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In-Vitro anti-inflammatory activity of *Sarcostemma acidum* Wight. & Arn. Indian herb by Human red blood cell membrane stabilization method

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Research Article

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Abstract

Somlata (*Sarcostemma acidum*) belonging to the family Asclepiadacea is grown in India, Europe and US is an underutilized crop. The herb is highly used by the rural and tribal people in curing various disorders like asthma, swelling, fever and cold, dyspepsia, inflammatory infection and gastric problem etc. It is found in dry rocky places in Bihar, Bengal, Konkan, Deccan, Tamil Nadu, Maharashtra, Madhya Pradesh and Kerala. The present study aimed at the evaluation of anti-inflammatory activity of Sarcostemma acidum. Ethyl acetate extract of *Sarcostemma acidum* was investigated for *In-vitro* Anti-inflammatory activity by human red blood cell membrane stabilization method. Four different concentration of extract were used for HRBC membrane stabilization method among which concentration at 1 mg/ml showed 30 % protection, con. 2 mg/ml showed 42.8 % protection, con. 4 mg/ml showed 54.0% protection and con.6 mg/ml showed 67.6% protection of HRBC in hypotonic solution. All result show that ethyl acetate extract showed significant membrane stabilizing action on human red blood cell when compared to standard drug Indomethacin which showed 69.6 % protection of HRBC in hypotonic solution. The activity may be due to the presence of one or more phytochemical constituents present in the extract.

Keywords: Membrane, stabilization, anti- inflammatory, *Sarcostemma acidum*

Introduction

The use of medicinal plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally.

The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs. *Sarcostemma acidum* Wight. & Arn. (Syn. *S. brevistigma*) commonly known as somlata (H), soma, somavalli (S), moon plant (E) belongs to family Asclepidacea is a perennial leafless, jointed trailing shrub with green, cylindrical, fleshy glabrous, twining branches having milk white latex, leaves reduced to scales, opposite, flowers white or pale greenish white, fragnant, in umbels on branch extremities, fruits follicles, tapering at both ends, seeds flat, ovate, comose. The plant is bitter, acrid, cooling, alternate, narcotic, emetic, antiviral and rejuvenating. Whole part of the plant is used in vitiated condition of pith, dipsia, viral infection, hydrophobia, psychopathy and general debility. It is distributed in various parts of India. It is found in dry rocky places in Bihar, Bengal, Konkan, Deccan, Tamil Nadu, Maharashtra, and Kerala.[1,2]

Phytochemical Studies have revealed the presence various Pregnane glycoside which are diglycosides and triglycosides. Some important pregnane glycosides are:- Sarcogenin (triglycoside), Brevobiose (disaccharide), Sarcobiose (nonreducing disaccharide), Tigmobiose (non reducing disaccharide), Brevine (triglycoside), Brevinine (Pregnane ester diglycoside).[3-6]

A fraction of this plant extract has been reported to have larvicidal potential against second and fourth instar larvae of the laboratory –reared mosquito species, cluex quinquefasciatus, anti-allergic and anti-inflammatory activities. It also inhibited the contractions induced by acetylcholine and histamine on isolated guinea pig ileum, and produced broncho-spasmolytic activity. It is traditionally used to reduce vitiations of pitta in the treatment of pyschosis, depression and fatigue. According

to folklore the whole part of the plant is used in the treatment of asthama, Juice given to children to get relief from cold, latex dropped in eyes in case of cataract for remedy [7-10]

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995) which are aimed at host defense and usually activated in most disease condition. Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process.

Inflammations can be uncomfortable and painful. But using natural remedies for inflammation is much better than using any kind of chemical drugs to suppress the symptoms. This is because, like other symptoms, such as fever, they are actually a sign of the body healing itself of a problem, for example an infection. An area of the body is inflamed because the immune system is sending additional "combat forces" there to try to fight the enemies, so to speak. By using artificial and chemical means to suppress an inflammation, we are directly interfering with the body's attempts to tackle and repair the problem which it is facing. Such attempts to merely treat symptoms, without considering the deeper underlying issues and attempting to achieve true and ultimate healing, is a problem not just unique to anti-inflammatory drugs, but to chemical drugs in general. The present study was carried out to evaluate the *in-vitro* anti-inflammatory activity of *Sarcostemma acidum* by HRBC method.

