Anti-inflammatory Activity of *Curcuma Amada* Roxb (Rhizome) Extract on Albino Mice

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Abstract

Inflammation is one of the complex biological processes exhibited by body tissues against harmful stimuli given by convinced pathogens, irritants and or damaged cells. Acute and chronic are two main types of inflammations found. First one includes body's first line response against harmful stimuli and which is followed by the increased movement of plasma and leukocytes around inflamed regions leading to oedema and characterized by redness in specified area. Prolonged inflammation is known as chronic inflammation. By the other hand Anti-inflammatory compounds are those who lower or minimize inflammation when applied internally or externally. Steroidal drugs are well known for their quick biological response, but they also known to provoke lot of side effects. In search of less harmful and less toxic compounds, phyto medicines are found to be very effective and promising drugs without side effects. By the last decade *Zingiberaceae* family has gained lot of phytochemical interest due to secondary metabolites with different biological activities in it. Belonging to the same family member *Curcuma amada* is traditionally used as carminative and stomachic. Literature survey revealed the presence of multiple chemical constituents in these rhizomes. However, very few references were available for the pharmacological evaluationof topical anti-inflammatory activity. Hence it was, decided to screen rhizome extract for its topical anti-inflammatory activity on albino mice by modified mercury displacement method.

KEYWORDS: Albino mice, *Curcuma amada*, Inflammation, Topical anti-inflammatory activity,

Introduction

Inflammation is one of the complex biological processes exhibited by body tissues to harmful stimuli given by convinced pathogens, irritants and or damaged cells. There found to be five classical signs of inflammation theyare heat, pain, redness, swelling and loss of function (Marina *et al.*,2008). Therefore Inflammation on the other hand describes only body's immune-vascular response, whatever the cause may be. Inflammation is a series of series of biochemical events which propagates and matures the body's response, involving the local vascular system, the immune system, and various cells within the injured tissue. From this one may consider as progressive tissue destruction by the harmful stimulus and compromise the survival of the organism. There are mainly two types of inflammations found they are acute and chronic.First one includes body'sfirst line response against harmful stimuli and

whichis followed by the increased movement of plasma and leukocytes around inflamed regions leading to swelling (oedema) and characterized by redness in specified area.Prolonged inflammation is known as *chronic inflammation* which leads to a progressive shift in the type of cells present at the site of inflammation leading to detrimental diseases like hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Therefore inflammation normally regulated by the body. (Mohini*et al*., 2011; Wu *et al*., 2000).

Anti-inflammatory drugs are those who lower or minimize inflammation (swelling) when applied internally or externally. Steroidal drugs are well known for their quick biological response, but they also known to provoke lot of side effects (Dama and Jadhav, 1998) Therefore research is being made for finding non-steroidal drugs(Ahmad and Ahmad, 1991)Ibuprofen, Diclofenac and many more are some common examples of non-steroidal anti-inflammatory drugs (NSAIDS) group famous for rapid response against inflammation. But recently they also found show lethal allergic responses. In search of less harmful and less toxic compounds, phytomedicines are found to be very effective and promising drugs without side effects.

By the last decade *Zingiberaceae* family has gained lot of phytochemical interest due to presence of secondary metabolites with different biological activities(Dama and Jadhav, 1998). Numbers of plants from this family are noted, listed in Ayurveda and Unani (Golap and Bandyopadhyaya, 1984). Being the same family member *Curcuma amada* is traditionally used as carminative and stomachic. Literature survey revealed the presence of multiple chemical constituents in these rhizomes. However, very few references about the evaluation of pharmacological activity of the extract are available indicating other biological activities (Wu *et al*., 2000). Hence it was, decided to screen rhizome extract for its topical (External) anti-inflammatory activity on albino mice (taken as an animal mode) by using modified mercury displacement method. Very less information was available about its topical anti-inflammatory in spray and ointments formulations.

Materials and Methods

Collection and identification of plant

Fresh rhizomes were collected directly from the field and were authenticated by the Botany group at D.B.F Dayanand college of Arts and science Solapur(M.S).

Extraction and partial purification of plant material

Nearly 1 kg of dried samples were cut into small pieces and immersed in methanol. Extracts were drawn (1L approximately) at the intervals of 72 hours till the sticky substance was obtained (Dama*et al*., 2016; Policegoudra, 2008).The collective extracts were concentrated under reduced pressure when the crude extract was obtained as orange, thick and fragrant liquid after column chromatography with Hexane: Acetone solvent fractions. TLC and preparative TLC of the crude extract (n-hexane-acetone, 6:4) showed a streak orange colour line.(Dama andJadhav 1998).

Anti-inflammatory activity

The anti-inflammatory activity of formulations was evaluated by the carrageenan-induced mice hind paw oedema method. Here 0.1ml of 1% carrageenan was injected in sub planter region of left hind paw. Subsequently after 30 min of injection swelling was noted. The crude extract was suspended in 90% alcohol and 5 % DMSO for topical application to mice.The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IEAC Ref. No. 7/AB/2017) (Reg. No. 1822/PO/RcBiBt/S/15 /CPCSEA) was obtained.Healthy albinomice of either sex weighing between 20-25 g were identified and obtained from the disease free animal house of Aarya Biotech at Dhule (M.S). All animals were housed in institutional animal house under standard conditions with free access to food and water Marina *et al.*, (2008).

Thirty six mice were divided into 6 groups of 6 mice each for various treatments as shown in Table 1Wu*et al.*, (2000). The paw volume was measured initially and at time interval of 1to 4 h after topical (External) application.Mercury displacement method was used for measuring paw volume. The percentage inhibition of inflammation was calculated using following formulae

Percentage inhibition = [(C-T)/C] x 100]

Where, C = control paw oedema(carrageenan induced) , T = test paw oedema (Spray applied).

Spectral analysis

The spectroscopic analysis of this extract was carried for the determination of bio actives as suggested by Pathade*et al.*, (2009). 1H NMR Spectrometer using CdCl3 as internal solvent. All pharmacological screening of the crude extract was carried out using standard protocols developed and designed by Dama*et al*., (2016)

Statistical analysis

Statistical analysis will be carried out using one way ANOVA for getting significant values($p< 0.05$) as discussed by Dama*et al*., (2016). (SAS 2003).

RESULTS

Extraction and partial purification of plant material

Total of 84.2g, (0.976%) of crude drug was extracted from 1 kg of dried sample.Six fractions from Colum chromatography were isolated (A-F) as argued by Tariq *et al*(2016). Among six, fractions E was chosen for the anti-inflammatory activity similar kind of work has been discussed by Dama*et al.*, (2016). Accordingly the same fraction was chosen for further spectral study. Fraction E had shown following characteristic features in spectroscopic studies.

Anti-inflammatory activity

The extract as well as Diclofenac in spray form showed anti-inflammatory activity when tested topically. In this case anti-inflammatory activity was dose-dependent and

found to be statistically significant at the less concentration, 25mg/kg, (Table 1). The anti-inflammatory activity of Diclofenac, a standard reference drug, was also found to be significant as discussed by Mohini*et al.*, (2011)

Table 1 Anti-inflammatory activity of *Curcuma amada* rhizome extract on mice left paw at 1-4 hour time interval

Animal (n=6)	Average paw volume in hour (Mercury displacement in ml) with Mean ± S.D				Average% Inhibition After 4 hr	Significance ($p<0.05$)
	1	2	3	4		
Group I Carrageenan	0.30± 0.3	0.30±0.05	0.29±0.05	0.27±0.05	-	-
Group II Diclofenac (10mg/kg body weight)	0.28±0.00	0.27±0.03	0.26±0.02	0.23±0.03	80.0±0.2	Yes
Group III Curcumin std. (25mg/kg body weight)	0.26±0.02	0.26±0.03	0.25±0.03	0.24±0.05	76.5± 0.3	Yes
Group IV *Curcuma amada** (25mg/kg body weight)	0.27±0.06	0.27±0.05	0.27±0.05	0.26±0.03	68.5± 0.1	Yes

