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IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF TURMESAC® ON CHEMICALLY INDUCED INFLAMMATION IN EXPERIMENTAL MODELS

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ABSTRACT
The objective of the present study was to evaluate the anti-inflammatory activity of Turmesac® on chemically induced inflammation in experimental animals. Oral administration of Turmesac® in two different doses (250 and 500 mg/kg body weight) by using 5% croton oil induced ear edema and 1% histamine induced paw edema test. Diclofenac sodium (10mg/kg) was used as a standard drug. The anti-inflammatory activity was evaluated using croton oil induced ear edema model in mice and histamine-induced paw edema model in wistar albino rats. The anti-inflammatory activity was found to be dose dependent on croton oil induced ear edema model. The Turmesac® has shown significant ($P < 0.05$) inhibition of ear edema, 58.65% on 2hr and 57.25% on 4hr at the doses of 500 mg/kg, respectively. Similar patterns of paw edema inhibition was seen in histamine induced paw edema model at the doses of 250mg/kg and 500 mg/kg, respectively. In conclusion, the present study demonstrated potent anti-inflammatory activity of Turmesac® in both acute (croton induced ear edema and histamine induced paw edema) inflammation models.

KEYWORDS: Turmesac®, anti-inflammatory activity, croton oil, histamine paw edema, Diclofenac sodium.

INTRODUCTION
Natural products provide a repertory for the discovery of new leads drugs that can be used in treating different types of illnesses such as cancer, inflammation and liver diseases. More than half of these all pharmaceutical products were discovered from natural compounds or their derivatives.[1] In the United States and Europe, approximately 65% of patients use herbal medicines against liver disease, due to their wide availability, low toxicity, pharmacological activity, chemical diversity and low side effects compared to synthetic drugs.[2–4] *Curcuma longa* commonly known as Turmeric has been used as a medicinal herb in India from ancient times. It played a great role in the day-to-day life of ancient Indians as a treatment for wounds, ~~s~~tomach ache, cough, poison *etc*. In addition, it is used for dyeing clothes and in the worship their god and goddesses. This plant has acquired great importance of the modern world with its anti-inflammatory, anticancer, antioxidant, and a variety of other medicinal properties.[5] The rhizomes of *C. longa* contain approximately 2% volatile oils, composed mainly of α and ß-turmerone, monoterpenes[6] (Leung AY et al., 1996), 5% curcuminoids, mainly curcumin[7] (Budavari S, 1996), demethoxycurcumin, bis-demethoxycurcumin and dihydrocurcumin, minerals, carotene and vitamin C.[8] (Kapoor LD, 1990). *C. longa* contribute to the anti-inflammatory and immune-stimulatory activities of NR-INF-02.[9] Therefore, this study has been conducted to evaluate the anti-Inflammatory activity of Turmesac® on chemically induced inflammation in two different animal models.

MATERIALS AND METHODS
Collection of Samples
Turmeric rhizomes were collected from Maharashtra, March 2019, India, and then authenticated by a botanist, R&D division, Star Hi Herbs Pvt. Ltd, Jigani, Bengaluru, Karnataka, India. The turmeric rhizomes were carefully cleaned, dried, powdered, and stored in an airtight container until the extraction procedure.

Preparation of the Turmesac®
Turmesac® is manufactured and registered by Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India.

Drugs and Chemicals Used
Croton oil and histamine was purchased from Hi Media, Mumbai, India. Standard anti-inflammatory drug diclofenac sodium was purchased from Recon, Bangalore, India.

Animals
The studies were carried out using 30 Swiss albino mice (30-35gm each) and 30 male wistar albino rats(180-

200g). They were obtained from the animal house, Bharathi College of pharmacy Mandya, Karnataka, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (Temperature 25 ± 2^0 C) with dark and light cycle (12/12 h). All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment (1135/PO/Re/S/07/CPCSEA).

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Croton oil-Induced Ear Edema.

This experimental procedure was performed using the method of Hosseinzadeh et al.[10] Swiss Albino mice of either sex weighing 30-35 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I Control (normal saline 10 ml/kg), Group II Negative control (2.5% Croton oil), Group III diclofenac sodium (10mg/kg) was used as a standard drug, IV Turmesac® 250 mg/kg and Group- V Turmesac® 500 mg/kg b.wt. The edema was induced in each mouse by applying 50 µL croton oil to the inner surface of the right ear. Ninety minutes after croton oil daubing, the mice were executed by cervical dislocation, and both ears were removed and weighed.[11]

Histamine-Induced Inflammation

The anti-inflammatory activity of the Turmesac® was determined using histamine induced rat paw edema method.[12] Wister albino rats of either sex weighing 180-200 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group -I Control (normal saline 10 ml/kg), Group - II Negative control (histamine), Group III Diclofenac sodium (10mg/kg), IV Turmesac® 250 mg/kg and Group V – Turmesac® 500 mg/kg. b.wt. Diclofenac sodium served as the reference standard anti-inflammatory drug. Turmesac® were administered to rats at 1 hr before the induction of inflammation. Edema was assessed as the difference in paw volume between the control and 0.5, 1, 2, 3, and 4 h after the administration of the inflammatory agent inhibition.

