

Anti -Inflammatory action of Acacia Catechu seed extract

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ABSTRACT

Aim: This study aimed to evaluate the anti -inflammatory action of Acacia Catechu seed extract. **Materials and methods:** The estimation of anti-inflammatory action was done using protein denaturation method. **Results:** The inhibitory efficacy of the Acacia was better when compared to diclofenac sample which was used as a standard drug. **Discussion:** Having the property of inhibiting the denaturation of protein and being more effective than diclofenac, Acacia catechu seed extract can be considered a favorable candidate for controlling the production of autoantigens.

Keywords: Acacia Catechu seed extract, protein denaturation, anti-inflammatory action, diclofenac, inflammation.

Introduction

The body immediately responds to damaged tissues and cells which are caused by pathogens, certain stimuli such as chemicals or even by physical injury by inflammation ^[1, 2]. Lately, inflammation has been described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" ^[3]. Inflammation is of two types: acute and chronic inflammation. Acute inflammation is a short term response that usually results in healings.

For example; leukocytes enter the area which is damaged, eliminate the stimulus and subsequently cure the tissues. Contrarily, chronic inflammation is a long time, and unregulated response that involves acute inflammation, tissue destruction and also attempts at tissue repair ^[4]. Therefore, to treat any diseases with inflammatory reactions, natural products with anti-inflammatory action can be chosen. Acacia catechu is such a product with impeccable properties.

There are about 1300 species of Acacia (family Mimosaceae),

and its exudates, leaves, seeds, heartwood, and bark are used in numerous ways ^[5]. Gum Acacia (gum arabic) has been used for its medicinal properties for approximately 2500 years. It is employed as a demulcent (soothing and protectant agent) and emulsifier, and is also employed to increase viscosity of solutions and suspensions. It is also used in the food, soft drink, textile, tanning, cosmetic, and confection industries. The traditional preparation of betel quid (paan masala), which consists of Piper betel leaves, A. catechu paste, chopped Areca nut, lime, and various spices with or without tobacco is done by using Acacia Catechu heartwood extracts ^[6]. Betel chewing is used to produce euphoria, a sense of well-being, heightened sense of alertness, and psycho- stimulation.

The Acacia Catechu is also known by the names like Cutch tree, Terra Japonica as well as Black Catechu. It is also called Khair in hindi and Khadira in Sanskrit. This herb was previously known as Kat or Cacho. Especially in the early 16th century, this tree was widely exported from India to China, Persia and Arabia. The main application of this plant was in dyeing and tanning. In the 17th Century, Japan introduced this plant to countries in Europe. The tree of Acacia catechu is widespread all around the Indian country. The main areas for its habitat in the country are the eastern slopes of the Western Ghats and the Himalayan tracts. This tree is deciduous having short hooked spines that grow to the height of 9 to 12 meters. There is a lot of significant therapeutic purposes of Acacia Catechu seed extract which includes Astringent, Bactericidal, Refrigerant, Stimulant Masticator, Expectorant and anti-inflammatory actions.

It has been recently reported that Acacia Catechu bark extract may help to stop the bodies' pain trigger mechanism. It

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possesses significant pharmacological and nutritive values. Acacia bark is a brown hardened woody with a rusty appearance containing tannins and Gallic acid. Acacia bark is commonly used in maintaining dental and oral hygiene. Its fresh twigs are used to protect gums and teeth. It can also be considered useful as an external application for mouth ulcers and even reducing gingival inflammation [7-9]. In rural areas, it is applied in case of leprosy.

To examine the constituents of A. Catechu seed extract, many studies have been carried out. A study done by [10] showed that when aqueous extract of the heartwood and the leaves of A. Catechu were subjected to high -performance liquid chromatography coupled with electrospray ionization mass spectroscopy, the primary constituents which are revealed include catechins which by definition are gallic acid (polyhydroxylated benzoic acid) derivatives and polymers. Catechins, epicatechin, epicatechin-3- O -gallate, and epigallocatechin-3- O - gallate. p are the most predominant catechins. Other secondary products present in the extracts include flavonol glycosides, flavonal dimers, and caffeine.

Li et al. [11] also found other constituents that have been identified in aqueous extracts of A. catechu including rhamnetin, 4-hydroxyphenol, 3,3',5,5',7-pentahydroxyflavane, fisetinidol, 5-hydroxy-2- [2-(4- hydroxyphenyl) acetyl]-3-methoxybenzoic acid, and (2S,3S)-3,7,8,3',4'-pentahydroxyflavane.

Thus, this study specifically analyses the anti-inflammatory action of Acacia Catechu seed extract in vitro.

Materials and Methods:

Chemicals:

Diclofenac sodium containing dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Co. India. The other chemicals used in this study were of analytical grade, and purchased locally.

