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Evaluation of anti-inflammatory activity and potency of herbal formulation consists of different proportions of *Curcuma longa* and *Boswellia serrata* by using Cotton pellet granuloma and xylene induced mice ear edema model

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Abstract: Inflammation is one of the pathological healing processes of human body, but the sign and symptoms which are produced during healing process can't be tolerable. In order to get relive mostly the allopathic drugs like NSAIDS (Non-steroidal Anti-inflammatory Drugs) are prescribed. But these allopathic drugs produce different side effects and toxicity like gastric up set, peptic ulcer and gastric bleeding. Hence in order to avoid the unwanted effects while preventing the inflammatory process the current preclinical practice has been carried out by taking alcoholic extract of *Curcuma longa* and *Boswellia serrata* in different proportions (1:1, 1:2 and 2:1) respectively. The study has been carried out by using cotton pellets granuloma, one of the sub-acute model and xylene induced mice ear edema one of the acute model. The herbal formulation has significantly ($p \leq 0.05$) decrease in wet weight and dry weight of cotton pellets and suppressed the development of ear edema induced by xylene in mice as compared to the vehicle control group.

Keywords: Dexamethasone, Prostaglandin, Xylene, Nuclear receptor.

Introduction:

Inflammation is a localized protective response elicited by injury or destruction of tissues, which serve to destroy, dilute or sequester both the injurious agent and injured tissue. It is a complex reaction usually necrotic, cells that consist of vascular response, migration and activation of leucocytes and systemic reaction. The inflammatory response is closely related to the process of repair. During repair, the injured tissue replaced through regeneration of native parenchymal cells, by filling of the defect with fibrous tissue.

Although clinical features of inflammation were describe in an Egyptian papyrus (dated around 3000 BC), Celsus a roman writer of the first century AD, first listed the four cardinal signs of inflammation; redness (*rubor*), heat (*calor*), swelling (tumour) and pain (dolor). A fifth clinical sign, loss of function (functioleasea) was later explain by Virchew.[1]

The cellular component involves the movement of white blood cells from blood vessels into the inflamed tissue. The white blood cells, or leukocytes, take on an important role in inflammation; they extravasate (filter out) from the capillaries into tissue, and act as phagocytes, picking up bacteria and cellular debris. (Wiki)

Anti-inflammatory agents are use in the treatment of inflammation. There are two main types of anti-inflammatory agents; namely Glucocorticosteroids and Non- Steroidal Anti-inflammatory Drugs (NSAIDs). The NSAIDs is so named because they do not belong

to the steroidal groups and eases discomfort by blocking the pathway of an enzyme that creates prostaglandins (hormones that cause pain and swelling). By doing so, the drugs lessen the pain in different parts of the body. While steroids, specifically glucocorticoids, reduce inflammation by binding to cortisol receptors. These drugs are often referred to as corticosteroids. (Wiki)

Many therapeutic agents from synthetic source have merits and equal demerits which limits their usefulness on long term basis. Same in case of NSAIDs also which shows mild side effects includes stomach ulcers, bleeding ulcers, kidney dysfunction, constipation, dizziness and headaches. Comparatively, the use of herbal and other naturally based medicine has a long history with minimum or no side effects. However, the utilization of whole plant, plant crude preparation, isolation of active constituent that has biological activity are used as folk remedies for various disease shows the way for new alternative treatment. The investigation of bioactive natural product has in recent years assumed a greater sense of urgency in response to the expanding human population and its demand for good health.[2]

Curcuma longa and *Boswellia serrata* is such a medicine in routine use with valuable pharmacological activities. A plant of Indian origin, *Curcuma longa* L (Zingiberaceae) has a rhizome of bright orange color under a fine light brown cell layer. It is in common use as a spice in Asian cultures, where it is consider a magical plant because of its organoleptic properties and undoubted therapeutic and protective effects, especially for the skin and liver. Turmeric (*Curcuma longa*) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. There are a great number of papers in the literature relating the activity of compounds extracted from *Curcuma longa* being potent inhibitors of inflammation. [7] Its use as a remedy for hypercholesterolemia, arthritis, indigestion and liver problem has been known since long. The continuing research of this plant indicates that turmeric and its active principle have unique antioxidant, anti-mutagenic, anti-tumorigenic, and anti-carcinogenic, anti-inflammatory, anti-arthritic, anti-microbial, and hypercholesterolemia potentials.

