

**PRELIMINARY ANTINFLAMMATORY STUDY OF DIFFERENT EXTRACTS
OF *BUXUS WALLICHIANA* BAILL WOOD**

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Summary

Buxus wallichiana Baill, wood was evaluated for preliminary anti inflammatory screening by Carrageenan induced rat paw edema model. Petroleum ether, chloroform, methanol, and aqueous extracts of *Buxus wallichiana* Baill were studied at two dose levels (300 and 600 mg/kg). Diclofenac was used as standard. Pet ether and chloroform extracts showed significant reduction in paw edema at 600 mg/kg dose. Pet ether and chloroform extracts at 300 mg/kg and 600 mg/kg showed statistically significant reduction of paw edema at 0.5 and 1 h only. Methanol and aqueous extracts does not showed reduction in paw edema.

Key words: *Buxus wallichiana*, anti-inflammatory studies, methanol, Diclofenac sodium.

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Introduction

Buxus wallichiana Baill, commonly called as Himalayan boxwood, it belongs to family Buxaceae. *Buxus wallichiana* mainly found at high mounts, shady place and cold climates. Boxwood is an evergreen monoecious tree growing to height 6mts with variable form and leave shape (Fleming, 1999; Chopra, et al.,1992).Traditionally *Buxus wallichiana* was used as bitters tonic, diaphoretic, anti-rheumatic, vermifuge, antihelmentic, analgesic, purgative diuretic, antiepileptic, antileprotic and in hemorrhoids. The bark of *Buxus wallichiana* was used as hair growth stimulant (Chopra, et al., 1992; Kritikar and Basu, 1989; Husain, et al., 1992). Phytochemical reported are alkaloids buxemenol E (Kvaltinova, et al.,1991), buxaltine H, Buxiramin D, buxatine, buxandrine F, buxidine F (Husain, et al., 1992), (+)-16a, 31-diacetylboxadine (Ata et al., 2002), semperviraminol, buxamine F (Atta-ur-Rahman et al., 1999). Steroidal alkaloid buxemenol E from *B. sempervirens* was found to produce hypotensive effect in rat attributed by central and peripheral activation of muscranic receptor and also by partial inhibition of *acetylcholinestrerase* enzyme (Kvaltinova, et al., 1991).

Materials and Methods

Collection of plant material and extraction

The wood of *Buxus wallichiana* was collected from the Doddabetta region of Nilgiris district and identified by Dr. Rajan, Botanist from Government Arts College, Ootcamund, Tamilnadu. The specimen was preserved in college herbarium, voucher no. SKVCP 15. The collected wood was shade dried and grinded to a coarse powder. Successive extraction was done with petroleum ether, chloroform, methanol, and water respectively soxlet extraction. Preliminary phytochemical screening of all the extracts was carried out (Kokate, 1986).

Animals

Wistar albino rats weighing about 150-250 g of either sex were acclimatized to the experimental room at temperature 23 ± 2 °C, controlled humidity conditions (50-55%). They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. The study was conducted after obtaining ethical committee clearance from the institutional animal ethical committee of S.K.V.C.P.

Acute toxicity (OECD guidelines 423 adoption)

Wistar albino rats of either sex divided into two groups of six animals each. Group one received 0.5 % CMC (02 ml/kg, orally) and served as control, while other group received MEBW at 2000 mg/kg body weight respectively. Immediately the animals were observed for 6 h for continuously for behavior and thereafter daily for 14 days for mortality.

ANTI-INFLAMMATORY STUDIES

Carrageenan-induced rat paw edema

The method described by Winter *et al.*, (4) was used. Carrageenan (0.1 ml of 1 % suspension) was injected into the supplantar region of the right hind paw of each rat. Pet ether, chloroform, methanol and aqueous extracts of *Buxus wallichiana* (300 and 600 mg/kg. p.o.) was administered to rats 1 h before carrageenan administration. Control rats received 1ml/kg normal saline, and diclofenac sodium (25 mg/kg. p.o.) was used as reference drug. The paw volume was measured using Plethysmometer (UGO Basile 7140, Samitek Instrument, Italy) before and after injection of phlogistic agent at 0.5, 1, 2, 3 and 5 h. Mean increase in paw volume was noted and percentage of inhibition was calculated.

$$\% \text{ edema reduction} = \frac{\text{Mean edema of control} - \text{Mean edema of treated}}{\text{Mean edema of control}} \times 100$$

Results

Acute toxicity studies

The oral administration of spray dried aqueous extract of *B. wallichiana* caused neither any behavioral changes nor mortality up to 2000 mg/kg. So the LD₅₀ of *B.wallichiana* was thus found to be more than 2000 mg/kg.

Carrageenan Induced Paw Edema in Rats

In carrageenan paw edema model only pet. ether extract of *B. wallichiana* (PEBW) at 600 mg/kg showed statistically significant reduction in paw edema when compared to the control at all time intervals. PEBW 300 mg/kg and chloroform extract *B. wallichiana* (CEBW) 600 mg/kg showed statistically significant reduction of paw edema at 0.5 and 1 h only. The standard drug diclofenac showed statistically significant reduction in all time

intervals in the study (Table 1). It is interesting to note that the PEBW 600 mg/kg showed significant % reduction of edema, i.e. 75 and 58 at 0.5 and 1 h respectively, while diclofenac exhibited at the same time of intervals 38 and 49 % (Table 2) reduction of edema only respectively. But at 3rd and 5th h diclofenac showed 62% of edema inhibition, while CEBW 600 mg/kg although produced significant reduction of paw volume, showed around 35 % of edema inhibition at 3rd and 5th h (Table 2).

