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IN VITRO EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *AGERATUM CONYZOIDES* LEAVES BY HUMAN RED BLOOD CELL (HRBC) MEMBRANE STABILIZATION METHOD

Amandeep Singh*[1], Deepak Nanda[1], Ashok Kumar[1], Abhishek Bhardwaj[1]

[1]*School of Pharmaceutical Sciences, Jigyasa University, Dehradun-248197, Uttarakhand, India.

ABSTRACT

Ageratum conyzoides, family Asteraceae is a straight, grassy, annual, 30 to 80cm long, stems are covered with fine white hairs, leaves are opposite, puberty with long petioles and trichomes of glands which were used for *in vitro* assessment of the anti-inflammatory activity. Medically it is used for the treatment of burn, colic, collyrium, dyspepsia, emetic, eye problems, lithontriptic as purgative, sleep-sickness, sore throat, syphilis, uterine disorders, wound, etc. This study used the *in-vitro* HRBC method to identify the anti-inflammatory property of the aqueous, alcoholic, and hydroalcoholic extracts of the leaves and analyzed them. The result of this study revealed that alcoholic extracts (200mg/ml) of Ageratum conyzoides showed significant anti-inflammatory activity. This anti-inflammatory activity of Ageratum conyzoides was compared with the potency of a standard drug (diclofenac sodium).

KEYWORDS

Ageratum conyzoides, HRBC, Inflammation and Diclofenac.

Author for Correspondence:

Deepak Nanda,

School of Pharmaceutical Sciences,

Jigyasa University, Dehradun, Uttarakhand, India.

Email: dean.pharmacy@hzu.edu.in

INTRODUCTION

Inflammation is a response to living tissues injury and includes systemic and local reactions[1]. Inflammation is generally produced by stimuli as pathogenic, irritants and damaged cells, produce vascular tissue response known as inflammation[2]. Despite our dependence on modern medicine and tremendous progress in synthetic medicine, a large number of the world's populations (80% of people) cannot afford to rely on pharmaceutical industry to afford products and the use of traditional medicines, which are primarily derived from plant material. The fact is well recognized by the WHO which

recently produced a list of medicinal plants listing over 20000 species. There are many important medicinal plants with a wide range of medicinal, biological activities and interesting phytochemical constituents. The main action of anti-inflammatory agents is the inhibition of Cyclooxygenase enzymes that are responsible for the converting Arachidonic acid to prostaglandins.

Ageratum conyzoides L. is an annual herb with medicinal effect in various diseases such as common wound and the burned one, antimicrobe, arthrosis, headache, and dyspnea. The further medicinal effect has been reported such as anti-pneumonia, pain killer, antiasthma, antispasmodic, haemostatic, gastrointestinal disorder, gynaecological disorder, antileprosy, and many other skin diseases[3].

Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti-inflammatory property of various extracts of Ageratum conyzoides. Thus, Human red blood cell membrane stabilization (HRBC method) has been used as a method in estimating the anti-inflammatory property. Ageratum contains many bioactive compounds including flavonoids, alkaloids, coumarins, essential oils, chromenes, benzofurans, terpenoids and tannins. The main plant chemical found in the leaves include: 6, 7-dimethoxy-2, 2-dimethylchromene, 6-demetoxyageratochromene, 6-vinyl-demethoxy-ageratochromene,

ageratochromene, alpha-cubebene, alpha-pinene, alpha-terpinene, beta-caryophyllene, beta-cubebene, beta-elemene, beta-farnesene, beta-myrcene, beta-pinene, beta-selinene, beta-sitosterol, cadinene, caryophyllene-oxide, conyzorigin, coumarin, dotriacontene, endo-borneol, endo-bornyl-acetate etc. In certain parts of India, the leaves of these plants were traditionally used in the treatment of inflammation. The present study aimed to authenticate that traditional information by *in vitro* anti-inflammatory screening[4-6].

MATERIAL AND METHODS

Preparation of extracts

Fresh leaves of *Ageratum conyzoides* were collected from FRI, Dehradun and were authenticated by a botanist. The leaves were dried in shade and powdered to a coarse form. It was then successively extracted with methanol, ethanol, water and hydro alcohol using a continuous cold maceration process. The extracts were concentrated under reduced pressure and preserved at low temperature.

Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Reference standard diclofenac sodium was obtained as a gift sample from Arbro pharmaceutical, New Delhi, India. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

Methods

Determination of Ash Value of *Ageratum conyzoides*

The ash of any organic material is composed of its non-volatile inorganic components. Controlled incineration of crude drug results in an ash residue consisting of inorganic material (metallic salts and silica). This value varies within fairly wide limits and is, therefore, an important parameter for the purpose of evaluation of crude drugs. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash and the water-soluble ash[7].

Determination of Total Ash

Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at as low a temperature as possible (about 450°C) to remove all the carbons. At higher temperature, the alkali chlorides may be volatile and may be lost by this process. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash – which is derived from the plant tissue itself and non-physiological ash- which is the residue of the adhering material to the plant, e.g., sand and soil. Indian Pharmacopoeia (IP), 2006, prescribes suitable methods for the determination of ash values.

Method I

Unless otherwise stated in the Individual monographs, weigh accurately 2-3g of the air-dried crude drug in the tarred platinum or silica dish and incinerate at a temperature not exceeding 450° C until free from carbon, cool and weigh. If carbon-free ash cannot be obtained in this way, exhaust the charred mass in hot water, collected the residue on an ashless filter paper, incinerate the residue, and filter paper until the ash is white or nearly white. Calculated the percentage of ash with reference to the air-dried drug.

Method II

Heated the silica or platinum crucible to red hot for 30 minutes, allowed to cool in a desiccator, and weigh. Unless otherwise specified in the individual monograph, weighed accurately about 1g of the substance being examined and evenly distribute in the crucible. Dried at 100°C to 105°C for 1 hr and ignited to constant weight in a muffle furnace at 600±25°C. Allowed the crucible to cool in a desiccator after each ignition. The material should not catch fire at any time during the procedure. If after prolonged ignition carbon-free ash cannot be obtained, proceed as directed in method I. Ignite to constant weight. Calculated the percentage of ash with reference to the air-dried substance.

Acid Insoluble Ash

Ash insoluble in hydrochloric acid is the residue obtained after extracting the sulfated or total ash with HCl, calculated with reference to 100g of drug. For the determination of acid-insoluble ash as prescribed in IP 1996, the method (I) is used unless otherwise directed in the individual monograph.

Method I

Boiled ash with 25ml of 2M HCl for 5 minutes, collected the insoluble matter in a gooch crucible or on an ashless filter paper, wash with hot water, ignites, cool in a desiccator and weigh. Calculated the percentage of acid-insoluble ash with reference to the air-dried drug.

Method II

Placed the ash, as described or as directed in the individual monograph, in a crucible. Add 15ml of water and 10ml of hydrochloric acid, boil for 10 minutes and allow to cool. Collected the insoluble matter on an ash-less filter paper, washed with hot water until the filtrate is neutral, ignite to dull redness, cooled in a desiccator, and weighed. Calculated the percentage of acid-insoluble ash with reference to the air-dried drug**.**

Water Soluble Ash

Water-soluble ash is that part of the total ash content which is soluble in water. It is a good indicator of either previous extraction of the water-soluble salts in the drug or incorrect preparations. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of Total ash with water.

As described in the IP 1996 to determine the water-soluble ash, boiled the ash as described before for 5 minutes with 25ml of water. Collected the insoluble matter in a crucible or an ash-less filter paper, washed with hot water and ignited for 15 minutes for a temperature not exceeding 450°C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference of weight represents the water-soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug[7,8].

Determination of Extractive Values

This method determines the number of active constituents in a given quantity of medicinal plant material when extracted with solvents. It is employed for that material for which no chemical or biological assay method exists. According to Indian Pharmacopoeia 1996 and British Pharmacopoeia 1980, the determination of water-soluble and alcohol soluble extractives is used as a means of evaluating crude drugs which are not readily estimated by other means. Such extractive values provide an indication of the extent of polar, medium polar, and non-polar components present in the plant material[7].

Extraction Process of *Ageratum conyzoides* from Different Chemicals

The leaves of Ageratum conyzoides were air-dried and then extracted by water, ethanol, methanol and hydroalcoholic by cold maceration process. Then extract the filter by using filter paper. The filtrate is

placed in a china disc and evaporates the filter. Finally collected the crude extract. Calculated its percentage yield.

