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Research Article

# In vitro study of anti-inflammatory activity of *Albizia julibrissin*

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## ABSTRACT

*Albizia julibrissin* is a plant for future, commonly known as mimosa. Recently, various research works are carrying out to find the unknown pharmacological properties of this plant. In this study we found out that the ethanol and hydro alcoholic extracts of the leaves of *Albizia julibrissin* (Family: Fabaceae) possess a significant anti-inflammatory action while comparing with diclofenac sodium as standard. HRBC membrane stabilization was taken as the screening procedure for obtaining the results. In this particular method inhibition of membrane lysis was taken as the measure of anti-inflammatory property. The haemoglobin content in the supernatant solutions was estimated using spectrophotometer at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in presence of distilled water as 100%. Finally the data obtained which showed 1000 µg/ml solution of ethanol extract of the plant possess 60.87% of percentage inhibition of membrane lysis, whereas std diclofenac sodium showed 69.56% of inhibition at 50 µg/ml.

***Key words:*** *Albizia julibrissin*, Anti-inflammatory, HRBC Membrane.

## INTRODUCTION

Inflammation can be induced by many different stimulating factors, including physical damage, precursor chemicals, microbial invasion and immune responses[1-3]. Generally speaking, controlled inflammation is a beneficial response that can defend and protect the body from harmful factors, but if the body's regulation of inflammation is dysfunctional, then inflammation will have an adverse effect on the body, such as the emergence of chronic inflammation and a series of chain reactions. A large number of inflammatory mediators lead to harmful effects on the body[4-6], including excessive degeneration, exudation, necrosis, or the formation of abnormal granulation formation, that result in different degrees of injury to the body[7,8]. Because inflammation involves many inflammatory mediators and pathways that lead to a wide range of changes in pathology, it is difficult to target the desired area when treating inflammation. The current treatment of inflammatory disorders involves the extensive use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids[9]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non-selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes[10,11]. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates[12,13]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs[14]. *Albizia julibrissin* Durazz (family-Fabaceae) is a species of legume in the genus Albizia, native to south-western and eastern Asia, from Azerbaijan east to China, Korea, Japan and Abyssinia. The genus is named after the Italian nobleman Filippodegli Albizzi, belonging to the famous Florentine family Albizzi, who introduced it to Europe in the mid-18th century. It is also known by its common names such as silk tree or mimosa[15].The plant is used in traditional Chinese medicine to treat depression and anxiety. The antianxiety[16], antidepressant[17], anti-angiogenic[18], anti-tumor[19], LDL oxidation[20] and sedative[21] properties of the plant were scientifically proved. Phytochemical studies showed the plant contain flavonoids, hyperoside, quercitrin[20], saponins[19] etc. In the present study different doses of ethanol and hydroalcoholic

extracts of *Albizia julibrissin* roots were investigated for anti-inflammatory activity.

## MATERIALS AND METHODS

The fresh leaves of *Albizia julibrissin* was collected from Kasargod district in Kerala, India in October 2011 and authenticated by Dr. Madhusaudanan Nambudiripad T.A. (M.D.) Dept. of Dravyagunavijnana, Govt. Ayurveda College, Pariyaram, Kannur. The plant leaves were dried in shade for several days. The dried plants were pulverized to a coarse powder, sieved through sieve no: 24.

### ETHANOL EXTRACT

The dried powders 100gm of the drug was taken in a 2000ml conical flask. It was extracted upto 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the mare was pressed and its filtrate dried in hot air oven at 45˚C to a semisolid mass. It was stored in airtight container in a refrigerator below 10˚C.

### HYDROALCOHOLIC EXTRACT

The shade dried powdered leaves (500g) were exhaustively extracted with a mixture of 70:30 proportions of ethyl alcohol and water using a soxhlet apparatus. The extract was concentrated in vacuum to a syrupy consistency.

