



Science

IN VITRO ANTI – INFLAMMATORY ACTIVITY OF THE FLOWERS OF NERIUM OLEANDER (WHITE)

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Abstract

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Natural products are rich source for discovery of new drugs because of their chemical diversity. A natural product from medicinal plants plays a major role to cure many diseases associated with inflammation. The conventional drug available in the market to treat inflammation produces various side-effects. In this present study anti-inflammatory potential of *Nerium Oleander* was investigated. Based on the resulted the ethonolic extract of the flowers showed the highest anti inflammatory activity. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by *Nerium oleander* and reference drug diclofenac sodium.

Keywords: Inflammation; *Nerium Oleander*; Nsaids; Egg Albumin; Diclofenac Sodium.

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1. Introduction

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of

diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our aging society. Rheumatoid arthritis and osteoarthritis are the major inflammatory diseases affecting people worldwide. Several classes of drugs, such as corticosteroids, NSAIDs, and biologics, are used to treat the inflammatory disorders. All these drugs possess several adverse effects and biologics are expensive to be used. Corticosteroids have long been used for the management of rheumatoid arthritis, but they suffer from some serious adverse effects, such as Cushing's habit (appearance with rounded face, narrow mouth, supraclavicular hump, obesity of the trunk with relatively thin limbs), hypertension, hyperglycemia, muscular weakness, increased susceptibility to infection, osteoporosis, glaucoma, psychiatric disturbances, growth arrest, etc [1]. Similarly, the side effects associated with the use of NSAIDs, such as gastrointestinal ulceration and bleeding, and platelet dysfunction, are due to the inhibition of COX-1-derived prostanoids, whereas inhibition of COX-2-dependent prostaglandin (PG) biosynthesis accounts for the anti-inflammatory, analgesic, and antipyretic effects. Thus, the selective COX-2 inhibitors (coxibs) were developed with better therapeutic activity during the last decade.

Inflammatory Diseases

Inflammatory cells produce a highly complicated mixture of growth and differentiation cytokines as well as biologically active metabolites. In addition, they possess the ability to generate and release a spectrum of reactive oxygen species (ROS), Reactive nitrogen species (RNS) and free radicals during oxidation. Among inflammatory cells, polymorphonuclear leukocytes are particularly at generative and releasing ROS and RNS, including superoxide, hydrogen peroxide, nitric oxide radical [2]. The reactive oxygen species can injure cellular biomolecules such as nucleic acids, proteins, carbohydrate and lipids, causing cellular and tissue damage, which in turn augments the state of inflammation. Therefore, compounds that have activities toward these radicals and / or suppressive activities on lipid peroxidation may thus be expected have therapeutic potential for several inflammatory diseases [3].

Medicinal Plants

In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions. Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms.

Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. *Nerium oleander* L. is a small evergreen shrub of 2-5m in height belongs to the family Apocynaceae. Oleanders are drought tolerant evergreen plants of this family. *Nerium oleander* L. shows terminal flower clusters that are available in different colours. The leaves are 5 to 20cm long, narrow, acuminate in the apex. Flowers are produced in terminal heads. Each flower is about 5cm in diameter with 5 petals. *Nerium oleander* L. contains Nerin and alkaloids. Oleandrine which has a cardio stimulatory action (Oleandrin is a cardiac glycoside). Essential oil is extracted from the flowers and anti-tumor activity is effectively done on the cell lines Ehrlich

Ascites Carcinoma (EAC). Ether extraction of the plant shows anti-convulsant activity. Ethanolic extract of leaves and roots shows effective action against gram positive and gram negative bacteria and fungus, and shows analgesic activity. Methanolic flowers extract exhibit Hepatoprotective activity.

2. Materials and Methods

Preparation of Plant Extract

Fresh flowers (2kg) of *Nerium oleander* L. collected from Thanjavur district during June were extracted with 85% methanol (4X500ml) under reflux. The alc. Extract was concentrated *in-Vacuo*. The present study focused on the phytochemical analysis and anti-inflammatory activity of methanolic extracts of *Nerium oleander* in different concentration. Different concentrations of *Nerium oleander* White flower extract (100, 200, 300, 400 and 500 µg/ml) was chosen for *in vitro* anti-inflammatory activity.

Determination of *In-Vitro* Anti-Inflammatory Activity

Inhibition of albumin denaturation:

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al.* (2012) [4].