Boswellia serrata (Family – Burseraceae) is known a Frankinconso in English and Olibanum in Arabian. This tree, abundantly growing in dry hilly tracts of India, Yeils Oleo – gum – resin. Which has been used for variety of theraputic purposes – (1) Cancer (2) Inflammation (3) Arthritis (4) Asthma (5) Hyperlipidemia (6) Psoriasis (7) Colitis (8) Crohn's diseases.

The alcoholic extract of salai guggal was reported to possess anti-inflammatory and anti- arthritic activity in animals which is due to boswollic acid. Boswollic acid selectively inhibits leukotrine synthesis by inhibiting 5 – LOX enzyme. *Boswellia serrata* shows analgesic and psychopharmacological effect.

This present study emphasis to evaluate the anti-inflammatory activity of herbal formulation containing two different extracts of *Curcuma longa* and *Boswellia serrata* in different proportions (1:1, 1:2 and 2:1) respectively.

Materials and Methods:

Animals[3]

Male albino Swiss mice and albino Wistar rats of either sex were obtained from the colonies maintain 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°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 Registration No-160/1999/CPCSEA has been approved to carry out the animal experimental work.

Standard Drugs

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

Preparation of herbal formulation doses

Herbal formulation doses were prepared in 1% DMSO (Dimethyl Sulphoxide) and 1% Tween-20 as a solution and administered to the respective doses.

Acute oral toxicity study

As per the OECD guidelines, the herbal formulation at different doses up to 1000mg/kg was administered and the animals were observed for behavioral changes, toxicity and motilityup to 48h.

Statistical Analysis

The data are 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 Dennet's't'-test.

Anti-inflammatory activity

1. Cotton pellet granuloma model

Animals

Animal	:Albino Wistar rat
Sex	:Either Sex
Weight	:150-180 gm
Animals per Group	:10
Number of groups	:5

Experimental Design for Cotton pellet granuloma model

Group-I: Vehicle control received 1% DMSO and 1% Tween-20 (dose: 10 ml/kg).
Group-II: Animals treated with Dexamethasone (dose: 0.5 mg/kg).
Group-III: Animals treated with formulation-1 (1:1) (dose: 90 mg/kg).
Group-IV: Animals treated with formulation-2 (1:2) (dose: 90mg/kg).
Group-V: Animals treated with formulation-3 (2:1) (dose: 90 mg/kg).

1.1 Experimental Procedure: [3]

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 sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat.

After recovering from anesthesia, animals were treated orally with vehicle control (1% DMSO and 1% Tween-20), dexamethasone and various doses of herbal formulation 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} C for 24 hrs. The percentage inhibition of the dry weight of the granuloma were calculated and compared.

Percentage inhibition (%)

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

2. Xylene induced mouse ear edema model

Animals

Animal	:Albino Swiss mice
Sex	:Male
Weight	:18-27 g
Animals per Group	:6
Number of groups	:5

Chemicals / Drugs

Xylene : Chemlabs, Bangalore.

Experimental Design for Xylene induced mouse ear edema model:

Group-I: Vehicle control received 1% Tween-20 and 1% DMSO (dose: 10 ml/kg).
Group-II: Animals treated with Diclofenac (dose: 50 mg/kg).
Group-III: Animals treated with formulation-1 (1:1) (dose: 126 mg/kg).
Group-IV: Animals treated with formulation-2 (1:2) (dose: 126mg/kg).
Group-V: Animals treated with formulation-3 (2:1) (dose: 126 mg/kg).

2.1 Experimental procedure[4, 9]

The effect of extract on acute edema was assessed by using xylene induced ear edema in mice. One hour after oral administration of herbal formulation and Diclofenac, 50µl of xylene was applied to the anterior and posterior surfaces of the right ear under light ether anesthesia. The left ear was considered as control. Four-hour later xylene application mice were sacrificed by cervical dislocation and both ears were removed. Ear lobes were punched out in circular disc using metal punch (6 mm diameter) and weighed. The difference in the weight of discs from right treated and left untreated was calculated and was used as measure of edema.[7] The level of percentage inhibition was calculated using the formula.

