ORIGINAL ARTICLE

Anti-inflammatory and antinociceptive activities of *H. schulli* seed extracts

Dayanand M. Kannur, Mukta P. Paranjpe[1], Prerana P. Dongre, Subhash T. Kumbhar[2], Kishanchand R. Khandelwal[3]
Department of Pharmacognosy and Phytochemistry, [2]Pharmacology, SCES' Indira College of Pharmacy, [3]JSPM, Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune, Maharashtra, India, [1]Institute for Pharmaceutical Technology, TU Braunschweig, Mendelssohnstr.1, 38106 Braunschweig, Germany

Background: *H. schulli* aerial parts have been attributed with anti-inflammatory and analgesic properties in the folklore medicine. There has been no systematic study carried out till date to substantiate the anti-inflammatory and analgesic properties of the seeds of *H. schulli*. **Aim:** The aim of our study was to carry out the evaluation of the anti-inflammatory and analgesic properties of the seeds of *H. schulli*. **Materials and Methods:** In this study, *H. schulli* seeds were extracted with ethanol and hexane and carrageenan-induced paw oedema, Eddy's hot plate test, tail immersion method were used to screen the anti-inflammatory and analgesic actions of the seeds. **Statistical Analysis:** The results were statistically interpreted using Student's "*t*" test. **Results:** The results obtained clearly indicate that *H. schulli* seed extracts have the ability to decrease the carrageenan-induced foot paw oedema. The seed extracts enhanced the pain threshold capacity in both the models, thereby producing analgesia. **Conclusions:** We can thereby conclude that *H. schulli* seed extracts posses' anti-inflammatory and antinociceptive activity.

Key words: Anti-inflammatory, antinociceptive, *H. schulli*, tail flick

INTRODUCTION

H. schulli (K. Schum) Heine has been used in traditional practice for many years. *H. auriculata* (K. Schum) Heine (synonym: *Asteracantha longifolia* Nees, *Barleria auriculata* schum, *Barleria longifolia* linn) Acanthaceae, is a wild herb commonly found in moist places on the banks of rivers, ditches and paddy fields throughout India [Figure 1]. *H. schulli* (Syn: *Asteracantha longifolia* (L.) Nees), Acanthaceae, finds mention in Ayurvedic treatise like "Sushruta Samhita" and "Charak Samhita" as Rasayan or rejuvenator.[1,2] *H. schulli* is described in the Ayurvedic literature as Ikshura, Ikshugandha and Kokilasha, "having eyes like the Kokila or Indian Cuckoo". They are also constituent of Ayurvedic formulations as "Seethaveryam", "Mathuravipaka", "Strirativallabhpugpak" and "Rativardhanyog" described in ancient text to improve sexual behaviour and as a general tonic.[3-5] It is classified in the Ayurvedic system of medicine and is used for the treatment of a number of conditions including premeham (diabetes) and athisaram (dysentery).

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.104934

Synonyms

Asteracantha longifolia, H. auriculata (Schumach.) Heine., *H. spinosa* T. Anders.

Vernacular Names

Sanskrit: Iksura; Bengali: Kuliyakhara; Gujarati: Ekharo; Hindi: Talmakhana; Malyalam: Nirmuli; Marathi: Kokilaksha, Talimakhana; Tamil: Golmidi and Urdu: Talmakhana.

Geographical Sources

The plant is widely distributed throughout India, SriLanka, Burma, Malaysia and Nepal.

General Description

H. schulli is found throughout India, especially in marshy places. It is an erect, perennial plant growing 1-1.5 m in height. The stems are sub-quadrangular and numerous. The flowers are bluish purple in the axils of leaves, amidst the spines. The seeds are slimy to taste. The plant flowers during October to December. It is a spiny, stout, annual herb, common in water-logged places. Leaves are subsessile, oblong–lanceloate or linear lanceolate, and spines are yellowish brown, 2-3 cm long, flowers 2-3 cm long, purple–blue, bilabiate, in whorls,[6] linear oblong, compressed about 8 cm long, pointed, four to eight seeded [Figure 2].[7] Seed are ovate, flat or compressed, 0.2-0.25 cm long and 0.1-0.15 cm wide, hairy but appearing smooth; when soaked in water, they immediately get coated

Address for correspondence: Prof. Dayanand M. Kannur, Department of Pharmacognosy, SCES's Indira College of Pharmacy, Tathawade, Pune, Maharashtra, India. E-mail: dmkannur@rediffmail.com
Received: 12-06-2012; **Accepted:** 31-07-2012

Figure 1: *Hygrophila schulli* plant

Figure 2: Seeds of *Hygrophila schulli*

with mucilage, light brown: Taste slightly bitter and odour is not distinct.

