

Short Communication

LYSOSOMAL MEMBRANE AND PROTEIN STABILIZATION BY *DALBERGIA SISSOO* (FAMILY: FABACEAE): *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Objective: Plants of the genus *Dalbergia* are reported to be useful in the treatment of arthritis, gonorrhoea and rheumatic pains. Present study was aimed to investigate the *in vitro* anti-inflammatory activity of ethanol extract from *Dalbergia sissoo* leaves (EDS) and to support its traditional use.

Methods: EDS was investigated for its *in vitro* anti-inflammatory activity in human red blood cell membrane stabilization (HRBC) method and protein denaturation method. Diclofenac sodium was used as the standard drug.

Results: The EDS and diclofenac sodium showed a concentration dependent stabilization toward HRBC membrane with 314.3±0.01 and 34.91±0.01 µg/ml; 50% protection, respectively. EDS and diclofenac sodium also showed dose dependent protein denaturation with IC₅₀ values 719.9±0.04 and 428.4±0.02 µg/ml, respectively.

Conclusions: EDS possessed noticeable *in vitro* anti-inflammatory effect against the HRBC membrane stabilization method and denaturation of albumin. Further authoritative studies are necessary to make certain the mechanisms and constituents behind its anti-inflammatory actions.

Keywords: *Dalbergia sissoo*, *In vitro* anti-inflammatory models, HRBC membrane stabilization, Protein denaturation.

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body's response to inactivate or destroy the invading organisms, to remove the irritants, and set the stage for tissue repair. It is triggered by liberating chemical mediators or signaling molecules from injured tissue and migrating cells [1]. The mechanism of inflammation is attributed, in part, to release of reactive oxygen species from activated neutrophil and macrophages. This overproduction leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes [2].

Dalbergia sissoo Roxb. ex DC. (*D. sissoo*) additionally, called Indian Rosewood (family: Fabaceae), a deciduous tree, occurs throughout the Sub-Himalayan tract from the Indus to Assam, and in the Himalayan valleys, ascending up to about 1500 m. Plants of the genus, *Dalbergia* is reported to be useful for the treatment of arthritis, gonorrhoea and rheumatic pains. Analgesic, antipyretic and anti-inflammatory activity of *D. sissoo* has been studied on different animal models [3, 4]. Leaves of the plant have the potency to inhibit diarrhoea and peristalsis [5]. *D. sissoo* plant is known to possess diverse phytochemical, most of which are observed to have health benefits. The leaves are known to contain biochanin A 7-O-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [6] and biochanin A 7-O-[β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [7]. A water-soluble polysaccharide was purified and isolated from *D. sissoo* leaves [8].

Recently, traditional medicine worldwide has been re-evaluated by extensive research on different plant species and their active therapeutic principles. Medicinal plants are proved to be useful for the management of many inflammatory diseases and are usually devoid of serious adverse effects [9]. The present study was aimed to evaluate anti-inflammatory activity of ethanol extract from *Dalbergia sissoo* (EDS) by using *in vitro* models.

The leaves of *D. sissoo* were collected from the lands of Balchandpur (Uttar Pradesh, India) in June 2011. Plant materials were taxonomically identified and authenticated by Dr Anamika Tripathi, Associate Professor, Hindu College, Moradabad, Uttar Pradesh, India, as *D. sissoo* Roxb. ex DC. with registration no. HC/Bot/PERL-25. Leaves of *D. sissoo* were washed with distilled water (H₂O) to remove dirt. It was further shade-dried and after that coarsely

powdered. This coarse powder was defatted with petroleum ether, and subsequently extracted with ethanol using soxhlet apparatus (Borosil, India). The extract was concentrated at reduced temperature and pressure using a rotary evaporator. A yield for ethanol extract was found as 13.19% (w/w). EDS was further investigated for anti-inflammatory activity through lysosomal membrane stabilization (HRBC membrane stabilization method) and protein denaturation method as *in vitro* models.

In vitro anti-inflammatory activity was extensively studied by the human red blood cell (HRBC) membrane stabilization method [10, 11]. In this; blood was collected from a human volunteer who had not taken any non steroidal anti-inflammatory drugs (NSAIDs) for two weeks prior to this experiment and mixed with equal volume of Asever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl). The resulting volume was centrifuged at 3000 rpm. The packed cells were washed with isosaline (0.9% w/v NaCl), and a 10% suspension was made. The reaction mixtures consist of the EDS with different concentration (25, 50, 100, 200, 400 and 800 µg/ml) and to each concentration one ml phosphate buffer, 2 ml hyposaline, and 0.5 ml HRBC suspensions were added. These were incubated at 37 °C for 30 min and centrifuged at 3000 rpm for 20 min. The haemoglobin content in the supernatant solution was estimated spectrophotometrically in triplicates at 560 nm. Diclofenac sodium with different concentration (10, 20, 40, 60, 80 and 100 µg/ml) was used as the reference standard and a control was prepared without the extract or standard. The percentage haemolysis was calculated by assuming the haemolysis produced within the control group as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula:

$$\text{Percentage Protection} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

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 EDS so that final concentrations became 50, 100, 200, 400, 800 and 1600 µg/ml. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37±2 °C in a BOD incubator for 15 min and then heated at 70 °C for five minutes. After cooling, their

absorbance was measured in triplicates at 660 nm (SHIMADZU, UV 1800) by using the vehicle as blank. Diclofenac sodium in the final concentrations (50, 100, 200, 400, 800 and 1600 µg/ml) were used as the reference drug and treated similarly for the determination of absorbance in triplicates [1]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbance of control}} \times 100$$

The extract concentration for 50% inhibition (IC_{50}) was determined by the dose-response curve.

