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An investigation on anti-inflammatory potency on various extracts of stem bark of *Bauhinia variegata* Linn., by carrageenen induced paw edema method.

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ABSTRACT

Bauhinia variegata Linn., (Caesalpiniaceae) commonly known as kanchanar, is widely used in Ayurveda. It is a medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India. The various parts of the plant traditionally used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, analgesic, anthelmintic, antileprotic, skin diseases, wound healing, antitumor, in obesity, stomatitis, antidote for snake poisoning, flatulence and as carminative. They are useful in vitiated conditions of pitta and kapha. A thorough survey of literature indicates that there is less work done on the chemical investigations of the plant. Preliminary phytochemical screening on petroleum ether, chloroform, ethanolic and aqueous extracts of stem bark shows the presence of steroids, triterpenoids, alkaloids, glycosides, tannins, saponin, flavonoids, and resins. The earlier findings that the plant extracts are rich in flavanoids having the ability to exert anti-inflammatory effects and hence the present study was undertaken to evaluate the comparative study of anti-inflammatory activity on various extracts of stem bark of *Bauhinia variegata* Linn., by carragenen induced paw edema method using plethysmometer in albino rats.

Key words: *Bauhinia variegata* Linn., anti-inflammatory activity, paw edema method.

INTRODUCTION

The practice of traditional medicine is extensive using natural products for curing common to chronic element globally; these plants still represent an enormous cradle of natural antioxidants that might serve as leads for the development of novel drugs. Numerous anti-inflammatory, neuroprotective, digestive, hepatoprotective, and antinecrotic medicines have lately been exposed to have an antioxidant and/or radical scavenging mechanism as part of their activity [1-3]. Inflammation is a tissue-reaction to infection, irritation or foreign substance. Aging is also considered to be a inflammatory response. It is a part of host defense mechanism. There are several tissue factors or mechanisms like release of histamine, bradykinin and prostaglandins are known to be involved in the inflammatory reactions. The characteristics of inflammation are reddening, swelling (edema), soreness and the corresponding histopathological changes [4].

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 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.

Chronic inflammation can lead to diseases, such as atherosclerosis, rheumatoid arthritis, hay fever sometimes even cancer.

Inflammation may be due to Pathogens, chemical irritants, physical injury or burns, hypersensitivity reactions, ionizing radiation, foreign bodies like dust or allergens, stress, trauma and alcohol.

Process of inflammation: The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mastocytes (see fig -1). These cells present on their surfaces certain receptors named pattern recognition receptors (PRRs), which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs).

At the onset of an infection, burn, or other injuries, these cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow cause the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia, dolor).

The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function (*functio laesa*) is probably the result of a neurological reflex in response to pain. In addition to cell-derived mediators, complement system activated by bacteria, and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma act in parallel to initiate and propagate the inflammatory response [5].

Mechanisms which serve to terminate inflammation include production and release of transforming growth factor (TGF) beta from macrophages, production and release of Interleukin 10 (IL-10), up-regulation of anti-inflammatory molecules such as the Interleukin 1 receptor antagonist or the soluble tumor necrosis factor receptor (TNFR), production of anti-inflammatory lipoxins and apoptosis of pro-inflammatory cells [6].

Acute and chronic inflammations are complex process that can be induced by a variety of means. All the steroidal and non-steroidal anti-inflammatory drugs currently available are probably poly-component in that they are able to modulate more than one mediator or cellular event concerned with the inflammatory response. The discovery of non-steroidal anti-inflammatory agents has overcome the human suffering such as arthritis.

The inflammatory reactions are readily produced in rats in the form of paw edema with the help of irritants. Carrageenan-induced paw edema is the most commonly used method to induce inflammation.

***Bauhinia variegata* Linn.**, is a small to medium-sized deciduous tree with a short bole and spreading crown, attaining a height of up to 15 m and diameter of 50 cm. In dry forests, the size is much smaller. The bark is light brownish grey, smooth to slightly fissured and scaly. Inner bark is pinkish, fibrous and bitter. The twigs are slender, zigzag; when young, light green, slightly hairy, and angled, becoming brownish grey.

Leaves have minute stipules 1-2 mm, early caducous; petiole puberulous to glabrous, 3-4 cm; lamina broadly ovate to circular, often broader than long, 6-16 cm diameter; 11-13 nerved; tips of lobes broadly rounded, base cordate; upper surface glabrous, lower glaucous but glabrous when fully grown.

