Research Article **ISSN: 2349 - 4492**

Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: www.ajrbps.com

ASSESSMENT OF *IN VITRO* ANTI INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *IPOMOEA BATATAS* TUBERS

T. Mercy Margaret*[1], P. Krishna[2], B. Revathi[2], D. Eswar Tony[1], M. Sathish Kumar[1], A. Narendra Babu[1]

[*1]Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India.
[2]Department of Pharmacology, Priyadarshini College of Pharmaceutical Education and Research, Guntur, Andhra Pradesh, India.

ABSTRACT

The sweet potato *(Ipomoea batatas)* is a dicotyledonous plant that belongs to the family convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable. Literature survey on the plant showed that leaves have a high content of polyphenolics - anthocyanins and phenolic acids, with at least 15 biologically active anthocyanins with medicinal value. The aim of the present study is to prepare the aqueous extract of *Ipomoea batatas* and to evaluate *invitro* anti-inflammatory activity by membrane stabilizing method. Phytochemical analyses of *IBAE* showed the presence of phenols, flavonoids, tannins, anthraquinones, and reducing sugars. It has been shown that the anti-inflammatory activity is may be because of phenols and flavonoids. The aqueous extract, IBAE has shown significant membrane stabilization at 200mg/ml indicated by the reduced absorbance.

KEYWORDS

Ipomoea batatas, Anti-inflammatory, Membrane stabilizing method and Sweet potato.

Author for correspondence:
T. Mercy Margaret,
Department of Pharmacology,
Chalapathi Institute of Pharmaceutical Sciences,
Guntur, Andhra Pradesh, India.

Email: pharma.mercy@gmail.com.

INTRODUCTION

Natural products from plants may become a new source for development of drugs. Although hundreds of plant species have been tested for pharmacological properties[1]. The vast majority of have not been adequately evaluated. Plants with their vast arrays of secondary metabolites form a reservoir of low molecular weight organic compounds that is largely untapped as a source of pharmaceuticals.

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The medicinal value of plants lies in some chemical

substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. In the recent years, Screening of medicinal plants for their biological activities and phyto chemicals are important for finding potential new compounds for therapeutic uses.

The plants are one of the most important sources of medicines. India is known due to availability of several thousands of medicinal plants in the different bioclimatic zones anti-inflammatory diseases. An array of drugs are available in the market to treat these disorders but only very few are free from toxicity. It is very important that profound research with ethno botanical plants possessing anti-inflammatory and analgesic, properties can definitely open up new vistas in inflammatory disorders [2]. Purified natural compounds from plants can serve as template for the synthesis of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value.

The sweet potato *(Ipomoea batatas)* is a dicotyledonous plant that belongs to the family convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable. Literature survey on the plant showed that leaves have a high content of polyphenolics - anthocyanins and phenolic acids, with at least 15 biologically active anthocyanins with medicinal value [3-5].

The aim of the present study is to prepare the aqueous extract of *Ipomoea batatas* and to evaluate *invitro* anti-inflammatory activity by membrane stabilizing method [6-12].

MATERIALS AND METHODS

Plant Material Collection and Authentication

The tubers of *Ipomoea batatas* was collected and authentified by Prof. V. Jaya. MSc, M.Phil, Botanist, Guntur.

Preparation of *Ipomoea batatas* aqueous extract

Fresh tubers will be collected, shade – dried and powdered mechanically. About 100 gm of tubers powder will be extracted with 200 ml of water allow for 24hr. Filter twice by watmans filter paper at room temperature for 4 h using a mechanical shaker. The extract will be dried at 40^{o}C under vacuum under reduced pressure. Thus, the prepared extract is used for further pharmacological evaluation.

Phytochemical Analysis

The aqueous extract is subjected to phytochemical analysis using conventional protocol like alkaloids, flavonoids, carbohydrate and glycosides gums and mucilage, fixed oils, saponins and proteins.

