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Effect of aqueous extracts of *Achyranthes aspera* Linn. on experimental animal model for inflammation

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Abstract

Background:

Achyranthes aspera is known as Chirchita (Hindi), Apamarga (Sanskrit), Aghedi (Gujarati), Apang (Bengali), Nayurivi (Tamil), Kalalat (Malyalam) and Agadha (Marathi) in our country. It possesses valuable medicinal properties and used in treatment of cough, bronchitis and rheumatism, malarial fever, dysentery, asthma, hypertension and diabetes in Indian folklore. Present study was designed to evaluate anti-inflammatory activity of an aqueous extracts of *Achyranthes aspera* (AEAA).

Materials and Methods:

AEAA leaves and whole plant (i.e. Aqueous extracts of *Achyranthes aspera* leaves (AEAAAL)/Aqueous extracts of *A. aspera* whole plant (AEAAW) were studied in albino mice using carrageenan induced left hind paw edema. Both extracts were subjected to preliminary phytochemical analysis and acute toxicity of the extracts was also studied using Organization for Economic Co-operation and Development OECD guidelines 423.

Results:

Acute toxicity study confirmed toxic dose of AEAA to be more than 2,000 mg/kg. Flavonoids, alkaloids, saponins and triterpenoids were the major constituents found in extracts. AEAA reduced the edema induced by carrageenan by 35.71-54.76% on intraperitoneally administration of 400 mg/kg and 800 mg/kg as compared to the untreated control group. Diclofenac sodium at 10 mg/kg inhibited the edema volume by 42.85%. The results indicated that the AEAA 800 mg/kg body weight shows more significant ($P < 0.01$, $P < 0.001$) anti-inflammatory activity when compared with the standard and untreated control respectively.

Conclusion:

Both AEAA exhibit promising anti-inflammatory activity attributed to flavonoids, alkaloids, saponins and triterpenoids phytoconstituents.

KEY WORDS: *Achyranthes aspera*, anti-inflammatory, high Performance thin layer chromatogram HPTLC, plethysmographic method

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started considering the ancient healing systems such as Ayurveda, Siddha, and Unani, because of the adverse effects

associated with synthetic drugs. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances.[1] In India, a small proportion of wild plants have been investigated both phytochemically and pharmacologically.[2]

Achyranthes aspera Linn. (Amaranthaceae) is a small erect annual shrub found distributed throughout the tropical and subtropical regions. It is commonly found in India, Baluchistan, Sri Lanka, tropical Asia, Africa, Australia, and America.[3] This wild tropical plant is known by different names such as *Chirchita* (Hindi), *Apamarga* (Sanskrit), *Aghedi* (Gujarati), *Apang* (Bengali), *Nayurivi* (Tamil), *Kalalat* (Malayalam),[4] and *Agadha* (Marathi) in India.

The plant is reported to have several medicinal properties and used as emmenagogue, purgative, diuretic, antimalarial, antihyperlipidemic, estrogenic, antileprotic, antispasmodic, cardiogenic, antibacterial, and antiviral agents in traditional systems of medicine. It is also used as antiasthmatic antitussive and in the treatment of snakebite, hydrophobia, urinary calculi, rabies, influenza, otorrhea, piles, bronchitis, diarrhea, renal dropsies, gonorrhea, and abdominal pain.[5–8]

Earlier phytochemical studies reported that it contains saponins, alkaloids (betaine, achyranthine), amino acids, steroids (stigmasterol), triterpenoids (oleanolic acid and its glucoside), phenolic content (indole acetic acid oxidase), and flavonoids. It has also been reported to have antiarthritic, antirheumatic activity as per folklore practice.[9] The present study was therefore designed to corroborate this claim of anti-inflammatory potential of the plant extracts on different phases of inflammation and to further deduce the phytochemicals responsible for this potential.

MATERIALS AND METHODS

Collection of plant material and preparation of extract

Plant material was collected from local areas of Pune, authenticated by Botanical Survey of India, Pune (MRZAA1 date: 16/9/09). Leaves and whole plant were pulverized separately using an electrical grinder and extracted with distilled water at 100°C for 4 h, centrifuged at 5,000 g for 15 min, and filtered using Whatman No.1 filter paper. The filtrate was vacuum evaporated to yield 9.4% leaf and 6.6% whole plant extract, respectively.

