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RESEARCH ARTICLE

In-vitro Anti-inflammatory and Anti-Arthritic Activities of Fruits of *Vernonia anthelmintica* Willd. (Asteraceae)

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ABSTRACT:

Hydroalcoholic extract of fruits of *Vernonia anthelmintica* Willd. (Family: Asteraceae) (HAEVA) was assessed for its anti-inflammatory activity and anti-arthritic activity by in vitro methods. In vitro anti-inflammatory activity and anti-arthritic activity were evaluated using HRBC (Human Red Blood Cell) membrane stabilization method and inhibition of protein denaturation method respectively. The present finding exhibited concentration dependent HRBC membrane stabilization and inhibition of protein denaturation activity of HAEVA. The maximum membrane stabilization was found to be 89.92% and maximum inhibition of protein denaturation was found to be 98.57% at 1000µg/ml dose. The extract showed HRBC membrane stabilization at a concentration range of 100-1000µg/ml significantly ($p<0.01$) while inhibition of protein denaturation activity at a concentration range of 250-1000 µg/ml significantly ($p<0.01$). The results obtained in the present study indicate that HAEVA can be a potential source of anti-inflammatory agents.

KEYWORDS: *Vernonia anthelmintica*, Asteraceae, anti-inflammatory, anti-arthritic.

INTRODUCTION:

Inflammatory diseases are still one of the most important heath problems in the world. It is a body defense reaction in order to eliminate or limit the spread of injurious agent.[1,2] Rheumatoid arthritis is a chronic, systemic inflammatory disease predominantly affecting the joints and peri-articular tissues. The screening and development of drugs for their anti-inflammatory activity is still in progress and there is hope for finding anti-inflammatory drugs from indigenous medicinal plants.[3-5]

Vernonia anthelmintica Willd. (Family- Asteraceae) is an annual plant**.** It is commonly known as kalijiri and found throughout India.[6,7] According to Unani system of medicine, the seeds are anthelmintic, used for asthma, inflammatory swellings, etc.[6,7] *Vernonia anthelmintica* contain fixed oil, main active principle delta-7-avenasterol and other sterols, alkaloid, flavanoids and other phenolic compounds, etc.[6-10]

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Traditional use in medicine and phytoconstituents of pharmacological interest of *Vernonia anthelmintica* fruits has instigated the investigations for possible anti-inflammatory and anti-arthritic activities.

MATERIALS AND METHODS:

Plant material:

The fruits of the plant *Vernonia anthelmintica* were collected from Rajkot district of Gujarat, India. The plant material was identified by Faculty in botany, Biology Department, Gyanyagna College of Science and Management, Rajkot and a voucher specimen (Voucher No. AIP/12/01) has been retained in Department of Pharmacognosy, Atmiya Institute of Pharmacy, Rajkot, Gujarat.

Reagents and chemicals:

Bovine serum albumin (Chiti Chem Corporation, Baroda, Gujarat, India), all the reagents were of analytical grades. Indomethacin capsules (25 mg) and Diclofenac sodium tablets (50 mg) standard non-steroidal anti-inflammatory drugs were purchased from a pharmaceutical shop at Rajkot, Gujarat, India**.**

Preparation of *V. anthelmintica* extracts:
The dried fruits of the plant *V. anthelmintica* were ground into powder using an electric grinder. Thousand gram of dried fruits powder were exhaustively extracted by maceration with aqueous ethanol (70%). Thereafter, filtered and extract was concentrated on water bath to a dry residue and kept in a desiccators (yield 24.6%, w/w).

Anti-inflammatory activity:
Membrane stabilizing activity assay:
The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (50,100,250,500,750 and 1000 µg/ml) and 1 ml of 10% human red blood cells (HRBCs) suspension, instead of test sample only saline was added to the control test tube. Indomethacin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The percentage inhibition of haemolysis was calculated as follows: % membrane stabilization = [{Ac-At}/Ac] ×100.Where, Ac is the absorbance of control, At is the absorbance of test sample.[11]

Anti-arthritic activity:
Inhibition of albumin denaturation:
The reaction mixture was consists of 2ml test extracts of different concentrations (50, 250, 500 and 1000 µg/ml) and 3ml of 1% aqueous solution of bovine albumin fraction, pH (6.4) of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm using UV-Visible Spectrophotometer. Diclofenac sodium (200 µg/ml) was used as reference standard while no drug was added as control. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows: % inhibition= [{Ac- At}/Ac] ×100. Where, Ac is the absorbance of control, At is the absorbance of test sample.[12]

Data analysis:
Data are presented as means ±S.E.M. of measurements. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. All data were analyzed using the GraphPad Prism 5 Demo computer software. Statistical differences were considered to be significant at $P < 0.01$.

RESULTS:
HAEVA was effective in inhibiting the heat induced hemolysis of HRBCs at different concentrations (50 to 1000µg/ml) as shown in Table 1. HAEVA showed dose dependent membrane stabilizing activity over all the concentration ranges. It showed the maximum RBC membrane stabilization 89.92% at 1000µg/ml.

Table 1. Effects of the *V. anthelmintica* fruits extract in membrane stabilizing activity assay

Test sample (Concentration in µg/ml)	Mean absorbance	% membrane Stabilization (Mean ±S.E.M.)
Control	0.834	-
Indomethacine (200 µg/ml)	0.015*	98.24±0.10
HAEVA (50 µg/ml)	0.646	22.51±0.14
HAEVA (100 µg/ml)	0.409*	50.89±0.11
HAEVA (250 µg/ml)	0.256*	69.33±0.21
HAEVA (500 µg/ml)	0.168*	79.88±0.10
HAEVA (750 µg/ml)	0.093*	88.80±0.08
HAEVA (1000 µg/ml)	0.084*	89.92±0.07

Values are expressed as mean ± S.E.M.,(N=3). *Significantly different from control ($P < 0.01$).

