

**Evaluation of Anti-Inflammatory Activity of Ethanolic Extract of
Gum *Boswellia Serrata***

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Summary

The anti-inflammatory activity of ethanolic extract of *gum Boswellia serrata* was determined in carrageenan induced rat paw oedema. The result indicate that ethanolic extract of gum of *Boswellia serrata* inhibited rat paw oedema significantly ($p < 0.01$) as compared to standard drug Diclofenec sodium ($p < 0.01$) and untreated control group. The present research work is focused to compare herbal extract with allopathic sub. Containing recent NSAID- Diclofenec. Diclofenec was used to test the sensitivity of the system as a standard drug. Topically applied extract will be standardised with physical methods and biological methods. During the physical methods, the following characters were find out of average pH of F1=6.48, F2=7.48, Viscosity Of F2 Is 760,000 Centipoises Or 7600 Poise Spread ability of F1=6 and F2= 2.25 Pedal inflammation of control is 0.3 ± 0.0064 , F% oedema inhibition is 4 days, F2 % oedema inhibition is 2 days. Granuloma pouch observations were made after 5 days F1=16.12, F2=67.52. Formulation F₂ inhibited oedema formation in formaldehyde induce models of acute inflammation. As compared to the F1 formulation.

Keywords- *Boswellia serrata*, carrageenan, Diclofenec. Granuloma pouch, Pedal inflammation

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Introduction

Herbal medicines are precious and valuable gift of nature. Of late, there is an upsurge in commerce of herbal medicines, cosmetics due to the “back othe nature” movements. Global estimate indicate that 80% of about four billion population cannot afford the products of western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. This fact is well compiled in inventory of medicinal plants listing over20, 000 species. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world’s population, although many such countries spend 40-50% of there total health budget on drugs.¹

Annual herbal drug production has been estimated at around 200 crores and is expected to reach Rs.4000 crores by the year 2000 herbal remedies are more effective, easy available, low cost and comparatively being devoid of serious toxic effects popularises herbal remedies.

The basis of allopathic system is diagnosis it is based on a balancing of all inputs and information. Symptoms, sign and results of various investigations provide these. This lack to increasing the number of investigations, costly often times, sometimes unnecessary. The increasing cost, increasing unfounded claims- unaccompanied by increasing success has led to dissatisfaction with the allopathic system.

Anti-inflammatory diseases including different types of rheumatic diseases are very common through the world. Although rheumatism is one of the oldest known diseases of mankind and affects a large population the world .The greatest disadvantage in the presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation.

Therefore, the search for screening and development of drugs for their anti-inflammatory activity is unending problem and there is much hope of finding anti-inflammatory drugs from indigenous plant. Recent reviews revealed that pant species of 96 genera belonging to 56 families have exhibited anti-inflammatory activities.

Today’s the first pharmaceutical line of defence against arthritis is the NSAID’s. The NSAID’s block release of prostaglandins, which tirigger inflammation. There are several NSAID’s are available.

The plant *Boswellia Serrata* (Salai Guggal) belongs to family Burseraceae is mostly used as the anti-inflammatory activity. The most important derivative of *Boswellia Serrata* tree is the *Boswellia Gum Resin*. *Boswellia* is the gummy resin of the *Boswellia* tree. It is native to India and used for centuries by Ayurvedic doctors. It is an Ayurvedic plant that contains anti-inflammatory terpenoids called boswellic acids^{2,3}.

Material and Methods

Plant Material

The *Boswellia Serrata* (Salai Guggal) gum exclude for the proposed study were collected and they were authenticated by department of Botany of Annamalaie University, Chidamabarm. The freshly collected gum of *Boswellia Serrata* was shade dried and then powdered to coarse size. About 500 gm of leaves powder of *Boswellia Serrata* was subjected to extraction with (ethanol 95 %). After extraction, the solvent was distilled off and the extracts were concentrated on water bath. Then prepare the formulation and formulations were evaluated for anti-inflammatory activity.

Preparation of Polyherbal formulation

Collection of Samples:

Anti-inflammatory activity preparation of topical formulation from the *Boswellia serrata* extract

Preparation of Polyherbal formulation

The quantity of ethanolic extracts of *Boswellia serrata* extract required for formulating herbal drug formulation (Table 1) are calculated on the basis of human dose of powder form and percentage practical yield of respective crude drugs. Two formulations are prepared Ointment base and considered as Lower dose and higher dose formulation

Evaluation Parameters

The semi-solids are needed to be evaluated for their physicochemical properties. Some. Of the important physicochemical properties to be evaluated is pH.

pH Measurement^{4,5,6,7,8,9}

pH has been considered one of the important physicochemical characters of the semi-solid vehicles. To have satisfactory absorption of drug across the membrane (lipid barrier) the drug should remain in the unionized form. Since dissociation is directly depended on pH, pH can be said to have an effect on absorption. pH of the vehicle could also modify skin keratin and thereby alter skin hydration. Above pH 10 water diffusion rate increases and water binding capacity decreases, as the buffer extract water binding material and dissolves the keratin. It has been shown that between neither pH 1 and 10 the tissue neither swells nor hydrates. An average figure for the pH of the skin is 5.5. pH also affects on the stability of the active ingredients.