Phytochemical study

Both aqueous extracts of *A. aspera* (AEAA) were subjected to preliminary phytochemical analysis with reference to standard protocol for the detection of various constituents such as alkaloids, glycosides, saponins, steroids/triterpenoids, tannins, flavonoids, carbohydrates, proteins and amino acids.[10]

High Performance Thin Layer Chromatogram (HPTLC) profile

AEAA 29.2 mg was dissolved in 29.2 mL of methanol and samples of 5 µL, 10 µL, and 20 µL were applied as 8 mm-wide bands, under a continuous flow of nitrogen, using a CAMAG LINOMATE V automatic sample applicator. The sample was applied with a 100 µL syringe (Hamilton, Bonaduz, Switzerland) at a constant application rate of 0.1 µL/s and the distance between adjacent bands was retained at 15 mm. The plate was developed using the solvent system chloroform: toluene: methanol (4:4:1 [v/v]) and scanned by a densitometer (CAMAG) at 254 nm.

Experimental animals

Swiss albino mice of either sex (25–30 g) were used for the study. The animals were housed under uniform standard laboratory conditions. They were provided with food and water *ad libitum*. The animals were acclimatized for 7 days before experiments were performed. The experimental protocol was approved by Institutional Animal Ethics Committee.

Acute oral toxicity test

The acute oral toxicity study for AEAA was carried out according to OECD guidelines 423.[11] Swiss albino mice were fasted overnight, with water also being withheld. The AEAA was administered at a dose

of 2,000 mg/kg. Animals were observed individually during the first 30 min and periodically during 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days.

Anti-inflammatory study

Anti-inflammatory activity of the AEAA was studied using the carrageenan-induced left hind paw edema (Plethysmographic method). Animals were grouped with 6 animals in each group and received treatment as control (NS), standard, i.e. Diclofenac 10 mg/kg, aqueous extracts of *A. aspera* leaves (AEAAL) 400 mg/kg, AEAAL 800 mg/kg, aqueous extracts of *A. aspera* whole plant (AEAAW) 400 mg/kg, and AEAAW 800 mg/kg intraperitoneally. One hour after administration of drug/extracts, 0.01 mL of 1% carrageenan (Sigma Aldrich, Chemical) suspension was injected subcutaneously into the plantar surface of the left hind paw. The volumes of albino mouse hind paws in the test, control, and standard groups were measured using a Plethysmometer (Make: Ugobasile) at 0 min, 45 min, 2 h, 4 h, and 5 h after the induction of inflammation, and edema was expressed as an increase in paw volume due to carrageenan injection.^[12]

Statistical analysis

Data was analyzed using Vassarstats software. Analysis was performed using ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant. Percent inhibition in paw edema was calculated using the following formula:

% inhibition = $100 (1 - V_t/V_c)$, where V_c = edema volume in control and V_t = edema volume in test groups.

RESULTS

Acute oral toxicity test

Acute toxicity study did not reveal any signs or symptoms of toxicity or mortality during the observation period, at the dose of 2,000 mg/kg. Hence, the starting dose selected for anti-inflammatory evaluation was $1/5^{\text{th}}$ of 2,000 mg/kg (i.e., 400 mg/kg).

Phytochemical analysis

Primary phytochemical screening of extracts determined the presence of alkaloids, glycosides, tannins, saponins, carbohydrates, and flavonoids as major constituents in AEAA; triterpenoids, however, were detected only in AEAAW, whereas steroids were found absent in both the extracts ^[Table 1]. HPTLC at 254 nm showed the presence of 14 components in AEAAW and 12 in AEAAL with their retardation factor (R_f) values. Component with R_f 0.20, 0.23, 0.35, 0.45 0.58, 0.65, and 0.86 matched with standards ^[Figure 1]. Component numbers 2 and 5 showed maximum concentration with low R_f , i.e. 0.03 and 0.17, respectively. These unknown components appear to be alkaloids and saponins, since volatile plant components such as flavonoids and phenolic content have higher R_f with less solubility compared to alkaloids and saponins, which have maximum and intermediate solubility, respectively, in all three solvents, with least volatility and low R_f .

Anti-inflammatory study

The results obtained are summarized in ^[Table 2]. As shown, standard and all the doses of AEAA exhibited significant anti-inflammatory activity at 2 h, 4 h, and 5 h ($P < 0.01$, $P < 0.001$) with marked percentage inhibition in paw edema compared to control at each time point measured; 800 mg/kg dose of both the extracts showed stronger activity in this regard. Maximum percentage inhibition in paw edema was observed at 4 h (35.71-54.76%). When this activity was compared to standard, 800 mg/kg in the AEAAW group exhibited significantly stronger anti-inflammatory activity ($P < 0.01$, $P < 0.001$). All extract groups, except for the 400 mg/kg leaf extract group, exhibited significant anti-inflammatory activity in 45 min ($P < 0.01$, $P < 0.001$), which was not observed with the standard group.