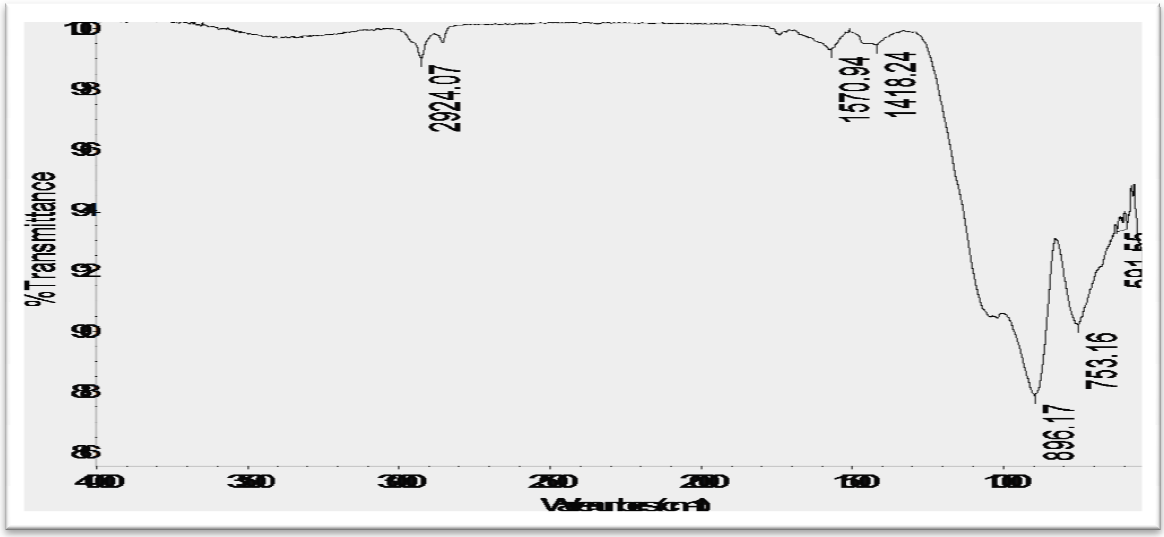
*Crude drug concentration 25 mg /kg body weight

A B

Figure 1. Anti-inflammatory activity on albino mice
(A-Administration of drug B-Evaluation of activity by mercury displacement)
Spectral analysis of E fraction

The UV spectrum showed maximum absorption at λ= 240.8 nm (weak chromo-phore) (Figure 2).FT-IR structural analysis revealed board peaks in the range of 3359 cm^{-1} and 1105 cm^{-1} shows the presence of OH group and phenolics and alkaloids in

fraction as also shown by Dama*et al.*, (2016).Thus spectral data of the crude extract showed the absence of conjugated chromophore and presence of hydroxyl, carbonyl, ester and olefinic functionalities in it.Its 1H NMR spectrum showed less prominent signals around with carboxyl, hexane impurity earlier discussed by Jadhav andDama., (1997).



Thu Dec 17 15:39:45 2015 (GMT+05:30)
Peak values :

Spectrum:	*Curcuma amada*		
Region:	4000.12	525.03	
Absolute threshold:	99.627		
Sensitivity:	50		
Peak list:			
Position:	591.55	Intensity:	93.509
Position:	753.16	Intensity:	90.203
Position:	1418.24	Intensity:	99.441
Position:	1570.94	Intensity:	99.283
Position:	2924.07	Intensity:	99.001