Plant collection and extract preparation

Acacia Catechu seed (ACS) was collected during the month of December 2015 from Hosur, Tamil Nadu, India, authenticated by Green Chem Lab, Bengaluru, Karnataka, India. The seeds were shade dried, and milled to fine powder. This seed powder was passed through 100 mesh sieve, and 2.5 kg of powdered ACS were extracted with 10 L of ethanolic, at 65°C, for 1 h. After 1 h of extraction, the extract was altered and collected. The marc, an insoluble residue was extracted repeatedly with 10 L of ethanolic, twice. The extract was evaporated in a Buchi rotary evaporator (Switzerland) at 65°C, to obtain 150 g of powder extract. The w/w yield of the prepared extract was 6%.

Method:

Evaluation of Invitro Anti-Arthritic Activity Inhibition of Protein Denaturation method (Mizushtma: 1966)

Concentration of test substance: 1000 to 200µg/ml

Standard: Diclofenac sodium

Chemicals Required: Bovine serum albumin, 1N HCl, Phosphate buffer (pH 6.3)

Instrument: Incubator, Spectrophotometer - 660nm

The following 4 solutions were used:

1. **Test solution** (0.5ml) consisted of 0.45ml of bovine serum albumin (5%w/v aqueous solution), 0.05ml of the test solution in various concentration, and pH which was adjusted to 6.3 by using a small amount of 1N Hcl. For 20 minutes, the samples were incubated at 37°C, and for 3 minutes, they were heated at 57°C. After cooling, 2.5ml of Phosphate buffer (pH 6.3) was added to them.
2. **Test control solution** (0.5ml) contained 0.45ml of Bovine serum albumin (5% aqueous solution), 0.05ml of distilled water, with pH adjusted to 6.3 by using a small amount of 1N Hcl. For 20 minutes, the samples were incubated at 37°C, and then they were heated at 57°C for 3 minutes. After cooling, 2.5ml of phosphate buffer (pH 6.3) was added to them.
3. **Product control** (0.5ml) consisted of 0.45ml of distilled water and 0.05ml of test solution in different concentrations with pH adjusted to 6.3 by using a small amount of 1N Hcl. For 20 minutes, the samples were incubated at 37°C, and then they were heated at 57°C for 3 minutes. After cooling, 2.5ml to phosphate buffer (pH6.3) was added to them.
4. **Standard solution** (0.5ml) consisted of 0.45ml of bovine serum albumin (5% w/v aqueous solution and 0.05ml of diclofenac sodium solution in various concentrations with pH adjusted to 6.3 by using a small amount of 1N Hcl. The samples were incubated at 37°C for 20 minutes, and then they were heated at 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer (pH6.3) was added to the sample.

The percent inhibition of Protein denaturation was calculated as follows:

Percent Inhibition

$$= 100 - \frac{OD \text{ of test solution} - OD \text{ of product control}}{OD \text{ of test control}} \times 100$$

The control represented 100% protein denaturation. The result was compared with diclofenac sodium treated sample.

Results

The results of the study were as follows.

Table 1: Percent Inhibition for Acacia Catechu Seed Extract (Test)

S.No.	Concentration ($\mu\text{g/ml}$)	% of Inhibition
1	200	99.9
2	400	99.6
3	800	99.96
4	1000	99.81

Table 2: Percent Inhibition for Diclofenac Sodium (Standard)

S.No.	Concentration ($\mu\text{g/ml}$)	% of Inhibition
1	200	99.88
2	400	98.27
3	800	99.52
4	1000	99.45

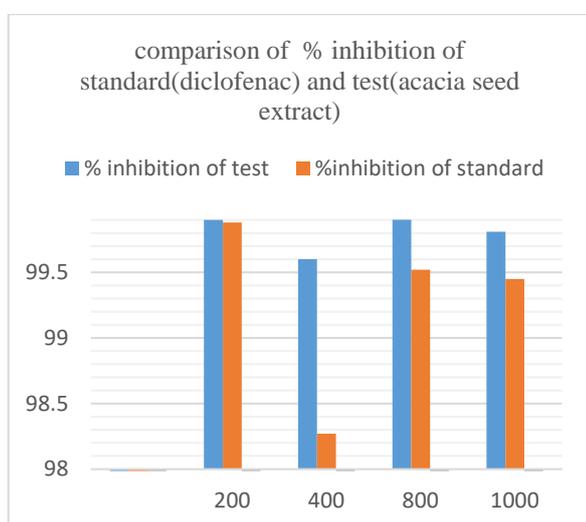


Figure 1: Comparison of % Inhibition of Standard(Diclofenac) And Test (Acacia Seed Extract)

