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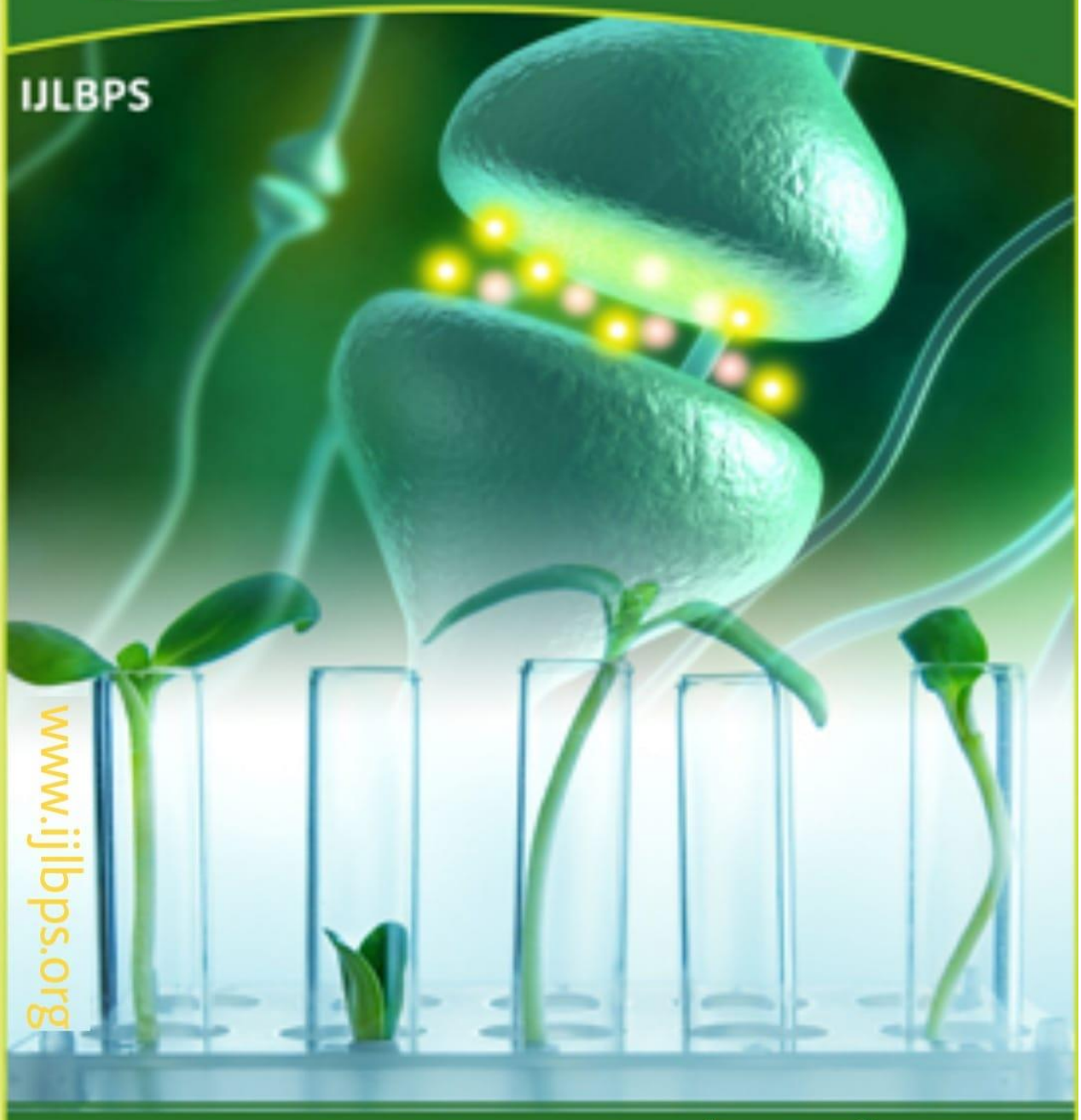

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Vitex leucoxylon Linn. leaf extracts inhibit inflammation in vitro by stabilizing red blood cell membranes.

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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), Me mbrane stabilization

Introduction

One definition of inflammation is "the response to damage of the living microcirculation and associated tissues," while another defines it as "the series of changes which happens in a living tissue when it is harmed provided that the injury is not of such a degree as to immediately destroy its structure and vitality." 2. An array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair 3 comprise the inflammatory response to tissue damage, which is triggered in most disease situations and serves as a host defensive mechanism.Since the membrane of HRBCs (human red blood cells) is similar to that of lysosomes, 11 the extract's ability to stabilize HRBC membranes suggests that it may also stabilize lysosomal membranes. Anti-inflammatory medications or plant extracts may be evaluated in vitro by measuring their ability to prevent the lysis of human red blood cell (HRBC) membranes in response to hypo tonicity.Since the increased use of synthetic chemicals in cancer treatment has resulted in numerous side effects and undesired dangers, this has resulted in a return to natural resources and economic within the study of the poor. This research was conducted because there is anecdotal evidence that Vitex leucoxylon Linn. leaf extract has anti-inflammatory properties.

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An important medicinal plant, Vitex leucoxylon Linn., (Synonym - Wallrothia leucoxylon Linn.), also known as the five-leaved chaste tree and Nirnochi in tamil, may be found growing along the edges of rivers, streams, and ponds all across India. While additional research is needed, Vitex has already shown promise as a drug of significant pharmacological relevance. Fourth, it may be found all throughout the Eastern Ghats and the Deccan plateau in India. 5. The leaflets of its 5-foliate, obovate to oblanceolate leaves are smooth, and the tree itself may grow up to 20 meters in height. Cream colored flowers develop corymbiform dichromatomous cymes in the axillary region. The fruit is an ellipsoid drupe that has a deep purple color and contains 4 seeds.

Traditional medicine relies on V. leucoxylon leaves to treat headaches, fevers, and catarrh.6. The anti-inflammatory and wound-healing properties of the crude alcoholic extract of the leaves have been studied in an acute inflammation model5, and general pharmacological studies have found that aqueous and ethanolic extracts of the leaves of V. leucoxylon have anti-psychotic, anti-depressant, analgesic, anti-inflammatory, anti-parkinsonian, and anti-microbial activities7,8. The roots are said to be used as a febrifuge, and both the roots and the bark are astringent. Additional research into the Vitex family's potential as a source of Hepatoprotective drugs is warranted. 4. V. leucoxylon9's leaves and bark have yielded the isolated compounds -sitosterol, dimethyl terphthalate, vitexin, isovitexin, agnuside, and aucubin.

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 roomtemperature with stirring for every 15min. The hydro alcoholic extract of *Vitex leucoxylon* Linn (HAVL) andethanolic 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 studythe 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% NaClin water) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a10% (v/v) suspension was made with isosaline.

Various concentrations of extracts were prepared (50, 100, 200, 400, 800 and 1000 µg/ml) using distilledwater and to each concentration 1 ml of phosphatebuffer (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^{0}C for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimatedspectrophotometrically at 560 nm.

Prednisolone (200 µg/ml) was used as referencestandard 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 extracellularactivity of these enzymes is said to be related to acuteor chronic inflammation. The extracellular activity of these enzymes

is said to be related to acute or chronic inflammation. The main action of anti-inflammatoryagents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acidto 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 cellularrelease 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 thatof 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 presenceof 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*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 ofinflammatory disorders and pain.

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