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Evaluation of anti-inflammatory property of the roots of *Borassus flabellifer*

Natarajan K, Sangeetha R*

Department of Biochemistry, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai – 600 117, Tamil Nadu, India

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ABSTRACT

Inflammation is a defense mechanism triggered by several agents and is observed as a co-morbid condition in various diseases such as cancer and diabetes. Chronic inflammation may lead to organ damage and mortality, and hence synthetic inflammatory agents are administered to cure inflammation. These synthetic drugs induce undesirable side effects, and hence natural agents are preferred. Plants have significant anti-inflammatory potential, and this study intended to assess the anti-inflammatory potential of the hydroalcoholic extract of the root of *Borassus flabellifer* (REBF). The evaluation was done using in-vitro models like inhibition of hemolysis and denaturation of proteins. The activity of the extract was compared with that of diclofenac, the standard drug of choice. The study results revealed that 800 μg of REBF inhibits hemolysis by 50%. The IC_{50} of REBF in manifesting hypotonicity induced hemolysis and heat-induced hemolysis was 2.26 mg and 820 μg. The IC_{50} of REBF required to inhibit protein denaturation was 2.1 mg. All the assay models reveal that the effect of REBF is comparable to that of diclofenac, and thus REBF is a potent anti-inflammatory agent.

*Corresponding Author

Name: Sangeetha R
Phone: 9884204394
Email: sara_dna@yahoo.co.in

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INTRODUCTION

Inflammation is not only a body's defense response but is also associated with several disorders such as arthritis, cancer, and asthma. As a defense response, inflammation is triggered by factors such as infection, ischemia, injury, physical shock, or antigen-antibody reactions. Inflammation, when uncontrolled, can pose various inconveniences by promoting injury to tissues. Few diseases and disorders may be triggered by the chronic inflammatory responses of the body, and thus chronic inflammation has been reported to a major contributing factor to the increasing mortality rate across the world.

At present, inflammation is treating using non-steroidal anti-inflammatory drugs, steroidal drugs, and immunosuppressants (Amri *et al.*, 2017). The use of these drugs presents adverse side effects like gastrointestinal bleeding and peptic ulcers (Singh *et al.*, 2010). Hence, the search for natural medicines that can assist in the treatment of inflammation and pain is inevitable, and researchers have substantiated the anti-inflammatory potential of natural compounds with scientific proof (Azab *et al.*, 2016).

The use of plant parts and plant products to treat inflammation is in practice since ancient years. However, research continues to identify newer plants with anti-inflammatory properties or to establish traditional folklore claims of the anti-inflammatory potential of plants. This study was designed to assess the anti-inflammatory potential of the root of *Borassus flabellifer*.

MATERIALS AND METHODS

Plant material

The roots of *B. flabellifer* were collected from the Chennai district, Tamil Nadu, India, during the month of January 2019. The plant was taxonomically identified by Dr. P. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai (Voucher specimen - PARC/2019/4112).

Plant extract

The roots of *B. flabellifer* were shade dried and mechanically ground to a coarse powder. The coarse powder was subjected to exhaustive cold maceration in 70% ethanol for 72 h, filtered, concentrated in a rotary evaporator and stored at 4 °C for further study (REBF)

In vitro anti-inflammatory activity

The potential of REBF in ensuring the stabilization of the human red cell membrane was assessed using methods such as heat and hypotonicity induced hemolysis. Blood was obtained from a healthy volunteer who had refrained from using NSAID for 2 weeks prior to blood collection. The blood sample was mixed with equal volumes of Alsever solution and centrifuged at 3,000 rpm. The packed cells were harvested and washed with 0.9% isosaline, and a 10% human red blood cell (HRBC) suspension was prepared (Sangeetha and Arulpandi, 2019).

Inhibition of Hypotonicity induced hemolysis

The inhibition of hypotonicity induced hemolysis was studied following the method of Gandhian et al., 1991. Varying concentrations of REBF were added to tubes containing 1mL phosphate buffer (pH 6.5), 2mL of 0.36% hyposaline and 0.5mL of HRBC suspension. These tubes were then incubated for 30 min at 37 °C.