Chemical constituents found are fixed oils with linoleic and oleic acid, palmitic, stearic myristic acids,[8,9] polysaccharides[10] like D-Xylose, D-Glucose, L-Arabinose and D-Mannose,[11] Xylose, Uronic acid,[12] Histidine, Lysine, Phenylalanine [13] and β-Sitosterol [14] are found.

H. schulli aerial plants are traditionally used in folklore medicine as well as Ayurvedic formulations with claimed anti-inflammatory as well as antibacterial properties; it is also a proven hepatoprotective agent.[15,16]

MATERIALS AND METHODS

Plant Material

Seeds of *H. schulli* were collected from the local market in and around Pune, and were further authenticated by the Botanical Survey of India, Pune *(V No. Kannur 1)*.

Preparation of Extracts

The plant material was dried and the seeds of *H. schulli* were coarsely powdered and extracted with 95% ethanol and hexane separately in a soxhlet extractor. The extracts were filtered and concentrated on a rota evaporator. The alcoholic extract was then evaporated to get a sticky reddish brown extract (*H. schulli* ethanolic extract [HSE]) (yield 22.8% w/w). The hexane extract was evaporated to get a pale reddish yellow oily extract (*H. schulli* hexane extract [HSH]) (yield 35.64% w/w).

Animals

The animal use protocol was approved by the Animal Ethics Committee of SCES' Indira College of Pharmacy, Pune, India (Animal Eths Comm/ICP/IAEC/09 - 10/P-14) and was in accordance with the International Standard on the care and use of experimental animals. Healthy adult Swiss albino mice weighing between 30 and 40 g were used for antinociceptive studies and Wistar albino rats weighing between 120 and 140 g of either sex were used for the anti-inflammatory studies. Animals were housed under standard conditions and were fed *ad libitum* with commercial pellet diet and had free access to water.

Extracts and Standard Drugs

In all the three models, the animals were divided into four groups of six animals each, the first group served as control (distilled water and 1% CMC), the second and third groups were treated with HSE (200 and 400 mg/kg p.o.), respectively, the fourth and fifth groups were treated with HSH (200 and 400 mg/kg p.o.) and the sixth group served as standard. In anti-inflammatory activity, the standard drug used was Indomethacin (10 mg/kg b. w.),[17] administered by the intra-peritoneal route. In the analgesic activity, in both models, Pentazocine (10 mg/kg b. w.)[18,19] was used as a standard drug and administered by the intra-peritoneal route.

Screening of Anti-Inflammatory Activity

Carrageenan-induced paw oedema in rats

In the carrageenan-induced paw oedema,[20-22] all the animals received their respective doses at a fixed time once daily for 5 days, except for the sixth group where the standard drug was injected 1 h prior to the administration of carrageenan. On the fifth day, 1 h after the drug administration, 0.1 mL carrageenan (1%) suspended in normal saline was injected into the subplantar region of the right hind paw.

The paw was immediately immersed in the Plethysmometer [23] up to the tibiotarsal articulation and the paw volume was measured,[24] which served as reading for 0 h. The readings were taken similarly and paw volumes were measured after every 60-min intervals for the next 4 h. The average paw swelling in the groups of the HSE-treated animals were compared with the control and that of the standard group. Mean increase in paw volume was determined and expressed as mean paw displacement volume in millilitres.

The % inhibition was calculated at the end of the fourth hour using the formula[25]:

$$I = 100 [1 - (a - x)/(b - y)]$$

Where "I" is the % inhibition, "a" is the mean volume at time = t of the test group, "x" is the mean volume at time = 0 of the test group and "b" is the mean volume at time = t of the control group and "y" is the mean volume at time = 0 of the control group.