RESULTS

Croton oil-Induced Ear Edema

The topical application of croton oil caused an increase in the weight of the ears of the animals due to the development of edema, as can be observed in the control group (Fig. 1 and Table-1). The edema was induced in each mouse by applying 50 µL croton oil to the inner surface of the right ear. Ninety minutes after croton oil daubing, the mice were executed by cervical dislocation, and both ears were removed and weighed. The Turmesac® reduced the edema significantly when compared to the control ($P < 0.05$ and $P < 0.01$, respectively). Diclofenac sodium10mg/kg body weight used as a standard anti-inflammatory drug. Turmesac® (250 and 500 mg/kg) showed a percentage of inhibition edema reduction of 44.65% (P< 0.5) and 57.25% (P< 0.01) respectively. Percentage inhibition was calculated by using the following formula.

% inhibition = (Difference of ear weight in control group - Difference of ear weight in test group)/Difference of ear weight in control group ×100.

Histamine-Induced Inflammation

Inflammation was induced in rats by the injection of 0.1 mL 0.1% histamine in normal saline into the subplantar tissue of the right hind paw in rats. Test drugs were administered to rats at 1 hr before the induction of inflammation. Control group received 10 mL/kg body weight of distilled water orally. Edema was assessed as the difference in paw volume between the control and 0.5hr, 1hr, 2hr and 4hr after the administration of the inflammatory agent, inhibition. The results were given in the following Table-2 and Figure-2.

Table 1: The effect of Turmesac® on Croton oil induced ear edema. Turmesac® (250 and 500 mg/kg) significantly inhibited Croton oil induced ear edema in mice in a dose-dependent manner, at the interval of 2hr and 4 hr.

Treatment				% inhibition	
	0hr	2hr	4hr	At 2hr	At 4hr
Control	0.306±0.091	0.306±0.019	0.306±0.069	-	
Croton oil	0.308±0.023	0.786±0.039	0.786±0.059	-	
Standard (Diclofenac sodium10mg/kg p.o)	0.310±0.051	0.320±0.029**	0.326±0.019**	59.28%	58.52%
Low dose (250mg/kg p.o)	0.310±0.032	0.425±0.091*	0.435±0.039*	45.92%	44.65%
High dose (500mg/kg p.o)	0.305±0.09	0.325±0.079**	0.335±0.029**	58.65%	57.25%

All values are expressed as mean ± SD; **= P < 0.01, *= P < 0.05 v/s croton oil control

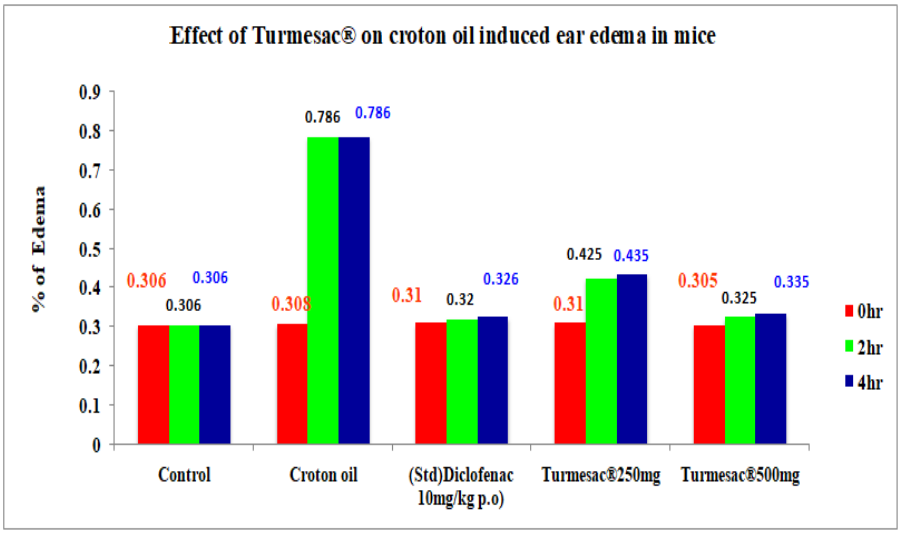

Figure 1: Effect of Turmesac® on croton oil induced ear edema in mice.

Table 2: The effect of Turmesac® on Histamine induced paw edema. Turmesac® (250 and 500 mg/kg) gave significant reduction of histamine-induced paw edema after 1hr, thus, confirming an NSAID-like property.