Test for Alkaloids

Aqueous extract is treated with dilute hydrochloric acid and filtered. The filtrate will be treated with various alkaloid agents.

Mayer's - Test

Aqueous extract is treated with Mayer's reagent. Appearance of cream color indicates the presence of alkaloid.

Dragendorff's - Test

When little amount of the sample is treated with the Dragendrorff's reagent, the appearance of reddish brown precipitate indicates the presence of alkaloid.

Hager's -Test

Aqueous extract is treated with the Hager's reagent; the appearance of yellow color precipitate indicates the presence of alkaloid.

Quinoline Alkaloids Test

Little amount of extract is added with glacial acetic acid gives reddish brown fumes and with concentrated sulphuric acid gives blue fluorescence in UV light.

Test for Carbohydrates and Reducing Sugar

A small quantity (300 mg) of alcoholic extract was dissolved in 4ml of distilled water and filtrate. The filtrate will be subjected to carbohydrates and reducing sugar.

Molisch's Test

A small portion of the filtrate will be treated with molisch's reagent and sulphuric acid. Formation of violet ring indicates the presence of carbohydrate.

Fehling's-Test

The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown precipitate indicates the presence of reducing sugar.

Benedict's Test

The extract will be treated with Benedict's reagent. The appearance of reddish orange precipitate indicates the presence of reducing sugar.

Barfoed's Test

The extract will be treated with Barfoed's reagent and heated. The appearance of reddish orange precipitate indicates the presence of non reducing sugar.

Test for Steroids

Libermann Burchard Test

When the extract will be treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of sulphuric acid, appearance of green color indicates the presence of steroids.

Test for Proteins

Biuret's-Test

When the extract is treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, appearance of violet color was observed.

Millon's-Test

When the extract is treated with millon's reagent, appearance of pink color indicates the presence of proteins.

Test for Tannins

When the extract is treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins. When the extract is treated with aqueous bromine solution, appearance of white precipitate indicates the presence of tannins.

Test for Phenols

When the extract is treated with neutral ferric chloride solution, the appearance of violet color indicates the presence of phenols. When the extract is treated with 10% sodium chloride solution, the appearance of cream color indicates the presence of phenols.

Test for Flavanoids

5 ml of the extract solution will be hydrolyzed with 10% v/v sulphuric acid and cool. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of dilute sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

Shinoda's Test

The extract will be dissolved in alcohol, to that one piece of magnesium followed by conc. HCI were added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

Test for Gums and Mucilage

The extract will be treated with 25 ml of absolute alcohol, and then solution is filtered. The filtrate will be examined for its swelling properties.

Test for Glycosides

When a pinch of the extract is dissolved in the Glacial acetic acid and few drops of ferric chloride solution is added, followed by the addition of indicates the presence of glycosides.

Test for Saponins

Foam Test

1ml of the extract is diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube is seen.

Evaluation of anti inflammatory activity

The anti inflammatory activity of tubers extract of *Ipomoea batatas* was determined by HRBC membrane stabilization method blood was collected from healthy volunteers and the collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) and 10%v/v suspension was made with isosaline. The assay mixture contained the drug.1ml phosphate buffer (0.15M pH 7.4), 2ml of hypo saline (0.36%) 0.5ml of HRBC

suspension. Diclofenac was used as the reference drug.

Instead of hyposaline 2ml of distilled water was used as control. All the assay mixtures were incubated at 37°c for 30mins and centrifuged. The haemoglobin content in the supernant solution was estimated using colorimetry at 560NM. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula:

$$\% \text{ protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

METHODOLOGY

Group I: Control (Normal saline).

Group II: Standard 5mg/ml (Diclofenac-1ml+1ml of phosphate buffer+2ml hyposaline+0.5 ml of HRBC suspension).

Group III: Extract (1ml of sample+1ml of buffer+2ml hyposaline+1ml of HRBC suspension).

Group IV: Extract (2ml of sample+1ml of buffer+2ml hyposaline+ 1ml of HRBC suspension).