Screening of Analgesic Activity

Eddy's hot plate test

Analgesic activity of the extracts and Pentazocine was determined by Eddy's hot plate test. In all the groups, the basal reaction time was recorded by observing hind paw licking or jump response in animals when placed on a hot plate [26] maintained at constant temperature (55°C). Normally, animals show such response in 6-8 s. A cut-off period of 15 s was observed to avoid damage to the paws. The reaction time of animals on the hot plate was recorded at 0, 30, 60, 90 and 120 min after the drug administration. Percentage increase in basal reaction time (index of analgesia) at each time interval was calculated.

Tail immersion method

The basal reaction time was recorded by observing tail flick response [27] in animals when the tail was immersed in water maintained at constant temperature (55°C). Normally, animals show such response in 3-5 s. A cut-off period of 10 s was observed to avoid tissue damage. In all the four groups, the reaction time of animals in hot water was recorded at 0, 30, 60, 90 and 120 min after the drug administration. Percentage increase in basal reaction time-flicking/removal of tail (index of analgesia) at each time interval was calculated.

Statistical Analysis

Comparison between control and drug-treated groups was made by Students "*t*" test.

RESULTS

Anti-Inflammatory Activity

Carrageenan-induced paw oedema in rats

In our studies, the animals in the negative control group exhibited severe oedema formation that lasted for more than 4 h. It was observed that the *H. schulli* HSE extract reduced the inflammation in all the animals at 200 mg/kg as well as 400 mg/kg [Figure 3]. The results clearly indicate that the HSE extract at a dose of 400 mg/kg was more effective and significant in controlling the inflammation. The HSE 400 group showed reduction in inflammation after the 60-min interval. The hexane fraction was far more efficient in reducing the inflammation as exhibited by HSH 400. The capacity of the hexane extract in comparison with the ethanolic extract to control inflammation at different time intervals clearly reflects the higher potency of hexane extract at both the doses. In comparison with negative control, the standard drug exhibited highly significant action in controlling the oedema and severely restricting the inflammation since the onset of inflammation. The % reduction of inflammation in all the groups at 240 min was highly significant.

Analgesic Activity

Eddy's hot plate method

Antinociceptive activity of the extract at both the doses and Pentazocine was determined by Eddy's hot plate test [Figure 4]. The basal reaction time was recorded by observing hind paw licking or jump response in animals when placed on a hot plate maintained at constant temperature (55°C). Both the extracts at different doses exhibited comparatively good analgesic action. The extract-treated groups showed onset of action after 60 min in comparison with the standard drug. HSE and HSH extracts at 400 mg/kg exhibited higher

