Anti inflammatory and wound healing activity of *Curcuma aromatica* salisb extract and its formulation

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Abstract

Anti-inflammatory and wound healing activity of topical application of Curcuma *aromatica* Salisb. rhizome extract and is cream formulations in Arachidonic acid -induced ear inflammation and excision wound model was confirmed in albino mice. The extraction of these rhizomes was carried out by ethanol. The ethanol extract and formulations exhibited significant anti-inflammatory activity in arachidonic acid - induced ear inflammation. It also showed significant wound healing activity in excision wound model. Thus, resultant anti-inflammatory activity might be due to effects on several mediators and arachidonic acid metabolism involving cyclo-oxygenase pathway resulting in prostaglandin synthesis.

Key Words: Anti-inflammatory, Arachidonic acid, Excision wound model.

Introduction

Curcuma aromatica salisb. which belongs to the Zingiberaceae family, is a perennial herb. Traditionally it is used as a anti-inflammatory agent. Many reports have suggested useful pharmacological properties of *Curcuma* drugs such as anti-inflammatory [1]. anti-tumor [2] and immunological effects [3]. Traditionally, since the pharmacological effects of curcuminoids, especially curcumin, have been investigated, such as radical scavenging [4], the inhibition of nitric oxide (NO) [5, 6] anti-inflammation [7], anti-tumor [8], anti-allergy [9] and anti-dementia [10]. Pharmacological studies of other *Curcuma* species were very few, because botanical origins of *Curcuma* drugs could not be easily identified due to similarity of morphology, and variety of naming derived from used parts and producing areas. The present investigation deals with anti-inflammatory and wound healing activity of *Curcuma aromatica* salisb. rhizomes.

Experimental Section

The dried rhizomes of *Curcuma aromatica* Salisb. (Family: Zingiberaceae) were obtained from local market at Pune. The sample was identified by comparing the botanical description available in the literature and the same was authenticated by Agharkar Research Institute, Pune. Glyceryl monostearate (Ranbaxy Pvt. Ltd.Goa) as a gift sample. Methyl paraben &Propyl paraben (Get-Rid Pvt. Ltd, Poona College of Pharmacy), CMC- sodium salt pure (Merck chemicals, Bombay, India) and white soft paraffin and liquid paraffin. Other the minor chemicals of sq. grade were obtained from quiligens. Arachidonic acid (Sigma Chemicals Co., St. Louis, USA), Phenidone (Fluka, Switzerland), Acetone (Merck Limited, Mumbai, India.). Standard povidone-iodine cream (Betadine, Win Medicare Ltd.)

The Pharmacological work was carried out as per Committee for Purpose of Control and Safety on Experiments on Animals (CPCSEA) guidelines for maintenance of experimental animals. Protocols for Pharmacological evaluation for anti-inflammatory activity and wound healing activity are approved by Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune.

Swiss albino mice were used from animal house facilities at Poona College of Pharmacy. They were housed in propylene cages in an air-conditioned area at 25±2º C temperature with 10:14 hour light and dark cycle. They were given Amrut brand balanced animal feed and water ad *libitum*. The optimum condition for experiments was decided on the basis of pilot experiments carried out using three animals per group. For further experiment a group of at least six animals was used for individual treatment. For anti-inflammatory study the thickness of mouse ear was measured by using a micrometer (Digitrix mark II, Japan).

The Results of all experiment were expressed in Mean ± SE. The data were analyzed using one–way analysis of variance (ANOVA) followed by Dennett's test. P.values <0.05 were considered significant.

Extraction procedure

At a time, 200gm air-dried rhizome of *Curcuma aromatica Salisb*. were reduced to fine powder and extracted in Soxhlet apparatus till completely exhausted with Ethanol (solvent). The extraction process is carried out 10 hours for complete extraction. The extracts were concentrated under reduced pressure to a dry residue. Weight of extract was recorded. The yield of extract for *Curcuma aromatica* was found to be 8.55 %.

Preparation of formulations [11]

Both o/w and w/o cream formulation were prepared. The Glyceryl monostearate, liquid paraffin, white soft paraffin was weighed accordingly and melted at 60°C and then add Propyl paraben and ethanol extract 1 gm or 2 gm for 1% and 2% cream formulation respectively. CMC-sodium and Methyl paraben was added in the previously weighed quantity of purified water (0.1 N HCL is added to adjust pH between 4-5 in case of o/w formulation) and was heated to 60 °C on water bath both are mixed and triturate to make cream. This was then homogenized by mechanical stirrer.