The result from the HRBC membrane stabilization method was shown in fig. 1 and fig. 2. The EDS showed a concentration dependent anti-inflammatory activity, and the protection percent increased by an increase as the concentration of the EDS. From the dose-response curve, plotted as log [EDS (µg/ml)] against percentage response keeping the haemolysis produced within the control group as 100%, 50% percentage response were found at 314.3 ± 0.01 µg/ml and similarly, for diclofenac sodium it was 34.91 ± 0.01 µg/ml.

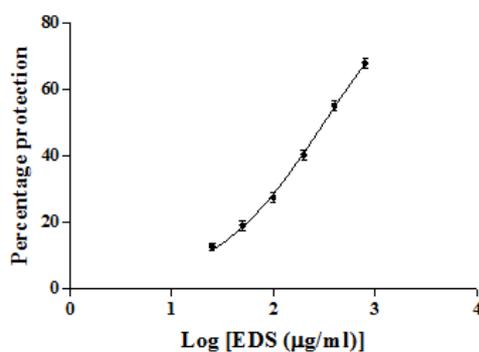


Fig. 1: Dose response curve for EDS in human red blood cell (HRBC) membrane stabilization method (n=3)

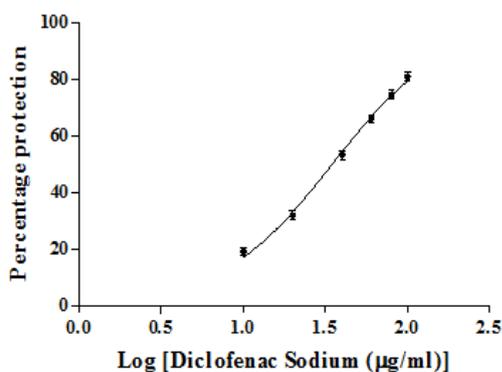


Fig. 2: Dose response curve for Diclofenac Sodium in human red blood cell (HRBC) membrane stabilization method (n=3)

EDS showed a dose dependent protein denaturation or stabilization throughout concentration range, i.e., 50, 100, 200, 400, 800 and 1600 µg/ml towards denaturation of egg albumin (fig. 3). The diclofenac sodium also showed the dose depended inhibition of protein denaturation in the range 50, 100, 200, 400, 800 and 1600 µg/ml and presented in the fig. 4. The IC_{50} values for EDS and diclofenac sodium are presented in the table 1.

The EDS exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [12], and its stabilization implies that the EDS may as well stabilize lysosomal membrane [13]. Injury to lysosomal membrane usually

triggers the release of phospholipase A_2 that mediate the hydrolysis of phospholipids to produce inflammatory mediators [14]. Stabilization of the membranes from these cells inhibits lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response. This might have contributed to the anti-inflammatory activity of the EDS.

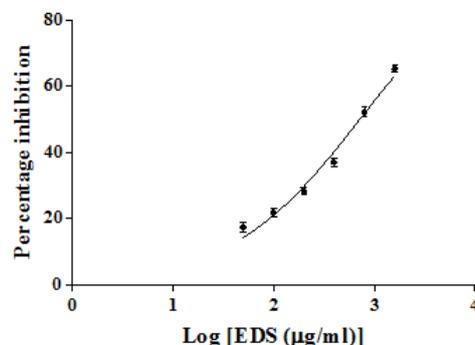


Fig. 3: Dose response curve for EDS in protein denaturation method (n=3)

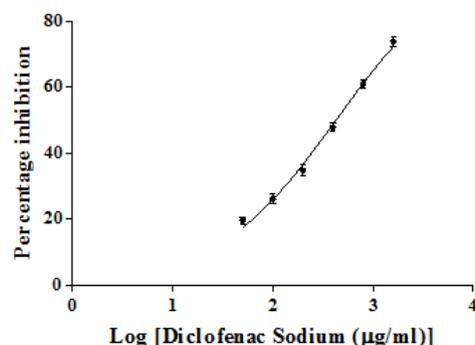


Fig. 4: Dose response curve for Diclofenac Sodium in protein denaturation method (n=3)

Table 1: The IC_{50} values for EDS and Diclofenac sodium in protein denaturation method (n=3)

Treatment	IC_{50} (µg/ml)
EDS	719.9 ± 0.04
Diclofenac Sodium	428.4 ± 0.02

Protein denaturation method was selected for *in vitro* assessment of anti-inflammatory property of EDS. Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. Production of autoantigens in said diseases may be due to denaturation of proteins *in vivo* [15, 16]. Agents, who can prevent protein denaturation, would be worthwhile for anti-inflammatory drug development. The increments in absorbances of test samples with respect to control samples indicate stabilization of protein, which implies the inhibition of heat-induced protein (albumin) denaturation by EDS and diclofenac sodium [1]. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (6.2-6.5) [17]. Hajare *et al.* [3, 4] studied the anti-inflammatory activity for the *D. sissoo* by conducting animal experiments, and in the relationship of our *in vitro* findings of the human red blood cell (HRBC) membrane stabilization method and denaturation of albumin.

CONCLUSION

Therefore, from the results of the present preliminary study, it can be resulted that EDS possessed noticeable *in vitro* anti-inflammatory

effect against the human red blood cell (HRBC) membrane stabilization method and denaturation of albumin. Further authoritative studies are necessary to make certain to the mechanism and constituents behind its anti-inflammatory actions.

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CONFLICT OF INTEREST

Declared None

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