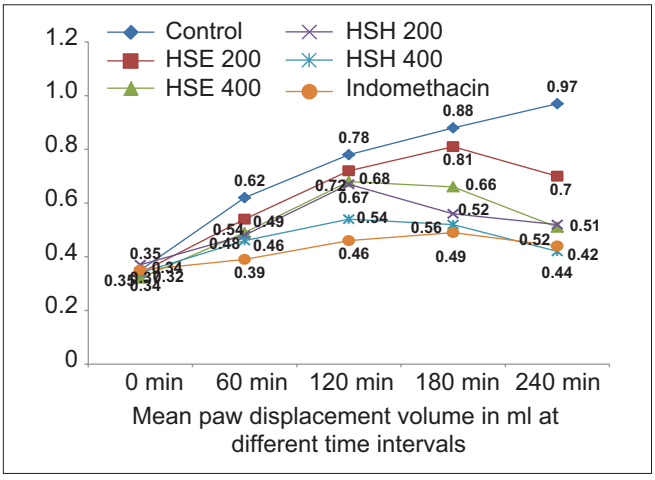

Figure 3: Effect of HSE extracts on carrageenan-induced paw oedema in rats

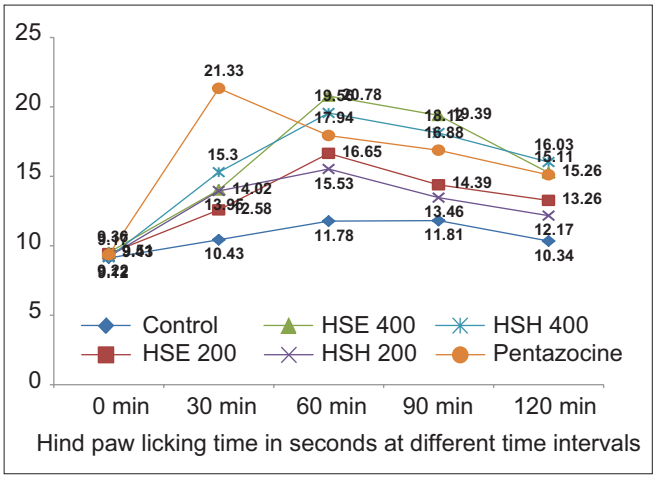

Figure 4: Antinociceptive activity by Eddy's hot plate method

analgesic tolerance, and the reflex time was enhanced significantly in comparison with the 200 mg/kg groups. The standard drug Pentazocine exhibited the most significant pain reducing action. Increase in the basal reaction time was high in the standard group in comparison with the extract-treated and the control groups.

Tail immersion method

The basal reaction time was recorded by observing tail flick response in animals when the tail was immersed in water maintained at constant temperature (55°C) [Figure 5]. HSE 200 and HSE 400 extracts exhibited significant increased basal reaction time and comparatively better analgesic action in comparison with untreated animals. The analgesic activity exhibited by HSH 400 was equally significant; HSH 200 exhibited moderate analgesic action. Pentazocine exhibited the onset of action at 30 min, whereas the onset of action in the extracts was observed after 60 min. In extract-treated animals, it was noted that the analgesia duration was prolonged in comparison with the standard drug. This could be due to delayed absorption and greater retention time of the drug.

DISCUSSION

The present study demonstrates that oral administration of the ethanolic and hexane extracts of *H. schulli* seeds at varied doses produces consistent antinociceptive and anti-inflammatory effects in different models of pain and inflammation. The above results clearly indicate that *H. schulli* seeds posses the anti-inflammatory and analgesic action as claimed in the literature. Carrageenan-induced paw oedema is a commonly used primary test for the screening of new chemical entities as well as various herbal extracts for anti-inflammation. Carrageenan-induced paw oedema is believed to be biphasic, where the first phase (1-2 h) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin.[18] The study results clearly indicate that the *H. schulli* extracts significantly reduced the carrageenan-induced paw oedema in rats. Hence, it may be inferred that the mechanism of action may be due to inhibition of histamine, serotonin or prostaglandin synthesis. Preliminary phytochemical screening has revealed the presence of alkaloids, flavonoids and steroids in the both the extracts. The anti-inflammatory potentials of alkaloids, steroids and flavonoids have been well reported in various studies.[28-30] Therefore, the anti-inflammatory activity of the extracts may be due to the presence of alkaloids, steroids and flavonoids; this has also been mentioned in the earlier studies.[31] Although anti-inflammatory activity of the aerial parts and roots of *H. schulli* has been proven,[31] this work is novel in proving the anti-inflammatory efficacy of the seeds. Even the analgesic action of the seeds has been investigated at different doses for the first time.