Arachidonic acid induced topical ear inflammation [12]

In control group an edema was induced on the right ear by topical application of 2mg/ear of Arachidonic acid in 20µl of acetone. The left ear acts as a control received the vehicle acetone. Control group received only Arachidonic acid treatment. Group 2 received Phenidone 1mg/ear were used as a positive control applied to right ear simultaneously with AA. Group 3 & 4 received Extract 0.5 & 1mg/ear dissolved in acetone (20µl), applied on right ear simultaneously with AA. Group 5 & 6 received 1% w/o and 2% w/o cream formulation (0.05 g/ear) respectively simultaneously with AA. Left ear act as a control received w/o cream base. Group 7 & 8 received 1% o/w and 2% o/w cream formulation (0.05g/ear) respectively simultaneously with AA application. In all group left ear act as a control receiving equal volume of vehicle. The edema was measured initially and after 1 hour, after challenges of phlogestic agent to assess an increase in the ear thickness**.**

Excision wound model [13]

The parameter studied includes wound closer, time of epithelization and scar size. The percentage wound closure of original wound area in different group of mouse were recorded at the intervals of 4^{th}, 8th, 12^{th} and 16^{th} day of post wounding. For the excision wound study, Swiss albino mice weighed 25-30g were used. The animal divided in to nine groups of six animals each. Group 1 served as control, Group 2 & 3 receiving o/w and w/o cream base respectively. Group 4 receiving standard povidone-iodine cream and group 5 receiving extract (5mg/animal). While group 6 & 7 receiving o/w 1% and o/w 2% test cream formulation and group 8 & 9 receiving w/o 1% and w/o 2% cream formulation respectively. The animals were kept fasting overnight prior to the initiation of experiment.

An excision wounds was inflicted by cutting away approximately 2 cm diameter full thickness of shaved skin of a predetermined area on the anterior-dorsal side of each mouse, under light Ether anaesthesia, in semi aseptic condition. The wound was left undressed. The animals were housed individually in separate cage and were treated once daily. The observation of percent wound closure were made on 4, 8, 12, 16^{th} post wounding day. The rate of contraction of wounds was measured by tracing the wound surface on to a transparent paper and then measuring the surface area using graph paper and expressed as percent of original wound size.

The wounds were monitored for complete epithelization. Epithelization time was measured in days from wounding day (0^{th} day) till the day eschar totally separated itself with no raw wound left behind. The wound was also examined for scar features.

Table: 1 Anti-inflammatory activity of *Curcuma aromatica* extract and formulations on arachidonic acid induced topical inflammation

Treatment	Difference in ear thickness (mm±S.E)	% Inhibition of inflammation
Control	0.176±0.005	--
Std drug (phenidone) 1mg/ear	0.047±0.005***	73.29
Extract (0.5mg/ear)	0.092±0.007***	47.72
Extract (1mg/ear)	0.068±0.005***	61.36
1% w/o cream formulation	0.095±0.008***	46.02
2% w/o cream formulation	0.055±0.007***	68.75
1% o/w cream formulation	0.108±0.003***	38.63
2% o/w cream formulation	0.075±0.003***	57.38

n = 6 < 0.001***----- More Significant

Table: 2 Effect of *Curcuma aromatica* extract and formulations on wound healing in albino mice

	4th day	8th day	12th day	16th day
Group	% Area closer Mean ± S.E.	% Area closer Mean ± S.E.	% Area closer mean ± S.E.	% Area closer Mean ± S.E.
Control	19.76 ± 1.83	50.54 ± 1.57	78.60 ± 0.43	85.92 ± 0.54
Base o/w cream	23.86 ± 0.67^{ns}	54.41 ± 1.02^{ns}	78.91 ± 0.34^{ns}	87.42 ± 0.98^{ns}
Base w/o cream	28.17 ± 0.35***	56.31 ± 0.73***	77.46 ± 0.44^{ns}	84.79 ± 0.43^{ns}
Standard cream	43.07 ± 1.24***	79.09 ± 0.51***	96.01 ± 0.22***	99.12 ± 0.0***
Extract 5mg/animal	45.64 ± 1.96***	75.18 ± 0.87***	88.52 ± 0.68***	96.13± 0.69^{ns}
o/w 1% cream formulation	38.32 ± 1.57***	62.18 ± 1.71***	75.06 ± 0.85***	83.59 ± 0.66^{ns}
o/w 2% cream formulation	44.41 ± 1.94***	66.34± 0.62***	80.94± 0.74*	89.15± 0.94^{ns}
w/o 1% cream formulation	44.50 ± 1.21***	73.10± 0.76***	91.63± 0.37***	97.47± 0.51^{ns}
w/o 2% cream formulation	47.92 ± 1.18***	79.87 ± 0.70***	96.60 ± 0.17***	99.23 ± 0.0***

