Available online on 20.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

In-Vitro anti-inflammatory activity of *S. xanthocarpum and A. officinarum* herb by Human red blood cell membrane stabilization method

Chandra Shekhar Singh[1*], Shailesh Gupta[2], Alok Pal jain[1]

SRK University, NH-12 Hoshangabad Road, Misrod, Bhopal, India

ABSTRACT

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. *Solanum xanthocarpum* herb is highly used by the rural and tribal people in curing various disorders. The aim of the current investigation is evaluation of anti-inflammatory activity of *solanum xanthocarpum* extract and *Alpinia officinarum. In-vitro* anti-inflammatory study performed by percentage inhibition of Human red blood cell (HRBC) membrane stabilization method. Four different concentration of extract 1mg/ml, 2 mg/ml, 4 mg/ml and 6 mg/ml were used for each extract. Among which ethanolic extract of *S. xanthocarpum* at concentration 6 mg/ml showed 50.1 % protection of HRBC in hypotonic solution and *A. officinarum* extract at concentration 6 mg/ml showed 56.89 while combination of extract (1:1 ratio) at concentration 6 mg/ml showed 67.89 % protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 70.0 % protection at concentration 2.5 mg/ml

Keyword: Natural remedies, anti-inflammatory, Human red blood cell (HRBC) membrane stabilization, hypotonic solution.

Article Info: Received 04 May 2019; Review Completed 08 June 2019; Accepted 11 June 2019; Available online 20 June 2019

Cite this article as:

Singh CS, Gupta S, jain AP, *In-Vitro* anti-inflammatory activity of *S. xanthocarpum and A. officinarum* herb by Human red blood cell membrane stabilization method, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):663-666
http://dx.doi.org/10.22270/jddt.v9i3-s.2948

***Address for Correspondence:**

Chandra Shekhar Singh, SRK University, NH-12 Hoshangabad Road, Misrod, Bhopal, India

INTRODUCTION

Medicines derived from plant sources are widely used in traditional cultures globally and now-a-days they are getting popular as natural alternatives to synthetic chemicals. In the last few decades the use of herbal medicine has increased exponentially. Recently it is getting popular in developing and developed countries owing to its natural origin and lesser side effects

Solanum xanthocarpum (Solanaceae) and *Alpinia officinarum* (Zingiberaceae) is an important medicinal herb in Ayurvedic medicine. Various studies indicated that S. *xanthocarpum* possesses antiasthmatic, hypoglycemic, hepatoprotective, antibacterial, analgesic and insect repellent properties. Although the results are very encouraging and indicated that some of the constituents of the plant like solasodine and diosgenin are important therapeutically, the herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects. . In India it is largely found in UP, Punjab, Bihar, Bengal, Uttaranchal, & other north east states. It grows generally in March- April and produce fruits in May- June. It can grows on any type of soil but hot and dry region is more suitable Various traditional claims like immunomodulation, anti-inflammatory, antiallergic, antianaphylactic and antitumor effects of the plant are still remain to be validated scientifically while Alpinia officinarum belong to the ginger family and commonly used for its anti-inflammatory, antihyperlipidemic bioactivity, anticancer, dysmenorrhea, osteoblast, anti-influenza virus activity, antibiotic resistance, antimicrobial effect.[1-5]

MATERIAL AND METHODS

Collection of plant material

The plant *Solanum xanthocarpum* and *Alpinia officinarum* were collected from Bhopal and was authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P., India.

Preparation of plant powder

The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed

through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts

About 250 gm of S. *xanthocarpum dried* fruit and *250 gm A. officinarum* dried root powder of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. Ethanol solvent is used for S. *xanthocarpum* extraction and methanol solvent for *A. officinarum.* The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.

***In-vitro* Anti-inflammatory activity of different Extracts of *S. xanthocarpum and A. officinarum*:**

Ethanol extract of S. *xanthocarpum* and methanolic extract of *A. officinarum* were investigated for *In-vitro* Anti-inflammatory activity by human red blood cell membrane stabilization method. Four different concentrations of extracts: 1mg/ml, 2mg/ml, 4mg/ml and 6mg/ml were used for anti-inflammatory study.

Preparation of drug

Standard drug (Indomethacin, 2.5 mg/ml) and extracts (1.0 - 6.0 mg/ml) were prepared in isosaline (0.85% NaCl) to final concentration.

Preparation of Suspension (10% v/v) of Human Red Blood cell

The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cells were washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation was repeated five times until the supernatant was clear. Then, Human erythrocytes suspension (10% v/v) was prepared as reported by Oyedapo et al., 2004.