DISCUSSION

Carrageenan-induced edema serves as an index of acute inflammatory changes, determined from differences in the paw volume measured immediately after carrageenan injection and then periodically. It is believed to be biphasic; the first phase (1 h) involves the release of serotonin histamine and kinin and the second phase (over 1 h) is mediated by prostaglandins and cyclooxygenase products.[13]

In the present study, 400 and 800 mg/kg of AEAA showed maximum inhibition in paw edema at 4 h (35.71-54.76%). A similar study performed by Vijaya Kumar *et al.* showed 32-40.5% inhibition with dose range 50-200 mg/kg.[2] Since acute toxicity study confirmed the toxic dose of AEAA to be more than 2,000 mg/kg, we have evaluated higher doses of AEAA and our study revealed a dose-dependent increase in anti-inflammatory activity. ED₅₀ for AEAAAL was found to be 1,200 mg/kg and 562 mg/kg for AEAAW [Figure 2], which is reflected in the results. AEAAW was found to exhibit stronger anti-inflammatory activity than AEAAAL.

For validity demonstration of the carrageenan-induced paw edema, Diclofenac 10 mg/kg was used as standard, and as expected it significantly ($P < 0.01$, $P < 0.001$) reduced edema of phase 2, i.e. at 2 h and 4 h. Comparable anti-inflammatory activity was observed with both extracts against phase 2; however, both the extracts (except for AEAAAL 400 mg/kg may be due to inadequate systemic bioavailability at this dose) exhibited significant anti-inflammatory activity against phase 1 response as well; this indicates it also inhibits serotonin- and histamine-induced inflammatory reaction. From these findings, it can be hypothesized that the anti-inflammatory activity of AEAA is due to the inhibition of release of serotonin and histamine from mast cells and by inhibition of the cyclooxygenase pathway. This mode of action supports its use in asthma/allergy and arthritis treatment in traditional medicine.[6]

On phytochemical screening, AEAA showed the presence of alkaloids, saponins, and flavonoids as major constituents in AEAA, which is in agreement with the study conducted by Sutar *et al.*[14] however, triterpenoids are revealed as additional constituents in AEAAW and this may be the reason for the stronger anti-inflammatory activity of the extract. HPTLC confirmed the presence of gallic acid, luteolin, apigenin, p-coumaric acid, chlorogenic acid, ursolic/oleanolic acid, and quercetin in AEAA. Hence, the significant anti-inflammatory activity of AEAA could be due to the presence of flavonoids, alkaloids, and saponins, which may exert predominant inhibition of inflammatory mediators from phlogogenic stimuli and potential inhibitory action on exudate formation.[9] Further studies to purify, isolate, and standardize the phytoconstituents for making them available for human use are warranted. This evaluation could be an important finding globally, as inflammation has become a common disease condition with “poor availability of safe drug therapies”.

CONCLUSION

Both AEAA exhibit promising anti-inflammatory activity, which is attributed to flavonoids, alkaloids, saponins, and triterpenoids phytoconstituents found in extracts. This abundantly available plant may serve as an important addition in therapeutic armamentarium of chronic inflammatory diseases such as arthritis.

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Footnotes

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Conflict of Interest: None declared.

REFERENCES

1. Shankar D, Ved DK. “A balanced Perspective for Management of Indian Medicinal Plants” Indian Forester. 2003;129:275–87.
2. Vijaya Kumar S, Sankar P, Varatharajan R. Anti-inflammatory activity of roots of *A. aspera*. Pharm

Biol. 2009;47:973–5.

3. The Wealth of India; a dictionary of Indian raw materials and industrial products. 1ST ed. III-C. New Delhi: CSIR; 1985. pp. 66–7.

4. Dwivedi S, Dubey R, Mehta K. *Achyranthes aspera* linn. (Chirchira): A magic herb in folk medicine. Ethno Leaf. 2008;12:670–6.

5. Elumalai EK, Chandrasekaran N, Thirumalai T. *Achyranthes aspera* leaf extracts inhibited fungal growth. Int J Pharmtech Res. 2009;1:1576–9.

6. Goyal BR, Goyal RK, Mehta AA. Phyto-pharmacology of *Achyranthes aspera*: A Review. Pharmacogn Rev. 2007;1:143–50.

7. Bhosale UA, Radha Y, Pophale P, Zambare M, Somani RS. Antinociceptive evaluation of an ethanol extract of *Achyranthes aspera* (Agadha) in animal models for nociception. Int J Phytomed. 2010;2:440–5.

