

Research article

Antinociceptive evaluation of an ethanol extract of *Achyranthes aspera* (agadha) in animal models of Nociception.

Bhosale Uma¹, Radha Yegnanarayan², Pophale Prachi³, Mandar Zambare⁴, R.S. Somani⁵

***Corresponding author:**

Bhosale Uma

¹Associate Professor,
Department of Pharmacology,
SKNMC, Pune-41, India
email: umabhosale2000@gmail.com
Fax :020-24106274;
M.:09226767554

² Professor and Head Department
of Pharmacology, SKNMC,
Narhe (Ambegaon) Pune-41,
India

³ Animal House In-charge
SKNMC, Narhe (Ambegaon)
Pune-41, India

⁴ M. Pharm. Student SCOP,
Vadgaon pune-41, India

⁵Professor in pharmacology
SCOP, Vadgaon pune-41, India

Abstract

Achyranthes aspera Linn., known as Chirchira (Hindi), Agadha (Marathi) is an indigenous herb found in India. It is the basic composition of many traditional remedies. The herb has been reported to have variety of activities like antifertility, antihyperlipidemic, antidiabetic, immunomodulatory, anticarcinogenic, diuretic and cardiogenic, anti-inflammatory, antifungal and antibacterial activity. Present study was designed to evaluate the antinociceptive activity of ethanolic extract of *A. Aspera* (EEAA) and to find the phytochemical responsible for this activity with possible mode of its activity. The antinociceptive activity of ethanol extract of *Achyranthes aspera* was investigated in albino mice. The pharmacological assays used were the tail flick, hot plate and the formalin-induced pain tests. The extract was given intraperitoneally at a dose of 400 mg/kg (our earlier study revealed no activity at the dose 200mg/kg). Pentazocine (10mg/kg body weight i.p.) was used as standard. Data analyzed by ANOVA test followed by Dunnett's test. All the results were expressed as Mean (\pm SEM). $P < 0.05$ was considered significant. For formalin test the percent inhibition was calculated by using formula $(C-T)/C \times 100$ (%). Phytochemical screening revealed presence of triterpenoid saponins possessing oleanolic acid as aglycone, viz. A & B, alkaloid achyranthine, water soluble base betaine and steroids. In the tail flick & hot plate test extract treated animals showed significant analgesic activity at 30, 60, 90 and 120min. Extract (400 mg/kg i.p.) reduced the formalin induced pain in both phases (i.e. neurogenic and inflammatory) by 58.8% and 92.7%, respectively. Ethanol extract of *A.Aspera* exhibit central as well as peripheral antinociceptive activity.

Key words: *Achyranthes aspera* Formalin Hot plate Tail flick.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. India is a land of rich biodiversity; total

number of lower and higher plants in India is about 45,000 species. [1]

Achyranthes aspera Linn. (Amaranthaceae), known by different names such as Chirchita (Hindi), Apamarga (Sanskrit), Aghedi (Gujarati), Apang (Bengali), Nayurivi (Tamil), Kalalat (Malyalam)[2] and Agadha (Marathi) in our

[doi:10.5138/ijpm.2010.0975.0185.02063](https://doi.org/10.5138/ijpm.2010.0975.0185.02063)

©arjournals.org, All rights reserved.

country; is a small herb found all over India possessing valuable medicinal properties useful in cough, bronchitis and rheumatism, malarial fever, dysentery, asthma, hypertension and diabetes [3, 4, 5]. In Chinese traditional medicine, the hot water extract of the plant has been used as an anti-arthritic and to alleviate arthritic pain [6].

Previous studies have reported that the herb has antifertility, antihyperlipidemic, immunomodulatory, antidiabetic, anticarcinogenic, diuretic and cardiogenic [7], anti-inflammatory [8], antifungal [1] and antibacterial [9] activities. Various activities of *A. aspera* have been reported but there is hardly any report on its antinociceptive activity.

Hence present study was designed to evaluate analgesic (antinociceptive) activity of an ethanol extract of *Achyranthes aspera* (EEAA) in animal models for nociception. And to further evaluate the phytochemical responsible for this activity with possible mode of its action.

Materials and Methods

Present study has been conducted in the Department of Pharmacology at Smt. Kashibai Navale Medical College, Pune. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

Plant Material

The leaves of *Achyranthes aspera* were procured from Empress Garden, Koregaon Park Pune and were identified by Botanical Survey of India (BSI), Pune (specimen voucher no: MRZAA1 date: 16/9/09). The leaves were washed under running water, shade dried and the dehydrate leaves powdered to a fine texture and 100 g of the dried leaves was repeatedly extracted with 95% ethanol for 10 days.

