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Comparative Evaluation of Anti-Inflammatory activity of different Extracts of *Boswellia serrata* in Wistar Albino Rats

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Abstract: Inflammation is the local response of living mammalian tissues to injury due to any agent. It is the body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removel of the necrosed cells and tissues, but the sign and symptoms which are produced during healing process can't tolerable. The present study was aimed to investigate the anti-inflammatory activity of Commercial and Hexane extracts of *Boswellia Serrata* in wistar albino rats. In this study both acute and sub-acute inflammation models were used to evaluate the anti-inflammatory activity of *Boswellia Serrata*. In acute model Carrageenan was used to induce inflammation in rat hind paw and in sub-acute inflammation Cotton pellets induced granuloma was performed. The extracts has significantly ($p \leq 0.05$) decrease in paw edema volume and wet weight and dry weight of Cotton pellets as compared to the untreated vehicle control group.

Key words: Diclofenac sodium, Dexamethasone, Cotton pellet granuloma, *Boswellia Serrata.*

INTRODUCTION:

Inflammation if fundamentally a protective response, the ultimate goal of which is to rid the organism of both the initial cause of cell injury (e.g., Microbes,toxins) and the consequences of such injury (e.g., necrotic cells and tissues).

Although clinical features of inflammation were described in an Egyptian papyrus (dated around 3000 BC), Celsus, a Roman writer of the first century AD, first listed the four cardial signs of inflammation: rubor (redness), tumor (swelling), Calor (heat), and dolor (pain). A fifth clinical sign, loss of function functioleasea) was later added by Virchow.[1]

The enzyme, phospholipase A_2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A_2 Converts phospholipids in the cell membrane in to arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation.[2,3]

Inflammation is terminated when the offending agent is eliminated and the secreted mediators are broken down or dissipated. In addition, there are active anti-inflammatory mechanisms that serve to control the response and prevent it from causing excessive damage to the host.

Many anti-inflammatory drugs (both NSAID's and corticosteroids) have been developed but the safety profile studies have shown that none of them is clearly safe.[4] They show wide ranges of adverse effects due to adverse reaction of synthetic and chemical medicines being observed round the globe, herbal medicines have

made a come back to improve our basic health needs. The use of herbal and other naturally based medicine has a minimum or no side effects. However the utilization of whole plants, plant crude preparation, isolation of active constituents that has biological activity are used as folk medicines for various disease shows the way for new alternative treatment. [5]

Salai guggal, an oleo-gum-resin from *Boswellia Serrata, (*Familly-Buseraceae) is also known as Frankincense in English and Olibanum in Arabian. This tree, abundantly growing in dry hilly tracts of India, yields oleo-gum-resin which has been used for variety of therapeutic purposes, such as 1)cancer,[6] 2)inflammation,[7] 3)arthritis,[8] 4)asthma, [9]5)psoriasis,[10] 6) colitis,[11] 7) crohn's diseases,[12]and 8) hyperlipidemia[13]. This present study emphasis to comparative evaluation of anti-inflammatory activity of Commercial and Hexane extracts of *Boswellia Serrata* in different doses (45, 90 and 180 mg/kg) respectively.

MATERIAL AND METHODS:

ANIMALS:

Wistar albino rats of either sex were obtained from the colonies maintains at Central Animal Facility, Natural Remedies Pvt. LTD, Bangalore, and housed three animals per cage with paddy husk as bedding. Animals were housed at temperature of 25±2^{o}C and relative humidity of 30-60%. A 12:12 h light and dark cycle was followed. The animals were allocated to different treatment groups and each animal in a group was recognized by mark of picric acid on the fur. Animals had free access to pellet feed and purified water *ad libitum.* Institutional Animal Ethic Committee (IAEC) Registration No-32/SASTRA/IAEC/RPP has been approved to carryout the animal experimental work.