Material and Methods

Collection of plant material

The plant *Sarcostemma acidum* was collected from Botanical Garden of National Botanical Research Institute, Lucknow, U.P and was authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P., India and Voucher specimen No. SG/KN/3210 was deposited in our department.

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 dried powder stem of plant was subjected to soxhlation. It was fist defatted with petroleum ether then exhaustively extracted with ethyl acetate solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. 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.

Preparation of drug

Standard drug (Indomethacin, 2.5 mg/ml) and extract (1.0 -6.0 mg/ml) was prepared in isosaline (0.85% NaCl) to final the 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 cell was washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. Then, Human erythrocytes suspension (10% v/v) were prepared as reported by (Oyedapo et al., 2004).[11]

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 used.

1. **Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) ,1ml of phosphate buffer (pH7.4), 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.

2. **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.

3. **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.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. 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.[12-14]

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

Where:
A_1 = Absorption of hypotonic buffered saline solution alone
A_2 = Absorption of test sample in hypotonic solution

Results and Discussion

During inflammation, lysosomal hydrolytic enzymes are released into the sites which causes damages of the surrounding organelles and tissues with attendance variety of disorders (Sadique et al., 1989). Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation (Kalyanpur et al., 1968; Lee and Thong, 1970; Swingle, 1974; Kumar and Sadique, 1987; Pal and Chaudhuri, 1992; Oyedapo et al., 1999). 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 ethyl acetate extract of the stem of Sarcostemma acidum was studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Four different concentration of extract were used among which concentration at 6 mg/ml showed 68 % protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 69.6 % protection. The activity may be due to the presence of one or more phytochemical constituents present in the extract. The result obtained have been supported by Photomicrographical pictures of the HRBC (Fig 1- 3). The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membraneis analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is importantin 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 extra cellular release (Murugasan, 1981).

Table 1: *In-Vitro* anti-inflammatory activity of *Sarcostemma acidum* Wight. & Arn. by red blood cell membrane stabilization method

Treatment	Con(mg /ml)	Absorbance(560 nm)	% of Inhibition
Control	-	0.250±0.29	-
Ethyl acetate extract	1.00 2.00 4.00 6.00	0.175±0.12[a] 0.143±0.23[a] 0.115±0.44[c] 0.081±0.39[b]	30.0 42.8 54.0 67.6
Indomethacin (standard drug)	2.50	0.070±0.18[b]	72.0

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.

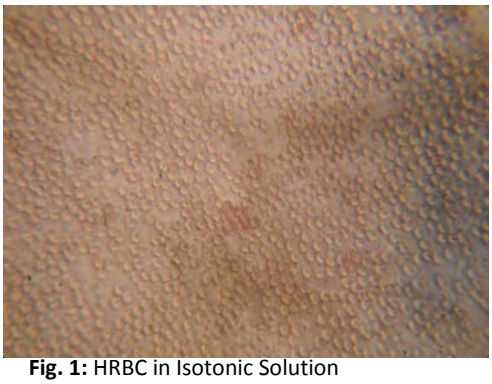

Fig. 1: HRBC in Isotonic Solution

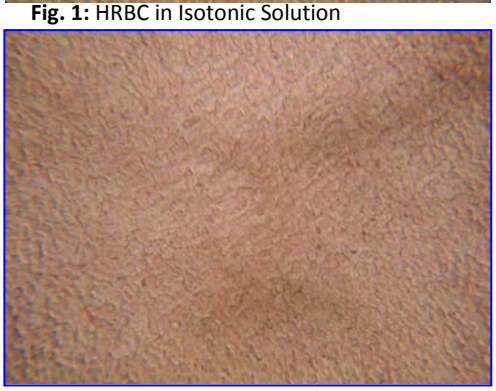

Fig. 2: HRBC in Hypertonic Solution -Control (Lysis of Hypertonic induced HRBC membrane)

