In vitro anti-inflammatory activity of *clerodendrum infortunatum* leaves.

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Abstract

The present study was carried out to evaluate the *in vitro* anti-inflammatory activity of hydroethanolic extract of leaves of *Clerodendrum infortunatum* plant. The HRBC membrane stabilizing activity and inhibition of protein denaturation (Anti-arthritic activity) activity was undertaken using freshly drawn human blood and bovine serum albumin, respectively.

The extract at 10, 20, 40, 80 and 100 µg/ml concentrations showed 46.67, 49.67, 48.33, 56.00 and 62.67 percent of membrane stabilization, respectively. At the same concentrations of extract the percent inhibition of protein denaturation were 25.71, 32.5, 46.07, 57.5 and 65.36.

Many biologically active phytoconstituents including flavonoids plays a major role in neutralizing the oxidative damage and reduces inflammatory responses. The presence of such pharmacologically active principles including flavonoids in *Clerodendrum infortunatum* plant may be responsible for demonstration of *in vitro* anti-inflammatory activity which supports the use of this plant in traditional system of medicine in inflammatory conditions and therefore requires further systematic efforts for *in vivo* studies.

Keywords: *Clerodendrum infortunatum*, *In vitro* anti-inflammatory activity, HRBC membrane stabilization, Inhibition of protein denaturation.

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Introduction

Although inflammation helps to clear infection and other noxious stimuli and initiates repair, the inflammatory reaction and the subsequent repair process can themselves cause considerable harm. There are strong associations between chronic inflammation and cardiovascular diseases, diabetes, cancer, arthritis, dementia and many other chronic diseases of ageing.

In inflammation lysosomal membranes releases enzymes into cytosol which causes damage to surrounding tissues and produces various disorders [1]. Inflammation is a complex process involving pain along with increased vascular permeability, increased protein denaturation and membrane alteration. Denaturation of protein is caused by inflammatory conditions like arthritis. Protein denaturation assays and membrane stabilization assays are frequently used to evaluate *in vitro* anti- inflammatory activities. These *in vitro* studies are helpful in developing an understanding of the mechanism of anti-inflammatory activity of herbal constituents.

Among the natural products found in plants, flavonoids and their glycosides constitute one of the largest classes of natural compounds known. Flavonoids are very common and widespread secondary plant metabolites [2]. They have a wide range of biological and physiological activities and serve as chemotaxonomic marker compounds.

Clerodendrum infortunatum is a gregarious perennial shrub belongs to a very large and diverse *Clerodendrum genus*. Its medical applications are described in Ayurveda, Unani and even in Homeopathic system of medicine. The plant have been reported to be used by tribes in colic, scorpion sting, snake bite, tumour and certain skin diseases, also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy.

Therefore, the present study was planned to evaluate membrane stabilizing and anti-arthritic activity of *Clerodendrum infortunatum* leaves extract with *in vitro* assays.

Materials and Methods

Extraction of plant material

The properly cleaned shed dried leaves of the plant were those a powdered and defatted with N-Hexane then refluxed for 70% hydroethanolic extract to obtain the desired flavonoid rich fraction.

HRBC membrane stabilizing activity

The HRBC membrane stabilizing activity was carried out by the using hepranized blood collected freshly from healthy human volunteer who has not used any NSAIDs for past 15 days. It was washed thrice with isotonic saline and later on centrifuged at 3000 rpm for 10 minutes. The packed blood cells were again washed with isotonic saline solution and a 10% v/v HRBC suspension was made by using isotonic phosphate buffer [3]. The extract and standard drug dexamethasone were diluted and used in concentrations of 10, 20, 40, 80 and 100 µg/ ml for the experiment.

The positive control extract or dexmethasone at the volume of to 0.5 ml in different concentrations of 10, 20, 40, 80 and 100 µg/ ml were mixed with 1 ml of PBS, 2 ml of 0.36% hyposaline solution and 0.5 ml of 10% HRBC suspension. Distilled water served as negative control. After incubation period of 10

minutes it was centrifuged at 3000 rpm for 10 minutes and supernatant was observed for absorbance at 560 nm with UV spectrophotometer.