(pH Meter model CI-46, Manufactured by Toshniwal Instruments Manufacturing Pvt. Ltd.) By using standard buffer at different pH 4 and 9.2, pH meter was calibrated.

Buffer solution A – pH 4:

Dissolve 10.21gm of potassium hydrogen phthalate in sufficient carbon dioxide free water to produce 1000 ml.

Buffer solution -pH 9 .2:

Dissolved 3.814 gm of borax in sufficient. Carbon dioxide free water to produce 1000 ml.

The pH of topical formulation was measured by Deeping the dried electrode of digital pH meter in the test compounds. (Table 2)

Spreadability Determination:

Semi-solids posses the particular property that they readily deform when we apply them to the skin, yet they cling to the body, generally until washed or wiped off.

Spreadability was measured, using the spreadability apparatus. An excess of semi-solid was placed between two slides. A weight of 1000 gm. was allowed to rest on the slide for 5 minutes to expel the air between .the slides and make a uniform film of semi-solid .Excess of semi-solid was carefully removed from the edge of the slide. The bottom slide was anchored and top slide was subjected to pull of 80 gm weigh. The time in seconds required to separate completely the slide was noted. In this way spreadability in terms of second was determined for each formulation under testing. The data was tabulated. (Table 3)

Viscosity Determination^{10,11,12,13}

Semi-solids possess the particular property that they readily deform when we apply them to the skin, yet they cling to the body, generally until washed or wiped off. Rheological properties of the semi-solids are required to be assessed with respect to its patient usage eg. the ease of removal of. The preparation from a tube without spillage or spreadability and adherence to the

skin. Consistency effect on the release of the drug from the preparation is also considered important. Brookfield Dial reading viscometer Model RVT-230V. (Table 4)

Test Animal

The experimental protocol was submitted and approved by Institutional Ethical Committee, Wister albino rats (150-200 g) of approximate same age were employed in this investigation. The animals were fed with standard pellet diet and water and ad libitum. They were housed under standard conditions of temperature 22⁰ C (\pm 3⁰ C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage. The animals received the drug treatments by oral gavages tube.

Chemicals

Diclofenec sodium, Carrageenan were obtained as a gift sample from Merck, India and the other chemicals and reagents used were of analytical grade.

Acute toxicity studies

Acute toxicity studies were carried out on Wister albino rats according to method proposed by Ghosh. The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 4000 mg/kg of body wt. in rats of either sex with no toxic reaction being observed.

Anti-Inflammatory Activity of Topical Formulation.

Acute inflammation:

Pedal Inflammation Induced by Chemical Agents:

It was produced by injecting 0.1 ml of the phlogistic agent underneath the planter aponeurosis of the hind paw of the rat. Diclofenec was used to test the sensitivity of the system as a Standard drug. A variety of chemical agents has been used to induce oedema in the feet of rodents. Anti-inflammatory agents may then be detected by their ability to diminish or prevent the oedema.

The following phlogistic agents were used:

- a) 3.5 % Formalin --0.05 ml.-b) Histamine 1 mg/ml
- c) 1% W/v solution of Carrageenan
- d) 0.5% W/W solution of 5-HT e) Fresh egg white 0.5% w/w solution.

In this test, formalin causes injury to the cells and thereby initiates the release of substance causing capillary hyper permeability. The activity of test substance, capable of inhibiting inflammation in the rat. Foot after formalin, may well owe to their antiferbrinolysin action. It is also possible that inhibition of formalin induced inflammation is owing to inhibition of the action of bradykinin released by the injured cell.

Formalin

In the method of North over and Subramanian (1961-1962) preliminary experiment showed that 0.05 ml of 3.5 % formalin in 0.9% sodium chloride produce a sub-maximal degree of swelling.

For this method, wistar rats of either sex weighing about 150-200 gm were used. They were divided into 4 groups of 6 animals each. The treatment was as follows:

Group 1: Control receiving normal saline

Group 2: Allopathic formulation (FA)

Group 3: F1

Group 4: F2

To a swollen foot of the test rat, test drug was applied in sufficient amount, (1 gm) secured in place by means of an adhesive tape (Johnson brand) and animals were left with applied test drug for a specified number of days. The paw volumes were measured for each group at 24 hours, 48 hours, and 72 hours up to decline after the formalin treatment.

Calculations: The % oedema was calculated by following

Formula:-

$$\% \text{ Oedema} = (1-t/c) \times 100$$

Where T = mean paw volumes in drug treated group

C = mean paw volume in control group

Interpolation for F 1 and F 2 and the Allopathic formulation Potency estimated (Table 5)

Chronic Inflammation

Granuloma Pouch:

The Granuloma pouch was introduced used as a means of assaying drugs. (Tolksdorf, 1959; Wilhelmi, 1960). The wall of the Granuloma pouch consists chiefly of cellular inflammatory exudates appearing in response to chemical injury, but thereafter these disappear, and mononuclear cells of various types predominate, including lymphocytes, monocytes, macrophages, histiocytes and plasma cells.