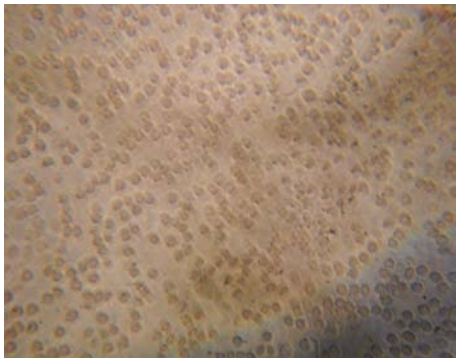

Fig. 3: RBC in Hypertonic solution with Plant extract (6mg/ml) (Protection of Hypertonic induced HRBC membrane lysis)

Some of the NSAIDs are known to posses membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Iwueke,2006).

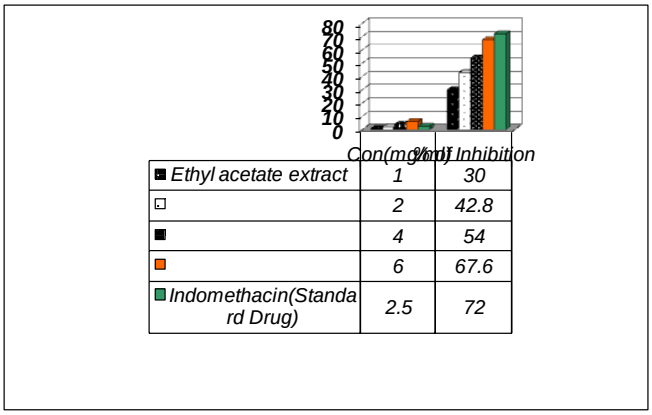

Fig. 4: % Inhibition of haemolysis of Ethyl acetate ext. and Std drug, Indomethacin

The study also provides a strong evidence for the use of the leaves *S. acidum* in folkloric treatment as anti-inflammatory agent. 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.

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References

1. Gupta S. and Kohli S.,Physicochemical analysis and anatomical studies of aerial part of *Sarcostemma acidum* W. & Arn., Nutracost, Mario Donati, Italy., May/June 2011, 17-21
2. Gupta S., Kohli S. International Journal of Pharmacy and Life Sciences, 2010, 1(3), 170-173.
3. K.G.Lalitha and M.G. Sethuraman, Phytochemical and pharmacological evaluation of the flowers of *Sarcostemma brevistigma wight*, Oriental Pharmacy and Experimental Medicine, 9 (3) (2009) 00-00.
4. Khare D. P., Tiwari S.S, Khare A. and Khare M.P. (1980). "Structure of brevobiose". Elsevier Scientific Publishing Company, 79, 279-286.
5. Khare D. P., Khare A. and Khare M.P. (1980). "Structure of sarcobiose". Elsevier Scientific Publishing Company, 81, 275-283.
6. Khare D. P., Khare A. and Khare M.P. (1980). "Structure of tigmobiose". Elsevier Scientific Publishing Company, 79, 287-292.
7. Rahuman A.A., Bagavan C., Kamaraj E., Zahir A.A. *Parasitol Res*, 2009, 104, 1365-1372
8. Saraf M.N., Patwardhan B.K. *Indian Drugs*, 1988, 26, 49.
9. Saraf M.N., Patwardhan B.K. *Indian Drugs*, 1988, 26, 53.
10. Bharath R. Suryanarayana B. *Ethnobotanical Leaflets*, 2009, 13, 281-292.
11. O. Oyedapo, A. Akinpelu *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,Vol 2(4),46-51
12. Vduvu R. and K.S. Lakshmi ,In vitro and in vivo anti-inflammtory activity of leave of symplocos cochinchnensis Moor ssp laurina, Bangladesh J Pharmacol,2008,3,121-124
13. 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
14. M. Sangeeta *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.

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests