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A *Curcuma angustifoila* : Phytopharmacological Study for the Treatment of Inflammation

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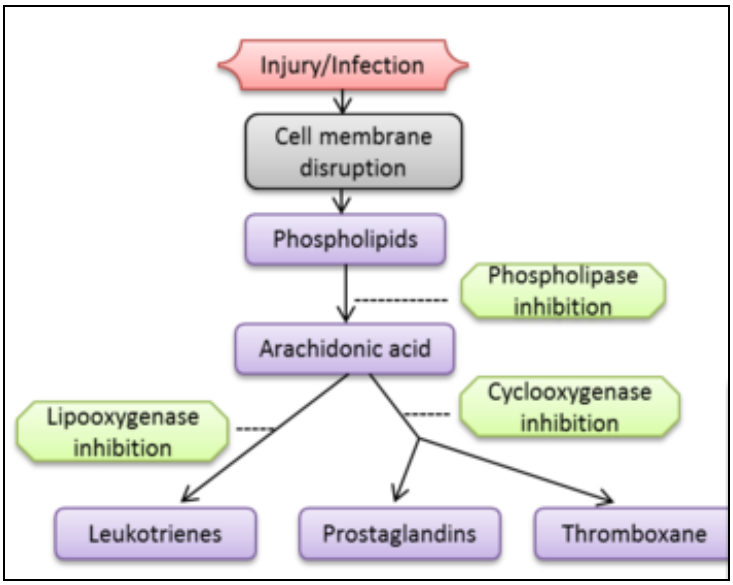
ABSTRACT

Inflammation is a complex biological response of vascular tissues to adverse stimuli such as bacteria, damaged cells, and irritants. It is distinguished by redness, swollen joints, joint discomfort, stiffness, and the absence of joint characteristics. NSAIDs are currently used to treat inflammation. The majority of NSAIDs are carboxylic acid derivatives, such as aspirin, carboxylic and heterocyclic acid derivatives (indomethacin), propionic acid derivatives (ibuprofen, ketoprofen, flurbiprofen), and phenylacetic acid derivatives (phenylacetic acid) (diclofenac). These organic acid-containing medicines block arachidonic acid (AA) from reaching the active site of the enzyme, effectively stopping the cyclooxygenase process. Unfortunately, despite the fact that NSAIDs have great anti-inflammatory properties, serious adverse effects such as GI ulceration, perforation, blockage, and bleeding have limited their therapeutic use. Hydroalcoholic extract rhizomes of *Curcuma angustifolia* administered at doses of 200 mg/kg p.o. and 400 mg/kg p.o. inhibited carrageenan-induced paw oedema by 61.36 percent and 70.45 percent, respectively, while Diclofenac sodium at a dose of 10 mg/kg inhibited carrageenan-induced paw oedema by 88.75%. As a result, the development of effective anti-inflammatory medications derived from natural sources is currently being considered.

INTRODUCTION

An intricate biological process that occurs when viruses, damaged cells, or irritants cause harm to vascular tissues including inflammation. It can be identified by redness, swollen joints, pain in the joints, stiffness, and lack of any joint characteristics. At the moment, NSAIDs are used to treat inflammation. These pills unfortunately increase the risk of blood clots, which can lead to heart attacks and strokes. An ordinary, protective response to tissue injury brought on by toxic chemicals, microbiological agents, or physical trauma is inflammation.



Causes of Inflammation

Types of Inflammations

Inflammation can be divided mostly into two categories:

a. Acute inflammation: It is connected to enhanced capillary infiltration, vascular permeability, and leukocyte emigration.

b. Chronic inflammation: It is connected to fibroblast activation, proliferation (angiogenesis), and fibrosis as well as the infiltration of mononuclear immune cells, macrophages, monocytes, and neutrophils.

NSAIDS (Non-Steroidal Anti-Inflammatory Drugs)

Patients with osteoarthritis and other painful conditions have relied on non-steroidal anti-inflammatory drugs (NSAIDs) as their go-to pain reliever for years. Non-steroidal anti-inflammatory medicine prescriptions make up the majority of the most often prescribed medications in the USA, accounting for 5% of all doctor visits. Rofecoxib, marketed as a selective inhibitor of cyclo-oxygenase-2 (COX-2), was taken off the market in 2004 after results of a randomized placebo-controlled trial revealed an increased risk of cardiovascular events linked to the medication. This location is supported by further studies and a comprehensive meta-analysis [1-2].

MATERIAL AND METHOD

Raw materials, including plants, animals, and minerals, are used to make crude pharmaceuticals. Their physical and chemical features should be accurately defined and characterized, and their quality should be upheld.

Plant collection: In the month of January 2019, rhizomes of *Curcuma angustifolia* were gathered from the neighborhood in Bhopal, Madhya Pradesh.

Extraction of plant material: Plant material was extracted using a soxhlet apparatus with ethanol and water (80:20 v/v) hydroalcoholic for 48 hours, filtered, and dried in a vacuum evaporator at 400°C. The plant material was dried powdered rhizomes of Curcuma angustifolia.

Determination of percentage yield

The percentage yield of each extract was calculated by using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Table 1: % Yield of hydroalcoholic extract

S. No.	Solvent	% Yield (w/w)
1.	*Curcuma angustifolia*	5.998

Phytochemical Screening: Using the standard procedures, phytochemical tests were conducted on all of the extracts.

1. *Identifying alkaloids*: Each extract was separately treated in diluted hydrochloric acid and filtered.

2. Mayer's Test: Filtrates have been subjected to Mayer's reagent handling (Potassium Mercuric Iodide). Alkaloids are present when a precipitate turns yellow, indicating their existence.

Wagner's Test: Filtrates had undergone Wagner's reagent treatment (Iodine in Potassium Iodide). Alkaloids are present because brown or reddish precipitate forms.

Dragendroff's Test: Dragendroff's reagent has been used to deal with the filtrates (answer of Potassium Bismuth Iodide). Alkaloids are present when red precipitate is formed.

Hager test: Filtrates were treated with Hager's reagent for the Hager test (saturated picric acid resolution). Alkaloids are present, as evidenced by the precipitate's yellow colour.

Carbohydrate detection: In my opinion, the extracts had been diluted in 5 ml of pure water and filtered. The presence of carbohydrates was checked for in the filtrates.

Molisch's Test: Two drops of alcoholic -naphthol solution were applied to filtrates in a test tube. The violet ring that forms on the junction indicates the presence of carbohydrates.

Benedict's Test: Filtrates were handled with Benedict's reagent and gently heated. The precipitate that is orange or red indicates that there are lowering sugars present.

Fehling's Test: Filtrates had been hydrolyzed with dilute HCl, neutralized with alkali, and heated with Fehling's A & B solutions. The presence of decreasing sugars is shown by the formation of red precipitate [3-5].

Table 2: Result of Phytochemical screening of the hydroalcoholic extract

S. No.	Constituents	*Curcuma angustifolia*
1.	Alkaloids	-ve
2.	Glycosides	-ve
3.	Flavonoids	+ve
4.	Diterpenes	+ve
5.	Phenolics	+ve
6.	Amino Acids	-ve
7.	Carbohydrate	+ve
8.	Proteins	-ve
9.	Saponins	+ve
10.	Oils and fats	-ve

3. Glycoside detection: After extracts were hydrolyzed with diluted HCl, they were tested for glycosides.

4. Modified Borntrager's Test: Extracts were handled with an answer containing ferric chloride and submerged in boiling water for about five minutes. The mixture was previously chilled and extracted using equal parts benzene. Once separated, the benzene layer was subjected to an ammonia solution treatment. The ammonical layer's formation of rose-crimson colour indicates the presence of anthranol glycosides.

Legal's Test: Sodium nitroprusside in pyridine and sodium hydroxide were used to treat the extracts. Purple to blood-red colour formation denotes the presence of cardiac glycosides.

5. Detection of saponins

Froth test: Extracts were diluted with distilled water to a volume of 20 ml and agitated for 15 minutes in a graduated cylinder. The presence of saponins is indicated by the formation of a 1 cm layer of froth.

Foam Test: 0.5 g of extract and 2 cc of water were once shaken to create foam. The presence of saponins is indicated if the foam formed lasts for 10 minutes [6-7].

6. Phytosterol detection

Salkowski's Test: Chloroform had been used to handle and filter the extracts. The filtrates were treated with a few drops of concentrated sulfuric acid, agitated, and then faced. Golden yellow coloration shows triterpenes are present.

Libermann Burchard's test: Chloroform has been used to treat and filter the extracts. A few drops of acetic anhydride were added to the filtrates before they were heated and chilled. Sulfuric acid used to be transported. The development of a brown ring at the junction is a sign of phytosterols[8].

7. Phenol detection

Test for ferric chloride: Extracts were subjected to a 3–4 drop treatment with ferric chloride resolution. Phenols are present when a bluish-black colour forms.

8. Tannin detection

Gelatin Test: A 1% sodium chloride-containing gelatin solution was added to the extract. The presence of tannins is shown by the formation of white precipitate.

9. Detection of flavonoids

Test using an alkaline reagent: Extracts were treated with a few drops of the sodium hydroxide solution. The formation of a bright yellow colour that becomes colourless when diluted acid is added indicates the presence of flavonoids.

Test with lead acetate: Extracts were treated with a few drops of lead acetate solution. The presence of flavonoids is indicated by the precipitate's yellow colour[9,10].

10. Protein and amino acid detection

Xanthoproteic Test: A few drops of concentrated nitric acid were used to handle the extracts. Proteins may be present, as shown by the formation of a yellow hue.

Ninhydrin Test: A 0.25 percent w/v ninhydrin reagent was added to the extract and heated briefly. Blue colour formation shows the presence of an amino acid.

11. Detection of diterpenes

Test with copper acetate: After extracts were dissolved in water, they were treated with three to four drops of a copper acetate solution. Diterpenes are present when the emerald-inexperienced colour forms [11].

Qualitative chromatographic Analysis [12-14]

Thin Layer Chromatography

The adsorption phenomenon is the foundation of thin layer chromatography (T.L.C.). In this form of chromatography, the stationary phase's surface is crossed by the mobile phase, which contains the dissolved solutes.

T.L.C. steps include:

1) Plate preparation

2) Plate activation

3) Chamber preparation and saturation

4) Illustration of an application and development

5) Rf Value detection and computation

Extract TLC was carried out and reported.

Preparation of Plates [15]

1. The most popular stationary phase, silica gel, was used for T.L.C. adsorption.

2. To lessen band broadening, the stationary phase should be composed of uniformly sized, microscopic particles that have a high surface area for interaction and a low void volume.

3. A slurry of silica gel and water was created.

4. Spread the slurry evenly around the plate to coat it.

5. The plate was air-dried for a while before being maintained for activation.

Activation of Plates

1. By baking the plates for 30 minutes at 100 to 110 °C.

2. For solutes to move linearly across the stationary phase, activation is required.

Preparation and Saturation of Chamber

1. Get the solvent system ready.

2. Poured it into the chamber and lined it with filter paper that had been moistened with the mobile phase to fill the chamber.

Application and Development examples

1. The sample, which might be in the range of a few g to mg, was applied to the plates with the aid of a capillary tube after the plates had been activated.

2. To a depth of about 0.5 cm, plates were inserted into the chamber containing the developing solvent.

3. Following the removal of the plates from the chamber, the solvent was evaporated in an oven while the mobile phase front was marked on the surface by scratching.

Detection and Calculation of Rf. Value [16-18]

The Rf Value of the spot was determined using the formula after the chromatogram had been generated.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Table 3: TLC of extracts

S. No.	**Toluene: Ethyl acetate: Formic acid (5:4:1) Quercetin (R_f value)**	**Toluene: Ethyl acetate: Formic acid (7:5:1) Gallic acid (R_f value)**
1.	0.714	0.678

Total phenolic and flavonoid content estimation[19-21]

Estimate of the total phenolic content:

The modified Folin-Ciocalteu technique was used to determine the extract's total phenolic content.

Procedure: Folin-Ciocalteu reagent was diluted with distilled water 1:10 v/v before being added to 2 ml of extract or standard, along with 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15 seconds before being left to stand for 15 minutes to develop the colour. To measure the absorbance, a spectrophotometer was used at 765 nm.

