EVALUATION OF IN VITRO ANTI ARTHRITIC ACTIVITY OF ACACIA AURICULIFORMIS A. CUNN. EX. BENTH. STEM BARK

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ABSTRACT

The present study was aimed to evaluate in vitro anti arthritic activity of ethyl acetate, ethanol and aqueous bark extracts of Acacia auriculiformis A. Cunn. (Family: Mimosaceae). The ethyl acetate, ethanol and aqueous extracts of the plant were subjected to preliminary phytochemical screening. Denaturation was induced by incubating the extracts with bovine albumin under controlled experimental conditions. Protein denaturation was calculated by determination of their absorbance. The results showed that the plant extracts showed anti arthritic activity in a concentration dependent manner and the activity was increased on increasing the concentration of extracts. Ethanol extract was found to be more effective than the other extracts. The present study reveals that stem bark of Acacia auriculiformis possess anti arthritic activity. The activity may be due to presence of phenols, tannins, and flavonoids present in the plant.

KEYWORDS: Anti arthritic activity, protein denaturation, Acacia auriculiformis.

INTRODUCTION

Inflammation is a normal protective response to tissue injury which involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair.[1] It is characterized by redness, swelling, pain, stiffness of joint and loss of joint function. Inflammation is associated with membrane alterations, increase in vascular permeability and protein denaturation.[2] Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immuno mediated response.[3] One fifth of the world’s elderly suffer with arthritis.[4] Cartilage is associated with
protection of the joint and allows it to move smoothly. Arthritis involves the breakdown of cartilage causing decreased amount of cartilage. As a result, the bones rub together causing pain, swelling and stiffness.[5] The normal joint lining is very thin having very few blood vessels but in the rheumatoid arthritis, the joints lining is crowded with white blood cells secreting chemical substances like interleukins and tumor necrosis factor alpha (TNF-alpha) that also produce pain, joint swelling and damage.[6] The current treatment of arthritis includes minimization of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs.[7,8] Due to adverse reactions of the NSAIDs and disease modifying anti-rheumatic drugs, the arthritic patients tend to search for other treatments that are effective and less toxic. Therefore, complementary and alternative medicines are commonly preferred by such patients.[7,9] Plants have been a rich source of medicines which are effective as well as devoid of adverse effects. *Acacia auriculiformis* A. Cunn. ex. Benth is a vigorously growing, evergreen and valuable plant of genus *Acacia*, belonging to family Mimosaceae. It has been reported to possess anti diabetic, antimalarial, antimicrobial, antioxidant, cestocidal, central nervous system depressant, hepatoprotective, wound healing, spermicidal, antimitagenic and chemopreventive activities. The bark of *Acacia auriculiformis* has been traditionally used to treat rheumatic disorders. Hence the present study has been performed as an attempt to prove the traditional use of this plant.

**MATERIAL AND METHODS**

**Plant material:** The stem bark of *Acacia auriculiformis* was collected from Panchkula, Haryana in the month of September and authenticated by Dr. Anjula Pandey, Principal scientist, National Herbarium of cultivated plants, NBGPR, New Delhi Ref. NHCP/NBGPR/2012-45. The plant material was air-dried at room temperature and ground into a coarse powder to use for the study.

**Drugs and Chemicals**

All organic solvents and other reagents were procured from SD Fine chemicals Ltd. Mumbai and were of analytical grade. Diclofenac sodium was obtained as gift sample from Horizon biocuticals Pvt. Ltd. Kala-amb (Himachal Pradesh).

**Preparation of extract**

The powdered plant material (100g) was defatted by extracting with petroleum ether and then successively extracted with ethyl acetate and ethanol in soxhlet extractor. The extracts
obtained from soxhalation were concentrated by distilling off the solvent and recovering the same. The total aqueous extract was prepared by cold maceration method. The drug was macerated with distilled water for 24 hours and then filtered. The marc obtained was again macerated with distilled water and filtered. The filtrates were combined and evaporated to dryness. The dried extracts were kept in dessicator. The percentage yield of ethyl acetate, ethanol and aqueous extracts were 5.73 % w/w, 7.56 % w/w and 3.94% w/w respectively. These extracts were further used for evaluation of in vitro anti arthritic activity.