8. Alam MT, Karim MM, Khan SN. Antibacterial activity of different organic extracts of *Achyranthes aspera* and *Cassia Alata*. J Sci Res. 2009;1:393–8.

9. Vetrichelvan T, Jegadeesan M. Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and subacute inflammation. Phytother Res. 2003;17:77–9. [PubMed: 12557252]

10. Khandelwal KR. Practical Pharmacognosy. 19th ed. Pune: Nirali Prakashan; 2008. pp. 149–56.

11. Organization for Economic Cooperation and Development (OECD). OECD guidelines for testing of chemicals test no. 423, acute oral toxicity. France: OECD Publishing; 2006. pp. 1–27.

12. Winter CA, Risley EA, Nuss Gw. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544–7. [PubMed: 14001233]

13. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethnopharmacol. 2006;104:410–4. [PubMed: 16303271]

14. Sutar N, Garai R, Sharma U, Goyal P, Yadav G. Pharmacognostic studies of the *Achyranthes aspera* leaves. Pharmacie Globale (IJCP) 2011;5:1–3.

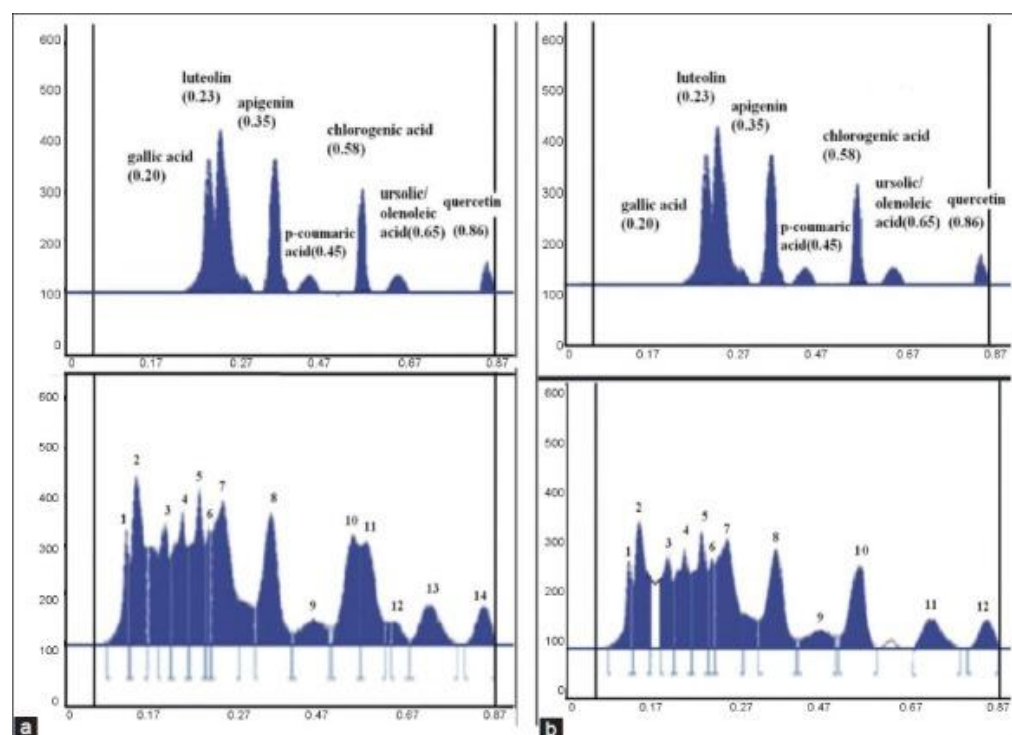
Figures and Tables

Table 1

Phytochemical tests	Phytochemicals	Whole plant	Leaves
Molisch's test/Benedict's test	Carbohydrate	+	+
Shinoda/Alkaline reagent test	Flavonoids	+	+
Legal's tests	Glycoside	+	+
Foam, Bromine water test	Saponins	+	+
Dragendorff's/Mayer's test	Alkaloids	+	+
Ferric chloride/Lead acetate test	Tannins	+	+
Salkowski's/Lieberman–Buchard tests	Triterpenoids	+	-
Salkowski's/Lieberman–Buchard tests	Steroids	-	-

Preliminary phytochemical analysis of *Achyranthes aspera*

Figure 1



Optimized HPTLC chromatogram of (a) Aqueous extracts *Achyranthes aspera* whole plant (b) Aqueous extracts *Achyranthes aspera* leaves at 254 nm

