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Original research article

Biphasic inhibition of Carrageenan-Induced Paw Edema Model in Rats by Curcuma longa Extract.

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ABSTRACT

Aim: This study aimed to investigate the anti-inflammatory activity of Curcuma longa (turmeric) alcoholic extract in a carrageenan-induced paw edema model in rats. **Materials and Methods:** The alcoholic extract of C. longa was prepared and subjected to phytochemical screening and acute oral toxicity studies. The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in rats. Animals were administered with the extract (200 mg/kg body weight) or vehicle control one hour before carrageenan injection. Paw edema was measured at various time intervals, and the percentage inhibition of edema was calculated. **Results:** The extract exhibited the presence of volatile oils, terpinolene, sugars, carbohydrates, phenols, resins, and alkaloids. Acute oral toxicity studies revealed no mortality or significant changes in behavior up to a dose of 2 g/kg body weight. In the carrageenan-induced paw edema model, the extract significantly reduced paw edema compared to the control group, with a maximum inhibition of 34.74% observed at 6

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

hours. **Conclusion:** The alcoholic extract of C.longa demonstrated significant anti-inflammatory activity in the carrageenan-induced paw edema model, supporting its potential use in the treatment of inflammatory conditions. Further studies are needed to optimize the dosage and elucidate the exact mechanism of action.

KEYWORDS: Curcuma longa, anti-inflammatory, carrageenan, paw edema, oral toxicity.

INTRODUCTION: Inflammatory diseases include different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and osteoarthritis. An array of drugs are available in the market to treat these disorders, but only very few are free from toxicity. Gastrointestinal problems associated with the use of anti-inflammatory drugs are still an enduring dilemma of medical world. Profound research with ethnobotanical plants possessing anti-inflammatory and analgesic properties can definitely open up new vistas in the treatment of inflammatory disorders. Purified natural compounds from plants can serve as template for the synthesis of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value. Hence drug derived from natural products, particularly from medicinal plants like C. longa are believed to be a vital source of chemical substances that have good potential therapeutic efficacy with fewer side effects[1].

Turmeric (Curcuma longa) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan EWC et al., 2009)[2]. It is native to tropical South Asia and needs temperatures between 20 °C and 30 °C (68 °F and 86 °F) and a considerable amount of annual rainfall to thrive. Curcuma longa is a perennial herb distributed throughout tropical and subtropical regions of the world. It is widely cultivated in Asian countries, mainly in India and China. As turmeric powder it has been in continuous use for its flavouring, as a spice in vegetarian and non-vegetarian food preparations and has digestive properties (Somchit, M.N et al., 2004)[3]. The traditional medicine in China uses C. Longa in abdominal pain. Religious ceremonies still use turmeric in many forms. The major components of turmeric are curcuminoids which include mainly curcumin (diferuloyl methane), dimethoxy curcumin and bisdimethoxycurcumin. These substances can be classified as Curcuminoids, the analogues of diarylheptanoids. Traditional Indian medicine claims the use of turmeric powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis[1]. Ammon H P T et al., (1992) reported turmeric powder has been extensively used for the treatment of sprains and swellings. Similarly in Ayurveda, turmeric

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

has been used for various medicinal conditions including rhinitis, wound healing, common cold, skin infections, liver, and urinary tract diseases and as blood purifier (Mukhopadhyay A et al., 1982, Aggarwal BB et al., 2004)[4,5&6].

It was found to be effective even when given by different routes of administration, including topically, orally and by inhalation. Based on the extensive use from time immemorial by ancient civilization till date by traditional healers and as household flavouring substance, concerns about toxicological profile will be the least. Hence this study is planned to test the pharmacological activity (anti-nociceptive & anti-inflammatory) of C. Longa extract, using scientifically approved animal model for inflammation, Carrageenan Induced Hind Paw Edema in Rats.

MATERIAL & METHODS

Plant Collection and Identification

The rhizomes of C.longa were collected from a traditional medicine shop. It was transferred immediately to the laboratory. The plant materials were shade-dried for one week. After drying the plant materials were used for experiments. The shade-dried plant material was powdered with the help of mixer grinder. The fine particles were separated and stored in a clean container. It was used for further analysis. The sample was authenticated by the Department of Pharmacognosy, C.L.Baid Mehta pharmacy college, Chennai. Samples were deposited at the Department of pharmacology, Meenakshi Medical College & Research Institute Kanchipuram, for future reference.

Preparation of Extract and its Fractions

The rhizomes of plant C.longa were shade-dried, and made into coarse powder. It was then passed through a 40 mesh sieve before extraction. A weighed quantity (1 kg) of coarse powder was soaked in 4.5 liters of absolute alcohol for 3 days at room temperature. This process was repeated thrice to get complete extraction. The extract was then evaporated to dryness under reduced pressure using rotary flash evaporator. The extract was weighed and subjected to various phytochemical and pharmacological evaluations.

Acute Oral Toxicity Study

Acute oral toxicity study was performed according to Organization for Economic Cooperation and Development (OECD 423) guidelines[7]. The acute oral toxicity method

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

followed is a stepwise procedure with 3 animals per step. This procedure results in the use of minimal number of animals while allowing for acceptable data which is based on scientific conclusion. The method is based on biometric evaluation with fixed doses (5, 50, 300, 2000 mg/kg body weight) and the results allow the substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of extracts which causes acute toxicity. The dose of the present study is based on the previous studies of curcuma longa by Srimal RC et al (1973), wherein positive results in rodent models with the dose of 50- 200 mg/kg observed. Similarly, dose selection was made by observing that Neha S et al., (2009) administered the dose of 100 and 200 mg/kg and the study exhibited positive results. Based on the positive effects exhibited by the above studies, dose of 200 mg/kg bd.wt was selected for the present study.

Procedure

Swiss albino Male rat weighing 100-150 g were used for the study. Tween 80 1% v/v was used as vehicle to suspend the ethanolic extract of C.longa. Rat were divided into control and test groups of three each. The test groups received a single oral dose of alcoholic extract of C.longa at a dose of 300 mg/kg and 2000 mg/kg bodyweight. Control group received 1%v/v of tween 80. Food was withheld for further 3 to 4 hours after oral administration of drugs. The rate of mortality was checked every hour and upto 24 hours and the study was extended upto 14 days.

Assessment of the Anti-inflammatory Activity.

Carrageenan Induced Hind Paw Edema in Rats[8]: (Gerhard vogel H et al., 2002)

Wistar male rats (100-150 g) six per group were used for the study. All the animals were fasted overnight and grouped as mentioned below. Edema was induced by injecting Carrageenan (0.1 mL of 1% w/v solution) subcutaneous into the sub-plantar surface of the right hind paw. Control group received 1%v/v tween 80 (1 mL /100 gm) orally. Indomethacin (10 mg/kg/i.p) was included as a reference drug 71 for comparison of paw diameter and was administered half an hour prior to carrageenan injection. The test group received orally alcoholic extract of C.longa (200 mg/kg/bd.wt) sixty minutes prior to carrageenan injection. The diameter of paw was measured by using digital vernier calliper immediately after administration of carrageenan and at 3 and 6 hr intervals.

Edema (ΔT) was calculated as follows:

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

$\Delta T = T_t - T_0$

T_t is the right hind paw thickness in mm at time t,

T_0 is the right hind paw thickness before sub-plantar injection.

Percentage (%) reduction of edema was calculated as follows: Mean edema in untreated control group – mean edema in drug treated group/ mean edema in untreated control group x 100 (C – T / C x 100) v. Statistical Analysis: The results were expressed as Mean ± S.E.M. The statistical comparison was performed using one-way analysis of variance (ANOVA). The study was approved by institutional animal ethics committee-MMCRI, Kanchipuraam- Tamil Nadu and the study was conducted during the period April 2010-2013.

RESULTS

Phytochemical Screening

The preliminary phytochemical screening of the extract shows the presence of volatile oils, terpinolene, sugars, carbohydrates, phenols, resins and alkaloids in the alcoholic extract.

