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M Nagendra
Assistant Professor
Department of Pharmacology,
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

SV Mani Deepika
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

D Ravi Shankar Babu
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

T Jyotshna
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

Dr. D Swarnalatha
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

Correspondence
M Nagendra
Assistant Professor
Department of Pharmacology,
Annamacharya College of
Pharmacy, New Boyanapalli,
Rajampet, Kadapa,
Andhra Pradesh, India

In vitro anti-inflammatory activity of aqueous extract of *Pithecellobium dulce*

M Nagendra, SV Mani Deepika, D Ravi Shankar Babu, T Jyotshna and Dr. D Swarnalatha

Abstract

Aqueous extract of whole plant of *Pithecellobium dulce* (Family: Fabacia) was assessed for its anti-inflammatory activity by *in vitro* methods. *In vitro* anti-inflammatory activity was evaluated using egg albumin denaturation assay, at 10mg concentrations. Diclofenac sodium, used as standard drugs. The results showed *Pithecellobium dulce* at Leaf, fruit significantly (P 0.05) activity.

Keywords: *Pithecellobium dulce*, anti-inflammatory

Introduction

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion^[1]

When tissue cells become injured they release kinins, prostroglandins and histamine. These work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This leads to increased blood flow to the injured site. These substances also act as chemical messengers that attract some of the body's natural defense cells a mechanism known as chemotaxis. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants.

Materials and Methods

Plant material

The whole plants *Pithecellobium dulce* were collected in fresh condition from kadapa region of rajampeta, Andhra Pradesh. Further identified by botanical survey of India (Rajampeta), kadapa. The plant was dried under shade then ground in to a uniform powder using a blender and stored in polythene bags at room temperature.

Preparation of extracts

The plant powder was loaded in to soxhlet extractor and subjected to extraction with water. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. Assessment of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation

According to previously reported protocol^[3], The reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of

varying parts of leaf, bark, fruit of the test extract, by which the concentrations (10mg) became Similar volume of double-distilled water served as control. Then the mixtures were incubated at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ in a biological oxygen demand incubator for 15 min and then heated at $70\text{ }^{\circ}\text{C}$ for 5 min. After cooling, their absorbance was measured at 660 nm (UV Spectrophotometer 1800) by using vehicle as blank. Diclofenac sodium were used as reference treated similarly for determination of absorbance. Test extracts were chosen such that, they remained the nearest possible to the standard therapeutic mode. The percentage inhibition of protein denaturation was calculated by using the following formula ^[4] Percentage inhibition = (Abs Control – Abs Sample) X 100/ Abs control

Statistical analysis

Results are expressed as Mean \pm SD. The difference between experimental groups was compared by One-way Analysis of

Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test) using the software Graph Pad Instat.

Results and Discussion

Inhibition of albumin denaturation Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 52.73% was observed Bark Extract at 10mg. Diclofenac is a standard anti-inflammation drug showed the maximum inhibition 51.96% at the concentration of 10mg compared with control (Table 1).

Table 1: Effect of *Pithecellobium dulce* on heat induced protein denaturation Treatment (s) Concentration (10mg)

s:no	Treatment and Concentration (10mg)	Absorbance at 660nm	% inhibition of protein denaturation
1	Control	0.241 \pm 0.002	-
2	Diclofenac sodium	0.123 \pm 0.006	48.97 \pm 0.337
3	Leaf extract	0.163 \pm 0.0002	32.513 \pm 0.327***
4	Bark extract	0.124 \pm 0.001	48.487 \pm 0.378
5	Fruit extract	0.178 \pm 0.001	24.873 \pm 0.438***

Table 1: Effect of Leaf extract, Bark extract, Fruit extract on heat induced protein denaturation Treatment (s) Concentration (10mg) Each value represents the mean \pm SD. N=3, Experimental group were compared with control ***p considered extremely significant.

compound of the plant *Pithecellobium dulce* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

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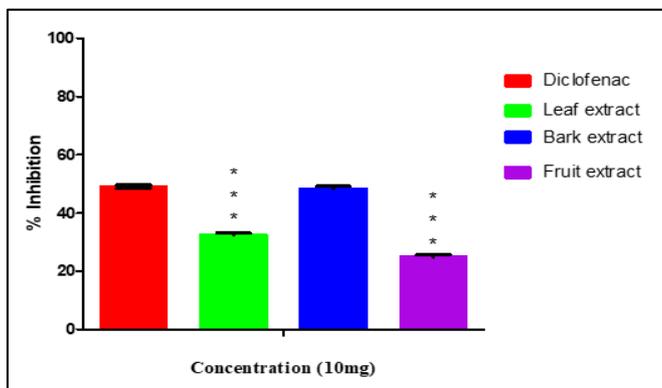


Fig 1: Effect of Leaf extract, Bark extract, Fruit extract on heat induced protein denaturation Treatment (s) Concentration (10mg).

Fig 1: Effect of Leaf extract, Bark extract, Fruit extract on heat induced protein denaturation Treatment (s) Concentration (10mg). Each value represents the mean \pm SD. N=3, Experimental group were compared with control ***p considered extremely significant.

Conclusions

In the present study, results indicate that the aqueous extracts of possess. *Pithecellobium dulce* anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation. This study gives on idea that the