It has been observed that the percent inhibitions varied for both the test and standard samples with varying concentrations. For instance, the percent inhibition for A. Catechu was 99.9 % whereas it was 99.88 percent for standard sample at a concentration of 200 $\mu\text{g/ml}$. Likewise, for 400 $\mu\text{g/ml}$ concentration, the percent inhibition was 99.6 and 98.27 for A. catechu seed extract and standard sample, respectively. The percent inhibition was 99.96 and 99.52 for the test and standard samples for a concentration of 800 $\mu\text{g/ml}$. For a concentration of 1000 $\mu\text{g/ml}$, the percent inhibition was 99.52 and 99.45 for the respective samples. Differences were seen in the inhibitory activity of varying concentrations. This explained the potent anti-inflammatory action of A. Catechu seed extract. Moreover, Protein denaturation is one of the important known cause of certain anti-inflammatory diseases. Electrostatic hydrogen and disulphide bonding were altered in denaturation mechanism. Auto antigen production may cause denaturation of protein in rheumatoid arthritis. Therefore, Acacia Catechu seed

extract possessing the property of inhibiting the denaturation of protein and being more effective than diclofenac, can be considered as a favorable candidate to be used to control the production of auto antigens.

Discussion:

This prospective study has suggested a positive anti-inflammatory action of Acacia Catechu seed extract against diseases like rheumatoid arthritis. Several studies have shown results similar to this study. For example, a study done by [12] showed that Acacia Catechu leaf extract has significant anti-inflammatory properties against rheumatoid arthritis. The results were the same as the present study's. In another study, the anti-inflammatory efficacy of A. catechu bark extract was explored. It was declared that Acacia Catechu Bark shows considerable anti-inflammatory activity. The ethanolic solvent form of Acacia Bark exhibits an inhibitory activity when examined at 200-1000 $\mu\text{g/ml}$ by inhibiting denaturation of protein and comparing its effect with diclofenac sodium as a standard drug. Denaturation of protein produced autoantigen in rheumatoid arthritis. The results of the current study demonstrated that ethanolic bark extract of Acacia Catechu can control the production of auto antigen, and inhibit the protein denaturation in rheumatoid arthritis. Therefore, it was made clear that even the leaf and bark extracts have significant potentials just like the seed extract [12, 13].

Not only Acacia, but also several other herbal extracts are being used for their remarkable anti-inflammatory properties. The results of different studies have been discussed below:

Lycopodium clavatum also known as club moss, has been reported to be used for anti-inflammatory actions in wound healing. According to a study carried out by [14], the extracts prepared petroleum ether, chloroform, ethyl acetate and methanol as well as the alkaloidal fraction from the aerial parts of Lycopodium clavatum using acetic acid. This preparation seemed to induce an increase in capillary permeability assessment in mice, revealing that only the chloroform extract and the alkaloid fraction displayed significant anti-inflammatory effects when compared to Indomethacin.

[15] conducted an investigation to assess the anti-inflammatory effect of X. caffra. qPCR was used to measure the LPS-stimulated expression of inflammatory enzymes and pro inflammatory cytokines, the mRNA expressions of inflammatory markers IL-6, iNOS, and TNF- α of the leaf extract. The most significant response was observed for IL-6, wherein the treatment at 312.5 $\mu\text{g/mL}$ induced a nearly 10-fold decrease in expression versus background expression and nearly 100-fold decrease in expression versus LPS induced cells which were not treated by X. caffra leaf extract. This demonstrated the anti-inflammatory effects of X. caffra clearly.

A study on animals was done to determine the anti-inflammatory efficacy of Mirabilis Jalapa [16]. Carrageenan and formalin-induced paw edema models in Wistar albino rats were used to assess the anti-inflammatory activity. In carrageenan-

induced paw edema model, the anti-inflammatory activity was found to be dose-dependent. The aqueous extract exhibited significant ($P < 0.05$) inhibition of paw oedema, 37.5% and 54.0% on 4th hour at the doses of 200 and 400 mg/kg, respectively. The results of the study demonstrated that aqueous extract of the leaves possess significant ($P < 0.05$) anti-inflammatory potential.

Beside Anti-Inflammatory Action, Acacia Catechu Has Also Been Known to Have Antihyperglycemic and Antinociceptive Activities [17, 18]. Although, Diabetes Mellitus as A Disease is Currently Affecting the Lives of Millions of People Globally, There Has Been No Known Total Cure for It in Allopathic Medicine. Moreover, The Disease Can Quickly Progress To Cardiovascular Disorders, Diabetic Retinopathy, And Diabetic Nephropathy. Therefore, Obtaining A Remedy From Khoyer, Even If Not Being Able To Cure The Disease But At Least Being Effective In Normalising The High Blood Sugar Levels As Seen In Diabetic patients, can be a boon to human beings.

Conclusion:

Thus, this study has explained the anti-inflammatory properties of Acacia Catechu seed extract which are because of the existence of flavonoids, phenols, polyphenols, and steroids in it [19]. Further studies are needed to recognize the active constituents which can account for the anti-inflammatory effects.

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