Statistical Analysis

The data's will be expressed as mean ± SD. The data of anti inflammatory activity will be analyzed by one way analysis of variance (ANOVA) followed by Dunnet's't' test. A *p* value less than 0.05 will be considered as statistically significant. A *p* value less than 0.05 will be considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening of *Ipomoea Batatas*

The revealed results of the preliminary phytochemical screening of the aqueous extract of *Ipomoea batatas* were shown Table No.1.

In Vitro Anti-Inflammatory activity of IBAE

This study shows the mean absorbance were significantly reduced ($p<0.05$) by treatment with IBAE at two dose levels 200mg/ml and 400mg/ml, when compared with control group. 400 mg/ml of IBAE group has shown very significant inhibition. There was significant difference in the mean values of 200 and 400mg/ml, shows the dose dependent activity of the extract. The results are shown in the Table No.2, Figure No.1.

DISCUSSION

Inflammation is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial or heterogeneous nature of the disease increases, so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries[5].

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. NSAIDS possibly induce the redistribution of lymphocytes which cause rapid and transient decrease in peripheral blood lymphocytes counts to effect longer term response[1]. HRBC method was selected for *invitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogues to the lysosomal membrane and its stabilisation implies that the aqueous extract of IBAE may as well stabilised lysosomal membranes. Stabilisation of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophill such as bactericidal enzymes and proteases which cause further tissue inflammation and damaged upon extracellular release[2]. The lysosomal enzymes released during inflammation produces a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic by inhibiting the lysosomal membrane [3].

Since HRBC membrane are similar to lysosomal membrane components, the prevention of hypo tonicity induced HRBC membrane lysis is taken as a measure of *invitro* anti-inflammatory activity of the aqueous extracts[4].

Phytochemical analyses of *IBAE* showed the presence of phenols, flavonoids, tannins, anthraquinones, and reducing sugars. It has been shown that the anti-inflammatory activity is may be because of phenols and flavonoids[7]. The aqueous extract, IBAE has shown significant membrane stabilization at 200mg/ml indicated by the reduced absorbance.

Table No.1: Preliminary Phytochemical Screening

S.No	Name of the test	Result
1	Carbohydrates	+
2	Proteins	_
3	Glycosides	_
4	Phenols	+
5	Flavonoids	+
6	Steroids	-
7	Terpenes	_
8	Tannins	+

(+)Indicates presence of that compound

(-) Indicates absence of that compound

Table No.2: *In Vitro* Anti-Inflammatory activity of IBAE

S.No	Groups	Treatment	Dose	Mean Absorbance	% Inhibition
1	1	Control	2 ml of distilled water	0.543±0.008	-
2	2	Standard	5mg/ml	0.138±0.004***	74.585
3	3	IBAE-1	200mg/ml	0.279±0.005**	48.618
4	4	IBAE-2	400mg/ml	0.210±0.005	61.325

Mean ±.S.E.M=mean values ±standard error of mean of three experiment ***P<0.001 P**<0.001, *P<0.05 compared to control group.