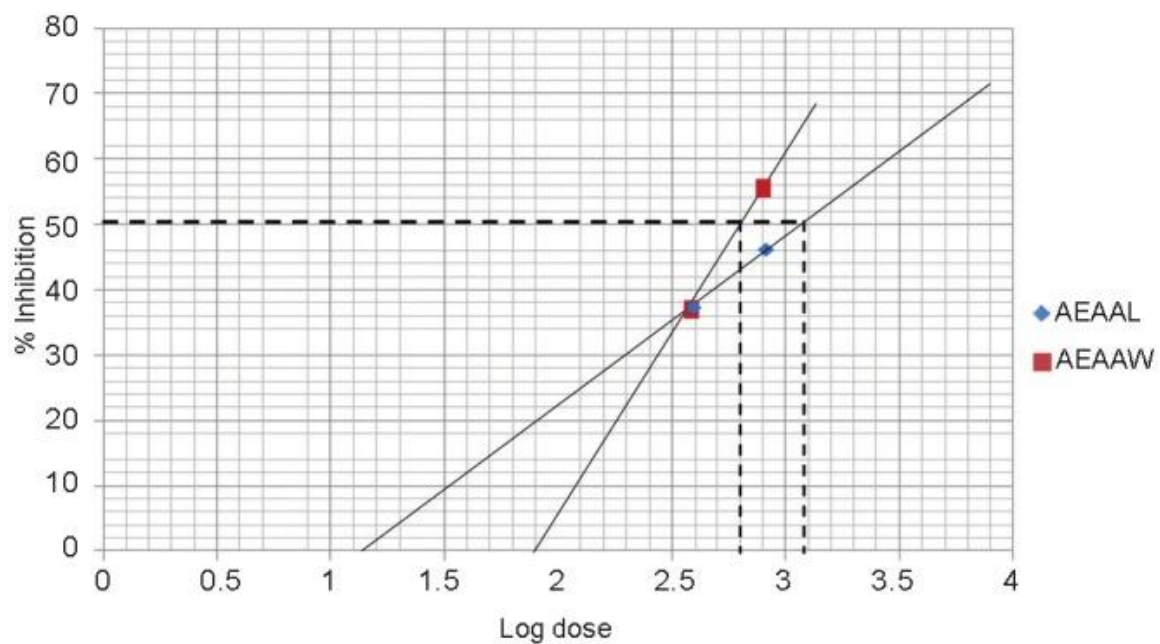
Table 2

Treatment groups	Left hind paw volume in mL (mean±SD)				
	0 min	45 min (%)	2 h (%)	4 h (%)	5 h (%)
Control (NS)	0.28±0.02	0.31±0.08	0.37±0.06	0.42±0.09	0.39±0.03
Diclofenac 10 mg/kg i.p.	0.26±0.03	0.29±0.04 (6.45)	0.27±0.01** (27.03)	0.24±0.01** (42.85)	0.26±0.02** (33.33)
AEAAL 400 mg/kg i.p.	0.26±0.03	0.29±0.01 (6.45)	0.28±0.01** (24.32)	0.27±0.02** (35.71)	0.27±0.02** (30.77)
AEAAL 800 mg/kg i.p.	0.29±0.01	0.28±0.01* (9.68)	0.26±0.02** (29.72)	0.23±0.01** (45.24)	0.24±0.01** (38.46)
AEAAW 400 mg/kg i.p.	0.29±0.03	0.28±0.04* (9.68)	0.28±0.04** (24.32)	0.26±0.04** (38.1)	0.29±0.02** (25.64)
AEAAW 800 mg/kg i.p.	0.30±0.02	0.25±0.03** (19.35) ^a	0.20±0.02** (45.95) ^b	0.19±0.02** (54.76) ^a	0.25±0.03** (35.90)

Figures in parentheses indicate the % anti-inflammatory activity. Values are expressed as mean±SD for *n*=6 animals in each group **P*<0.01, ***P*<0.001, compared to control. ^a*P*<0.01, ^b*P*<0.001, compared to standard (NS: Normal saline, AEAAL: Aqueous extracts of *Achyranthes aspera* leaves, AEAAW: Aqueous extracts of *Achyranthes aspera* whole plant, i.p.: Intraperitoneally)

Effect of aqueous extracts of *Achyranthes aspera* on carrageenan-induced mouse paw edema

Figure 2



Effect of aqueous extracts *Achyranthes aspera* leaves (AEAAL) and aqueous extracts *Achyranthes aspera* whole plant (AEA AW) on carrageenan induced paw edema. ED₅₀ log dose = 3.09 and 2.75 for AEAAL and AEA AW respectively. Dose = 1200 mg/kg for AEAAL and 562 mg/kg bodyweight for AEA AW

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