Calibration Curve of Gallic acid

Table 4: Preparation of calibration curve of Gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

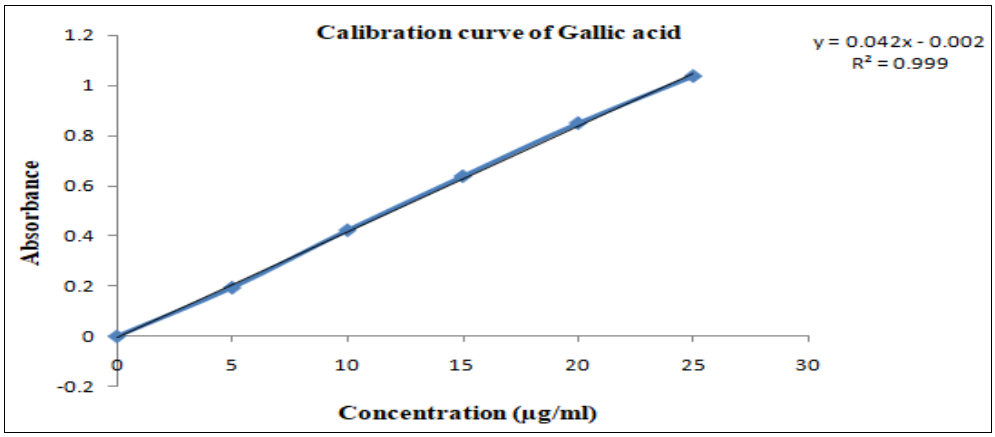


Figure 1: Graph of Estimation of Total Phenolic content

Estimation of the Total Flavonoids content

After adding 3 ml of extract or standard to 1 ml of a 2 percent AlCl3 methanolic solution, the mixture was allowed to stand for 15 minutes at room temperature. The absorbance was then measured at 420 nm.

Estimation of the total flavonoid content:[22-24]

The aluminum chloride method was used to determine the total flavonoid content.

Procedure: After adding 1 ml of a 2 percent AlCl3 methanolic solution to 3 ml of an extract or a standard, the mixture was allowed to rest for 15 minutes at room temperature before the absorbance at 420 nm was measured.

Total flavonoids content estimation

3 ml of extract or standard were combined with 1 ml of a 2 percent AlCl3 methanolic solution, which was then given 15 minutes to stand at room temperature. The absorbance at 420 nm was then determined.

Calibration Curve of quercetin

Table 5: Preparation of calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

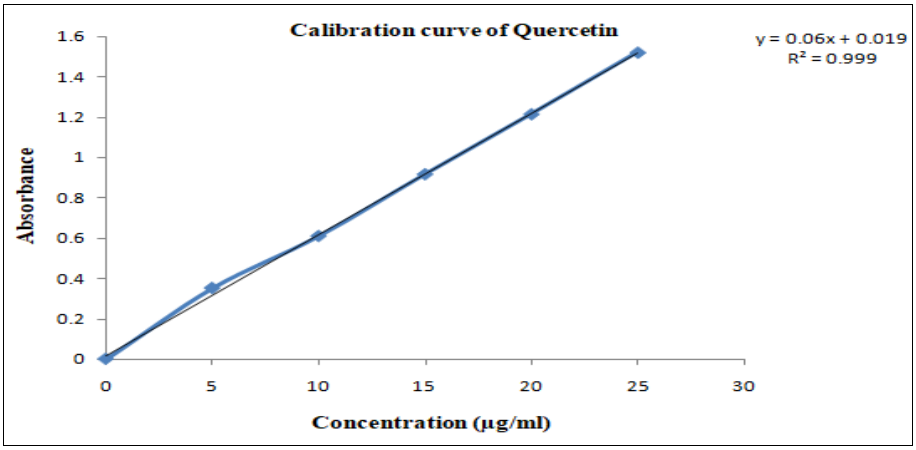


Figure 2: Graph of Estimation of Total flavonoid content

Table 6: Total Phenolic and Total flavonoid content of Hydroalcoholic extract

S. No.	Compound	*Curcuma angustifolia*
1.	Total Phenol (Gallic acid equivalent (GAE)mg/100mg)	0.412
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.964