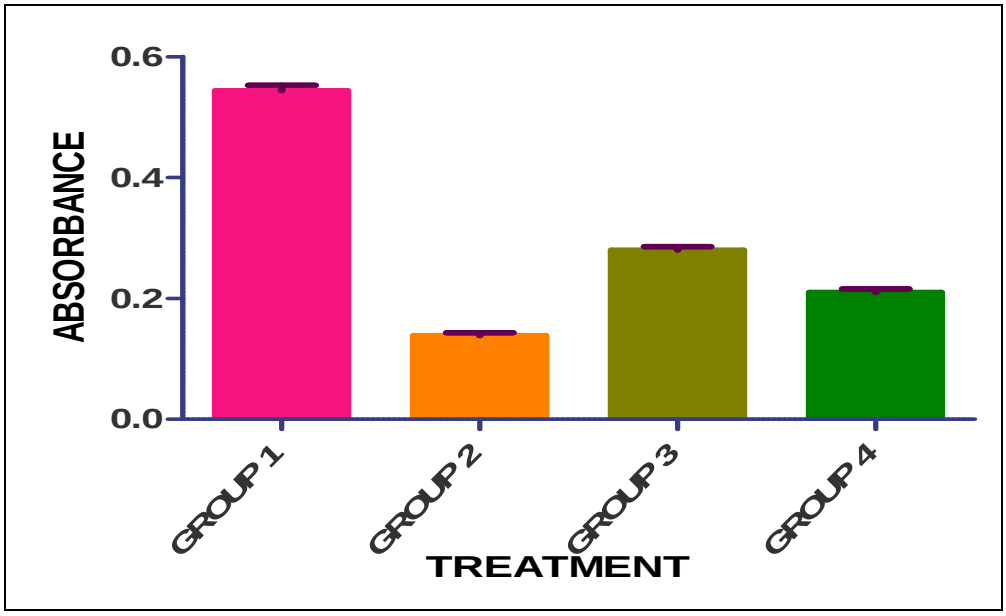

Figure No.1: *In Vitro* Anti-Inflammatory activity of IBAE

CONCLUSION

The study was concluded that the aqueous extract of *Ipomoea batatas* 200 mg/ml has shown significant anti-inflammatory activity (membrane stabilization property). The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogus to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane.

This study reveals that, the aqueous extract of *Ipomoea batatas* contains high amounts of phenolics and flavonoids may be responsible for anti-inflamatory activity.

Thus, further detailed studies are to be done for isolation of active constituents and identifying the possible mechanism for anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors are sincerely thanks to the, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India for providing the facilities to complete this research work.

BIBLIOGRAPHY

1. Eisenberg D M, Davis R B, Ettner S L, Appel S, Wilkey S, Van Rompay M, Kessler R C. Trends in alternative medicine use in the United States, *American Medical Association*, 280, 1997, 1569-1575.
2. Berman B M, Swyers J P, Kaczmarczyk J. Complementary and alternative medicine: herbal therapies for diabetes, *J Assoc Acad Mino Phys* 10, 1999, 10-14.
3. Umamaheswara Rao P, Anti inflammatory and antinociceptive activities of leaves of *Rumex dentatus* in rats, *Asian Pacific Journal of Tropical Biomedicine*, 3, 2008, 14-17.
4. Harvey A L. Natural products in drug discovery, *Drug Disc Today*, 13, 2008, 19-20.
5. Newman D J, Cragg G M. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*, 70, 2007, 461-477.
6. Badilla B, Arias Y, Mora G A and Poveda L J. Anti inflammatory and antinociceptive activities of *Loasa speciosa* in rats and mice, *Fitoterapia*, 74, 2003, 45-51.
7. Lee D Y. Anti- inflammatory effects of *Asparagus cochinchinensis* extract in acute and

chronic cutaneous inflammation, *Journal of ethnopharmacology*, 114, 2007, 234-240.

8. Abena A A, Ouamba J M, Keita A. Anti-inflammatory, analgesic and antipyretic activities of essential oil of *Ageratum conyzoides*, *Phytother Res*, 10, 1996, 164-165.
9. Akbar S. Pharmacological investigations on the ethanolic extract of *Salvia haemtodes*, *Fitoterapia*, 60, 1989, 270-272.
10. Akubue P I, Mittal G C, Aguwa C N. Preliminary pharmacological study of some nigerian medicinal plants, *J Ethnopharmacol*, 8(1), 1983, 53-63.
11. Vandana Panda and Madhav Sonkamble. Anti-ulcer activity of *Ipomoea batatas* tubers (sweet potato), *Functional Foods in Health and Disease*, 2(3), 2012, 48-61.
12. Kusano S. Antidiabetic activity of white skinned sweet potato (*Ipomoea batatas L.)* in obese Zucker fatty rats, *Biol Pharm Bull*, 23(1), 2000, 23-6.