***In vitro* Anti-inflammatory activity**

The human red blood cell (HRBC) membrane Stabilization method

The blood was collected from a healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with an equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100 and 200µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000rpm for 20 min. and the haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560nm. Diclofenac (100 and 200g/ml) was used as a reference standard and control was prepared by omitting the extracts[9,10].

RESULTS AND DISCUSSION

Determination of Ash Value of *Ageratum conyzoides*

The ash value of the Ageratum conyzoides was calculated and the total ash, acid insoluble ash, water-soluble ash, was found out to be, 80.6%, 11.28%, 9.18%.

Determination of Extractive Values

The extractive value of Ageratum conyzoides was calculated and the extract of ethanolic, methanolic, hydroalcoholic and water was found out to be 0.26gm, 0.23gm, 0.30gm, 0.32gm.

Extraction Process of *Ageratum conyzoides* from Different Chemicals

The percentage yield of Ageratum conyzoides was calculated and the percentage yield of water, ethanol, methanol and hydroalcoholic found out to be 6.5gm, 5.2gm, 4.6gm, 6gm.

***In vitro* anti-inflammatory activity**

Ageratum conyzoides extracts at different concentrations (100, 200mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of alcoholic extract at a concentration of 200mg/ml was higher than that of other concentrations. The results were tabulated in Table No.4.

Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause a rapid and transient decrease in peripheral blood lymphocyte counts to affect longer-term response. Phytochemical evaluation of the various extracts of Ageratum conyzoides reveals the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Here anti-inflammatory activity was performed based on the folklore information using two methods. HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane[11] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of the lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release. The result indicated that the leaves extract of Ageratum conyzoides at various concentrations has significant anti-inflammatory property. The present result indicates the efficacy of Ageratum conyzoides as an effective therapeutic agent in the treatment of acute inflammations. The result of the present study authenticity the folklore information on the anti-inflammatory property of the leaves extract of Ageratum conyzoides. Further and detailed studies are in process for the isolation of active constituent responsible for this property and for the identification of the possible mechanism of its anti-inflammatory property[12].

Table No.1: Different Ash values of A*geratum conyzoides*

S.No	Types of ash value	Observation(%)w/w
1	Total ash	80.6
2	Acid insoluble ash	11.28
3	Water soluble ash	9.18

Table No.2: Different Extractive value of *Ageratum conyzoides*

S.No	Types of extract	Weight of drug (gm)	Weight of empty china dish (gm)	Weight of china dish with dry extract (gm)	Extractive value w/v (gm)
1	Ethanolic extract	5	30.05	30.31	0.26
2	Methanolic extract	5	30.86	31.09	0.23
3	Hydroalcoholic extract	5	29.10	29.40	0.30
4	Water extract	5	29.41	29.73	0.32

Table No.3: Percentage yield of the different extract of *Ageratum conyzoides*

S.No	Solvent	% yield
1	Water	6.5
2	Ethanol	5.2
3	Methanol	4.6
4	Hydroalcoholic	6

Table No.4: % inhibition of different extracts of the *Ageratum conyzoides*

S.No	Type of extract	Concentration (µg/ml)	Absorbance	% Inhibition
1	Control	----	0.18±0.23	---
2	Water	100	0.0894±1.20	35.25±1.89
3	Water	200	0.0574±1.20	62.23±1.79
4	Ethanol	100	0.0790±0.31	40.12±1.05
5	Ethanol	200	0.0534±1.20	61.25±1.79
6	Methanol	100	0.0714±1.20	46.25±1.79
7	Methanol	200	0.0497±0.26	74.33±1.24
8	Hydroalcoholic	100	0.0894±1.20	31.25±1.54
9	Hydroalcoholic	200	0.0594±1.20	63.25±1.43
10	Diclofenac	50	0.0544±0.32	69.82±0.98
11	Diclofenac	100	0.0374±1.20	79.25±1.79

Plant of *Ageratum conyzoides*

ONCLUSION

The result indicated that the leaves extract of Ageratum conyzoides at various concentrations has significant anti-inflammatory properties. The present result indicates the efficacy of Ageratum conyzoides as an effective therapeutic agent in the treatment of acute inflammations. The result of the present study authenticates the folklore information on the anti-inflammatory property of the leaves extract of Ageratum conyzoides. Further and detailed studies are in process for the isolation of active constituent responsible for this property and for the identification of the possible mechanism of its anti-inflammatory property

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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