INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 3 | July - Sep -2017

Journal Home page: www.ijpar.com

Research article *Open Access*

Evaluation of anti-inflammatory activity of hydroalcoholic extract of *Ananas cosmosus* fruit peel by HRBC membrane stabilization

Y. Niharika*

Dept. of pharmacy, Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, India.

***Corresponding Author: Y. Niharika**

ABSTRACT

The aim of the study is to determine the in-vitro anti-inflammatory activity of the hydroalcoholic extract of *Ananas Cosmosus* fruit peel by HRBC membrane stabilization. Hypotonicity induced human red blood cell (HRBC) membrane stabilization method was performed to check the anti-inflammatory activity of *Ananas Cosmosus*. The extract was compared with a standard synthetic drug Diclofenac to check the anti-inflammatory activity. Haemolysis and protective activity of both the drugs were checked and analyzed. Results showed significant anti-inflammatory activity but was less compared to the standard drug. Inflammation has to be treated prior to the disease treatment since decreasing pain is first step in a treatment procedure. This is where the anti-inflammatory drugs act and eventually decrease the inflammation. Diclofenac being a synthetic drug can lead to a lot of side effects. *Ananas Cosmosus* being a natural drug has fewer side effects comparatively and can be used in combination with other drugs in future in giving and efficient anti-inflammatory drug.

Keywords: *Ananas Cosmosus*, Anti-inflammatory, Diclofenac, HRBC membrane stabilization, Hydroalcoholic extract.

INTRODUCTION

Inflammation is a pervasive form of defense that is broadly defined as a non specific response to tissue malfunction and is employed by both innate and adaptive immune systems to combat pathogenic intruders. Inflammation is a complex biological response of vascular tissues to harmful stimuli. The cause of tissue injury is attributed to trauma, autoimmune, microbial, heat and toxins. When tissue injury occurs, numerous substances are released which causes changes to the uninjured tissues. Some of the tissue products that cause the inflammation reaction include: histamine, serotonin, prostaglanidins, leukotriene, platelet-activating factor, bradykinin. These are the substances released from the cells in response to various stimuli to elicit normal physiological responses locally. An imbalance in the synthesis and release of the substances contribute significantly to pathological conditions such as inflammation.

Medicinal plants have been used for centuries as remedies for disease because they contain component of therapeutic values. According to

WHO, 80% of the world population continues to rely on herbal and other traditional medicines for their health care. The therapeutic effects of many medicinal plants are usually due to their anti-oxidant and anti-inflammatory properties. The photochemical such as flavanoids, phenols, tannins, and terpenoids are responsible for anti-oxidant and anti-inflammatory activity.

The main action of the anti-inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of the arachodonic acid to prostaglandins. Since human red blood cells (HRBC) membrane resemblances to the lysosomal membrane, the prevention of hypotonicity induced haemolysis of the HRBC membrane was taken as measure in estimating the anti-inflammatory activity. The currently used anti-inflammatory drugs such as NSAID's aminosalicylates and steroids to treat inflammation are associated with severe side effects. So there is a need to find safer anti-inflammatory compounds. The plant *Ananas Cosmosus* commonly called as pineapple is a tropical plant with an edible multiple fruit consisting of coalesced berries and it is the most economically significant plant in Bromeliaceae family. The present work aims at evaluating the anti-inflammatory activity of hydroalcoholic extract of *Ananas Cosmosus* fruit peel by HRBC membrane stabilization.

