SEM of 6 animals. * $p < 0.001$ compared to control (One way ANOVA followed by Tukey's multiple comparison test). Asp- Aspirin, HAA – aqueous extract of aerial parts, HAR- aqueous extract of root.

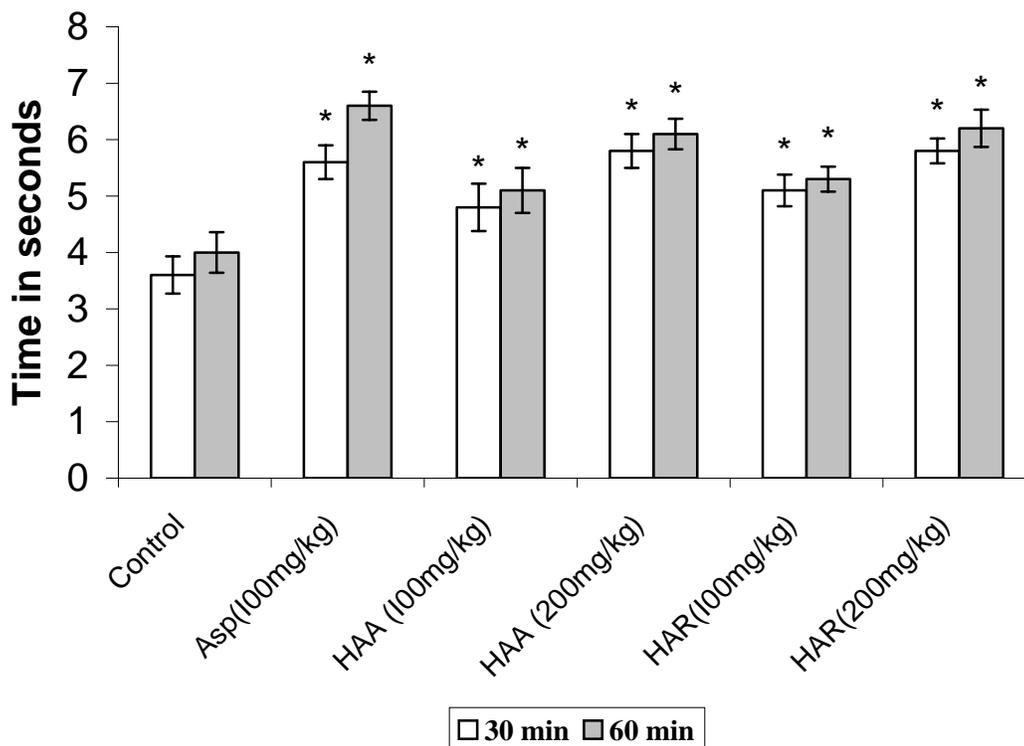


Figure 3 Effects of HAA and HAR on latency time of mice exposed to tail flick test. Data represent mean \pm SEM of 6 animals. * $p < 0.001$ compared to control (One way ANOVA followed by Tukey's multiple comparison test). Asp-Aspirin, HAA – aqueous extract of aerial parts, HAR- aqueous extract of root.

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