n = 6, < 0.1*-----Insignificant, < 0.001***----- More Significant, ns ----- not significant

Table: 3 Effect of *Curcuma aromatica* extract and formulations on epithelization time of wound

Groups	Time (in days) Mean ± S.E.
Control	20.67 ± 0.42
Base o/w cream	20.33 ± 0.56^{ns}
Base w/o cream	20.67 ± 0.42^{ns}
Standard cream	15.83 ± 0.31***
Extract	18.00± 0.36***
o/w 1% cream formulation	20.17 ± 0.48^{ns}
o/w 2% cream formulation	19.17± 0.31^{ns}
w/o 1% cream formulation	17.83± 0.31***
w/o 2% cream formulation	16.00 ± 0.26***

< 0.001***----- More Significant, ns ---- not significant

Table: 4 Effect of *Curcuma aromatica* extract and formulations on wound scar size

Groups	Area (sq.mm. %) Mean ± S.E.
Control	10.20 ± 0.08
Base o/w cream	9.03 ± 0.21^{ns}
Base w/o cream	7.69 ± 0.15***
Standard cream	7.14 ± 0.36***
Extract	7.74± 0.16***
o/w 1% cream formulation	7.93 ± 0.53***
o/w 2% cream formulation	7.31± 0.26***
w/o 1% cream formulation	6.91± 0.19***
w/o 2% cream formulation	5.85 ± 0.24***

< 0.001***----- More Significant, ns ----- not significant

Results and Discussion

Ethanolic extract and cream formulations found to show significant anti-inflammatory and wound healing activity in animal models.

Table no. 1 indicates that during the course of study it was found that difference in ear thickness, in case of control was 0.176±0.005. Percentage inhibition of inflammation in case of extract was found to be dose dependent i.e. inhibition of inflammation increases with increase in the dose, which was found to be 47.72 % with 0.5 mg/ear and 61.36 % with 1mg/ear respectively. Percentage inhibition of inflammation with all the formulation was found to be significant when compared with standard drug (phenidone). 2% w/o cream formulation shows highest inhibition of inflammation with respect to all cream formulations and it was comparable to standard drug (phenidone).

This topical model also showed that the left ear thickness was unchanged when treated with cream formulation and it concluded that the cream formulation are safe and does not show any irritation when applying it on skin.

The parameter studied includes wound closer, time of epithelization and scar size. The percentage wound closure of original wound area in different group of mouse were recorded at the intervals of 4^{th}, 8th, 12^{th} and 16^{th} day of post wounding. Table no. 2 indicates on 4^{th} day, reduction in wound size in control group was 19.76± 1.83 %. On the other hand in animal treated with test o/w 1% and 2% cream formulation (38.32±1.57 and 44.41±1.94 respectively). w/o 1% and 2% cream formulation (44.50±1.21 and 47.92±1.18), the rate of wound closure was increased significantly. A more significant increase in the rate of wound closure was observed on and after day 4, in animal treated test cream formulation

Table no. 3 indicates the mean period of epithelization in control group was 20.67±0.423 days. On the other hand, in animal treated with test o/w 1% and 2% cream formulation, the mean period of epithelization was 20.17 ± 0.477 and 19.17±0.307 days respectively. In case of w/o 1% and 2% cream formulation the epithelization time was 17.83±0.307and 16±0.258 days respectively. Thus a significant decrease in the period of epithelization was observed when compared to control.

Table no. 4 indicates that scars in control animals remained wide and poorly contracted while those in animals treated with test formulation were narrow and well contracted.

Conclusion

Ethanolic extract and cream formulations found to show significant anti-inflammatory and wound healing activity in animal models. It can be concluded that *Curcuma aromatica* is a medicinal plant with a wide range of biological activity, which can be used for the preparation of various formulations for treatment of inflammation, wound and microbial infections.

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