The contents of the tubes were centrifuged at 3,000 rpm for 20 min, supernatant harvested, and the hemoglobin content of the supernatant was estimated spectrophotometrically at 560 nm.

Inhibition of Heat-induced hemolysis

To tubes containing 1mL phosphate buffer (pH 6.5) and 0.5mL of HRBC suspension, varying concentrations of REBF were added. The tubes were kept in a boiling water bath at 60 °C for 30 min. The tubes were later cooled and centrifuged. The hemoglobin content of the supernatant was estimated spectrophotometrically at 560 nm.

Inhibition of denaturation of albumin

This inhibition study was done following the method of (Heendeniya *et al.*, 2018). Varying concentrations of REBF were added to tubes containing 1 mL phosphate buffer (pH 6.5) and 200 μl of egg albumin. The tubes were kept in a boiling water bath at 40 °C for 15 min. Incubation of 5 min in increasing temperatures up to 70 °C was done. The tubes were later cooled, and the contents were read spectrophotometrically at 660 nm. For all the assays, the reference standard used was Diclofenac (1mg/mL), and control was prepared by excluding the extract.

All the analyses were done in triplicates. The extent of inhibition of hemolysis or denaturation was calculated using the following formula,

Percentage inhibition =

$$\frac{1 - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical analysis

The results are recorded as Mean $\pm$ standard deviation. The IC_{50} values were calculated by regression analysis.

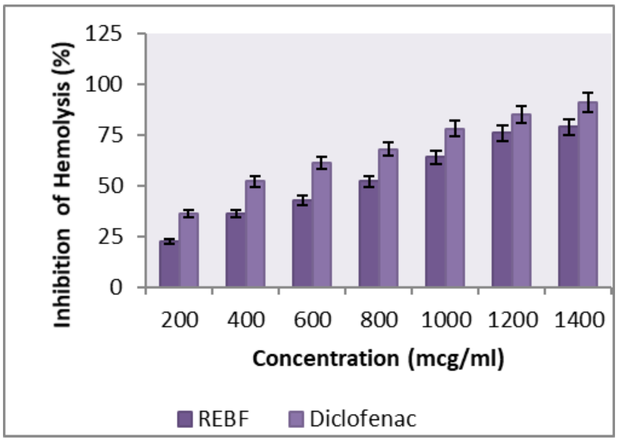
RESULTS AND DISCUSSION

Inflammation is a process triggered by various factors such as injury, exposure to physical and chemical agents, microbes, and free radicals. The responses to inflammation include heat, redness, edema, and pain. These responses set in due to several biochemical processes such as the release of lysosomal enzymes and inflammatory mediators, degradation of tissue proteins, cell migration, etc. These processes must be inhibited to prevent the onset of inflammation. Prevention and cure of inflammation require the usage of NSAIDs, which is associated with several side effects (Sangeetha and Arulpandi, 2019). Hence, safe and effective alternative medicines are always sought after.

Several plants have been reported to possess anti-inflammatory properties. Roots of certain plants are used traditionally to cure inflammation (Perianayagam *et al.*, 2006). This study was done to assess the anti-inflammatory effect of the root of *Borassus flabellifer* using various models.

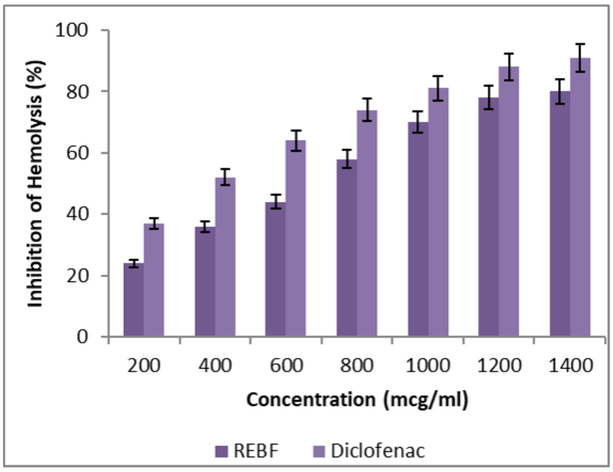
The anti-inflammatory potential of REBF was analyzed at different concentrations (200 – 1400 μg) and was compared with diclofenac, the standard anti-inflammatory drug. The inhibition of hemolysis and protein denaturation was observed to increase gradually with increasing concentrations of REBF. The extract REBF caused 50% inhibition of hemolysis and denaturation at 800 μg. The IC