Flower clusters (racemes) are unbranched at ends of twigs. The few flowers have short, stout stalks and a stalk like, green, narrow basal tube (hypanthium). The light green, fairly hairy calyx forms a pointed 5-angled bud and splits

open on 1 side, remaining attached; petals 5, slightly unequal, wavy margined and narrowed to the base; 5 curved stamens; very slender, stalked, curved pistil, with narrow, green, 1-celled ovary, style and dotlike stigma.

Pods dehiscent, strap-shaped, obliquely striate, 20-30 by 2-25 cm; long, hard, flat with 10-15 seeds in each; seeds brown, flat, nearly circular with coriaceous testa.

The generic name commemorates the Bauhin brothers Jean (1541-1613) and Gaspard (1560-1624), Swiss botanists. The two lobes of the leaf exemplify the two brothers. The specific name refers to the variegation of the flowers.

This plant has been widely reported to have several medicinal properties in traditional form of medicine. The beneficial properties are antimicrobial, anti-inflammatory, cytotoxic, hepatoprotective, anthelmintic, vulnerary, depurative and immunomodulatory activities. They are useful in vitiated conditions of *pitta* and *kapha*, diabetes, antioxidant, antitumor etc [7-8].

Since there is a paucity of information regarding the activity of *B. variegata* in inflammation, this study was undertaken to fill the lacuna in this regard.

MATERIALS AND METHODS

Collection and authentication of the plant:

The stem bark of *Bauhinia variegata* Linn., was collected from Chitradurga, Karnataka during May 2010. It was authenticated by Prof. V.T. Hiremath, Department of Botany, SJM College of Arts, Commerce & Science, Chitradurga, Karnataka. A voucher specimen no 108-A is deposited in the herbarium of S.J.M. College of Pharmacy, Chitradurga, Karnataka, India.

Preparation of Crude Extracts:

The stem barks of *B. variegata* were collected during May-2010. They were dried in shade. The dried stem bark was powdered (500 gms). The powdered material was refluxed successively with the solvents petroleum ether (40⁰-60⁰, E- Merck Mumbai, India), Chloroform (50⁰ – 70⁰, E-Merck Mumbai, India) and Ethanol (E- Merck Mumbai, India) in a soxhlet extractor for 48 hrs in batches of 350g each. Every time, before extracting with the next solvent the marc was dried. After extraction with ethanol lastly, marc was kept in closed jar in distilled water with little chloroform (as preservative) for 48 hrs with occasional shaking, then extract obtained by pressing the marc with tincture press.

All the extracts were concentrated in vacuum using rotary flash evaporator (Buchi-Flawil, Switzerland). The solvents were removed completely over the water bath and finally dried in the desiccator. The extracts so obtained from each of the solvents were labeled, weighed and the yield was calculated in terms of grams percent of the weight of the stem bark powder.

Preliminary Phytochemical screening:

Qualitative analysis was carried out to identify the major natural chemical groups such as alkaloids, glycosides, steroids, triterpenoids, saponins, tannins, phenolic compounds and flavanoids [9, 10].

Acute toxicity studies as per OECD Guidelines 425 [11].

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labelling. LD₅₀ (medium lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD₅₀ value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood [12]. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD₅₀. Depending on the out come for the previous animal, the dose for the next is increased or decreased,

usually by a factor of 3.2. This sequence continues until there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/kg should be taken only when justified by a specific regulatory need.

Healthy Wistar rats weighing between 150-180 g were used to carryout acute toxicity studies by the 'staircase' method. All successive extracts of *B. variegata* stem bark in 0.5% tween 80 was administered orally by gavages in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality. Observations include changes in skin and fur, mucous membranes and also muscle spasm, convulsion, motor activity and behavioral patterns.

Selection of dose of the extract

The stem bark extracts of *B. variegata* bark was found to be non toxic up to the dose of 2000 mg/kg and did not cause any death, therefore it is considered as safe. The biological evaluation was carried out at 200 mg/kg and 400 mg/kg dose levels.

Procedure:

Healthy Wistar rats of either sex weighing around 150-200g were selected for the study. All the experimental animals were maintained at standard laboratory condition as per animal ethical committee guidelines.