Assay of Membrane stabilizing activity

The HRBC membrane stabilizing activity assay was carried out as reported by Sadique et al., 1989; Oyedapo et al., 2004 using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline.

To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were prepared[1-2]

a) **Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH7.4), and 1ml of test extract (1mg/ml – 6 mg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

b) **Test control** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

c) **Standard solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Indomethacin (2.5mg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline.

The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times (A_1 - A_2/A_1)$$

Where:

A_1 = Absorption of hypotonic buffered saline solution alone

A_2 = Absorption of test sample in hypotonic solution

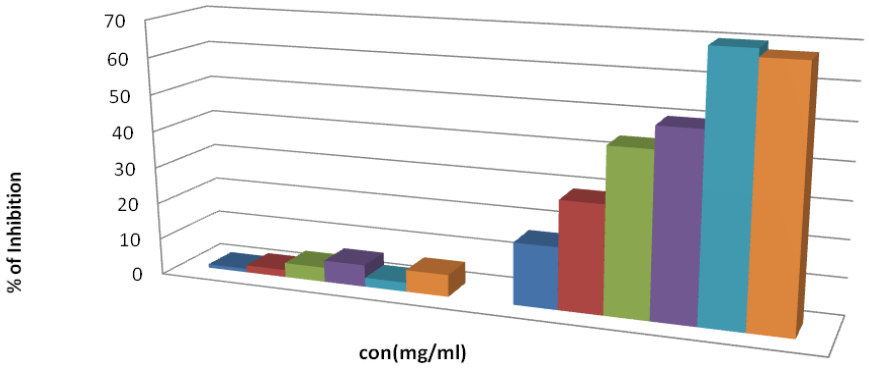
RESULT AND DISCUSSION

In-vitro* Anti-inflammatory activity of Extracts of *S. xanthocarpum and A. officinarum

During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damages of the surrounding organelles and tissues with attendance of variety of disorders. Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. In the present study, stabilization of erythrocyte membranes exposed to both heat and hypotonic induced lyses was employed due to its simplicity and reproducibility. The ethanolic extract of the root of *S. xanthocarpum and* Methanolic extract *A. officinarum* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Four different concentration of extract 1mg/ml, 2 mg/ml, 4 mg/ml and 6 mg/ml were used for each plant extract. Among which ethanolic extract of *S. xanthocarpum* at concentration 6 mg/ml showed 50.1 % protection of HRBC in hypotonic solution and *A. officinarum* extract at concentration 6 mg/ml showed 56.89 while combination of extract (1:1 ratio) at concentration 6 mg/ml showed 67.89 % protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 70.0 % protection at concentration 2.5 mg/ml (Table 1 & 2, figure 1& 2) .The activity may be due to the presence of one or more phytochemical constituents present in the extract.

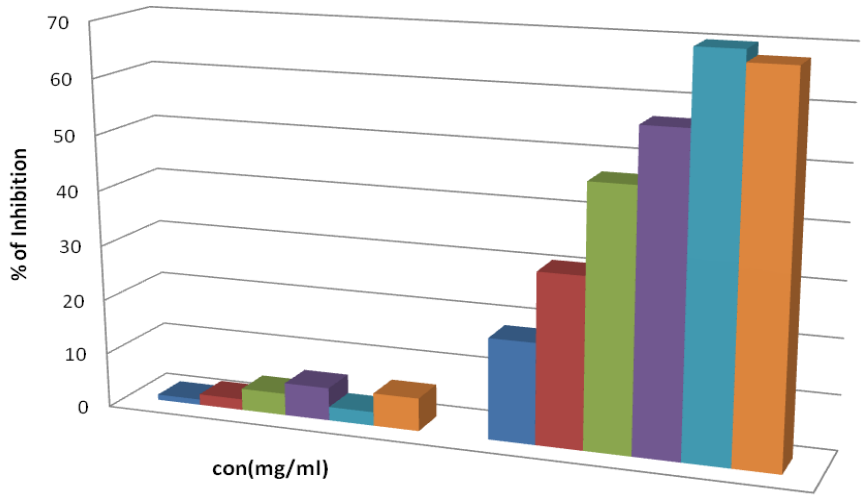
The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

Fig 1: In-Vitro anti-inflammatory activity by membrane stabilization method



	con(mg/ml)	
S. xanthocarpu extract	1	16.8
	2	29.2
	4	44.1
	6	50
Indomethacin(Standard Drug)	2.5	70
Extract of S. xanthocarpu and A. officinarum in 1:1 ratio	6	67.89