Phytochemical study

Freshly prepared ethanol extract of *Achyranthes aspera* was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol. [10]

Experimental animals

Wistar albino mice of either sex weighing 35-40gms, bred in Central Animal House (CAH) facility of the SKN Medical College, Pune were used for the study. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. They were acclimatized to laboratory conditions before the experiment. Each animal was used once in every experiment and all experiments were carried out in daylight.

Acute toxicity study

Acute toxicity study was carried out according to the OECD (Organization for Economic Co-Operation and Development) Guidelines No. 423.

Test methods

Animals were divided into various groups in such way that 6 animals were there in each group. Animals treated with 5% Gum Acacia Suspension (0.1 ml p.o.) served as control. Pentazocine (10mg/kg i.p.) [11] and Aspirin (100mg/kg p.o.) [12] served as standards and animals in test group were treated with *A. Aspera* ethanolic extract (400 mg/ kg i.p.) respectively. Each animal was treated with respective drug 30 min before experimentation. Following are the details of experiments performed,

Tail Flick Response Method

The activity was screened according to previously reported method with minor modifications [13]. Prescreened animals (reaction time: 3-5sec) were used to assess the tail flick latency by the analgesiometer. A cut off period of 15 secs was observed to avoid damage to the tail. The reaction time was recorded at 30, 60, 90, 120 and 180 min after the administration of drugs.

Hot Plate Method

The test is performed using Eddy's hot plate maintained at a temperature of $55 \pm 1^\circ\text{C}$. Prescreened animals (reaction time: 3-5sec) were used to assess the reaction time at 30, 60, 90, 120 and 180 min after the administration of drugs. A cut off period of 10 secs is observed to avoid damage to the paws [14].

Formalin Test

20 microgram of 2.5% formalin solution was injected subcutaneously under the surface of the right hind paw for the induction of pain. [15] Animals were treated with respective drugs 1hr before the formalin injection. Immediately after the formalin injection the time of pain reactions were registered that the animals remained licking or biting the paw during the first phase (0-5 min i.e. neurogenic) and the second phase (15-30 min i.e. inflammatory) of the test. Percent inhibitions of time pain reactions were calculated by the formula:

$$\% \text{ Inhibition} = (C-T)/C \times 100$$

where C and T indicate non-treated (vehicle) and drug-treated, respectively. [16]

Statistical Analysis

For thermal tests data was analyzed by ANOVA test followed by Dunnett's test. All the results were expressed as Mean (\pm SEM). $P < 0.05$ was considered significant.

Results**Phytochemical screening**

Phytochemical screening of ethanol extract revealed presence of triterpenoid saponins (A & B) possessing oleanolic acid as aglycone, alkaloid achyranthine, water soluble base betaine and steroids, while flavonoids and tannins were found absent.

Acute toxicity study

The results of acute toxicity study showed no clinical signs of toxicity and mortality in the A.Aspera treated animals. Lethal dose was calculated and was found to be more than 2000 mg/kg. $1/5^{\text{th}}$ of this lethal dose (400 mg/kg) was taken as effective dose for the study since $1/10^{\text{th}}$ of lethal dose showed no activity in our earlier study. [17]

Tail Flick Response and Hot Plate Method

Pentazocine (10mg/kg) and A.Aspera leaf extract (400mg/kg) treated groups showed significant analgesic activity at 30, 60, 90 and 120min when compared to control. (Table-1, Table- 2).

Table 1: Mean reaction time in hot plate method (cut off time 10 secs.)

Groups (n=6)	Dose	Mean reaction time (in secs.) \pm SEM					
		Basal	30 mins	60 mins	90 mins	120mins	180 mins
Control	5% gum acacia	3.5 \pm 0.22	3.3 \pm 0.21	3.5 \pm 0.57	3.5 \pm 0.57	3.7 \pm 0.33	3.5 \pm 0.42
Standard (Pentazocine)	10mg/kg	3.7 \pm 0.33	7.7 \pm 0.66*	7.5 \pm 0.42*	7.5 \pm 0.42*	4.8 \pm 0.30*	3.3 \pm 0.21
Test II (A.Aspera extract)	400 mg/kg	3.3 \pm 0.42	8.0 \pm 0.57*	7.3 \pm 0.49*	7.3 \pm 0.49*	4.8 \pm 0.30*	3.2 \pm 0.17

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean \pm S.E.M. (ANOVA test followed by Dunnett's t-test). Significance variation against control at, * $p < 0.001$.

Table 2: Tail flick latency in tail flick method (cut off time 15 secs.)