Procedure: ^{14, 15}

Female Wister rats weighing about 150-200 gm were used. They were divided into 3 groups of 6 animals each. The treatment was as follows

Group 1: Control receiving normal saline

Group 2: Allopathic formulation (FA)

Group 3: F1

Group 4: F2

Pouches were produced by injecting 25 ml of air subcutaneously in the clean shaven skin on the back of each animal, after breaking all the connecting tissue strands till needle moved freely sideways. This was followed by injection of 0.5 ml. of 1% sterile croton oil in ground-nut-oil in the pouch through the same needle. The puncture hole was sealed with a tint. Benzoin seal animals were lightly anaesthetized with ether throughout the procedure. The drugs were applied once a day for 4 days, beginning one day prior to the administration of croton oil.

After four days of the daily drug treatment, the rats were sacrificed on the fifth day; the pouches were excised, trimmed. Volume and character of the exudate in the pouch was determined by aspirating the exudate with a syringe. (Table 6)

Results

Table 1: Quantity of plant extracts used for preparing herbal formulations F1 and F2

Allopathic formulation	Diclofenec diethyl ammonium salt	1.16% w/w
	equivalent to Diclofenec sodium	1 % w/w in gel
Herbal Formulation (F1)	<i>Boswellia serrata</i> extract	7.5 % w/w
	Ointment base	q.s.

Herbal Formulation (F2)	<i>Boswellia serrata</i> extract	8.5 % w/w
	Ointment base	q.s.

pH Measurement:

Table 2: pH measurement.

Sr. no	Formulation	pH			Average
		1	2	3	
1	F1	6.48	6.49	6.49	6.48
2	F2	7.48	7.48	7.49	7.48

Spreadability determination

Table 3: Spreadability determination

Sr. no.	Formulation	Time in sec			Average
		1	2	3	
1	F1	6	6	6	6
2	F2	2	2	3	2.25

Viscosity Determination F2

Table 4: Viscosity Determination F2

Speed	Dial reading D.R	Factor F	Viscosity =D.R.×F Centipoises
6	92	1M	92,000
3	78	2M	156,000
1.5	67	4M	268,000
0.6	51	10M	510,000
0.3	38	20M	760,000

Pedal inflammation

Table 5: Shows the Pedal inflammation

Group no	Treatment	Days	Mean paw oedema volume cm ±S.E.**	% oedema inhibition	ED50* days
1	Control	-----	0.3 ±0.0064	-----	
	FA	1	0.16 ±0.0120	26	2 days
		2	0.11±0.0070	48	
		3	0.05±0.055	77	

2	F1	1	0.18±0.0079	10	4 days
		2	0.16±0.0092	20	
		3	0.13±0.0123	35	
		4	0.09±0.092	55	
		5	0.07±0.0064	65	
		6	0.04±0.0064	80	
3	F2	1	0.15 ±0.0113	26	2 days
		2	0.10±0.0064	50	
		3	0.04±0.043	80	

FA- Allopathic formulation

*ED50 is the day which cause 50% inhibition in oedema

Granuloma pouch

Table 6: % inhibition Observations were made after 5 days.

Group no.	Treatment	Exudates (Mean) c.c. ±S.E.	Character of exudates	mortality	% inhibition
1	Control	0.93±0.2966	Watery yellow	Nil	----
2	FA	0.26±0.0568	Thicker yellow	Nil	59.65
2	F1	0.78±0.0789	Thicker yellow	Nil	16.12
3	F2	0.3±0.0663	Thicker yellow	Nil	67.75

Discussion

The authenticated and collected plant gum exclude of *Boswellia serrata*. Were shade dried and then powdered to coarse size. About 500 gm of leaves powder of *Boswellia serrata*. Was subjected to extraction with (ethanol 95 %). After extraction, the solvent was distilled off and the extracts. The present study explores the formulation for anti-inflammatory activity. During the physical methods, the following characters were find out of average pH of F1=6.48, F2=7.48 ,Viscosity Of F2 Is 760,000 Centipoises Or 7600 Poise Spread ability of F1=6 and F2= 2.25 Pedal inflammation of control is 0.3±0.0064, F% oedema inhibition is 4 days, F2 % oedema inhibition is 2 days. Granuloma pouch observations were made after 5 days F1=16.12, F2=67.52. Formulation F₂ inhibited oedema formation in formaldehyde induce models of acute

inflammation. As compared to the F1 formulation. Formulation F2 has shown significant anti-inflammatory activity than the F1 with compare of FA i.e. Diclofenec sodium as a standard drug. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme or act on central opioid receptors. Based on the results of the present study, it can be concluded that formulation F2 showed signification anti-inflammatory activity than F1 in rats.

Conclusion

In conclusion, we can confirm that the formulation F2 showed the potent anti-inflammatory activity as compared to the formulation F1 in rats.

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