Discussion

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent (Winter et al., 1962). This edema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins (Damas et al., 1986). The development of edema in the paw of the rat after the injection of carrageenan has been described by Vinegar et al. 1969 as a biphasic event. The initial phase, observed around 1 h, is attributed to the release of histamine and serotonin [16]; the second, accelerating, phase of swelling is due to the release of prostaglandin-like substances (Vinegar et al., 1969). It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents (Vinegar et al. 1969; Di Rosa et al., 1971). Our studies showed significant activity of PEBW at 600 mg/kg dose in the suppression of the first and second phases of carrageenan-induced inflammation may due to inhibition of the release of the early mediators such as histamine, serotonin and kinins (Winter et al., 1962). The action on the second phase may be explained by an inhibition of cyclooxygenase, a prostaglandin derivative (Seibert et al., 1994). PEBW at 300 mg/kg and CEBW at 600 mg/kg showed inhibition of first phase ie, release of the histamine, serotonin and kinins. From this it can be suggested that the steroidal components might be responsible for the antiinflammatory activity as methanol and aqueous extract did not show any presence of steroids and at the same hand did not also show any prominent anti-inflammatory activity. Methanol extract of *Buxus wallichiana* (MEBW) and aqueous extract of *Buxus wallichiana* (AEBW) does not exhibits antiinflammatory activity.

Table 1. Effect of various extract of *B.wallichiana* wood in carrageenan paw edema model in rats.

Treatment	Dose (mg/kg; p.o.)	n	Mean changes in paw edema vol (ml) at (h)				
			0.5	1	2	3	5
Control I	-	6	0.33 ± 0.05	0.52 ± 0.04	0.68 ± 0.06	0.97 ± 0.08	1.21 ± 0.08
Control II	-	8	0.36 ± 0.03	0.57 ± 0.03	0.75 ± 0.07	1.16 ± 0.07	1.23 ± 0.07
Diclofenac	25	8	0.24 ± 0.02 ^{##}	0.32 ± 0.03 ^{###}	0.36 ± 0.03 ^{###}	0.49 ± 0.04 ^{###}	0.50 ± 0.04 ^{###}
PEBW	300	6	0.14 ± 0.02*	0.36 ± .02***	0.64 ± 0.03	0.85 ± 0.03	1.10 ± 0.04
	600	6	0.09 ± 0.01***	0.23 ± 0.05***	0.36 ± 0.06***	0.61 ± 0.06***	0.83 ± 0.06***
CEBW	300	6	0.29 ± 0.04	0.53 ± 0.03	0.72 ± 0.04	0.96 ± 0.03	1.11 ± 0.04
	600	6	0.19 ± 0.03**	0.37 ± 0.05**	0.63 ± 0.07	0.91 ± 0.07	1.12 ± 0.08
MEBW	300	6	0.27 ± 0.03	0.55 ± 0.04	0.77 ± 0.05	1.03 ± 0.05	1.32 ± 0.06
	600	6	0.31 ± 0.01	0.46 ± 0.02 [#]	0.68 ± 0.07	1.03 ± 0.05	1.19 ± 0.06
AEBW	300	6	0.33 ± 0.02	0.58 ± 0.02	0.79 ± 0.03	1.17 ± 0.02	1.45 ± 0.05
	600	6	0.31 ± 0.04	0.48 ± 0.04	0.76 ± 0.05	1.12 ± 0.06	1.30 ± 0.05

Values are mean ± SEM, n= number of rats per group, *p<0.05, **p<0.01, *** p<0.001, vs. control I, # p<0.05, ## p<0.01, ###p<0.001 vs. control II; One Way Analysis of Variance (ANOVA) followed by Dunnett's t test. Control I- Olive oil (0.2 mg/100g b.w.); Control II- 1% CMC (0.3 ml/100g b.w.).

Table 2. Percentage of edema inhibition of various extracts of *Buxus wallichiana* in rat in carrageenan paw edema model.

Treatment	Dose (mg/kg; p.o.)	n	% of edema Inhibition at (h)				
			0.5	1	2	3	5
Diclofenac	25	6	33	45	52	58	60
PEBW	300	6	58	33	6	11	8
	600	6	76	58	48	36	32
CEBW	300	6	12	00	-7	2	6
	600	6	42	31	7	5	6
MEBW	300	6	25	4	-2.6	11	-7
	600	6	14	20	14	1	3
AEBW	300	6	8	-2	-5	-1	-18
	600	6	14	16	-2	4	-6

PEBW-petroleum ether extract of *Buxus wallichiana*; CEBW- chloroform extract of *Buxus wallichiana*; MEBW-methanol extract of *Buxus wallichiana*; AEBW-aqueous extract of *Buxus wallichiana*

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