Premilinary phytochemical screening
Various chemical tests were performed using dried ethyl acetate, ethanol and aqueous extracts to detect the presence of phytoconstituents like carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids and saponins.

*In vitro* Anti-arthritic activity
This activity was evaluated using albumn denaturation test. The different concentrations of plant extracts ranging from 100-500 µg/ml were prepared. Reaction mixture for each concentration was prepared which consisted of I ml test drug and 1 ml of 1% bovine albumin solution. These prepared solutions were incubated at 27 ±1°C for 15 minutes. Then, the reaction mixtures were kept at 70°C in a water bath for 10 minutes to induce denaturation. The solutions were cooled and turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as standard drug in the concentration of 100-500µg/ml and treated similarly as test extracts. Percentage inhibition of denaturation was calculated using control in which no drug was added. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by following equation.

\[
\text{% Inhibition of protein denaturation} = 100 \times \frac{A_1 - A_2}{A_1}
\]

Where:

A 1 = Absorbance of control
A 2 = Absorbance of test /standard sample with albumin solution

Statistical analysis: Data was analyzed by ANOVA followed by Dunnett’s t-test. The values were represented as mean ± S.E.M (standard error of mean).
RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the extracts revealed the presence of carbohydrates, phenols, tannins, saponins and flavonoids.

In vitro anti arthritic activity

The extracts were found to be effective as anti arthritic agent and showed significant activity as compared to the standard drug. The anti arthritic activity was also shown in a concentration dependent manner and the activity was increased on increasing the concentration of extracts. Hence, maximum activity was reported at the highest concentration taken for evaluation. Ethanol extract was found to be more effective than the other extracts and showed 78.23 ± 0.53 % inhibition of protein denaturation while 58.67 ± 0.45 % and 54.33 ± 0.70 % inhibition was shown by ethyl acetate and aqueous extract respectively at the concentration of 500 µg/ml which was the highest concentration evaluated. The percentage inhibition by the extracts at different concentrations and their comparison with the standard drug are shown in table 1. IC_{50} values for ethyl acetate, ethanol and aqueous extracts were 310.05, 60.50 and 361.10 µg/ml respectively which further confirmed that ethanol extract was most effective.

Table 1: Percentage inhibition of protein denaturation by standard drug (diclofenac sodium) and various plant extracts

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% inhibition of protein denaturation</th>
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<tbody>
<tr>
<td></td>
<td>Diclofenac sodium</td>
</tr>
<tr>
<td>100</td>
<td>64.02 ± 0.82**</td>
</tr>
<tr>
<td>200</td>
<td>73.31 ± 0.61***</td>
</tr>
<tr>
<td>300</td>
<td>76.83 ± 0.41***</td>
</tr>
<tr>
<td>400</td>
<td>79.65 ± 0.47**</td>
</tr>
<tr>
<td>500</td>
<td>82.85 ± 0.44**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M. (n=3). Data was analyzed by ANOVA followed by Dunnett’s t-test, *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

Tissue proteins denaturation is one of the well known causes of arthritic diseases. In certain arthritic diseases auto antigens may be produced due to denaturation of proteins.\textsuperscript{2, 10} Alteration of hydrogen, hydrophobic, disulphide and electrostatic bonds in proteins is the possible mechanism underlying denaturation.\textsuperscript{10} The agents preventing protein denaturation
would be beneficial for the development of anti arthritic drugs. Hence, in vitro anti arthritic activity of ethyl acetate, ethanol and aqueous extracts of *Acacia auriculiformis* A. Cunn. ex. Benth. stem bark was evaluated in terms of inhibition of protein denaturation method. The study showed that the plant extracts exhibit anti-arthritic activity. The major constituents of *Acacia auriculiformis* were found to be polyphenolic compounds which are well known to possess several biological properties. In the present study, the in vitro anti-arthritic activity of the plant can be attributed to its constituents like phenols, tannins and flavonoids.

**CONCLUSION**

The ethanol extract of *Acacia auriculiformis* showed better anti arthritic activity than the ethyl acetate and aqueous extracts. The future scope of study involves the isolation of phytoconstituents and mechanisms responsible for the anti arthritic activity.

**REFERENCES**