STANDARD DRUGS:

Dexamethasone Sodium (Dexona, Cadila Healthcare Ltd.), Diclofenac Sodium (Voveran, Novartis Pharmaceutical Ltd.) was used for experiments. All other experimental chemicals and solvents used were of analytical grade.

PREPARATION OF PLANT EXTRACTS DOSES:

Boswellia serrata were prepared in 1% Tween-20 and 1% DMSO (Dimethyl Sulphoxide) as a suspension and administered to the respective doses.

ACUTE ORAL TOXICITY STUDY:

As per the OECD 423 guidelines, the herbal extracts at different doses up to 2000 mg/kg was admistered and the animal were observed for behavioral changes, the animals were observed for a further 14 days for any signs for delayed toxicity. The Commercial and Hexane extracts of *Boswellia Serrata* has good margin of safety and did not shown the lethal effects on the animals up to the doses of 2000 mg/kg.

STATISTICAL ANALYSIS:

The data expressed as MEAN ± SEM for each treatment group. The data obtained for each response measure were subjected to one way analysis of variance (ANOVA) followed by Dunnet´s "t"- test.

ANTI-INFLAMMATORY ACTIVITY:

1. CARRAGEENAN INDUCED PAW EDEMA MODEL

ANIMALS:

Animal	:	Wistar Albino rats
Sex	:	Both Sexes
Weight	:	150-180 gm
Animals per Group	:	10
Number of groups	:	9

EXPERIMENTAL DESIGN FOR CARRAGEENAN INDUCED PAW EDEMA MODEL:

Group-I: Vehicle control received 1% Tween-20 and 1% DMSO (dose: 10 ml/kg).

Group-II: Animals treated with 1% carrageenan (dose: 0.1 ml)

Group-III: Animals treated with Diclofenac Sodium (dose: 50 mg/kg).

Group-IV: Animals treated with *B. serrata* – Commercial ext (dose: 45 mg/kg).

Group-V: Animals treated with *B. serrata* – Commercial ext (dose: 90 mg/kg).

Group-VI: Animals treated with *B. serrata* – Commercial ext (dose: 180 mg/kg).

Group-VII: Animals treated with *B. serrata* – Hexane ext (dose: 45 mg/kg).

Group-VIII: Animals treated with *B. serrata* – Hexane ext (dose: 90 mg/kg).

Group-IX: Animals treated with *B. serrata* – Hexane ext (dose: 180 mg/kg).

1.1 EXPERIMENTAL PROCEDURE:[14]

Carrageenan induced paw edema is a classical model for determination of acute phase inflammation. The rat paw edema was provoked by sub plantar injection 0.1 ml of 1% w/v of carrageenan in 0.9 % saline in right hind paw. The hind paw volume was measure by dipping the foot in Digital plethysmometer up to the lateral malleolus (Winter et. al. 1962). The displacement of sodium chloride solution was measure in the plethysmometer.

The initial paw volume was measure and recorded, it was considered as 0h reading. The drug or test substances like 1% Tween-20 and 1% DMSO (vehicle

control), Diclofenac Sodium, and various extract doses were administered orally 60 min before administration of carrageenan. 0.1 ml of 1% w/v of carrageenan in saline was injected into the right hind paw of rats. The hind paw volume was measured at 1h internal up to 4th hour of experiment.

The difference between paw volumes at various time intervals indicated the edema volume due to inflammation. The percentage inhibition produced by the drug and extracts were calculated by following formula,

Percentage inhibition of paw edema (%)

$$= \frac{\text{Control - Treated}}{\text{Control}} \text{ X } 100$$

2. COTTON PELLETS GRANULOMA MODEL:

ANIMALS:

Animal	:	Wistar Albino rats
Sex	:	Both Sexes
Weight	:	150-180 g
Animals per Group	:	10
Number of groups	:	8

EXPERIMENTAL DESIGN FOR COTTON PELLETS GRANULOMA MODEL:

Group-I: Vehicle control received 1% Tween-20 and 1% DMSO (dose: 10 ml/kg).