- Paw edema was induced according the method described by Winter *et. al* [13]. The animals were fasted for 12 hrs, before screening.
- Test samples were orally administered to the animals.
- Indomethacin at 10mg/kg body weight was administered to standard group.
- Control was administered with normal saline
- One hour later 0.1ml of 1% (w/v) of carrageenan suspension was injected to sub-plantar regions of the right hind paws of all the experimental rats.
- Measurement of the paw size of the animals was done hourly by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was measured using a meter rule.
- Measurements were carried out immediately before and 1–5 h following carrageenan injection.
- The inhibitory activity was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

C_t is the paw circumference at time after carrageenan injection (considered as volume)

C₀ is the paw circumference before carrageenan injection (considered as volume)

Statistical Analysis

The mean value ± SEM was calculated for each parameter. The results were analyzed statistically by ANOVA followed by Dunnet's test. The level of significance was fixed at p < 0.01.

RESULTS

The effect of all extracts of *B. variegata* (200 and 400 mg/kg) in carrageenan induced paw edema in rats is shown in Table - 1; Fig - 3. The ethanolic extract of stem bark at the dose of 400mg/kg prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity ($p < 0.05$). The ethanolic extract at the dose of 200 mg and 400 mg/kg reduced the edema induced by carrageenan by 58.9% and 61.3 % after 2 h. injection of noxious agent as compared to the control vehicle treated group. Diclofenac sodium at 10mg/kg inhibited the edema volume by 67.0%. On carrageenan induced acute inflammation model the ethanolic extract at both the doses produced better inhibition of paw edema. In case of other extracts, the petroleum ether extract exhibited significant anti-inflammatory activity at 2 h. compared to control group.

Fig - 1: Mediators of inflammation

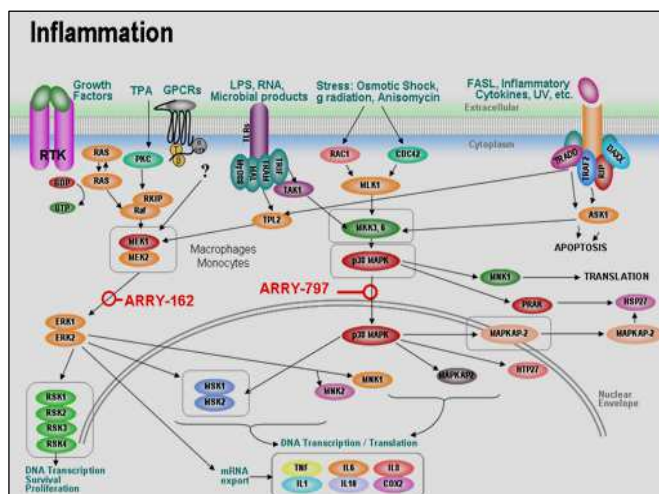


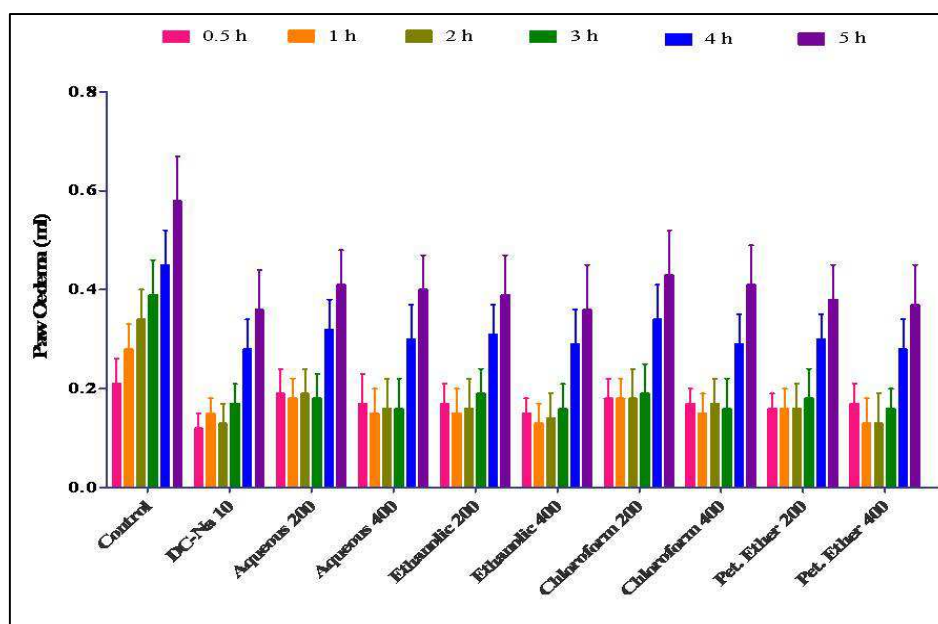
Fig - 2: Plethysmograph

Table - 1: Anti-inflammatory activity stem bark extracts of *B. variegata* on carrageenan induced rat paw oedema.