Anti-inflammatory activity

Wistar rats (180–200 g) were housed in groups of six (n = 6) and maintained in regulated conditions of humidity and temperature (25–2 °C, 55–65 percent). Regular rodent food and unlimited water were given to the rats. Prior to the experiments, rats were given a 7-day acclimatization period in the lab. From 8:00 AM until 5:00 PM, all studies were conducted in a quiet room. For each series of studies, a distinct group of rats (n=6) was used. The Ministry of Environment and Forests, Government of India, New Delhi, India, established the Institutional Animal Ethics Committee (IAEC) for the aim of controlling and supervising the use of experimental animals [25-29].

Toxicity study

Rats (n = 6) were used in the preliminary trials. To determine the range of doses that result in 0% to 100% animal death, Curcuma Angustifolia hydroalcoholic extract rhizomes were given orally in a variety of doses. The method used by the Organisation for Economic Co-operation and Development (OECD) for studying acute oral toxicity was followed. Animals were kept fasting and only given water. Extract was supplied orally for 4 days to different groups of rats

(n=6) in doses of 500, 1000, and 2000 mg/kg/p.o. The animals were monitored for mortality as well as any behavioural changes to assess a potential anti-inflammatory impact[30-32].

Table 7. Effect of Hydroalcoholic extract Rhizomes of *Curcuma angustifolia* on paw oedema induced by carrageenan in rats

Group	Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Group I	Control(Normal Saline)	0.1 ml NS	-	-
Group II	Negative Control(carragenan (1%w/v)	0.1 ml of 1% (w/v) treated with carragenan (1%w/v) in saline	0.88 ± 0.07	2
Group III	Positive control Diclofenac sodium10mg/kg I.P	10	0.17 ± 0.02	88.75
Group IV	HE CA(200mg)	200	0.34 ± 0.06	61.36
Group V	HE CA(400mg)	400	0.26 ± 0.03	70.45

Values are expressed as mean ± SD.*P < 0.05-significant compared to carragenan treated group.