Sl No	Constituents	Test	Observation	Inference & Intensity
1.	Alkaloids	Mayer's reagent	Milky precipitate	+
2.		Wagner's reagent	Reddish brown precipitate	+
		Picric Acid (1%)	Yellow precipitate	+
3.	Carbohydrates	Fehling A&B	Red precipitate	++
4.	Phenols	Liebermann's test	Deep green colour	+ +
5.	Protein	Ferric chloride test	No changes observed	-
6.	Glycosides	Foam test	No foam observed	-
7.	Saponin	Emulsion test	No emulsion	-

(+) denotes the presence of the respective class compound, (-) denotes the absence of the respective class compound

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

Acute oral toxicity study : There was no significant alteration in autonomic or behavioral responses in the rat treated with alcoholic extract of Curcuma longa. No mortality was recorded in these animals upto 14 days. Thus, the alcoholic extract was found to be non-toxic upto a dose of 2g /kg body weight.

Anti-inflammatory activity : Carrageenan induced hind paw edema

Intraplantar carrageenan administration increased the diameter of the paw significantly over the period of observation in vehicle treated control animals(Table 1).The increase was significantly less at all observation periods with Indomethacin treatment. A maximum of 50.42% inhibition was observed at six hours. In a similar fashion, treatment with alcoholic extract of Curcuma longa also attenuated the increase in paw diameter, due to carrageenan administration. A maximum of 34.74% inhibition was observed at 5th hour.

Table:1 Effect of Curcuma longa extract on Carrageenan induced paw edema

Groups (n=6)	1st hour (t)	2nd hour (t)	3rd hour (t)	4th hour (t)	5th hour (t)	%Decrease in paw volume at 5th hour
Vehicle (Tween 80, 1% v/v, p.o)	1.37±0.05	2.18±0.09	2.26±0.09	2.35±0.09	2.38±0.10	--
Diclofenac 10 mg/kg, s.c,	1.28±0.01	1.52±0.04*	1.41±0.03*	1.28±0.02*	1.19±0.01*	50.00
C.longa alcoholic Extract 200 mg, p.o	1.29±0.01	1.81±0.0*	1.82±0.0*	1.70±0.0*	1.60±0.0*	32.77

n- number of animals, t-time, *P < 0.05; (compared with one way ANOVA). Figures in parentheses represent percentage inhibition of inflammation)*

DISCUSSION

In India, Curcuma Longa has been in use as a culinary ingredient since 3000 BC. It is used as a food colouring for curry and as a preservative for food. As a medicine, it is used to treat a wide variety of ailments including abdominal pain, skin problems, muscular problems and arthritis. Curcuma Longa has also been used as a clothing dye and as a cosmetic. Indians are thought to consume between 8 to 20 mg per day of Curcuma Longa extract. India as a whole consumes 480,000 tons of turmeric annually. In China it has been used as a topical analgesic,

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

and for colic, hepatitis, ringworm infection and chest pain. In Europe it is used in many foods, as a colouring in cheese, margarine, beverages and cakes. In the recent past it has been used for dyspepsia, chronic anterior uveitis and Helicobacter pylori bacteria. It is generally recognized as safe by the FDA of the United States. (Ravindran PN et al., 2007)[9]. Based on the above traditional claims the present study was undertaken to scientifically validate the traditional claims of Curcuma longa with particular reference to its antinociceptive and anti-inflammatory effects.

Today, the classification into central and peripheral analgesics is definitively too simplified, but provides a guide for differentiation by pharmacological methods[10]. Mostly, rodents such as rat or rats are used for analgesic tests, but in some instances experiments in higher animals such as monkeys are necessary[11].

Acute toxicity of C.longa is investigated with the objective to identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality, according to regulatory guidelines (OECD). In acute toxicity testing no mortality was observed in rat even in a dose of 2 g/kg of alcoholic extract of C.longa, which indicates the safe nature of the extract as it correlates with the traditional use for centuries.

