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Anti-inflammatory Study of *Wrightia tinctoria* R.Br Stem Bark in Experimental Animal Models

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Abstract: In the present study, the bark of *Wrightia tinctoria* was investigated for anti-inflammatory activity by carrageenan- induced rat paw oedema and cotton pellet induced granuloma method. The various extracts showed inhibition of rat paw oedema and percent granuloma changes at dose of 200mg/kg when compared to control group. The activity was compared with that of standard drug diclofenac sodium (13.5 mg/kg /b w, p.o)
Keywords: *Wrightia tinctoria,* granuloma method, anti-inflammatory activity.

INTRODUCTION

In the traditional system of ayurvedic medicines, plant products either single drug or in combination with others which are considered to be less toxic and free from side effects compared to synthetic drugs[1]. Inflammatory diseases are major cause of morbidity of the working force throughout the world. Many drugs produced a dramatic symptomatic improvement in rheumatic processes, but all of them shared the common side effects like gastrointestinal irritation[2].

Wrightia tinctoria R.Br (Apocynaceae) bark commonly known as kalakuda, indrajau (in Marathi). It is a small deciduous tree, generally up to 7.5 m height, found in Rajasthan, Madhya Pradesh and peninsular region of India. The bark is light grey, scaly and smooth[3]. It is used in dropsy and bilious affections[4,5]. A literature survey revealed that no scientific investigation regarding anti-inflammmatory activity of the bark was mentioned to evaluate scientifically the potency of various extracts of *Wrightia tinctoria* based in its ethanobotanical clue.

MATERIAL AND METHODS

PLANT MATERIAL

For the present study, the plant material (bark part) was collected from TORANMAL region of Nandurbar District (MS) and the plant material was taxonomically identified at Department of Botany, SSVPS College, Dhule (Voucher no.11).

PREPARATION OF EXTRACT

The bark was shade dried and then powdered to particle size 40 #. 100g of powdered material was subjected to cold maceration with distilled water (250 ml) for 7 days and remaining powder (120g) was successively extracted with petroleum ether, chloroform and methanol in a Soxhlet apparatus. All the extracts were concentrated on rotary vacuum flash evaporator (Roteva, Equitron), at 50^0 and finally to dry residue. The yield of pet. ether, chloroform, methanol extracts was found to be 6.5%,3.16%,4.19% respectively.

The preliminary phytochemical investigations were performed using standard qualitative chemical tests[6-8]

and the phytoconstituents were identified as sterol, triterpenoids, and flavonoids

SCREENING OF ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity was assessed using the carageenan- induced rat paw oedema method and cotton pellets –induced granuloma method[9]. The ethical clearance was obtained by the Institutional Animal Ethics Committee (Registration no.651/02/c/CPCSEA) before the experiment. Albino Wistar rats of either sex (180-200g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard laboratory diet and water *ad libitum,* The animals were exposed to alternate cycle of 12 hour of darkness and light each.

Carrageenan induced rat paw oedema-the rats were divided into 6 groups (n=6).Group1 (control) received 1ml of distilled water orally. Group 2 received 13.5 mg/kg of diclofenac sodium orally.Group 3,4,5 and 6 received 200mg/kg of aqueous,petroleum ether, chloroform and methanol extracts respectively. After 1 h, rats were challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan into the subplanter side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection (0h) and followed by every hour for 5 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual oedema volume.

Cotton pellet induced granuloma- rats were divided into 6 groups (n=6).Pellets of cotton weighing 20mg ±1mg were sterilized in a hot air oven at 120^0 for 2 h, then implanted bilaterally in region of rat, under ether anesthesia according to the method of Goldstein et al (1976).All the groups of animals were given the various extracts at a dose of 200mg/kg b w, diclofenac (13.5mg/kg,bw,p.o.) throughout the experimental period of 7 days, where the control group received only the vehicle. On the 8^{th} day, the pellets were dissected out under the light anesthesia, dried overnight at 70^0 and weighed after cooling. Increase in the dry weight of the pellets was taken as measure of granuloma formation.

For Carageenan induced rat paw oedema, percentage inhibition of inflammation was calculated using formula, % inhibition=100(1-Vt/Vc) where Vt represents oedema volume in test compounds and Vc represents oedema volume in control whereas in cotton pellet induced granuloma, the average weight of the pellets of the control group as well as of the test group was calculated. The percent change of granuloma weight relative to vehicle control group was determined. The experimental results were expressed as the mean ±S.E.M and were analyzed by the method of analysis of ANOVA followed by Dunnets test. P-value of <0.05 was considered as stastically significant.