Groups (n=6)	Dose	Mean value of Tail Flick Latency (sec)±S.E.M.					
		Basal	30 mins	60 mins	90 mins	120mins	180 mins
Control	5% gum acacia	3.7±0.33	3.3±0.42	3.3±0.42	3.5±0.34	3.7±0.33	3.8±0.30
Standard (Pentazocine) Test II	10mg/kg	3.5±0.34	12.2±1.37**	10.3±0.66**	8.2±0.61**	5.2±0.48**	3.7±0.33
(A. Aspera extract)	400 mg/kg	2.7±0.33	8.7±1.58**	9.7±1.38**	7.3±0.61**	4.8±0.30*	3.0±0.26

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate Mean ± S.E.M.(ANOVA test followed by Dunnett's t-test). Significance variation against control at, *p<0.01, ** p<0.001.

Table-3: Observation table for formalin paw licking method:

Drugs (n=6)	Mean Licking time in secs (% inhibition)	
	First phase (0-5mins)	Second phase (15-30mins)
Group-I (5% gum acacia)	83.67	27.33
Group-II (Pentazocine 10mg/kg)	7.3 (91.3%)	5.3 (80.6%)
Group-III (Aspirin 100mg/kg)	32.7 (60.9%)	1.7 (93.8%)
Group-IV (A. Aspera extract 400 mg/kg)	34.5 (58.8%)	2.0 (92.7%)

Percent inhibitions of time pain reactions were calculated by the formula: $(C-T)/C \times 100$ (%), where C and T indicate non-treated (vehicle) and drug-treated, respectively.

Formalin Test

Extract (400 mg/kg i.p.) reduced the formalin induced pain in both phases (i.e. neurogenic and inflammatory) by 58.8% and 92.7%, respectively. (Table-3)

Discussion

Hot plate and tail flick tests model an acute thermal pain in mice. These are commonly used to assess narcotic analgesia, only the central analgesics increase the time of response in these tests. However chemical pain is inhibited by the both central and peripheral analgesics [18].

The formalin test is believed to represent a more valid model for clinical pain. The formalin test is a very useful method, not only for assessing antinociceptive drugs, but also helping in the elucidation of the action mechanism. The neurogenic phase is probably a direct result of stimulation in the paw and reflects centrally mediated pain with release of substance P while the late phase is due to the release of histamine, serotonin, bradykinin and prostaglandins. Drugs that act primarily on the central nervous system, such as narcotics, inhibit both phases equally while peripherally acting drugs such as anti-

inflammatory non-steroidal (NSAID) and anti-inflammatory steroidal only inhibit the late phase. [18] EEAA was able to block both phases of the formalin response although the effect was more pronounced in the second phase. Observations of our study strongly suggest that EEAA possess centrally and peripherally mediated antinociceptive properties.

The peripheral antinociceptive effect of EEAA may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators). Phytochemical screening of EEAA revealed presence of triterpenoid saponins possessing oleanolic acid as aglycone, alkaloid achyranthine and steroids. Achyranthine is reported to have anti-inflammatory activity and it has similar mechanism of action as that of steroids i.e. by inhibiting prostaglandin synthesis at phospholipase A2 and at the level of cyclooxygenase/PGE isomerase [19, 8] and triterpenoid oleanolic acid also reported to have inflammatory activity due to powerful inhibitory actions on cellular inflammatory processes such as the induction by IFN- γ of inducible nitric oxide synthase (iNOS) and of cyclooxygenase 2. [20, 21] Hence peripheral antinociceptive activity observed for EEAA may be due to presence of triterpenoid saponins, achyranthine and steroids in the extract. Anti-inflammatory drugs exhibit analgesic activity via inhibition of inflammatory mediators release responsible for inflammatory pain.

For central antinociceptive action however aminergic and GABAergic receptors mediated mechanisms may be considered [18] Triterpenoid saponins are reported to have agonistic/facilitatory activities at these receptors [22, 23] and presence of these phytochemicals in EEAA said to exhibit central antinociceptive activity.

Conclusion

From the obtained results we can conclude that EEAA possesses considerable antinociceptive

activity which is comparable with the standards. In vivo inhibition of pain in the thermal test indicates that EEAA exhibits central activity. Indeed, it was also observed in the formalin test that this extract exhibited a high peripheral antinociceptive activity. Triterpenoid saponins (central and peripheral), achyranthine and steroids (peripheral) may be the phytochemicals responsible for this antinociceptive activity.

Authors' contributions

All authors read and approved the final manuscript.

UB being principal investigator made substantial contributions to experimental design, data acquisition, analysis, interpretation and drafting the manuscript.

RY made substantial contributions to conception, have been involved in revising the manuscript critically for important intellectual content and have given final approval of the version to be published.

PP has been involved in experimentation and data acquisition. MZ and RS have contributed in extract preparation, phytochemical analysis and acute toxicity studies with the extract.

Acknowledgement

The authors are thankful to BSI Pune for identification. The authors thank to Dr. Jain, Principal Sinhgad College of Pharmacy Vadgaon & Dr. Bhore, Dean SKNMC for providing facilities to carry out of the experiments of this work.