$_{50}$ concentration of REBF required to inhibit heat-induced hemolysis was observed to be 2.26 mg, while diclofenac exhibited a similar effect at 2.5 mg Figure 1. Thus, REBF exerted an effect that was significant compared to that of the standard drug, diclofenac.



Percentage inhibition assay performed for REBF and diclofenac at concentrations 200 µg to 1400 µg. All values are expressed as Mean ± S.D (n = 3)

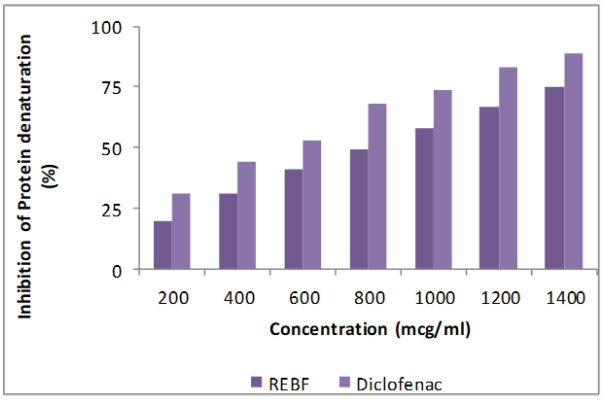
Figure 1: In-vitro Inhibition of Hypotonicity induced hemolysis



Percentage inhibition assay performed for REBF and diclofenac at concentrations 200 µg to 1400 µg. All values are expressed as Mean ± S.D (n = 3)

Figure 2: In-vitro Inhibition of Heat-induced hemolysis

The IC $_{50}$ of both REBF and diclofenac for inhibiting hypotonicity-induced hemolysis was 820, and 739 μg, respectivelyFigure 2, and these results indicate that REBF effective as diclofenac in exerting anti-inflammatory effect. The release of lysosomal enzymes during the inflammatory process culminates in several discomforts and disorders related



Percentage inhibition assay performed for REBF and diclofenac at concentrations 200 µg to 1400 µg. All values are expressed as Mean ± S.D (n = 3)

Figure 3: In-vitro Inhibition of Protein denaturation

to inflammation. The allopathic anti-inflammatory drugs, primarily the NSAIDs, exert their role by either inhibiting the lysosomal enzymes or by stabilizing the membrane of the lysosomes. To assess the potential of REBF in stabilizing the lysosomal membrane, the hypotonicity model using HRBCs was tried. The anti-inflammatory activity of the drugs is reflected in their potential to prevent hypotonicity-induced HRBC membrane lysis.

The IC $_{50}$ of REBF calculated to inhibit protein denaturation was found to be 2.1mg, and similar regression analysis shows that the IC $_{50}$ of diclofenac was 1.02 mgFigure 3. Denaturation of tissue proteins is correlated with pathological responses such as hypersensitivity, which leads to inflammatory diseases such as arthritis, and thus, any compound of plant origin which can inhibit protein denaturation will have significant therapeutic potential as an anti-inflammatory drug (Osman *et al.*, 2016).

CONCLUSION

The in-vitro assay models ascertain the anti-inflammatory potential of the hydroalcoholic extract of *B. flabellifer* root. The extract was able to prevent the damage to RBC membranes and promotes the stabilization of the membrane. This indicates that the extract might as well help in stabilizing the lysosomal membrane during any inflammation process.

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