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In Vitro anti- inflammatory activity of *Vitex leucoxylon* Linn. leaves by HRBC membrane stabilization

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Abstract

Various functions of hydro alcoholic extract and ethanolic extract of the *Vitex leucoxylon* Linn. leaves of were screened for anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method. The prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of the anti- inflammatory activity. All the fractions showed a biphasic effect on the membrane stabilization. Their activities are comparable to that of the standard drug prednisolone. However their activities decreased with time.

Key-Words: *Vitex leucoxylon* , Anti-inflammatory, Prednisolone, Human Red Blood Cell (HRBC), Membrane stabilization.

Introduction

Inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" [1], or "the reaction to injury of the living microcirculation and related tissues [2]. Inflammatory response to tissue injury involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [3] which are aimed at host defense and usually activated in most disease conditions.

HRBC or erythrocyte membrane is analogous to the lysosomal membrane [11] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the drugs or plant extracts.

Medicinal plants are therapeutically effective and culturally acceptable, since the increase in the use of synthetic chemicals in cancer therapy has lead to many side effects and undesirable hazards, which led to go back to natural resources and economically within the research of the poor people. So the present study was undertaken to establish the scientific evidence for anti-inflammatory activity of leaves of *Vitex leucoxylon* Linn.

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Vitex leucoxylon Linn., (Synonym - *Wallrothia leucoxylon* Linn.) commonly known as five-leaved chaste tree and Nirnochi in tamil belongs to the family Verbenaceae which is an important medicinal plant generally found on the banks of river, streams and ponds throughout India. Although more studies are necessary, *Vitex* exhibits proven potential to become of important pharmacological interest [4]. It is widely distributed in Eastern Ghats and Deccan plateau in India [5]. It is a small to large deciduous tree up to 20 m tall, grey bark, smooth leaves which are 5- foliated and leaflets obovate to oblanceolate. Flowers are cream coloured in axillary as corymbiform dichromatomous cymes. Fruit is ellipsoid shaped drupe which is dark purple in colour and 4 seeded.

The leaves of *V. leucoxylon* are used in traditional medicine for relieving headache, fever and catarrh[6]. General pharmacological studies revealed anti-psychotic, anti-depressant, analgesic, antiinflammatory, anti-parkinsonian and anti-microbial activities of aqueous and ethanolic extracts of leaves of *V. leucoxylon*[7,8] and have studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model[5]. The roots and bark are astringent and the roots are reported to be used as a febrifuge. The *Vitex* genus deserves additional evaluation as a provider of Hepatoprotective agents [4]. β- Sitosterol, dimethyl terphthalate, vitexin, isovitexin, agnuside and aucubin were isolated from the leaves or barks of *V. leucoxylon*[9].

Material and Methods

Plant material

The fresh leaves of *Vitex leucoxylon* Linn was collected during the month of September 2010 from Tirunelveli district, Tamil Nadu, India. The plant was identified and authenticated by Botanist, V. Chelladurai, C.C.R.A.S.Govt. of India, Tirunelveli. A voucher specimen has been deposited at C.L.Baid Metha College of Pharmacy for future reference. Fresh plant material was washed under tap water. The leaves were air dried to a constant weight. The dried leaves were homogenized to fine powder and powder was stored in airtight bottles.

Preparation of Plant Extract

Fresh leaves were collected and shade dried. Dried leaves were crushed and powdered coarsely with an electronic blender and about 200g of this powder was macerated with 95% ethanol separately for 72h at room temperature with stirring for every 15min. The hydro alcoholic extract of *Vitex leucoxylon* Linn (HAVL) and ethanolic extract of *Vitex leucoxylon* Linn (EVL) leaves was then evaporated on heating mantle at 60ºC till the semisolid mass was obtained and was stored in airtight containers in refrigerator below 10ºC and measured the yield of the extract. The percentage yield of HAVL and EVL was found to be 14% w/v respectively. The HAVL and EVL were freshly suspended in distilled water before use for further studies.

HRBC Membrane Stabilization Method

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the invitro anti-inflammatory activity [10]. Blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilised Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl in water) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline.

Various concentrations of extracts were prepared (50, 100, 200, 400, 800 and 1000 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension were added. It is incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm.

Prednisolone (200 µg/ml) was used as reference standard and a control (distilled water) was prepared omitting the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%. The percentage of HRBC membrane stabilization or hemolysis was calculated using the formula

% inhibition of Hemolysis= 100× OD1- OD2/ OD1

Where OD_1 and OD_2 are absorbance of prednisolone and test extracts respectively.

Results and Discussion

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG) [12].

The non-steroidal drugs (NSAIDs) act either by inhibiting these lysosomal enzymes or by stabilising the lysosomal membranes by means of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane [13].

