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Research Article

Elucidation of *in vitro* anti-inflammatory activity Of *Achyranthes aspera* (L.) By HRBC Membrane Stabilization Method

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Abstract

The whole plant extract of *Achyranthes aspera* were assessed for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The presence of flavanoids has been reported earlier in *Achyranthes aspera*. Since the flavanoids have remarkable anti-inflammatory activity, so the present work aims at evaluating the anti-inflammatory activity of *Achyranthes aspera.* Different concentrations (100,200, 300µg/ml) of extract were compared against standard Diclofenac sodium. Maximum stabilization in our study of aqueous extract is 98.85% and ethanolic extract is 98.95% at 300µg/ml. Therefore, our studies support the use of *Achyranthes aspera* in treating inflammation. Further investigations are anticipated to identify the active components and lead to their further clinical use.

Keywords: *Achyranthes aspera,* HRBC, Diclofenac sodium, Anti-Inflammatory

Introduction

Medicinal plants are a part and partial of human society to combat diseases, from the drawn of civilization (Anonymous, 1985). Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effect. The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. The World health Organization (WHO) has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Achyranthes aspera L. (Latjeera) Present communication, dealing with medicobotanical uses of A. aspera in treatment of gynaecological disorders, is part of an extensive study conducted in five districts of western Uttar Pradesh viz., Aligarh, Badaun, Bulandshahar, Farrukhabad and Hatharas (Kirtikar and Basu, 195). In indigenous system of medicine, whole plant exploited for the treatment of renal dropsy, bronchial affections and leprosy (Hawiaran and Rangaswami, 1993).Some pharmacological properties as diuretic, anti- inflammatory, antifungal, abortifacient, larvicidal, hypoglycemic, antifertility and anticancer were reported (Mishra et al., 1993). Literature survey reveled that chemical constituents like flavonoids, triterpenoids, polyphenolic compounds and steroids are responsible for antioxidant activity and these chemical constituents were reported in the methanolic extract of aerial parts of Achyranthes aspera L. (Tahiliani and Kar 2000). Acute lead poisoning occurs at high levels of exposure, causing symptoms of blindness, brain damage, kidney disease, convulsion and cancer (Vasudev Rao *et al*., 2006).

Inflammation

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. There are mainly two types of inflammation which are as follows acute inflammation is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes. Chronic inflammation is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proli-

feration (angiogenesis) and fibrosis. Inflammation is a common clinical conditions and rheumatoid arthiritis (RA) is a chronic debilitating autoimmune disorder, that affects about 1% of the population in developed countries (Cardinali and Esquifino, 2003).The classic signs of inflammation are local redness, swelling, pain, heat and loss of function (Pervical, 1999).

HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an *in vitro* measure of anti inflammatory activity of the drugs or plant extracts. Hence the present study demonstrates the *in vitro* anti-inflammatory activity of *Achyranthes aspera* by HRBC Membrane stabilization method.

MATERIALS AND METHODS:

Collection, Identification and Authentication of plant materials

The plant species namely *Achyanthes aspera* L. plant was collected by in and around Koothanallur, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997). The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the aqueous extract

The plant material (Whole plant) was shade dried and coarsely powdered with electrical blender. 200g of *Achyranthes aspera* was mixed with 1200ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extracts was prepared according to the methodology of ǀIndian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40^0C – 50^0C). The paste form of the extracts was put in an air tight container stored in refrigerator.

In vitro anti-inflammatory activity

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *in vitro* anti-inflammatory activity (Gandhisan *et al.,* 1991).

RESULTS AND DISCUSSION

Anti-inflammatory activity

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG) (Arun Shriwaikar *et al.,* 2011). The non-steroidal drugs (NSAIDs) act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membranes by means of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases, which cause further tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane (Seema Chaithaniya *et al*., 2011).

HRBC membrane is similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. All the fractions of ethanolic extract of *Achyranthes aspera* L. whole plant showed the biphasic effects on HRBC membrane stabilization. They showed increasing activity at low concentration levels and decreasing activity at high concentration levels. The activities of the various fractions are comparable to that of diclofenac sodium at the concentration of 100µg/ml. The anti-inflammatory activity of the extracts was concentration dependent. The results of the anti-inflammatory activity are presented in Table 1 .

Table 1: *In vitro* anti-inflammatory activity of whole plant extract of *Achyranthes aspera*

S.No	Concentration (µg/ml)	Aqueous extract		Ethanolic extract	
		Optical density at 560nm	% of protection	Optical density at 560nm	% of protection
1	Control	0.00	-	0.00	-
2	100	0.52	98.70	0.58	98.79
3	200	0.50	98.75	0.54	98.87
4	300	0.46	98.85	0.50	98.95
5	Diclofenac sodium (standard)	0.40	100.00	0.48	100.00

Aqueous and ethanolic extract at the concentration 300µg/ml showed significant anti-inflammatory activity was compared to that diclofenac sodium. Membrane stabilization may contribute to the anti-inflammatory effect. The *in vitro* study on whole plant extract of *Achyranthes aspera* showed the presence of significant anti-inflammatory activity. Further investigations are anticipated to identify the active components and lead to their further clinical use.

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