Group-II: Animals treated with Dexamethasone (dose: 0.5 mg/kg).

Group-III: Animals treated with *B. serrata* – Commercial ext (dose: 45 mg/kg).

Group-IV: Animals treated with *B. serrata* – Commercial ext (dose: 90 mg/kg).

Group-V: Animals treated with *B. serrata* – Commercial ext (dose: 180 mg/kg).

Group-VI: Animals treated with *B. serrata* – Hexane ext (dose: 45 mg/kg).

Group-VII: Animals treated with *B. serrata* – Hexane ext (dose: 90 mg/kg).

Group-VIII: Animals treated with *B. serrata* – Hexane ext (dose: 180 mg/kg).

2.1 EXPERIMENTAL PROCEDURE:[15]

This is sub acute model for inflammatory study. This method was adopted from D'Arcy (1960), which was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia, using blunted forceps subcutaneous tunnel was made and autoclaved cotton pellets (10±1 mg) were implanted in the axilla and groin region of the rat (D'Arcy et al. 1960).

After recovering from anesthesia, animals were treated orally with vehicle control (1% Tween-20 and 1% DMSO), Dexamethasone and various doses of extracts for consecutive seven days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed, freed from extraneous tissue and dried at $60^{o} \pm 1$ for 24hrs. The percentage inhibition of the dry weight of the granuloma were calculated and compared.

Percentage inhibition (%)

$$= \frac{\text{Control - Treated}}{\text{Control}} \text{ X } 100$$

RESULTS AND DISCUSSIONS:

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief of inflammation. To give a scientific validation to this plant an attempt was made to study the anti-inflammatory effect.

Carrageenan induced inflammation is a biphasic phenomenon.[16] The first phase of edema is attributed to release of histamine and 5-Hydroxy tryptamine, plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action.

The above acute model result was indicate that Commercial extract of *B.Serrata* at the dose levels of 45, 90 and 180 mg/kg showed reduction in paw edema volume at all time intervals as compared to carrageenan control but significant reduction in paw edema volume was noticed in 45 mg/kg at 1st and 3rd hour observation 24.13 % and 23.71 % respectively shown in Table -1 and Fig-1.

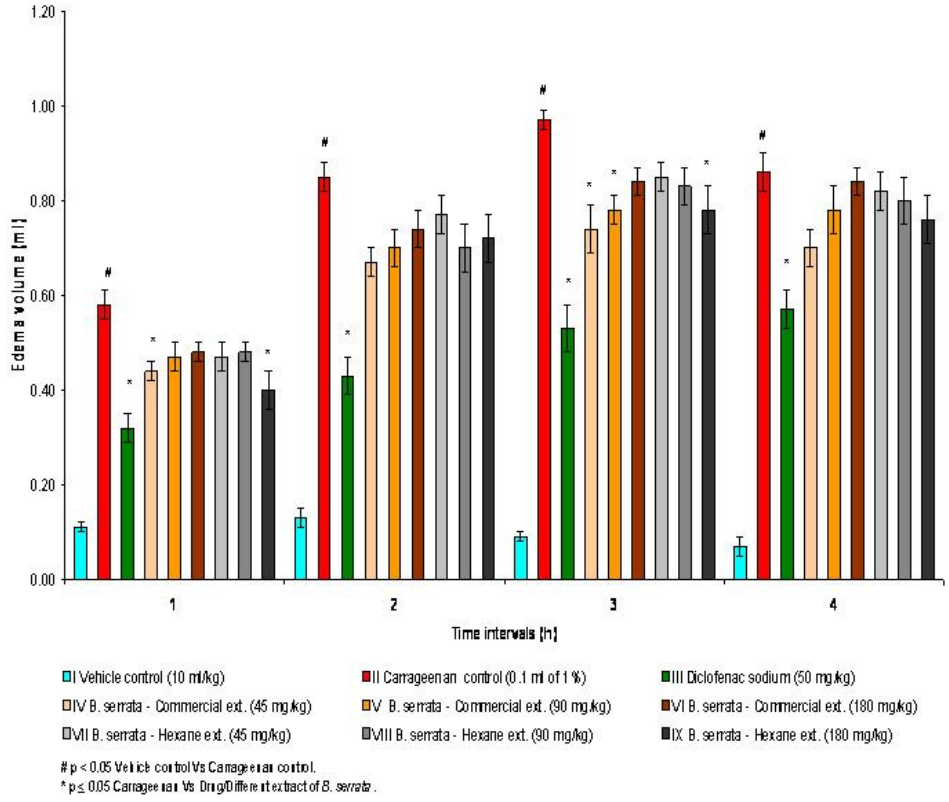
The groups treated with Hexane extract of *B.Serrata* at the dose levels of 45, 90 and 180 mg/kg showed reduction in paw edema volume at all time intervals as compared to carrageenan control but significant reduction in paw edema volume was noticed in 180 mg/kg body weight at 1st and 3rd hour observation 31.03 % and 19.59 % respectively shown in Table -1 and Fig-1.