Reagent

- 1) Egg albumin
- 2) Phosphate Buffer (pH 6.4)
- 3) Diclofenac sodium as standard

Procedure

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of *Nerium oleander* extract (100, 200, 300, 400 and 500 µg/ mL respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in a incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100- 500µg/ mL) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

The extracts concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

3. Results

In vitro anti-inflammatory activity of *Nerium oleander* (Egg albumin)

In the present investigation, the *in vitro* anti-inflammatory effect of *Nerium oleander* was evaluated against denaturation of egg albumin. The results are summarized in Table 1 and fig 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by *Nerium oleander* throughout the concentration range of 100 to 500 µg/mL. Diclofenac sodium (at the concentration range of 100 to 500 µg/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 1); however, the effect of diclofenac sodium was be higher when compared with NOF. This was further confirmed by comparing their IC₅₀ values. NOF possessed IC₅₀ value 236.03 µg/mL whereas that of diclofenac sodium was found to be 231.76 µg/mL.

Table 1: *In vitro* anti-inflammatory activity of *Nerium oleander* (Egg albumin)

S. No.	Concentration (µg/ml)	<i>Nerium oleander</i> “White”	Standard(Diclofenac sodium)
1	100	24.94 ± 1.74	22.36 ± 1.56
2	200	38.63 ± 2.70	46.05 ± 3.22
3	300	67.26 ± 4.07	63.15 ± 4.42
4	400	90.10 ± 6.30	80.26 ± 5.61
5	500	94.94 ± 6.64	97.36 ± 6.81
IC ₅₀		231.76	231.78

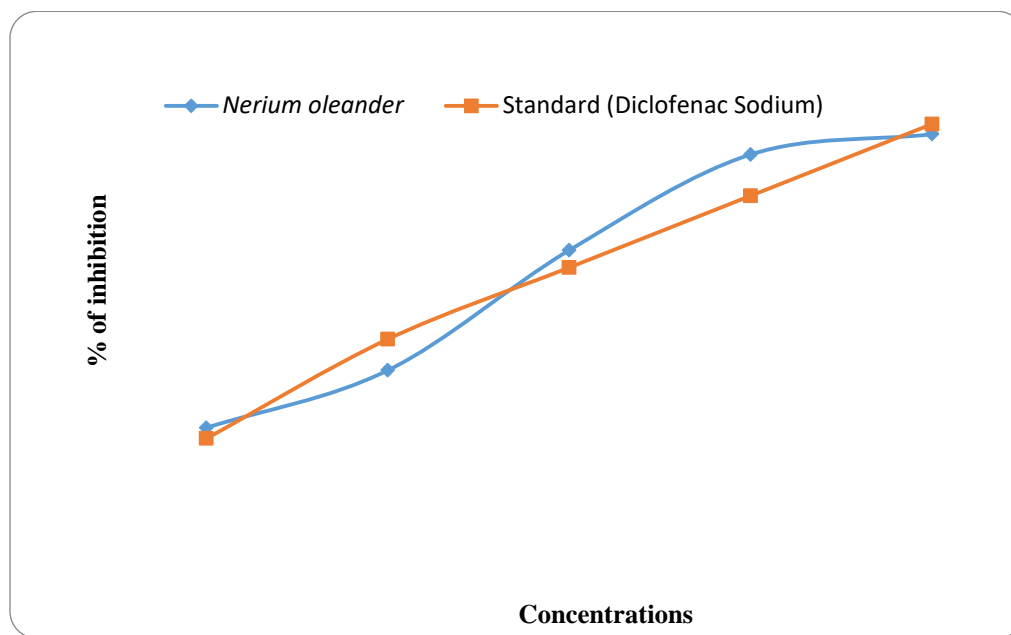


Figure 1: *In vitro* anti-inflammatory activity of *Nerium oleander* (Egg albumin)

4. Discussion

Antiinflammatory Activity of *Nerium Oleander*

It is believed that current drugs available such as opioids and non –steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side

effects and potency [5]. As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs [6]. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs [7].

In the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory activity of aqueous extract of flowers of *Nerium oleander*. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [8]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by *Nerium oleander* and reference drug diclofenac sodium [9]. *Nerium oleander* contains alkaloids, flavonoids, tannins and a phenolic acid are known to promote anti-inflammatory activity [10].

5. Summary and Conclusion

Anti-inflammatory drugs are presently available for the treatments of joint inflammation of various kinds and pathological state have undesirable side effects such as peptic ulcers. Therefore, plant remedies have become increasing popular and are often preferred to synthetically derived pharmaceuticals.

In the present study *in vitro* anti-inflammatory activity of *Nerium oleander* flower extract was studied. The ethanolic extract was screened for *in vitro* anti-inflammatory activity by protein denaturation method such as egg albumin at different concentrations. Throughout the studies flower extract showed marked anti-inflammatory activity. The anti-inflammatory activity was found to be concentration dependent and may be attributed to the presence of phytochemicals content in the flowers of *Nerium oleander*. Over all, It can be concluded that *Nerium oleander* possessed marked anti-inflammatory effect against the denaturation of protein.

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