

IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *VACCINIUM LESCHENAUULTII* WIGHT. (VACCINIACEAE) - AN ENDEMIC MEDICINAL PLANT SPECIES IN NILGIRIS, THE WESTERN GHATS, INDIA**Arumugasamy, K*, D. Sudhakaran, N. Kirthika, H. Abdul Kaffoor and R. Nantha Kumar**

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ABSTRACT

Berries of *Vaccinium* species have been a source of food and pharmaceutical ingredients. The ethanol, methanol and chloroform extracts of *Vaccinium leschenaultia* was investigated for anti-inflammatory activity. All the extracts (150 and 300mg/kg each) were found to significantly ($P < 0.05$) inhibit paw edema induced by carrageenan in rats. The results indicated that the methanol extract with the dose of 300mgkg^{-1} b.w. and the chloroform extract with the dose of 300mgkg^{-1} b.wt. showed maximum anti-inflammatory activity as compared to the reference drug, Indomethacin. *V. leschenaultia* could be used as a potential source of anti-inflammatory agent.

Keywords: *Vaccinium leschenaultii*, anti-inflammatory, Indomethacin.

1. INTRODUCTION

Traditional literature shows the use of herbal preparatins in the treatment of inflammation and pain. The search for new anti-inflammatory and analgesic drugs from the medicinal plant resources is intensifying since these shows no side effects (Chatterjee and Pal, 1984). Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Although it is a defense mechanism, the complex events and mediators that are involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa *et al.*, 2002). Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer or no side effects from medicinal plants origin is the need of the hour.

Vaccinium is a genus of shrubs or dwarf shrubs in the family Vacciniaceae. Most are edible and some are of commercial importance, including, the cranberry, bilberry, cocoberry and huckle berry. Acetone and methanol extract of *V. leschenaultii* leaf and fruit exhibited higher anti inflammatory and anti analgesic activity (Poornima *et al.*, 2005). Among the different species of *Vaccinium* (Bilberries) reported in India, *V. leschenaultii* (Indian cranberry) is considered to be one of the most potent for its medicinal properties. This plant has been used in treatment of several disorders such as mouth ulcer,

diarrhea and diabetics. Bilberry fruit extracts have been used for the treatment of diarrhea, dysentery, mouth and throat inflammations (Anon, 2002). Based on the medicinal properties of Vacciniaceae members, the present study was undertaken on *Vaccinium leschenaultii* Wt. an endemic medicinal plant from the Niligiris, the Western Ghats, India.

2. MATERIALS AND METHODS*2.1. Collection and extraction of plant material*

Vaccinium leschenaultii was collected during blooming season (January 2012) from nearby sholas of Ebanadu, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist.

In the acute toxicity studies the various solvents extracts did not cause any mortality even at the heights dose of 2000 mg/kg. Thus the selected plant extracts were safe and non toxic.

Based on acute toxicity studies two different doses were selected to assess anti inflammatory activity in rat models.

The unadulterated powdered material of the whole plant of *Vaccinium leschenaultii* was successively extracted with ethanol, methanol and chloroform in a soxhlet apparatus and concentrated to dryness. These extracts were made free of any solvent by distillation. The various solvent extracts were used as an emulsion in 5% suspension with gum acacia and administered orally at the dose of 150 and 300 mg kg^{-1} . The animals were grouped in cage in an air conditioned room at the temperature of $22 \pm 1^\circ\text{C}$ with 12 hour light and dark cycle. The

animals were maintained with pellet diet and water *ad libitum*. They were further segregated in to various groups. This experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA). Intra Gastric Catheter tube (IGC) was used for oral drug administration.

2.2. Anti inflammatory activity

2.2.1. Carrageenan-induced paw oedema in albino rats (Winter and Poster, 1957)

The wister albino rats were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysometrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with Indomethacin (10 mgkg⁻¹ b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage inhibition was calculated for all the extracts. Wister albino rats of 120-180 g were subjected to acute and sub acute toxicity studies. A dose of LD₅₀ was determined ideal for pharmacological studies. Percentage inhibition of paw volume was calculated by the following formula

$$\text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where

Vt- means increase in paw volume in rats treated with test compounds

Vc- means increase in paw volume in control group of rats.

2.3. Statistical analysis

The mean paw volume was expressed in terms of mean ± SEM and evaluated for statistical significance by ANOVA followed by Dunnett's t-test, P<0.05 was considered by statistically significant.