TABLE 1: EFFECT OF DIFFERENT EXTRACTS OF *B. SERRATA* ON PAW EDEMA VOLUME IN WISTAR ALBINO RATS

Treatment group	Edema volume (ml)			
	1 Hour	2 Hour	3 Hour	4 Hour
I: Vehicle control (10 ml/kg)	0.11 ± 0.01 (0.00)	0.13 ± 0.02 (0.00)	0.09 ± 0.01 (0.00)	0.07 ± 0.02 (0.00)
II: Carrageenan control (0.1 ml of 1%)	0.58 ± 0.03# (100)	0.85 ± 0.03# (100)	0.97 ± 0.02# (100)	0.86 ± 0.04# (100)
III: Diclofenac sodium (50 mg/kg)	0.32 ± 0.03* (45.00)	0.43 ± 0.04* (49.41)	0.53 ± 0.05* (45.36)	0.57 ± 0.04* (33.72)
IV: *B. serrata* - Commercial ext. (45 mg/kg)	0.44 ± 0.02* (24.13)	0.67 ± 0.03 (21.18)	0.74 ± 0.05* (23.71)	0.70 ± 0.04 (18.60)
V: *B. serrata* - Commercial ext. (90 mg/kg)	0.47 ± 0.03 (18.97)	0.70 ± 0.04 (17.65)	0.78 ± 0.03* (19.58)	0.78 ± 0.05 (09.30)
VI : *B. serrata* - Commercial ext (180 mg/kg)	0.48 ± 0.02 (17.24)	0.74 ± 0.04 (12.94)	0.84 ± 0.03 (13.40)	0.84 ± 0.03 (02.33)
VII : *B. serrata* - Hexane ext. (45 mg/kg)	0.47 ± 0.03 (18.97)	0.77 ± 0.04 (9.41)	0.85 ± 0.03 (12.37)	0.82 ± 0.04 (04.65)
VIII : *B. serrata* - Hexane ext. (90 mg/kg)	0.48 ± 0.02 (17.24)	0.70 ± 0.05 (17.65)	0.83 ± 0.04 (14.43)	0.80 ± 0.05 (06.98)
IX : *B. serrata* - Hexane ext. (180 mg/kg)	0.40 ± 0.04* (31.03)	0.72 ± 0.05 (15.29)	0.78 ± 0.05* (19.59)	0.76 ± 0.05 (11.62)

Percentage inhibition of paw edema values are presented in parentheses
Values are expressed as mean ± SEM; n=10, # $p \leq 0.05$ Vehicle control *Vs* carrageenan
* $p \leq 0.05$ Carrageenan *Vs* drug/test substance

Fig. 1: Effect of different extracts of *Boswellia serrata* on paw edema volume in Wistar albino rats



In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts which are basic sources of granuloma formation. Hence the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the *B.Serrata.*

The mean wet weight and dry weight of cotton pellets are presented in Table-2 and Fig-2 and 3, the standard drug dexamethasone showed significant anti-inflammatory activity by reducing both wet weight as well as dry weight of the cotton pellets when compared with vehicle control 52.37 % and 54.21 % respectively.