Treatment	0 hr	0.5hr	1hr	2hr	4hr
Group-I (control-only vehicle)	1.94±0.3	1.9±0.9	1.9±0.9	1.9±0.9	1.9±0.9
Group-II (Toxic control) vehicle + histamine	1.9±0.6	2.6±0.2	3.2±0.4	3.8±1.4	3.8±1.4
Group-III (Diclofenac sodium10mg/kg p.o + histamine)	1.9±0.7	2.3±0.9±0.4**	2.7±0.1**	2.2±0.6**	2.2±0.2**
Group-IV (Low dose (250mg/kg p.o) + histamine)	1.9±0.6	2.6±0.5*	3.0±1.2*	2.9±0.9*	2.8±0.7*
Group-V (High dose (500mg/kg p.o) + histamine)	1.9±0.8	2.1±0.6**	2.8±0.8**	2.3±0.6**	2.3±0.2**

All values are expressed as mean ± SD; **= P < 0.01 *= P < 0.05 v/s Histamine control

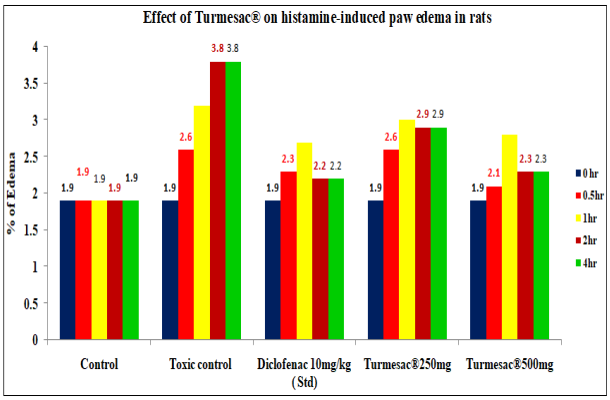

Figure 2: Effect of Turmesac® on histamine-induced paw edema in rats.

DISCUSSION

Acute inflammation is characterized by classical symptoms, such as heat, redness, swelling and pain. Edema (swelling) is therefore a good measure of inflammation and is useful for the quantification of skin inflammation induced by phlogistic agents such as croton oil. Croton oil-induced ear edema is a widely used method for studying the inflammatory process in skin, and for identifying anti-inflammatory agents that could be useful in the treatment of skin disorders.[13,14] In croton oil-induced ear oedema in mice, croton oil contains 12-O-tetracanoilphorbol-13-acetate (TPA) and other phorbol esters as main irritant agents. The TPA is able to activate protein kinase C, which activates other enzymatic cascades in turn such as cyclooxygenase 2 and inducible nitric oxide synthase (Aquila et al. 2009).[15] This cascade of events stimulates vascular permeability, vasodilation, polymorphonuclear leukocytes migration, histamine and serotonin release and moderate synthesis of inflammatory eicosanoids by cyclooxygenase and 5-lipoxygenase enzymes (Wang et al. 2001; Murakawa et al. 2006).[16,17] The effect of Turmesac® on croton oil-induced ear oedema is probably attributed to lipophilic substances that are able to penetrate through the skin barrier (Okoli et al. 2007).[18] Histamine is one of the inflammogens that contributes to acute inflammation and increase of vascular permeability.[19] The present study documents for the first time the ability of Turmesac® to suppress the histamine-induced inflammation in rats. Paw edema assay is a useful tool for investigating agents with potential anti-inflammatory capabilities. The present study showed that exposure of rat's paw to histamine triggered an elevation in fluid extravasations from micro vessels in the vicinity, an event that led to tissue swelling. The results showed that Turmesac® was able to significantly suppress histamine-induced paw edema in rats, and this may probably due to the inhibition of the H_1 receptor or other signaling molecules down its pathway. Histamine H_1 receptor expressed in endothelial cells of blood vessels is important for regulating vascular permeability[20], and increased permeability occurs once it is activated.

Vascular endothelial growth factors (VEGFs) are known as key regulators of permeability and binding to their receptors trigger an increase in vascular permeability.[21] Upregulation and downregulation of VEGF expression contributes to abnormal angiogenesis indirectly leading to vascular hyperpermeability.[22] Furthermore, overproduction of VEGF also contributes to progression to other diseases such as coronary disease[23] and cancer.[24] Ghosh and colleagues[25] demonstrated that histamine enhanced the production of VEGF during acute inflammation. VEGF release and its above-mentioned effects on vascular permeability and cellular infiltration could contribute to edema formation. Thus, it is very important to regulate VEGF production in cells either by suppression when it is overproduced or vice versa. Our results showed that the oral administration of Turmesac® in rats decreased the histamine-induced VEGF production, indicating that Turmesac® may have the potential to regulate vascular permeability by monitoring the expression of VEGF.

The overall results showed that Turmesac® exerted a significant anti-inflammatory activity in different models of inflammation.

CONCLUSION

In conclusion the Turmesac® showed significant anti-inflammatory effects in croton induced ear edema and histamine induced paw edema inflammation models.

Our results revealed that administration of Turmesac® inhibited the edema starting from the first hour and during all phases of inflammation, which is significantly inhibition of different aspects and chemical mediators of inflammation.

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