Administration of Curcuma longa extract has consistently reduced the paw edema in carrageenan rat model (Table-1). Carrageenan induced paw-edema has a biphasic effect[12]. The first phase (0-3hr) is due to release of histamine and serotonin, plateau phase is maintained by a kinin like substance (3 hours) and late phase (4.5-6 hr) of inflammation is attributed to prostaglandin[13]. C.longa has exhibited statistically significant inhibition at 3rd hour and at 6th hour. In the initial phase, the mast cells are activated and de-granulated releasing histamine and serotonin. These mediators increase the vascular permeability of blood vessel that facilitates the infiltration of neutrophils, accumulation of plasma fluids and proteins into the interstitial spaces. This is followed by the release of kinins after certain time. These events lead to the development of oedema in control rodents, whereas the same events are inhibited by standard Indomethacin 10 mg/kg, s.c and by C.longa. This observation indicates the potent anti-inflammatory effect of alcoholic extract of Curcuma longa. C.longa was challenged with Carrageenan induced paw-edema model which has gained greater importance and support over the years, because edema induced by carrageenan is reported to have been inhibited by majority of the steroidal and the non-steroidal anti-inflammatory

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

drugs. In the similar fashion, Alcoholic extract of C.longa demonstrated anti-inflammatory activity by inhibiting carrageenan induced paw edema in both the phases.

CONCLUSION

The alcoholic extract of C.longa on preliminary phytochemical screening revealed the presence of volatile oils, terpinolene, sugars, carbohydrates, phenols, resins and alkaloids. There was no significant alteration in autonomic or behavioral responses in the rat treated with the alcoholic extract of C.longa. No mortality was recorded in these animals upto 14 days. Thus, the alcoholic extract was found to be non-toxic upto a dose of 2 g/kg body weight in acute toxicity studies. Intraplantar carrageenan administration increased the diameter of the paw significantly over the period of observation in vehicle treated control animals, wherein the treatment with alcoholic extract of Curcuma longa attenuated the increase in paw diameter by a maximum of 34.74% inhibition as observed at 6^{th} hour. Anti-inflammatory activity at 200 mg/kg by significant biphasic inhibition on Carrageenan induced paw-edema. To conclude, the present study indicates the multifaceted action of anti-inflammatory activity of C.longa for therapeutic efficacy for inflammation and confirms the traditional claims by standardized scientific methods of evaluation.

REFERENCES

1. Govindarajan VS. Turmeric--chemistry, technology, and quality. Crit Rev Food Sci Nutr. 1980;12(3):199-301.

2. Chan, E.W.C. et al.; Lim, Y; Wong, S; Lim, K; Tan, S; Lianto, F; Yong, M. "Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species". Food Chemistry. 2009; 113 (1): 166–172.

3. Somchit, M.N., Sulaiman, M.R., Zurani., A., Samsuddin, L., Somchit, N., Israf, D.A., and Moin, S. 2004, I. J.Pharmacol. 36, 377.

4. Ammon HPT, Anazodo MI, Safayhi H, Dhawan BN, Srimal RC 1992. Curcumin: a potent inhibitor of Leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). Planta Med 58: 26.

5. Mukhopadhyay A, Basu N, Ghatak N, Gujral PK. Anti-inflammatory and irritant activities of curcumin analogues in rats.Agents Actions 1982 Oct;12(4):508-15.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE3, 2024

6. Aggarwal BB, Shishodia Suppression of the Nuclear Factor-{kappa}B Activation Pathway by Spice- Derived Phytochemicals: Reasoning for Seasoning. Ann N Y Acad Sci. 2004 Dec;1030:434-41.

7. Ecobichon, D.J. (1997) the Basis of Toxicology Testing. RC Press, New York, 4386 p.

8. Vogel, H. Gerhard. Drug Discovery & Evaluation, Pharmacological Assay, 2002, 2nd Edition, pp. 696, 697, 759-760, 772.

9. P.N. Ravindran, K. Nirmal Babu, K. Sivaraman, Eds. Medicinal and Aromatic Plants - Industrial Profiles, Volume 45. 2007. CRC / Taylor & Francis, Boca Raton, FL.. 54. 36-38.

10. Bannwarth B., Labat L., Moride Y., Schaeverbeke T. Methotrexate in rheumatoid arthritis. An update. Drugs. 1994; 47:25–50.

11. P F Vonvoigtlander, G J Kolaja and E M Block. Corneal lesions induced by antidepressants: a selective effect upon young Fischer 344 rats. Journal of Pharmacology and Experimental Therapeutics July 1, 1982, 222 (1) 282-286.

12. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 1971 May;104(1):15-29.

13. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998;38:97-120.

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