Percentage inhibition (%)

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

Results and Discussions:

Daily administration of herbal formulation (test substances) of different proportions for consecutive 7 days orally has shown significant percentage inhibition of inflammation as compare to control. The wet weight and dry weight of cotton pellets has reduced in the treatment groups. In case of wet weight and dry weight, herbal formulation of different proportions (1:1, 1:2, and 2:1) reduces the inflammation 20.64%, 24.43%, 22.26% and 17.98%, 21.86%, and 19.56% respectively for the dose level of 90 mg/kg as shown in Table no. 1 and Graph no. 1 and 2. All formulations at the dose of 90 mg/kg rat body weight, showed significant decrease in wet weight and dry weight of cotton pellets as compared to the vehicle control group. The formulation of *Curcuma longa* and *Boswellia serrata* (1:2) showed anti-inflammatory activity by maximum inhibition of 24.43 % and 21.86 % in wet weight and dry weight of

cotton pellet granuloma respectively as compared to vehicle control group.

The standard drug, Dexamethasone 0.5 mg/kg showed significant anti-inflammatory activity by reducing wet weight as well as dry weight of the cotton pellet (52.03% and 54.21%)
respectively when compared with vehicle control group. The mechanisem of action of Dexametasone is by acting on nuclear receptors and alter the protein synthesis and the herbal formulation may act by inhibiting the release of inflammatory mediators like prostaglandin and histamine.

Similarly in case of xylene model the mean ear edema of different groups is presented in Table -2 and graph no.3. The standard drug, diclofenac sodium at dose 50 mg/kg showed significant anti-inflammatory activity by reducing ear edema 57.98% as compared to vehicle control group. Treatment with all formulations at the tested dose of 126 mg/kg mice body weight showed significant decrease in ear edema when compared with vehicle control. The herbal formulation of different proportions (1:1, 1:2, and 2:1) reduces the inflammation 39.98%, 54.02%, and 43.94% respectively for the dose level of 126 mg.

Table-1: Effect of *Curcuma longa* and *Boswellia serrata* on Cotton pellet granuloma model.

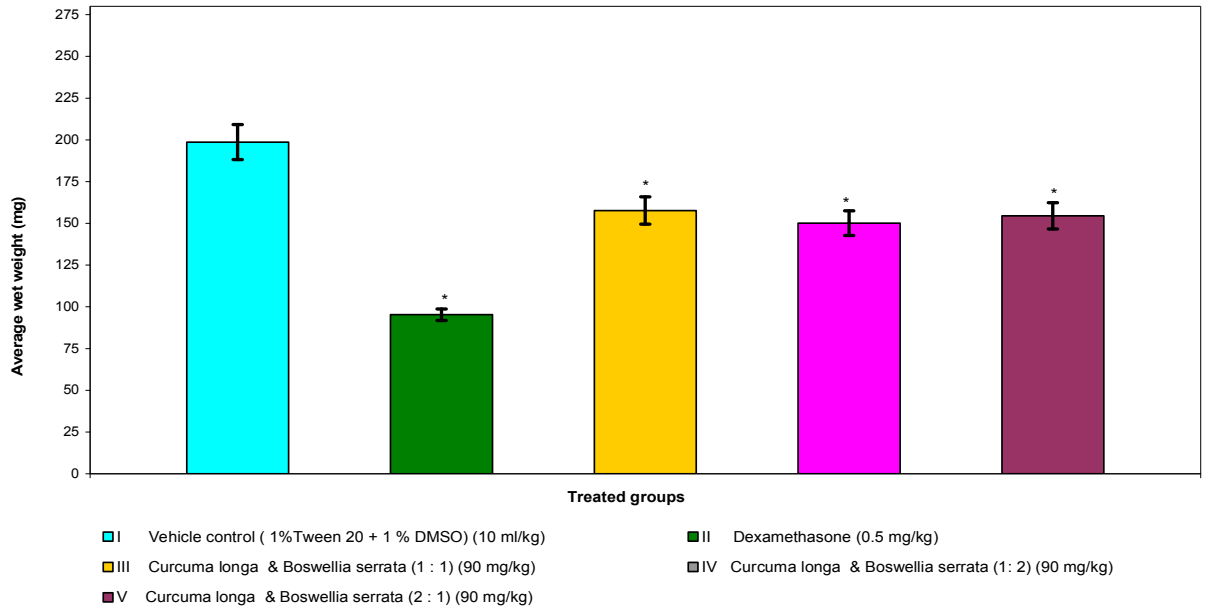
Treatment Groups	Mean Wet Weight of Pellet (mg)	Percentage inhibition	Mean Dry Weight of Pellet (mg)	Percentage inhibition
I Vehicle control (1% tween20+ 1% DMSO) (10 ml/kg)	198.68 ± 10.47	0	45.60 ± 2.04	0
II Dexamethasone (0.5 mg/kg)	95.30 ± 3.46*	52.03	20.88 ± 0.77*	54.21
III *Curcuma longa* and *Boswellia serrata*(1: 1) (90 mg/kg	157.68 ± 8.18*	20.64	37.40 ± 1.59*	17.98
IV *Curcuma longa* and *Boswellia serrata*(1: 2) (90 mg/kg	150.15 ± 7.43*	24.43	35.63 ± 1.52*	21.86
V *Curcuma longa* and *Boswellia serrata*(2: 1) (90 mg/kg	154.45 ± 7.89*	22.26	36.68 ± 1.39*	19.56