The Commercial extract of *B.Serrata* at the dose of 45 mg/kg showed significant decrease in wet and dry weight of cotton pellets and inhibition was noticed as compared to vehicle control group 19.87 % and 17.76 % respectively. Hexane extract of *B.Serrata* at the dose of 45 mg/kg showed significant decrease in wet and dry weight of cotton pellets and inhibition was noticed as compared to vehicle control group 19.62 % and 17.32 % respectively.

TABLE 2: MEAN WET WEIGHT AND DRY WEIGHT OF COTTON PELLETS OF DIFFERENT GROUPS AND PERCENTAGE INHIBITION

Treatment groups	Mean Wet Weight of Pellets (mg)	Percentage inhibition (%)	Mean Dry Weight of Pellets (mg)	Percentage inhibition (%)
I: Vehicle control (1% Tween 20 + 1% DMSO) (10 ml/kg)	199.68 ± 9.97	-	45.60 ± 2.04	-
II: Dexamethasone (0.5 mg/kg)	95.30 ± 3.46*	52.27	20.88 ± 0.77*	54.21
III: *B. serrata* - commercial extract (45 mg/kg)	160.00 ± 6.01*	19.87	37.50 ± 1.27*	17.76
IV: *B. serrata* - commercial extract (90 mg/kg)	172.26 ± 9.76	13.73	39.88 ± 1.93	12.54
V: *B. serrata* - commercial extract (180 mg/kg)	171.75 ± 7.95	13.99	39.70 ± 1.39	12.94
VI: *B. serrata* - Hexane extract (45 mg/kg)	160.50 ± 7.17*	19.62	37.70 ± 1.74*	17.32
VII: *B. serrata* - Hexane extract (90 mg/kg)	163.48 ± 8.35*	18.13	37.98 ± 1.28*	16.71
VIII: *B. serrata* - Hexane extract (180 mg/kg)	167.13 ± 12.00	16.30	38.23 ± 2.20*	16.16

Values are expressed as mean ± SEM; n=10,
*$p \leq 0.05$ Treated groups Vs Vehicle control

CONCLUSION:

Based on the results of the present study it can be concluded that Commercial extract of *B.Serrata* at the dose level 45 mg/kg and Hexane extract of *B.Serrata* at the dose level 180 mg/kg showed significant anti-inflammatory effect in wistar albino rats using carrageenan induced paw edema model.

In cotton pellet granuloma model both the Commercial and Hexane extract of *B.Serrata* at the dose of 45 mg/kg showed significant anti-inflammatory effect in wistar albino rats.

Fig- 2: Effect of commercial and hexane extracts of *Boswellia serrata* on the wet weight of cotton pellets