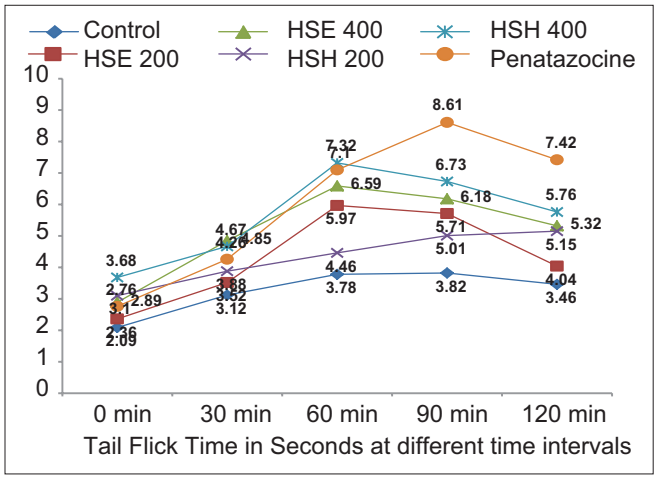

Figure 5: Basal reaction time in tail flick method

The extracts of the *H. schulli* seed produces analgesia at both the doses and acts as an analgesic agent. To screen the central analgesic response of the extracts, the tail flick (spinal analgesia and hot plate supraspinal analgesia) tests were used as reported in the literature. In the tail flick test as well as the hot plate test, it was observed that all extracts increased the analgesic baseline. These results indicate that the extracts develop spinal analgesic activity. The activity exhibited by the extract of the seed can be attributed to the presence of certain steroidal moieties in the seed extract. Our study clearly indicates that HSE and HSH enhance the tolerance to heat and increases the animal's reaction time, which reflects good analgesic activity against the hot plate test. In the case of the tail flick method too, the basal reaction time is increased in the HSE- and HSH-treated animals. The hot plate and tail flick tests are most sensitive to centrally acting analgesics. The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extracts increased the reaction time of animals towards the thermal source. From the above results in both the models, we suggest that *H. schulli* seeds have strong peripheral antinociceptive activity.

CONCLUSION

The above results confirm that *H. schulli* seeds possess significant anti-inflammatory and analgesic properties. The *H. schulli* seeds have been used in the Ayurvedic literature as a Rasayana and for analgesic and anti-inflammatory actions. Our studies hereby confirm the traditional claims that seeds of *H. schulli* possess anti-inflammatory as well as analgesic activity.

REFERENCES

1. Paranjpe P. Indian Medicinal Plants-Forgotten Healers. Delhi: Chaukhamba Sanskrit Pratishthan; 2005. p. 139.
2. Nadkarni AK. Indian Materia Medica. vol 1. Bombay: Popular Prakashan Private Limited; 1978. p. 667-9.

3. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants, National Institute of science and Communication. New Delhi: C.S.I.R Publication; 1956. p. 330-2.
4. Vijayakumar M, Govindarajan R, Rao GM, Rao ChV, Shirwaikar A, Mehrotra S, *et al*. Action of *H. auriculata* against streptozotocin-induced oxidative stress. J Ethnopharmacol 2006;104:356-61.
5. Chauhan NS, Dixit VK. *Asteracantha longifolia* (L.) Nees, Acanthaceae: Chemistry, traditional, medicinal uses and its pharmacological activities-A Review. Braz Phcog J 2010;20:812-17.
6. Kshirsagar AD, Ingale KG, Vyawahare NS, Thorve VS. *H. spinosa*: A comprehensive review. Pharmacogn Rev 2010;4:167-71.
7. Hussain S, Fareed S, Ali M. *H. auriculata* (K. Schum) Heine: Ethnobotany, phytochemistry and pharmacology. Asian J Tradit Med 2010;5:122-31.
8. Haq QN, Nabi MN. Studies on oil from seeds of H. spinosa. Bangladesh J Sci Ind Res 1978;13:29-32.
9. Mazumdar UK, Sengupta A. Triglyceride composition of H. spinosa seed oil. Ind. J Pharm Sci 1978;40:119-20.
10. Godbole NN, Gunde BG, Shrivastav PD. An investigation of oil from seed of *H. spinosa*. J Am Oil Chem Soc 1941;18:206-7.
11. Haq QN, Nizamuddin M, Rahman J. Studies on water soluble polysaccharides from seeds of *H. spinosa*. Bangladesh J Sci Ind Res 1985;20:59-63.
12. Ali MA. Chemical investigation on the seeds of *H. spinosa*. Pak J Sci Ind Res 1967;10:82-3.
13. Thanki RJ, Thaker KA. Studies on Amino acid composition of the seeds of plants *H. schulli* and C. trilocularies. J Inst Chem (India) 1980;52:23-4.
14. Tiwari RD, Srivastava KC, Sattasangi PD. Examination of fixed oil from seeds of *H. spinosa*. J. Univ. Bombay. J Appl Chem 1967;30:58-9.
15. Datte JY, Traore A, Offoumou AM, Ziegler A. Effects of leaf extract of *H. schulli* (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats. J Ethnopharmacol 1998;60:149-55.
16. Singh A, Handa SS. Hepatoprotective activity of Apium graveolens and H. Auriculata vs. Paracetamol and thioacetamide intoxication in rats. J Ethnopharmacol 1995;49:119-26.
17. Sagar MK, Upadhyaya K. Anti-inflammatory Activity of Root of Dalbergia sissoo (Rox.b) in Carrageenan-Induced Paw Edema in Rats. Phcog J 2010;2:427-30.
18. Matheus ME, Berrondo LF, Vieitas EC, Menezes FS, Fernandes PD. Evaluation of the antinociceptive properties from Brillantaisia palisotii Lindau stems extracts. J Ethnopharmacol 2005;102:377-81.
19. Nonato FR, Barros TA, Lucchese AM, Oliveira CE, dos Santos RR, Soares MB, *et al*. Antiinflammatory and antinociceptive activities of Blechnum occidentale L. extract. J Ethnopharmacol 2009; 125:102-7.
20. Kim JY, Hwang YP, Kim DH, Han EH, Chung YC, Roh SH, *et al*. Inhibitory effect of the Saponins derived from Roots of Platycodon grandiflorum on Carrageenan-induced inflammation. Biosci Biotechnol Biochem 2006;70:858-64.
21. Balasubramanian A, Kothai R, Krishnan S, Christina AJ. Antiinflammatory Activity of Morus indica Linn. IJPT 2005;4:13-15.
22. Ratheesh M, Helen A. Anti inflammatory activity of Ruta graveolens Linn on carrageenan induced paw edema in wistar male rats. Afr J Biotechnol 2007;10:1209-11.
23. Gupta GD, Gaud RS. Antiinflammatory activity of Tenoxicam gel on carrageenan-induced paw oedema in rats. Indian J Pharm Sci 2006;68:356-9.
24. Borgi W, Ghedira K, Chouchane N. Antiinflammatory activity and analgesic activities of Zizyphus lotus root barks. Fitoterapia 2007;78:16-9.
25. Turner RA. Screening methods in Pharmacology. New York: Academic Press; 1965. p. 163.
26. Harisha CR, Ashok BK, Achary R, Shukla VJ, Ravishankar B. Anti-inflammatory and Analgesic activities of root and stem of Cissus rependa vahl. Phcog J 2011;2:49-53.
27. Shang X, Wang J, Li M, Miao X, Pan H, Yang Y, *et al*. Antinociceptive and anti-inflammatory activities of Phlomis umbrosa Turcz extract. Fitoterapia 2011;82:716-21.
28. Mossa JS, Tariq M, Mohsin A, Ageel AM, Al-yahya MA, Al-said MS, *et al*. Pharmacological studies on aerial parts of Calotropis procera. Am J Chin Med 1991;19:223-31.
29. Singh RK, Acharya SB, Bhattacharya SK. Pharmacological activity of Elaeocarpus sphaericus. Phytother Res 2000;14:36-9.
30. al-Said MS, Tariq M, al-Yahya MA, Rafatullah S, Ginnawi OT, Ageel AM. Studies on Ruta chalepensis, an ancient medicinal herb still used in traditional medicine. J Ethnopharmacol 1990;28:305-12.
31. Patra1 A, Jha S, Murthy PN, Aher VD, Chattopadhyay P, Panigrahi G, *et al*. Anti-Inflammatory and Antipyretic Activities of *H. spinosa* T. Anders Leaves (Acanthaceae). Trop J Pharm Res 2009;8:133-7.

How to cite this article: Kannur DM, Paranjpe MP, Dongre PP, Kumbhar ST, Khandelwal KR. Anti-inflammatory and antinociceptive activities of *H. schulli* seed extracts. Int J Green Pharm 2012;6:212-6.

Source of Support: Nil, **Conflict of Interest:** None declared.