Inhibition of protein denaturation (anti-arthritic activity)

Bovine serum albumin as 5% aqueous solution was mixed drug with 0.5 ml of extract or standard drug dexamethasone at different concentrations of 10, 20, 40, 80 and 100 µg/ml. A product control was made by adding 0.45 ml of ethanol with 0.5 ml of test solution. The pH of all the solutions was adjusted to 6.3 by using 1% HCl [4]. The samples were incubated for 20 minutes at 37°C and further at 57°C for 3 minutes. The absorbance was measured with UV spectrophotometer at 416 nm.

Results

HRBC membrane stabilizing activity

The hypotonic solution induced Human RBC membrane further stabilizing activity was carried out using different concentrations of extract and standard dexamethasone. In this spectrophotometric study the results obtained are presented. The extract of infortunatum showed significant HRBC membrane stabilization activity at various concentrations when compared with standard drug dexamethasone [5-7]. The inhibition was concentration dependant and at 100 µg/ml concentration the inhibition of lysis was highest i.e. 62.67% (Table 1).

Table 1. *List of standard concentration drug de amethasone.*

Concentration (µg/ml)	Membrane stabilization (%)	
	Dexametha-sone	Extract
10	56.33	46.67
20	54.67	49.67
40	67	48.33
80	69.67	56
100	75.33	62.67

Inhibition of protein denaturation (Anti-arthritic activity)

The hydroethanolic extract of leaves of *C infortunatum* has the significantly inhibited the protein denaturation at various concentrations. It has shown 25.71, 32.5, 46.07, 57.5 and 65.36 percent inhibition at 10, 20, 40, 80 and 100 µg/ml concentrations, respectively (Table 2).

Table 2. *Protein denaturation of various concentrations.*

Concentration (µg/ml)	Percent inhibition (Dexamethasone)	Percent inhibition (*C. infortunatum*)
10	42.86	25.71
20	45.36	32.5
40	61.07	46.07
80	80.36	57.5
100	94.29	65.36

Discussion

The NSAIDs stabilizes the lysosomal membrane and or release inactivates the lysosomal enzymes. Similarly these drugs also stabilize RBC and prevent release of haemoglobin when subjected to hypotonic stress [8]. Therefore, HRBC membrane stabilization may prove a useful assay to assess anti-inflammatory activity *in vitro*. The 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.

Most biological proteins lose their biological function when the denatured. Denaturation of protein is a well-documented cause of inflammation. When BSA (Bovine Serum Albumin) is heated and is undergoing denaturation, it expresses antigens associated to Type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus [9].

The presence of biologically active phytochemicals in plants has been documented to be responsible for the pharmacological activities of medicinal plants [10]. In a series of experiments, the *phenyl propanoids: Isoeugenol* and *eugenol* present in the leaf oil of *Pimenta dioica* (L.) (Myrtaceae) demonstrated considerable anti-oxidant and is used for treating inflammation. Flavonoids such as quercetin are known to be effective in reducing acute inflammation. Certain flavonoids possess potent inhibitory activity against a variety of enzymes such as proteinkinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases.

In our previous work, the leaves of the plant C. infortunatum the demonstrated strong presence of flavonoids in preliminary pytochemical analysis and TLC. The extract was found to have 74.50 µg/ml of quercetin equivalent and Total Flavonoid Content (TFC) was estimated as 6.07% when determined by Aluminium chloride colorimetric method [11]. It was also found effective in DPPH scavenging action with IC50 value of 0.047 ± 0.003 mg/ml.

Conclusion

The results obtained in present *in vitro* study reveal that is the leaves of the plant *Clerodendrum infortunatum* possess anti-inflammatory activity. In previous studies the plant was found to have useful many active phytoconstituents including flavonoids, which plays a major role in neutralizing the oxidative damage. The findings in the study support the use of this plant in traditional system of medicine in inflammatory conditions and therefore requires further systematic efforts for *In vivo* studies.

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