3. RESULTS

The anti-inflammatory activity of ethanol, methanol and chloroform extracts of *V. leschenaultii* was evaluated by carrageenan induced rat paw oedema method. The extracts were tested at two different dose levels. Which were found to be statistically significant (Table 1). 150mg/kg b.wt. and 300mg/kg. b.wt. of ethanolic, methanolic and chloroform extracts of *V. leschenaultii* significantly

reduced the carageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. The dose effect of 300mgkg⁻¹ b.wt. of the ethanolic, methanolic and chloroform extracts of *V. leschenaultii* was more active than 150 mgkg⁻¹ b.wt.

At a dose of 300mgkg⁻¹ b.wt. the methanol and chloroform extract showed a percent inhibition of 86.89 and 61.71% respectively which are higher than that of reference drug. The ethanol extract at the same dose showed a lower percent inhibition of 39.49% with respect to reference drug. At 150mgkg⁻¹ b.wt. both the ethanol and chloroform extract shower a lower percent inhibition of 14.68 and 55.98% in comparison to the reference drug. However, the methanol extract at the same dose showed a higher value of 72.50% . Therefore it is clear that the methanol extract at both the doses of 150 and 300mgkg⁻¹ b.w. showed the maximum percent inhibition as compared to the other extracts of reference drug.

4. DISCUSSION

Anti-inflammatory effect of natural products has been frequently assessed through the method of carrageenan induced paw oedema. The inflammatory response is a physiological characteristic feature of vascularized tissues (Rang *et al*, 2007)The inflammatory response is a common feature in many diseases and its control is of relevance in the treatment of these pathologies. There are several herbal drugs used for the anti-inflammatory activity. Plants exhibiting anti-inflammatory activity reveal that species of 96 genera belonging to 56 families have exhibited such potential (Chawla *et al.*, 1987). Keeping in view the growing significance of anti-inflammatory related herbal medicines in global market, the present anti-inflammatory study was been carried out on *V. leschenaultii* (Vacciniaceae) Oedema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and second phase is due to kinin like substances (Vinegar *et al.*, 1969). The second phase is said to be promoted by prostaglandins of lysozymes. The second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and Indomethacin (Winter *et al.*, 1962). The present results of carragennan induced paw oedema model indicated dose dependent anti-inflammatory activity.

Table 1. Effect of *Vaccinium leschenaultii* extracts on the percentage inhibition of carrageenan induced paw oedema in rats.

Treatment	Dose mg/kg	Oedema volume (ml)				% Inhibition after 180 min
		0 min	60 min	120 min	180 min	
Control (Group-I)	Normal saline	39.63±2.16	85.11±4.15	103±2.33	123.31±9.33	-
Ethanol extract (Group-II)	150 mg/kg	35.08±1.57	74.20±3.60	94.12±3.18	105.46±5.43	14.68 %
	300 mg/kg	22.11±2.18*	39.73±4.05*	63.35±4.18*	74.77±3.58*	39.49 %
Methanol extract (Group-III)	150 mg/kg	28.32±1.95	38.43±2.18*	31.26±3.93*	34.24±2.87*	72.30 %
	300 mg/kg	27.02±1.84*	21.17±1.86**	17.47±2.69**	16.21±1.86**	86.89 %
Chloroform extract (Group-IV)	150 mg/kg	31.37±1.98*	71.37±2.67*	71.16±2.18*	54.41±1.69*	55.98 %
	300 mg/kg	29.31±1.89	53.43±2.68*	54.76±1.98*	47.33±1.90*	61.71 %
Indomethacin (Group-V)	10 mg/kg	25.71±1.69**	28.43±1.94*	49.11±1.69*	51.75±2.15**	58.13%

Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated rats.

Group I: Control rats given normal saline orally by using an Intra Gastric Catheter tube (IGC).

Group II: Rats given ethanol *V. leschenaultii* extract at the dose of 150 and 300 mg/ Kg b.wt. by IGC.

Group III: Rats given methanol *V. leschenaultii* extract at the dose of 150 and 300 mg/ b.wt. by IGC.

Group IV: Rats given chloroform *V. leschenaultii* extract at the dose of 150 and 300 mg/ Kg b.wt. by IGC.

Group V: Rats given Indomethacin at the dose of 10 mg/ Kg b.wt. by IGC.

5. CONCLUSION

In the present study *Vaccinium leschenaultii* methanol extract exhibited maximum and potent anti-inflammatory activity. The importance of this plant as phytotherapeutic for human health especially as an anti-inflammatory agent is proved in this study.

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