### ANTI-INFLAMMATORY ACTIVITY

The HRBC membrane stabilization method was used to study the anti-inflammatory activity[22]. Blood was collected from healthy volunteer who had not taken any NSAIDs for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in Table 2), 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclofenac (50µg/ml) was used as reference drug. Instead of hyposaline, 2 ml of distilled water was used as a control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solutions was estimated using spectrophotometer at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in presence of distilled water as 100%.

## RESULTS

The percentage yield obtained for ethanol extract and hydro alcoholic extract was found to be 15.78 % w/w and 19.23 % w/w respectively.

### PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening reveals that ethanol extract contains steroids, alkaloids, saponins, terpenoids, flavanoids, tannins and polyphenols and hydroalcoholic extract contains carbohydrates, steroids, alkaloids, saponins, terpenoids, flavanoids, tannins and polyphenols as active secondary metabolites. (Table No. 1)

**Table No. 1: Phytochemical screening of plant material *Albizia julibrissin***

| Phytochemical constituents | Ethanol Extract | Hydroalcoholic Extract |
|---|---|---|
| Carbohydrates | – | + |
| Steroids | + | + |
| Alkaloids | + | + |
| Saponins | + | + |
| Terpenoids | + | + |
| Flavonoids | + | + |
| Tannins | + | + |
| Polyphenols | + | + |

(+)=Present; (–)=Absent

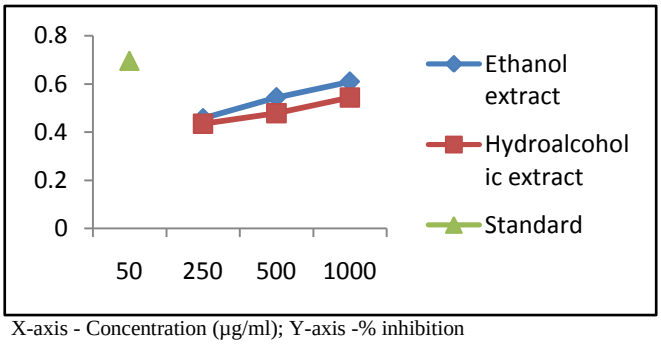
### ANTI-INFLAMMATORY ACTIVITY

The ethanol and hydro-alcoholic extracts of leaves of *Albizia julibrissin* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Since HRBC membrane similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. Ethanol extract at a concentration of 1000µg/ml showed 60.87% protection and hydroalcoholic extract showed 54.35% protection. Both are compared with diclofenac sodium which showed 69.56% of protection.

**Table 2: Invitro anti-inflammatory activity of ethanol and hydroalcoholic extract of *Albizia julibrissin***

| Treatment | Concentration (µg/ml) | Absorbance (540nm) | %Inhibition |
|---|---|---|---|
| Control | – | 0.46±0.12 | – |
| Ethanol Extract | 1000 | 0.18±0.06 | 60.87% |
| | 500 | 0.21±0.08 | 54.35% |
| | 250 | 0.25±0.07 | 45.65% |
| Hydroalcoholic Extract | 1000 | 0.21±0.20 | 54.35% |
| | 500 | 0.24±0.007 | 47.83% |
| | 250 | 0.26±0.002 | 43.48% |
| Std | 50 | 0.14±0.08 | 69.56% |

Values are expressed as mean ± SEM. n=6 animals in each groups.




X-axis - Concentration (µg/ml); Y-axis -% inhibition

**Figure 1.Anti-inflammatory activity of *Albizia julibrissin***

## DISCUSSION

Ethanol and hydro alcoholic extracts of leaves *Albizia julibrissin* showed biphasic effects on HRBC membrane stabilization. They showed an increased activity at higher concentration levels and decreased activity at lower concentration. The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The Diclofenac Sodium act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane[23]. The extracts may inhibit the process which may stimulate or enhance the reflex of the intracellular components.

## CONCLUSION

*Albizia julibrissin* is a plant with a variety of ethanobotanical uses. The plant's medical value has to be explored further. The pharmacological evaluation reveals that the plant extract of *Albizia julibrissin* has anti-inflammatory action. Further studies are needed to reveal the exact mechanism of anti-inflammatory activity.

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