LITERATURE REVIEW

1. V.Vijayakumar and Hindumathy has evaluated the anti inflammatory activity of *Strychnos Potatorum Linn* seed by HRBC Membrane stabilization. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti inflammatory activity. The anti inflammatory activity of hydroalcoholic extract was compared to that of the standard drug hydrocortisone. The percentage protection for the hydroalcoholic extract and hydrocortisone at a concentration of 100µg/ml showed 54.95±0.74 and 67.50±0.52. The hydroalcoholic extract of *Strychnos Potatorum* Linn seed has significant anti inflammatory activity.
2. T.K Mohamed Saleem and AK Azeem has evaluated the anti-inflammatory activity property of the aqueous and alcoholic extracts of *Gendarussa Vulgaris* Nees leaves by both in-vitro and in-vivo methods. In vitro method was estimated by HRBC Membrane stabilization method and in-vivo method was estimated on the carrageen induced paw oedema. The result showed that both the methods showed significant anti-inflammatory property. The alcoholic extracts at a concentration of 300µg/ml showed potent activity comparing with the standard drug Diclofenac sodium.
3. G.R.Rajalakshmi and Jyoti Harindran evaluated the anti inflammatory activity of aqueous and ethyl alcohol extract of *Wrightia tinctoria* by HRBC Membrane stabilization method. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity. The ethylalcohol extract at a concentration of 100µg/ml showed 70% protection of HRBC in hypotonic solution and compared with standard drug diclofenac which showed 73% protection.
4. G.Prakash Yoganandam and K.Ilango has evaluated the efficiency of Water, ethanol, methanol and ethyl acetate extracts of fruit peel of *Punica granatum*.L for anti-inflammatory activity by HRBC membrane stabilization method. Among the extract screened the methanol and ethyl acetate extracts exhibited better activity ($P<0.001$) than the other two extracts which may be due to the presence of higher phenolic content estimated by Folin-ciocalteu reagent.

MATERIALS AND METHODS

Plant material

The plant material was purchased from the local market in Visakhapatnam and it was authenticated by a botanist.

Reagents, chemicals and instrument

All the reagents and chemicals used in the study were of analytical grade. Ethanol was obtained from Leisha Pharma Solutions, Dextrose, Sodium citrate, Sodium chloride, Citric acid was obtained from SD fine chemicals, Mumbai. Reference standard Diclofenac was obtained from Yarrow chem. Products, Mumbai. Systronics UV-Visible spectrophotometer was used for the estimation of anti-inflammatory activity.

Preparation of plant extract

The peel were separated from the fruit and shade dried for one week. Dried peels were powdered using an electric mixer. The dried powdered peel was extracted successively with 70% alcohol and 30% water in a soxhlet extractor. The extract was concentrated by distilling the solvent and then evaporation to dryness on a water bath. The extract was stored in an air tight container for further use.

Figure:1 Soxhlet extractor

ANTI-INFLAMMATORY ACTIVITY

Preparation of blood samples for Membrane Stabilization assay

The in-vitro anti-inflammatory activity of hydroalcoholic extract of *Ananas Cosmosus* fruit peel was determined by HRBC membrane stabilization method. The blood was collected from healthy volunteer who was not taken any NSAID's for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate 0.05% citric acid & 0.042% sodium chloride in distilled water) and centrifuged at 3000rpm and the supernatant was removed. The cell suspension was washed with sterile isosaline solution (0.85%, P^H-7.2) and centrifuged at 3000rpm. This was repeated till the supernatant was clear and the packed cell volume was measured. The cellular component was reconstituted to a 10%v/v suspension with isosaline and was used in the assay.

Human Red Blood Cell (HRBC) Membrane Stabilization Method

The assay mixture contains 0.5mL of dilutions extract at different concentrations (50 to 250µg) prepared in isosaline solution, 1mL phosphate buffer (P^H -7.4), 2mL hyposaline solution (0.36%), 0.5mL of 10%v/v HRBC suspension. The mixture was incubated at 37˚c for 30minutes and centrifuged at 3000rpm for 20 minutes. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac was used as standard and a control was

prepared by distilled water instead of hyposaline solution to produce 100% haemolysis without plant extract. The percentage protection of HRBC was calculated by using the following formulae.

$$\% \text{ Protection} = 100 - \frac{(O.D\ of\ Sample)}{(O.D\ of\ Control)} x100$$

Figure:-2 Different Concentrations (50 to 250µg) of extract and standard

RESULT

Table 1: Percentage protection of HRBC membrane by diclofenac (standard drug) and hydroalcoholic extract of *Ananas Cosmosus* fruit peel

% Protection of HRBC membrane		
Conc. (µg/mL)	**Std. drug (Diclofenac)**	**Hydroalcoholic extract of *Ananas cosmosus***
50	69.81±1.44	58.26±0.71***
100	72.15±1.06	61.44±0.71***
150	74.17±0.8	64.36±0.94***
200	79.45±0.97	67.51±1.25***
250	83.57±0.53	72.86±1.05***

*Percentage protection of HRBC membrane values represented as Mean±SD n=3). SD=Standard deviation, statistically significant with*** *p<0.01 compared with standard drug*

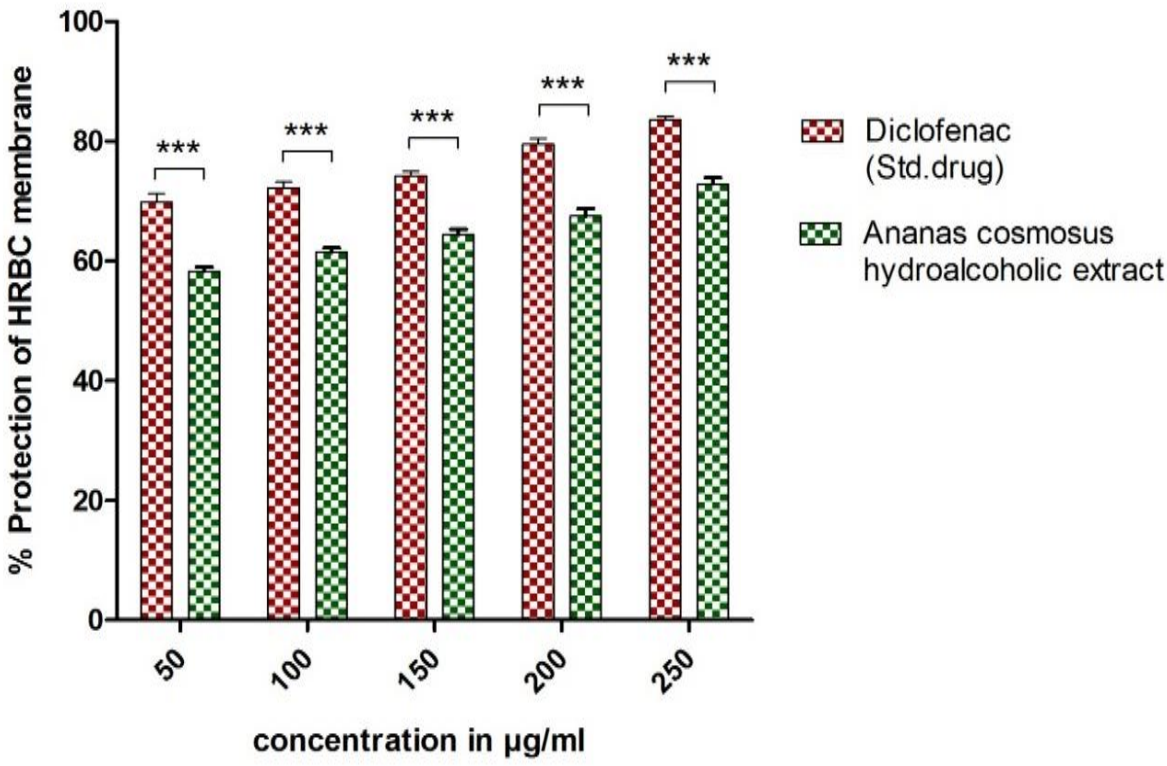

Figure 3: Effect of Diclofenac and Hydroalcoholic extract of *Ananas cosmosus* fruit peel on HRBC Membrane stabilization

DISCUSSION

The hydroalcoholic extract of *Ananas Cosmosus* exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane.

The lysosomal enzyme released during inflammation produce various disorders. The stabilization of the lysosomal membrane will inhibit the release of lysosomal constituents of activated neutrophils such as proteases and bactericidal enzymes which cause further tissue inflammation and damage upon extra cellular release. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the membrane. Since HRBC membranes are similar to lysosomal membrane components the prevention of hypotonicity induced lysis of HRBC membrane is taken as a measure of anti-inflammatory activity of drug. The sample was compared with standard to check the percentage protection of HRBC cells.