Groups	Dose (mg/kg)	Paw oedema (mean volume, ml)						Inhibition (%)	
		0.5 h	1h	2h	3h	4h	5h	2h	5h
Control		0.21 ±0.05	0.28 ± 0.05	0.34 ± 0.06	0.39 ± 0.07	0.45 ± 0.07	0.58 ± 0.09	--	--
D. Sodium	10	0.12 ± 0.03 ^a	0.15 ± 0.03 ^b	0.13 ± 0.04 ^b	0.17 ± 0.04 ^b	0.28 ± 0.06 ^b	0.36 ± 0.08 ^a	67.0	45.2
Pet Ether extract	200	0.16 ± 0.03 ^a	0.16 ± 0.04 ^b	0.16 ± 0.05 ^b	0.18 ± 0.06 ^b	0.30 ± 0.05 ^a	0.38 ± 0.07 ^a	59.6	37.5
	400	0.14 ± 0.04 ^b	0.13 ± 0.05 ^b	0.13 ± 0.06 ^b	0.16 ± 0.04 ^b	0.28 ± 0.06 ^b	0.37 ± 0.08 ^a	62.1	37.9
Chloroform extract	200	0.18 ± 0.04 ^a	0.18 ± 0.04 ^a	0.18 ± 0.06 ^a	0.19 ± 0.06 ^a	0.34 ± 0.07 ^a	0.43 ± 0.09 ^a	45.9	26.4
	400	0.17 ± 0.03 ^a	0.15 ± 0.04 ^b	0.17 ± 0.05 ^b	0.16 ± 0.06 ^a	0.29 ± 0.06 ^a	0.41 ± 0.08 ^a	48.4	25.3
Ethanol extract	200	0.17 ± 0.04 ^b	0.15 ± 0.05 ^b	0.16 ± 0.06 ^b	0.19 ± 0.05 ^a	0.31 ± 0.06 ^a	0.39 ± 0.08 ^a	58.9	36.7
	400	0.15 ± 0.03 ^b	0.13 ± 0.04 ^b	0.14 ± 0.05 ^b	0.16 ± 0.05 ^b	0.29 ± 0.07 ^a	0.36 ± 0.09 ^a	61.3	35.3
Aqueous extract	200	0.19 ± 0.05 ^a	0.18 ± 0.04 ^a	0.19 ± 0.05 ^a	0.18 ± 0.05 ^a	0.32 ± 0.06 ^a	0.41 ± 0.07 ^a	46.8	25.6
	400	0.17 ± 0.06 ^a	0.15 ± 0.05 ^b	0.16 ± 0.06 ^b	0.16 ± 0.06 ^a	0.30 ± 0.07 ^a	0.40 ± 0.07 ^a	48.5	26.1

Values are expressed as mean ± SEM, n = 6. ^aP < 0.05, ^bP < 0.01 as compared to control group.

D. Sodium = Diclofenac sodium, PE = Pet. ether extract, CE = Chloroform extract, EE = ethanolic extract, AE = Aqueous extract.

Table - 3: Anti-inflammatory activity stem bark extracts of *B. variegata* on carrageenan induced rat paw oedema

DISCUSSION

The anti-inflammatory activity was also observed up to 5 h. both ethanolic and pet. ether extracts treatments at both the doses exhibited significant activity comparable with the standard diclofenac sodium. Thus, the present results of anti-inflammatory activity of *B. variegata* substantiate the earlier findings that the plant extracts are rich in flavanoids having the ability to exert anti-inflammatory effects. The chloroform and aqueous extracts showed less significant activity.

CONCLUSION

The present research concluded that the various extracts of stem bark of *Bauhinia variegata* Linn., substantiate the earlier findings that the plant extracts are rich in flavanoids having the ability to exert anti-inflammatory effects, thereby justifying its use in indigenous system of medicine. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for anti-inflammatory drug formulation.

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