

**ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT OF  
*BACOLEPIS NERVOSA* DECNE. EX. MOQ. USING CARRAGEENAN  
INDUCED PAW OEDEMA IN ALBINO RATS**

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Article Received on  
15 April 2014,

Revised on 10 May 2014,  
Accepted on 03 June 2014

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**ABSTRACT**

The present study was conducted to evaluate the anti-inflammatory activity in ethanol extracts of stem and leaves of *Bacolepis nervosa*. The anti-inflammatory activity was assessed by carrageenan induced paw oedema in albino rats. The stem and leaf extracts of this plant were administered orally at doses of 150 and 300 mg / kg body weight and the study was compared with a standard drug indomethacin (10 mg / kg). The highest dose of *B. nervosa* stem and leaf extracts (300 mg / kg) produced a maximum inhibition of 82.28% and 79.64% at 3 hours after administration respectively. The anti-inflammatory activity is more effective in group V carrageenan induction with oral administration of *B. nervosa* stem extract of 300 mg/kg body weight

compared to standard drug and leaf extract. Further and detailed studies are in progress for the isolation of single entity responsible for anti-inflammatory activity and development of suitable formulations.

**KEYWORDS:** Carrageenan, *Bacolepis nervosa*, indomethacin, anti-inflammatory.

**INTRODUCTION**

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants.<sup>[1]</sup> It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is a stereotyped

response and therefore it is considered as a mechanism of innate immunity, which is specific for each pathogen.<sup>[2]</sup>

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 from the blood into the injured tissues. It is characterized by five cardinal signs. Redness and heat are due to increased blood flow at body core temperature to the inflamed site. Swelling is caused by accumulation of fluid and pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes.<sup>[3]</sup> Chronic inflammation on the other hand results in a progressive shift in inflammatory cells characterized by simultaneous destruction and healing of the injured tissue.

Inflammatory abnormalities are a large group of disorders that causes a variety of human diseases. Rheumatoid arthritis is one of the challenging disorders associated with inflammatory condition. The immune system is involved with inflammatory disorders. Non immune diseases with etiological origin in inflammation process include cancer, atherosclerosis and ischaemic heart disease. Since ancient times, inflammatory disorders and related diseases have been treated with plants or plant derived formulations.<sup>[4-5]</sup> Wide ranges of phytoconstituents were responsible including phenolics, alkaloids and terpenoids.<sup>[6]</sup>

*Bacolepis nervosa*, belonging to the family Periplocaceae is an endemic medicinal plant. The medicinal properties of this plant are not yet documented. However, to the best of our understanding there is no information in the literature about the anti-inflammatory activity of an ethanol extract of this plant. Therefore, this study was undertaken in order to investigate the anti-inflammatory activity of the plant using carrageenan induced paw oedema in albino rats.

## MATERIALS AND METHODS

### Collection of plant samples

The stem and leaves of *Bacolepis nervosa* Decne. ex. Moq. were freshly collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

**Preparation of plant extract for anti inflammatory activity**

The dried stem and leaves of *Bacolepis nervosa* were powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extracts of stem (BNS) and leaf (BNL) were used for anti-inflammatory activity.

**Animals**

Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature ( $25\pm 2^{\circ}\text{C}$ ) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

**Acute toxicity study**

Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study.<sup>[7]</sup> The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

**Anti – inflammatory activity****Carrageenan induced paw hind edema**

Albino rats of either sex weighing 150-200 grams were divided into six groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group - II and III – *Bacolepis nervosa* leaf (150 mg/kg and 300 mg/kg, p.o.), Group IV and V – *Bacolepis nervosa* stem (150 mg/kg and 300 mg/kg, p.o.), Group VI – Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug.

After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the

digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 60min., 120min., 180min., 240min., 360min., and 480min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula;

$$\text{Percentage inhibition} = [(V_c - V_t) / V_c] \times 100$$

Where,  $V_t$  the percentage represents the percentage difference in increased paw volume after the administration of test drugs to the rats and  $V_c$  represents difference of increased volume in the control groups.

### Statistical analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a  $P$  values of less than 0.001, 0.01 and 0.05 was taken as significant.