Since HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The results are reported in Table 2. All the fractions of ethanolic extract of the *V. leucoxylon* Linn. leaves showed biphasic effects on HRBC membrane stabilization. They showed increasing activity at low concentration levels but decreasing activity with higher concentrations. They have a critical concentration (50 µg/ ml) at which their activities are maximum. The activities of the various fractions are comparable to that of prednisolone at the concentration of 50 and 100 µg/ ml. Hence anti inflammatory activity of the extracts was concentration dependent.

The phytochemical investigation reveals the presence of constituents Flavonoids, carbohydrates, flavones, phenols in HAVL and EVL extract may be responsible for the anti inflammatory activity. Further work is in progress to isolate and identify the compounds responsible for the activity.

Conclusion

The results of this study have shown that the leaves of *V. leucoxylon* Linn possess anti-inflammatory and analgesic properties mediated by prostaglandin synthesis inhibition. Membrane stabilization may contribute to the anti-inflammatory effect. The study also provides empirical evidence for the use of the

leaves of *V. leucoxylon* Linn in folkloric treatment of inflammatory disorders and pain.

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References

1. Sanderson JB: A system of Surgery (1871). 2nd edition. London Long- mans: Green and Co.
2. Spector WG, Willoughby DA (1963. The Inflammatory Response. Bac- teriological Reviews, 27:117-149.
3. Vane JR, Botting RM (1995). New insight into the mode of action of anti-inflammatory drugs. Inflamm Res., 44: 1-10.
4. A K Meena, Uttam Singh, A K Yadav, B Singh, M M Rao (2010). International Journal of Pharmaceutical and Clinical Research, 2(1): 01-09.
5. Alluri V. Krishnaraju, Chundi B.M. Rao, Dodda Sundararaju, Krishanu Sengupta and Golakoti Trimurtulu (2009). Anti-Inflammatory Activity of *Vitex leucoxylon* L. Bark Extracts Against Freund's Complete Adjuvant Induced Arthritis in Sprague Dawley Rat. American Journal of Infectious Diseases , 5 (2): 68-73.
6. Chanda Y.R., (1982). The wealth of India:A dictionary of Indian Raw materials and Industrial products; Publication and Information Directorate, CSIR, New Delhi, pp: 520-521.
7. Makwana, H.G., B. Ravishankar, V.J. Shukla, N.P. Vijayan, C.K. Sasikala, V.N. Saraswathy and S.V. Bhatt, (1994). General pharmacology of *Vitex leucoxylon* linn leaves. Indian J. Physiol. Pharmacol., 38: 95-100.
8. Sarma, S.P., K.S. Aithal, K.K. Srinivasan, A.L. Udupa, V. Kumar, D.R. Kulkarni and P.K. Rajagopal, (1990). Antiinflammatory and wound healing activities of the crude alcoholic extract and flavonoids of *Vitex leucoxylon*. Fitoterapia, 61: 263-265.
9. Rao, R.V.K., T. Satyanarayana and R. Jena, (1997). Phytochemical studies on *Vitex leucoxylon* L. Indian Drugs, 34: 50-51.
10. Gandhisan R, Thamaraichelvan A and Baburaj (1991). Anti-inflammatory action of Lannea coromandelica HRBC membrane stabilization. , Fitotherapia ,62: 82-83.
11. Chou CT (1997). The anti-inflammatory effect of Tripterygium wilfordii Hook F on adjuvant induced paw edema in rats and inflammatory mediators release. Phytother Res, 11: 152-154.
12. Arun Shirwaikar, Sarala Devi, E N Siju (2011). Anti-Inflammatory activity of Thespesia populnea fruits by Membrane Stabilization. International Journal of PharmTech Research, 3 (4): 2060-2063.
13. Seema Chaitanya Chippada, Sharan Suresh Volluri, Srinivasa Rao Bammidi and Meena Vangalapati (2011). In vitro anti inflammatory activity of methanolic extract of centella asiatica by hrbc membrane stabilisation. Rasayan J. Chem.4(2): 457-460.

Table 1: Phytochemical screening of *Vitex leucoxylon* Linn. leaves

Constituents	HAVL	EVL
Carbohydrates	+	+
Proteins	-	+
Steroids	+	+
Phenols	+	+
Alkaloids	-	-
Tannins	-	-
Flavonoids	+	+
Gums and Mucilage	+	+
Flavones	+	+
Glycosides	-	+
Saponins	-	-
Trepenes	-	-

+ Present; - absent

Table 2: *In vitro* Anti-inflammatory activity of hydroalcoholic extract and ethanolic extract of the *Vitex leucoxylon* Linn. leaves

Treatment	Concentration (µg)	Absorbance	% of Inhibition
HAVL	1000	0.21±0.006	84.16
	100	0.20±0.001	90.97
	50	0.18±0.002	96.63
EVL	1000	0.24±0.002	83.43
	100	0.21±0.004	90.02
	50	0.14±0.001	99.03
Prednisolone	500	0.15±0.001	98.85
	100	0.13±0.001	97.23
	50	0.11±0.002	99.03

Values are expressed as mean ± SEM, n=6 in each groups