Fig 2: In-Vitro anti-inflammatory activity by membrane stabilization method



	con(mg/ml)	
A. officinarum	1	18.35
	2	30.87
	4	46.96
	6	56.89
Indomethacin(Standard Drug)	2.5	70
Extract of S. xanthocarpu and A. officinarum in 1:1 ratio	6	67.89

Table 1: *In-Vitro* anti-inflammatory activity of Ethanolic extract of *S. xanthocarpum* by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Extract of S. xanthocarpu	1.00	0.208±0.25 [a]	16.80
	2.00	0.182±0.22[a]	29.20
	4.00	0.147±0.28[b]	44.10
	6.00	0.123 ±0.42[c]	50.0
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	70.0

Values are expressed as X (Mean) ±SEM, n=3. (One way ANOVA followed by Student t-test). Statistically significance of [a]P < 0.05, [b]P<0.01, [c]P<0.001 and [d]NS in comparison to respective control.

Table 2: *In-Vitro* anti-inflammatory activity of Methanolic extract of *A. officinarum* by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Methanolic extract of *A. officinarum*	1.00	0.202±0.25 [a]	18.35
	2.00	0.175±0.12[a]	30.87
	4.00	0.143±0.23[a]	46.96
	6.00	0.110±0.44[c]	56.89
Extract of S. xanthocarpu and A. officinarum in 1:1 ratio	6.00	0.081±0.39[b]	67.89
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	70.0

Values are expressed as X (Mean) ±SEM, n=3. (One way ANOVA followed by Student t-test). Statistically significance of [a]P < 0.05, [b]P<0.01, [c]P<0.001 and [d]NS in comparison to respective control.

Table 3: *In-Vitro* anti-inflammatory activity of combination of extract by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Ethanolic extract of *S. xanthocarpum and* Methanolic extract of *A. officinarum* in 1:1 ratio	6.00	0.081±0.39[b]	67.89
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	70.0

ACKNOWLEDGEMENT

Special thanks to Dr. Alok Pal Jain, Principal, RKDF college of Pharmacy, Bhopal and Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

CONCLUSION

Ethanolic extract of *S. xanthocarpum and* Methanolic extract of *A. officinarum* in 1:1 ratio at concentration 6 mg/ml showed maximum 67.89 % protection of HRBC in hypotonic solution. Combination of both extract given synergist action which increased the therapeutic value. The activity may be due to the presence of one or more phytochemical constituents present in the extracts. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

REFERENCES

1. Khanna P and Nag TN. Effect of Phenylalanine and Tyrosine on growth and alkaloid production in Datura metel L. tissue cultures. Indian J. Pharma. 1972; 34(2):42-44.
2. Keeler RF, Young S, Brown D, Spina bifida, exencephaly, and cranial bleb produced in hamsters by the Solanum alkaloid solasodine. J. Res Commun Chem Pathol Pharmacol, 1976; 13(4):723-30.
3. Dixit VP and, Gupta RS. Antispermatogenic/ antiandrogenic properties of solasodine obtained from Solanum xanthocarpum berries on the male genital tract of dog. A histopathological approach. J Int J Androl, 1982; 5(3):295–307.
4. Manjunath BL & Shadaksharaswamy M. Reexamination of the alkaloids of the fruits of Solanum xanthocarpum, Journal of Mysore University, 1942; 3B:117.
5. Mukherjee PK. (2001).Quality Control Herbal Drug, Published by Business Horizon, Ist edition, 380.
6. Oyedapo O, Akinpelu A et al., Red blood cell membrane stabilizing potentials of extracts of Lantana camra and its fraction, International journal of plant physicology and biochemistry, October 2010; 2(4):46-51.
7. Gupta S, Kohli S and Dwivedi S, In-Vitro Anti-inflammatory activity of Sarcostemma acidum by HRBC Membrane Stabilization, International Journal of Pharmacy Teaching & Practices, 2011; 2(4):184-188
8. Vduvu R. and Lakshmi KS, In vitro and in vivo anti-inflammtory activity of leave of symplocos cochinchnensis Moor ssp laurina, Bangladesh J Pharmacol, 2008; 3:121-124
9. Gambhire M., Juvekar A., Wankhede S., Evaluation of anti-inflmmatory action of methanol extract of Barleria Cristata leave by in vivo and in vitro methods, The Internationl journal of Pharmacology 2009; 7
10. Sangeeta M et al., In-vitro anti-inflammatory and Anti-arthritic Activity of Leaves of Cleodendron Inerme, Research Journal of Pharmaceutical, Biological and chemical sciences 2009; 61.