References

1. Elumalai EK, Chandrasekaran N, Thirumalai T. et al. *Achyranthes aspera* Leaf Extracts Inhibited Fungal Growth. International Journal of Pharmtech Research. 2009;1(4):1576-1579.
2. Dwivedi S, Dubey R, Mehtaz K. *Achyranthes aspera* Linn. (Chirchira): A Magic Herb in Folk Medicine Ethnobotanical Leaflets. 2008;12: 670-676.

3. Girach RD, Khan ASA. Ethanomedicinal uses of *Achyranthes aspera* leaves in Orissa (India). International Journal of Pharmacognosy. 1992;30:113–115.
4. Satyavati GV, Raina MK, Sharma M. Medicinal Plants of India, Vol. I, Indian Council of Medical Research, Cambridge Printing Works, Delhi. 1976; pp 10–14.
5. Tang W, Eisenbrand G, Achyranthus bidentata Bl. in Chinese Drugs of Plant Origin, Springer-Verlag, Berlin. 1992;pp 13–17.
6. Nehete JY, Deshmukh VN, Shewale VV, Narkhede MR, Aurangabadkar VM. Quantitation of oleanolic acid in *Achyranthes aspera*. Roots and Leaves extracts by high-performance thin-layer chromatography. Int. J. Phar. Research and Dev. Online. 2009;7.
7. Goyal BR, Goyal RK, Mehta AA. Phytopharmacology of *Achyranthes aspera*: A Review. Pharmacognosy Reviews. 2007;1(1):143-150.
8. Neogi NC, Rathor RS, Shrestha AD, Banerjee DK. Studies on the anti-inflammatory and Anti-arthritis activity of ach.yranthine. Ind. J. Pharmac. 1969;1(3):37-47.
9. Alam MT, Karim MM, Khan SN. Antibacterial Activity of Different Organic Extracts of *Achyranthes aspera* and Cassia Alata J. Sci. Res. 2009;1(2):393-398
10. Khandelwal KR. Practical Pharmacognosy. (19th edn.) Nirali Prakashan, Pune;2008. p: 149-156.
11. Flecknell PA. Laboratory Animal Anesthesia: Post operative care. (2nd edn). Academic Press, Landon.1996, pp:127-157
12. Chakraborty A, Devi RKB, Rita S, Sharatchandra K, Singh Indian TIJ. Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. Indian J Pharmacol. 2004;36(3):148-150
13. Turner RA. Screening methods in Pharmacology. New York: Academic Press. 1965;pp: 100.
14. Turner RA. Screening Methods in Pharmacology. Academic Press, Landon, 1965 .pp.105-108.
15. Correa CR, Calixto JB. Evidence for participation of B1 and B2 kinin receptors in formalin induced nociceptive response in the mouse. Br. J. Pharm. 1993;110:193–198.
16. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30:103-114.
17. Bhosale UA, Yegnanarayan R, Pophale P, Zambare M, Somani RS. Antinociceptive Screening of an Ethanol Extract of *Achyranthes aspera* (Agadha): A Preliminary study. Inventi Rapid Ethnopharmacology. 2010;1(2).
18. De Souza ET, De Lira DP, De Queiroz AC, et al. The Antinociceptive and Anti-Inflammatory Activities of Caulerpin, a Bisindole Alkaloid Isolated from Seaweeds of the Genus Caulerpa Mar. Drugs 2009;7:689-704.
19. Goppelt-Struebe M, Wolter D, Resch K. "Glucocorticoids inhibit prostaglandin synthesis not only at the level of phospholipase A2 but also at the level of cyclo-oxygenase/PGE isomerase". Br. J. Pharmacol. 1989;98(4): 1287–95.
20. Giner-Larza EM, Manez S, Recio MC. et al. Oleanonic acid, a 3-oxotriterpene from Pistacia, inhibits leukotriene synthesis and has anti-inflammatory activity. Eur J Pharmacol. 2001;428:137-143.
21. Albená T, Dinkova-Kostova, Karen T. Liby. et al. Extremely potent triterpenoid inducers of the phase 2 response: Correlations of protection against oxidant and inflammatory stress. PNAS. 2005;102(12):4584–4589.
22. Chakraborty A, Amudha P, Geetha M, Singh NS. Evaluation of anxiolytic activity of methanolic extract of *Sapindus mukorossi* Gaertn in mice. Int. Jr. of Pharma and Bio Sciences. 2010;1(3):1-8.
23. Barua CC, Roy JD, Buragohain B, et al. Anxiolytic effects of hydroethanolic extract of *Drymaria Cordata* L Wild. Ind. J Expt. Bio. 2009;47:.969-973.