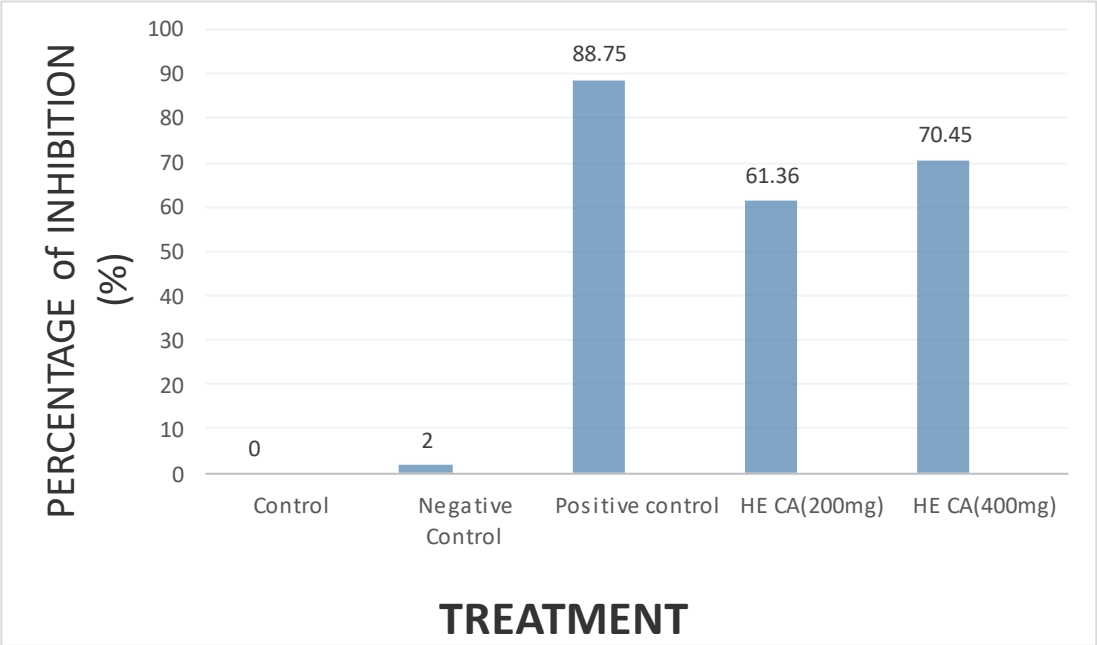


Figure 3: Effect of Hydroalcoholic extract Rhizomes of *Curcuma angustifolia*on

Carrageenan-induced hind paw oedema

Using an experiment to generate paw oedema in rats, carrageenan's anti-inflammatory efficacy was quantified. The rats were split into five groups of six each (plant extract was dissolved and administered per oral at different dose levels). Group 1 served as the usual control. Group 3 was a Positive Control and received Diclofenac Sodium (10 mg/kg, IP) as the Positive Control. Group 2 was treated as the Negative Control (0.1 ml of 1 percent (w/v) of carragenan was injected in saline in the subplanter region of the right hind paw). Curcuma angustifolia hydroalcoholic extract rhizomes (200 mg/kg, p.o.) were administered to Group 4. Curcuma angustifolia hydroalcoholic extract rhizomes (400 mg/kg, p.o.) were administered to Group 5. The right hind paw's subplantar area was injected with 0.1 ml of a 1 percent solution of carrageenan in saline to cause oedema[33-35].

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group

Vt- Edema volume of test group

Table 8 : DPPH scavenging activity and anti-inflammatory activity represented by IC50 (µg/ml)

Group	**Treatment**	**DPPH IC50 (µg/ml)**
Group I	Control	0
Group II	Negative control (carrageenan)	0.88 ± 0.03
Group III	Positive control (Diclofenac Sod.)	0.17 ± 0.02
Group IV	HECA(200)	0.34 ± 0.02
Group V	HECA(400)	0.26 ± 0.03

Values are expressed as mean ± SD.*P < 0.05-significant compared to treated group.

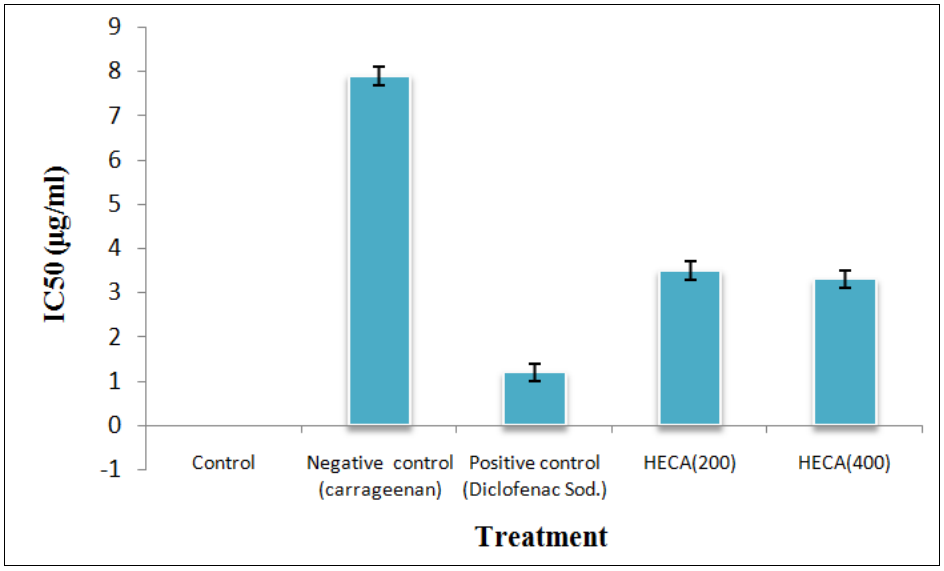


Figure 4: IC50 determination of Hydroalcoholic extract Rhizomes of Curcuma angustifolia of lipoxygenase (LOX) inhibition

Statistical Analysis

Graph Pad Prism for Windows was used to do all of the analysis. Mean standard error of the mean is how statistical analysis is always expressed (SEM). When appropriate, p0.05 was used to determine statistical significance in the one-way ANOVA comparison to the vehicle, which was then followed by Dunnett's test.