The assay was carried out using five different concentrations (50, 100, 150, 200, 250µg/ml), the extract showed significant anti inflammatory activity at all the tested concentrations and the results have been tabulated in the table-1. The results indicated that hydroalcoholic extract exhibited appreciable inhibition of HRBC membrane lysis in comparison with Diclofenac. There was a dose dependent increase in percentage protection of HRBC membrane by hydroalcoholic extract of *Ananas Cosmosus* fruit peel. The present study indicates that *Ananas Cosmosus* fruit could be useful in management of inflammation.

CONCLUSION

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Inflammation is the most common symptom for most of the diseases and it has to be treated prior to the disease treatment since decreasing pain is the first step in a treatment procedure. This is where

the anti-inflammatory drugs act and eventually decreases the inflammation.

The result of the present study indicates that the hydroalcoholic extract of *Ananas Cosmosus* fruit peel has showed significant membrane stabilization property. Thus this study confirms that the *Ananas Cosmosus* fruit peel has anti-inflammatory activity. The plant may contain essential herbal bioactive compounds which stabilizes the HRBC Membrane by inhibiting the hypotonicity induced lysis and further structural elucidation and characterization methodologies have to be carried out for the isolation of active constituent responsible for this property and to identification of the possible mechanism of its anti-inflammatory property.

ACKNOWLEDGEMENTS

My sincere thanks, heartfelt indebtedness and sincere gratitude to my guide Mr. G.Uma sankar for providing us the most valuable suggestions to carry out this work successfully and we are very much thankful to our esteemed college for providing a very cordial and conductive environment for this work.

REFERENCES

[1]. V.Vijayakumar, C.K.Hindumathy: In-vitro anti-inflammatory activity of *Strychnos Potatatorum* Linn seed by HRBC Membrane stabilization. *Indian Journal of Research in Pharmacy and Biotechnology. 1(3), 2013, 410-412.*

[2]. T.K Mohamed Saleem, AK Azeem: Anti-inflammatory activity of the leaf extracts of *Gendarussa Vulgaris* Nees. *Asian Pac J Trop Biomed. 1(2), 2011, 147-14.*

[3]. Rajyalakshmi G.R, Jyoti Harindran:Anti-inflammatory activity of *Wrightia Tinctoria* leaves by membrane stabilization. *International Journal of Pharma Sciences and Research. 3, 2012, 497-499.*

[4]. G.Prakash Yoganandam, K.Ilango: Evaluation of anti-inflammatory and membrane stabilizing properties of various extracts of *Punica Grantum L. Int.J.PharmTech Res. 2(2), 2010, 1260-1263.*

[5]. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan Shahima: Anti-inflammatory activity of the glandular extracts of *Thunnus Alalunga*. *For Med. 3(10), 2010, 412-420*

[6]. Chaitanya, Sandhya S, David Banji, Vinod K.R and Murali.S: HRBC Membrane Stabilizing Property of Root, Stem and Leaf of *Velutinum. International Journal of Research in Pharmaceutical and Biomedical Sciences ISSN. 2(1), 2011, 2229 -3701.*

[7]. Ncube NS, Afolayan AJ, Okoh AI: Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology 7(12), 2008, 1797-1806.*

[8]. Okoli CO, Akah PA, Nwafor SV, Anisiobi AI, Ibegbunam IN, Erojikwe O: Anti-inflammatory activity of hexane leaf extract of *Aspilia Africana . J Ethnopharmacol. 109(2), 2007, 219–225*.

[9]. O. H Nielson, P S Andersen, and S E Girardin: Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clin Exp Immunol 147(2), 2007, 227–235.*

[10]. Gandhisan R, Thamaraichelvan A. Baburaj: Anti-inflammatory action of *Lannea Coromandelica* HRBC membrane stabilization. *Fitotherapia. 62, 1991, 82–83*.

[11]. William B.Jensen, "The origin of Soxhlet extractor". 1(*8), 2007,12.*

[12]. Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S: In-vitro anti-inflammatory activity of leaf extracts of Basella Alba Linn. Var. alba. Int J Drug Dev Res. 3, 2011, 124–127.