Values are expressed as mean ± SEM; n=10
* $p \leq 0.05$ Treated groups Vs Vehicle control (1% tween 20 +1% DMSO)

Table-2 Effect of *Curcuma longa* and *Boswellia serrata* on xylene induced mice ear edma model.

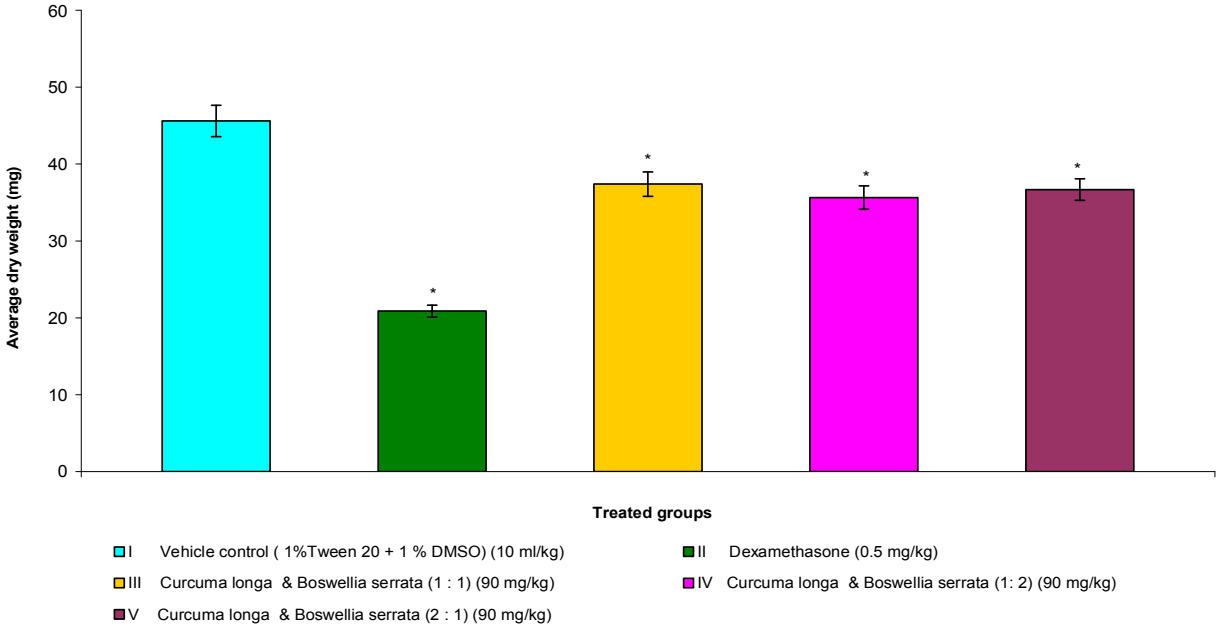
Treatment groups	Ear edema(g)	(%)inhibition
I. Vehicle control (10ml/kg)	8.33 ± 0.61	-
II. Diclofenac sodium (50 mg/kg)	3.50 ± 0.22*	57.98
III. *Curcuma longa* and *Boswellia serrata* (1: 1)(126 mg/kg)	5.00 ± 0.26*	39.98
IV. *Curcuma longa* and *Boswellia serrata* (1: 2) (126 mg/kg)	3.83 ± 0.31*	54.02
V. *Curcuma longa* and *Boswellia serrata* (2: 1) (126 mg/kg)	4.67 ± 0.21*	43.94