## RESULT AND DISCUSSION

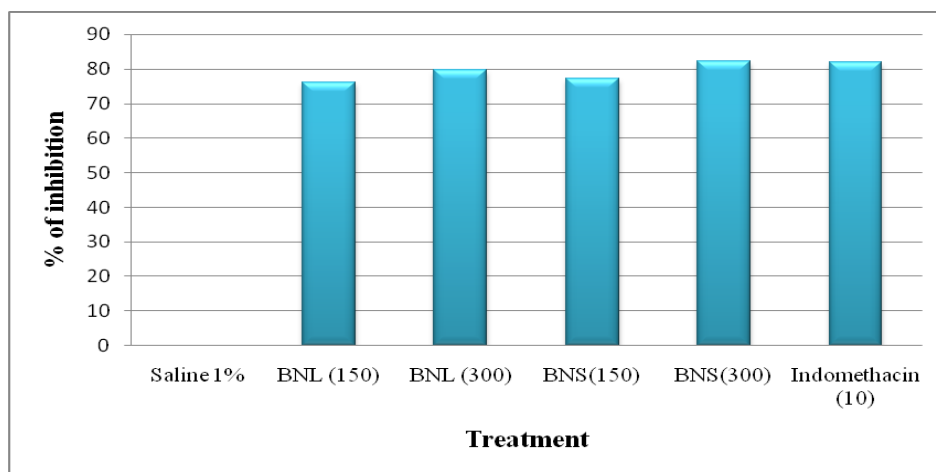
Carrageenan rat paw edema is the suitable method for evaluating the anti-inflammatory activity. The percentage inhibition of edema values of carrageenan induced rat paw edema was given in the Table 1. The BNL and BNS extracts in the doses of 150 and 300 mg/kg body weight showed 76%, 79.64%, 77.08% and 82.28% inhibition of edema and at 300 mg dose, the inhibition was higher in both the extracts (Fig. 1). The indomethacin (10 mg/kg) was used as standard anti-inflammatory drug. It exhibited a protective effect and the percentage of inhibition of edema was 81.82%. The ethanol extract of BNL and BNS at the dose level of 150 and 300 mg/kg decreases the edema significantly ( $p < 0.01$ ,  $p < 0.001$ ) at 3<sup>rd</sup> and 4<sup>th</sup> administration of the extract when compared to the control group.

**Table 1: Effect of BNL and BNS extracts on the Percentage of inhibition of Carrageenan induced paw edema in rats**

Group	Dose (mg/kg body weight)	edema volume (ml)				% inhibition after 180 min.
		0 min.	60 min.	120 min.	180 min.	
I	Saline 1%	34.80±1.28	88.56±1.93	119.54±2.13	129.51±1.84	-
II	BNL (150)	39.54±0.94	69.16±1.24ns	51.93±1.88**	31.83±1.26**	<b>76</b>
III	BNL (300)	43.56±2.41	58.33±1.56*	34.18±1.92***	26.36±0.84***	<b>79.64</b>
IV	BNS(150)	31.54±1.04	63.26±2.59*	48.15±1.04**	29.68±0.94***	<b>77.08</b>

V	BNS(300)	36.88±1.16	50.84±1.86**	39.80±1.04***	21.65±1.84***	<b>82.28</b>
VI	Indomethacin (10)	35.80±0.94	59.55±1.84**	39.63±1.08***	23.54±1.05***	<b>81.82</b>

Each Value is SEM  $\pm$  6 individual observations \*  $P < 0.05$ ; \*\*  $P < 0.01$  \*\*\*  $P < 0.001$ , Compared paw oedema induced control vs drug treated rats. ns – not significant.



**Fig.1: Effect of BNL and BNS extracts on the Percentage of inhibition after 180 min.**

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions such as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Inflammation has now the prime focused area of scientific research because majority of human population worldwide is getting affected by inflammation related disorders.

Carrageenan induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic.<sup>[8]</sup> The first phase (1-2hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin.<sup>[9-10]</sup> The carrageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non – steroidal anti inflammatory agents.<sup>[11]</sup>

Non steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immuno-suppressant drugs which have been usually used in the relief of inflammatory diseases worldwide for a

long time. They are associated with severe adverse side effects such as gastrointestinal bleeding and peptic ulcer.<sup>[12]</sup> Recently, many natural medicines derived from plants, marine organisms were considered effective and safer for the treatment of various diseases including inflammation and pain.<sup>[13]</sup>

The probable mechanism of anti-inflammatory action of extract may be due to its influence on the second phase of inflammation the cyclooxygenase pathway rather than lipooxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan.<sup>[14-15]</sup> There are also evidences that compounds inhibiting the carrageenan induced oedema are effective in inhibiting the enzyme cyclooxygenase.<sup>[16]</sup>

Phytochemical evaluation of the various extracts of *B. nervosa* revealed the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Stem and leaves of ethanol extracts of *B. nervosa* showed significant anti-inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of inflammatory mediators by the alkaloids and flavonoids,<sup>[17]</sup> glycosides or steroids<sup>[14]</sup> present in the extract.

The maximal anti-inflammatory activity was observed (82.28%) at 3<sup>rd</sup> hour in stem of *B. nervosa* compared to leaf of the same plant. GC-MS analysis of *B. nervosa* stem revealed the presence of thymol, eugenol, caryophyllene, phenol 2,5 bis (1,1 dimethyl ethyl), caryophyllene oxide, tumerone, 3,7,11,15, tetra methyl – 2 hexadecen-1-0, widdrol, phytol, piperine and 9,17 octa decadienol (Z).<sup>[18]</sup> Most of these compounds are derivatives of terpenoids, alkaloids and phenolic compounds which have the role of anti-inflammatory effect. Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors.<sup>[19]</sup>

## CONCLUSION

The ethanol extracts of stem and leaves of *Bacolepis nervosa* was screened for their anti-inflammatory activity. Stem of this plant showed potent anti-inflammatory activity at a dose of 300 mg/kg and lesser effect at 150 mg/kg as compared to the standard drug, indomethacin. Further and detailed studies are in progress for the isolation of single entity responsible for anti-inflammatory activity and development of suitable formulations.

## ACKNOWLEDGEMENT

The authors are thankful to Dr. R. Sampathraj, Honorary Director, Dr. Samsun Clinical Research Laboratory, Tirupur, for providing necessary facilities to carry out this work.

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