Figure 2. FTR Investigation

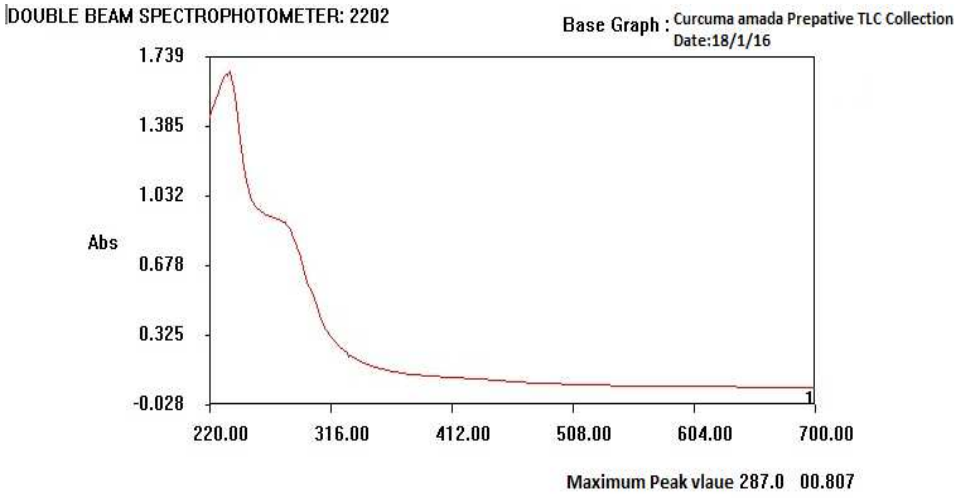


Figure 3. UV /VIS Investigation of partially purified extracts

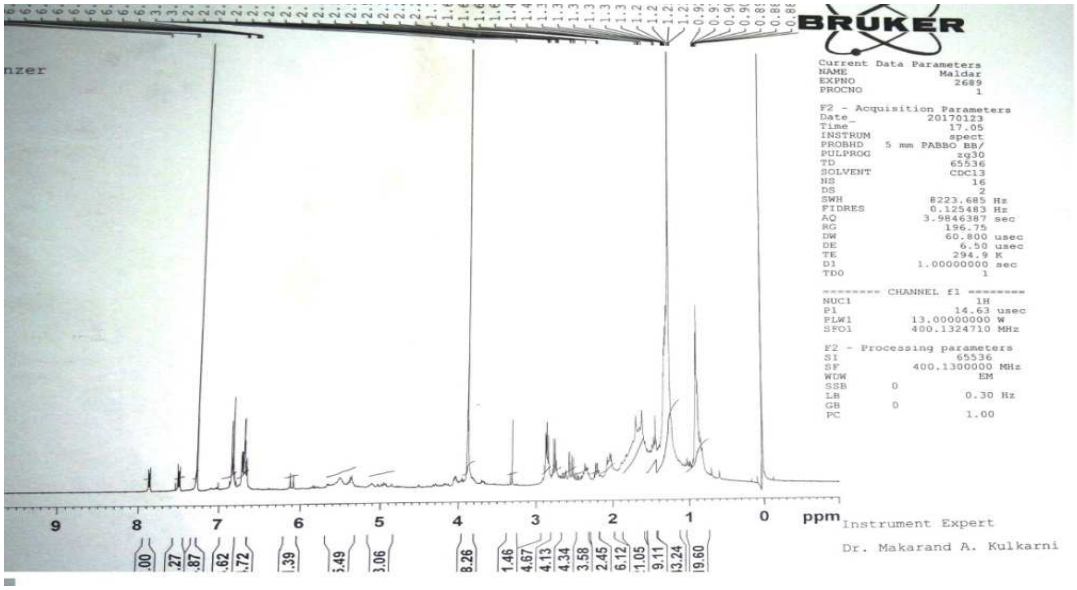


Figure 4.1H NMR Investigation of partially purified extracts

Statistical analysis

All observations and results were scrutinized using one-way analysis of variance. All Dunnett's test showed overall significance at $p< 0.05$.Here number of animals in each group was kept 6, whichalso statisticallysignificantSAS(2003).

Discussion

Being neglected *Curcuma amada* pose a high potentiality as topical anti-inflammatory drug for treating localized inflammationas it numerously has active secondary metabolites,as shown byGolap (1984). Synergistic effect with other known medicinal plants has to be studied Ahmad *et al*., (1991). It may be well formulated with other solubilizing agents like isopropyl alcohols for effective diffusivity across skin. Among all fractions of Colum, fraction 'E' shows maximum biological activity as also shown by Dama*et al*., (2016).Present investigation was intended to check topical anti-inflammatory activity only.Skin irritation on different area has not seen but when injected orally this drug may have allergic reactionpredicted by Tariq *et al*., (2016).Drug concentration may be the key factor for activityJadhav (1997).According to the study crude drug concentration of 25 mg/kg drug proves to be effective. This concentration may vary upon purification Marina *et al*., (2008). Till date no single literature has been known with the same attempts,So more attempts should have to be made to improve quality of results.This may proves a new effective way for effective drug delivery

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