Graph- 1: Effect of herbal formulations on the wet weight of cotton pellets

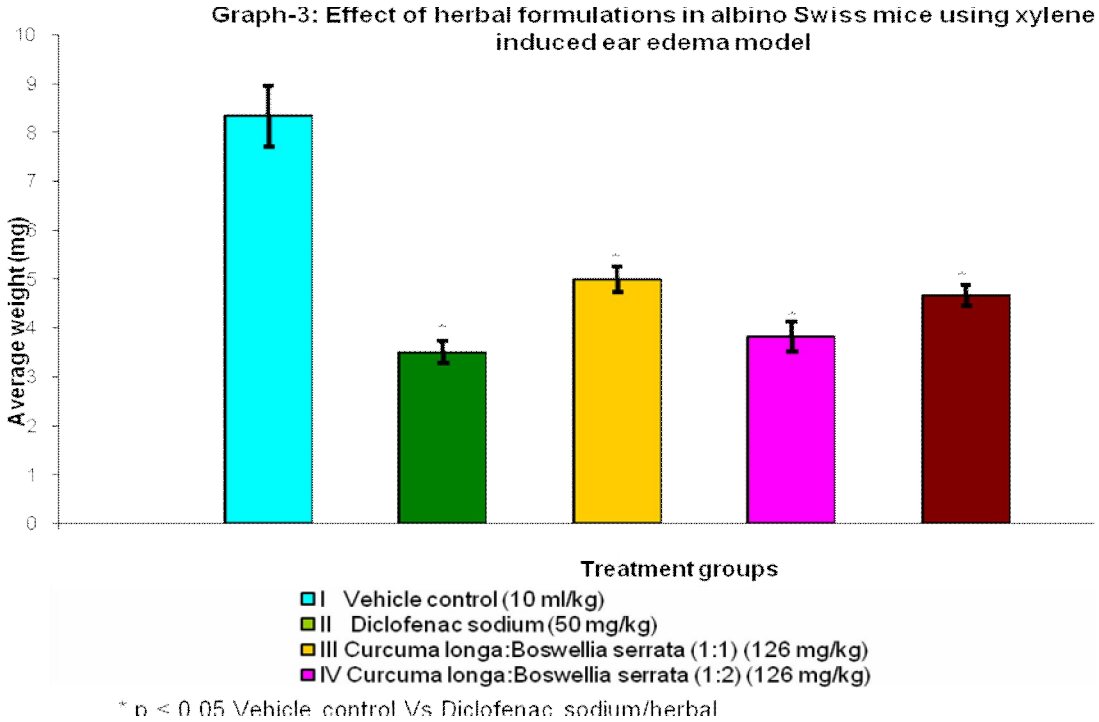


* p ≤ 0.05 Treated groups Vs Vehicle control

Graph-2: Effect of herbal formulations on the dry weight of cotton pellets



* p ≤ 0.05 Treated groups Vs Vehicle



Conclusion:

The herbal formulation containing *Curcuma longa* and *Boswellia serrata* in different proportions (1:1, 1:2, and 2:1) revealed significant anti-inflammatory activity and finally we concluded that ratio (1:2) exhibited better activity and higher potency than other two.

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