RESULTS AND DISCUSSION

The results obtained from Carageenan model indicated that, aqueous and chloroform extract treated group showed significant ($p<0.05$) reduction in paw oedema from 4 h to 5 h, while methanolic extract and diclofenac sodium treated group showed significant reduction ($p<0.05$) in paw oedema from 2 h to 5 h when compared to control group.

Carrageenan induced inflammation is a biphasic phenomenon. The first phase of oedema is attributed to the release of Histamine and 5-Hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances[10]. It appears from the study that aqueous , methanol extract and chloroform extract treated group might be inhibiting kinin and prostaglandin like mediators. The result of anti-inflammatory activity by carrageenan induced rat paw oedema have been shown in table no. 1

Results of cotton pellet induced Granuloma method indicated that, the weight of cotton pellet granuloma was significantly ($p<0.05$) reduced by all the extracts (200 mg/kg, b w, p.o) and diclofenac sodium (13.5mg/kg, bw, p.o).The percentage inhibition of chloroform extract was found to be 36.39%. Cotton pellet granuloma model is an indication of the proliferative phases of inflammation. Inflammation involved proliferation of macrophages, neutrophils and fibroblast, which are basic sources of granuloma formation.[9] Therefore the decrease in granuloma weight indicates suppression of the proliferative phases, which was effectively inhibited by *Wrightia tinctoria* in the present study. It appeared from the study that, the activity of chloroform extract of *Wrightia tinctoria* bark could be due to presence of flavonoid[10,11]. We are reporting the anti-inflammatory activity of the bark first time. The preliminary phytochemical investigation of all extracts indicated presence of steroids, triterpenoids and flavonoids. However, detailed phytochemical pharmacological study of bark is worthwhile to pin point the activity

.**TABLE 1**: **EFFECT OF VARIOUS EXTRACTS OF *Wrightia tinctoria* ON CARAGEENAN INDUCED RAT PAW OEDEMA**

Group	**Test material (Dose** mg/kg**)**	**Mean increase in paw volume±SEM(ml)**				
		1 h	**2 h**	**3h**	**4h**	**5h**
1.	Control	0.40 ± .011	0.43 ±0.009	0.50 ± .008	0.50 ±0.008	0.50 ±0.008
2.	Diclofenac (13.5	0.38±0.010 (5%)	0.38±0.010* (11.62%)	0.38±0.010* (24%)	0.38±0.010* (24%)	0.38±0.010 (24%)
3	Aqueous Ext. (200)	0.35±0.015 (12.5%)	0.41±0.021 (4.65%)	0.41±0.021 (18%)	0.37±0.017* (26%)	0.35±0.015* (30%)
4.	Pet. ether Ext. (200)	0.38±0.010 (12.5%)	0.40±0.010 (6.9%)	0.45±0.017 (10%)	0.45±0.018* (10%)	0.44±0.020* (12%)
5.	Chloroform Ext. (200)	0.37±0.011 (7.5%)	0.42±0.017 (2.3%)	0.42±0.017 (16%)	0.45±0.018 (10%)	0.40±0.023* (20%)
6.	Methanol Ext. (200)	0.32± 0.011 (20%)	0.38±0.010* (11.62%)	0.40±0.012* (20%)	0.38±0.010* (24%)	0.34±0.015* (32%)

***** Indicates significant anti-inflammatory activity at P<0.05 compared to control. All values are mean± SEM of sample size 6. All treatment are given orally. Values in parenthesis are percent inhibition of increase in paw volume.

TABLE 2 : **EFFECT OF VARIOUS EXTRACTS OF *Wrightia.Tinctoria* ON GRANULOMA WEIGHT**

Group	**Test material (Dose** mg/kg**)**	**Weight of dry cotton pellet granuloma**	**Percentage of inhibition (Mean±SEM)**
1.	Control	36.98±0.661	------
2.	Diclofenac (13.5)	17.15±1.35*	53.70
3.	Aqueous Ext.(200)	27.84±0.864*	24.71
4.	Pet.ether Ext (200)	32.02±0.755*	13.41
5.	Chloroform Ext (200)	23.52±0.442*	36.39
6.	Methanol Ext (200)	28.56±0.1.075*	22.76

*Indicates significant anti-inflammatory activity at P<0.05 compared to control and sample size of 6. All treatment given orally.

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