HAEVA showed significant effectiveness in inhibition of heat induced albumin denaturation (Table 2). The maximum percentage inhibition of protein denaturation was 98.57% at 1000 µg/ml of HAEVA. Diclofenac sodium, a standard anti-inflammation drug showed 87% inhibition at the concentration 200 µg/ml compared with control.

Table 2. Effects of the *V. anthelmintica* fruits extract on albumin denaturation

Test sample (Concentration in µg/ml)	Mean absorbance	%Inhibition (Mean±S.E.M.)
Control	0.093	-
Diclofenac sodium (200 µg/ml)	0.012*	87.14±0.62
HAEVA (50 µg/ml)	0.049	47.85±1.28
HAEVA (250 µg/ml)	0.016*	82.85±0.62
HAEVA (500 µg/ml)	0.005*	94.28±0.94
HAEVA (1000 µg/ml)	0.001*	98.57±0.35

Values are expressed as mean± S.E.M.,(N=3). *Significantly different from control ($P < 0.01$)

DISCUSSION:
Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage.[13] The erythrocyte membrane is analogous to the lysosomal membrane [13] and its stabilization implies that the extracts may as well stabilize lysosomal membrane. HAEVA may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

Protein denaturation is one of well documented causes of inflammation in conditions like rheumatoid arthritis.[14] Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins.[15,16] Some anti-inflammatory drugs have shown dose dependent ability to inhibit protein denaturation. Similar results were observed from many reports from plant extract.[17] Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study it can be stated that HAEVA is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

The present study showed significant anti-inflammatory and anti-arthritic activity of HAEVA. In conclusion, these findings rationalize the traditional usage of this plant as an anti-inflammatory and anti-arthritic agent. The plant contains many secondary metabolites such as flavonoids, phytosterols, phenolics, etc. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-inflammatory and anti-arthritic drug research. This established a significant scope to develop a broad spectrum use of *V. anthelmintica* in herbal medicine and as a base for the development of novel potent drugs against inflammations and arthritis.

REFERENCES:

1. Vane JR, Bolting RM. New insights into the mode of action of anti- inflammatory drugs. Inflammation Res 1995;44(1):1-10.
2. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethnopharmacol 2006;104(3):410-4**.**
3. Lavanya R, Maheshwari S, Harish G, Bharath J, Kamali S, Hemamalani D, et al. Investigation of *In-vitro* anti-inflammatory, anti-platelet and anti-arthritic activities in the leaves of *Anisomeles malabarica* Linn**.** Res J Pharm Biol Chem Sci 2010; 1:745-52.
4. Chandrashekhar KA, Sheikh S. *In vitro* antimicrobial, antioxidant, anti-arthritic and phytochemical evaluation of *Pscychotria flavida* Talbot- an endemic plant of western ghats. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5:214-8.
5. Yerramsetty N, Valluri K, Shaik R, Ramadosskarthikeyan. Anti-inflammatory activity of leaves of *Jatropha gossypifolia* L. by hrbc membrane stabilization method. Journal of Acute Disease 2013; 2:156-158.
6. Nadkarni KM. Indian Materia Medica. Vol. 1. Mumbai: Popular Prakashan; 1982.
7. Mashelkar RA editor. Wealth of India, First supplement series (Raw Materials). New Delhi: Council of Scientific and Industrial Research; 2008.
8. Khare CP. Indian Medicinal Plants An Illustrated Dictionary. Verlag Berlin: Springer; 2007.
9. Wu Jian FM, Fen Sheng CK. Studies on Chemical Constituent of *Vernonia anthelmintica* Willd. Acta chimica sinica [serial on the Internet]. 1991 Oct [cited 2006 Jun 12]. Available from: http://en.cnki.com.cn/Article_en/CJFDTOTALHXXB19911001.
10. Tian G, Zhang U, Zhang T, Yang F, Ito Y. Separation of flavonoids from the seeds of *Vernonia anthelmintica* Willd by high-speed counter-current chromatography. Journal of Chromatography A 2004; 1049(1-2):219-22.
11. Arawwawalaa LDAM, Arambewelaa LSR, Ratnasooriya WD. *Alpinia calcarata* Roscoe: A potent antiinflammatory agent. Journal of Ethnopharmacology 2012; 139:889–92.
12. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytotherapy 2011;3(3):43-51.
13. Chou CT. The anti inflammatory effect of *Tripterygium wilfordihook* on adjuvant induced paw edema in rats and inflammatory mediators release. Phytother Res 1997; 11:152-4.
14. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with same biologically active proteins. Journal of Pharmacy and Pharmacology 1968; 20(1):169-73.
15. Singh M, Soni P, Upmanyu N, Shivhare Y. *In-vitro* Anti-arthritic activity of *Manilkara zapota* Linn. Asian J Pharm Tech 2011; 1:123-4.
16. Kokila N, Radha R, Jayshree N. *In vitro* Antioxidant and antiarthritic activity of polyherbal formulation. IJPI'S Journal of Pharmacognosy and Herbal Formulation 2013; 13(3): 10-15.
17. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharmacol Sci 2010; 2(1):146-55.