***In vitro* cyclooxygenase (COX) and lipoxygenase (LOX) inhibition assays**

The extract of ethanol The initial test used rhizomes of *Curcuma angustifolia* extracts to look into the anti-inflammatory response by blocking COX-2 and LOX enzymes. When arachidonic acid and N,N,N', N'-tetramethyl-p-phenylenediamine (TMPD) were combined, the COX activity was measured on a spectrophotometer at 611 nm. The concentration inducing a 50% inhibition of the enzyme was used to measure the effectiveness of extracts and reference compounds to inhibit COX-2 isoenzymes (IC50). Monitoring of the rise in absorbance at 234 nm was used to measure the inhibition of LOX from Glycine max (type I-B; EC 1.13.11.12).In a nutshell, 50 l of the various extract concentrations were pre-incubated at 25 °C for 15 min. with 100 l of soybean LOX solution (1000 U/ml in borate buffer solution, pH 9) and 3000 l of borate buffer. 100 l of linoleic acid (the substrate) were introduced to each tube after the pre-incubation period to kick-start the reaction. The formula (E S)/E 100, where E represents the enzyme's activity without the test sample and S represents the enzyme's activity with the test sample, was used to calculate the inhibition

percentage of LOX. Reference compounds included quercetin. From the displayed graphs of enzyme inhibition (percent) against the concentrations of the extracts, IC50values—the inhibitory concentration of the extracts needed to reduce the enzyme's activity by 50%—were calculated [36-37].

RESULT AND DISCUSSION

In a carrageenan-induced paw edoema model, the hydroalcoholic extract of Curcuma angustifolia's rhizomes and the usual medication were evaluated against carrageenan control. While diclofenac sodium at a dose of 10 mg/kg reduced carrageenan-induced paw edoema with a percentage inhibition of 88.75 percent, hydroalcoholic extract Rhizomes of *Curcuma angustifolia* demonstrated 61.36 percent and 70.45 percent inhibition, respectively. The HECA at a concentration of higher dose was shown to have a promising COX-2 inhibitory response when compared to control among all the herbal extracts examined for in vitro COX-2 inhibitory effect 68. Table 7,8 shows the COX-2 inhibition of the different extracts. By examining the COX-2 inhibitory activity of the HECA at a concentration of higher dose, it was possible to examine the role of COX-2 inhibition. The results were encouraging and revealed the HECA's potential for COX-2 inhibitory activity. As a result, it may be said that COX-2 inhibition may be one of the mechanisms underlying HECA68's anti-inflammatory effects. Numerous inflammatory and allergic illnesses have been linked to the pathophysiology of LOXs, according to research69. It is widely known that throughout the inflammatory process, reactive oxygen radicals are created. Plant lipoxygenase 2 is known to be inhibited by antioxidants (such as polyphenolics and flavonoids). It was discovered that quercetin has the strongest LOX inhibitor activity. The IC50 value was 1.2 0.7 ng/mL. However, it was shown that HECA (200) had the lowest LOX inhibitor activity69 (3.5 1.9). The purpose of this study is to develop more potent anti-inflammatory and antioxidant products. For the first time, anti-inflammatory activity is shown here. It is attributed to the antioxidant and phenolic components' capacity to suppress the pro-inflammatory mediator [38].

CONCLUSION

When vascular tissues are exposed to damaging stimuli like pathogens, damaged cells, or irritants, their complicated biological reaction includes inflammation. One of the best test methods for evaluating anti-inflammatory medications is carrier-induced acute inflammation. A biphasic curve depicts the progression of edoema in carragenan-induced edoema over time.

In order to assess the impact of non-steroidal anti-inflammatory drugs, which largely inhibit the cycloxygenase involved in prostaglandin synthesis, carrageenan-induced paw edoema is used. 200 mg/kg and 400 mg/kg p.o. of Curcuma angustifolia hydroalcoholic extract demonstrated 61.36 percent and 70.45 percent inhibition, respectively, while 10 mg/kg of diclofenac sodium prevented carrageenan-induced paw edoema with a 74.0 percent inhibition rate.

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