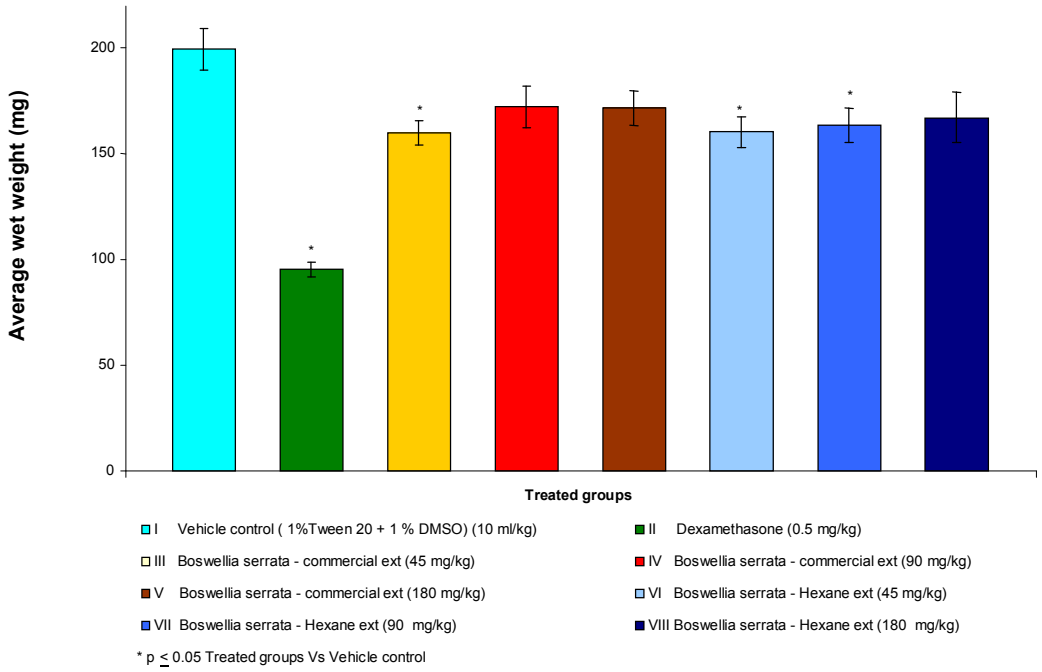
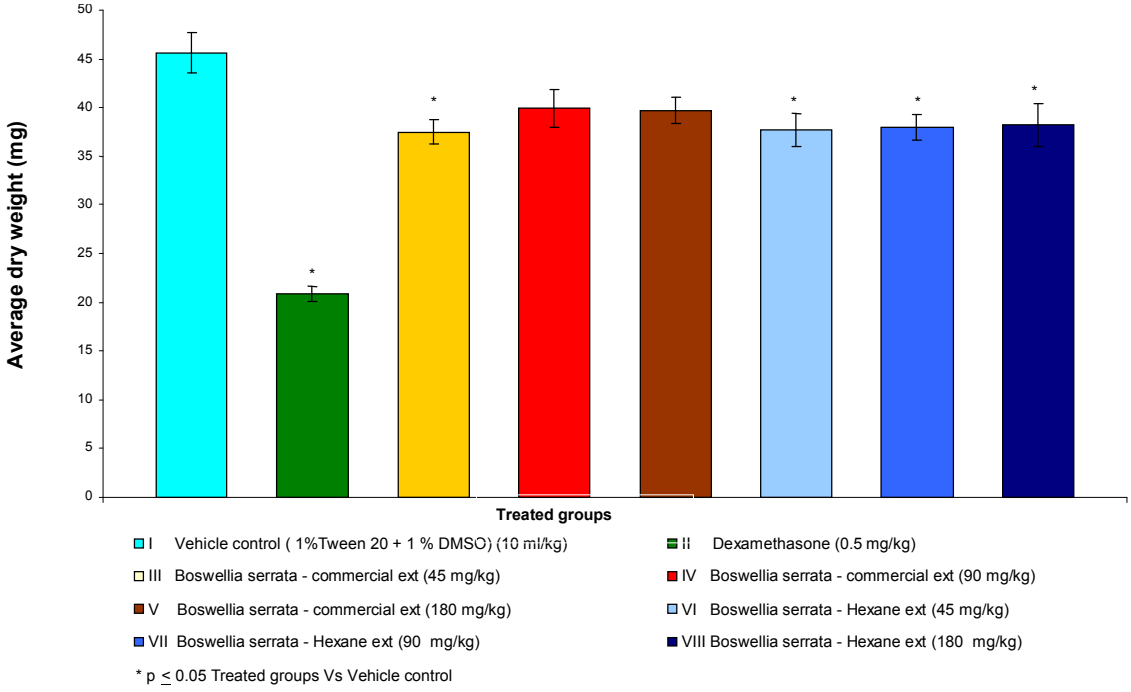


Fig- 3: Effect of commercial and hexane extracts of *Boswellia serrata* on the dry



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