Curcuma longa Linn (Fam : Zingiberaceae) has been used in indigenous medicine for decades since ancient times. Studies of turmeric have revealed numerous pharmacological activities including antioxidant, anti-inflammatory, antiparasitic, antimutagenic, anticancer, chemoprotective, hepatoprotective, antimicrobial and antiviral properties. Turmeric has been known for centuries, and is now known to be one of the useful requirements for over the past years as one of the useful requirements for most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. The anti-inflammatory activity of turmeric has been known for centuries, and is now known to be due to the component curcuminoids. These compounds have been reported to regulate a number of inflammatory mediators and enzymes such as transcription factors, cytokines, protein kinases and cyclooxygenases.

**INTRODUCTION**

Natural pigment is a vital quality attribute of foods, and plays an important role in sensory and consumer acceptance of products. Curcumin, is a naturally occurring polyphenolic phytoconstituent, isolated from the rhizomes of Curcuma longa Linn (Fam : Zingiberaceae). It is an important permitted natural colorant used in food, nutrition and pharmaceutical preparations. Curcumin is chemically (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione. It has a pKa1, pKa2, and pKa3 value of 7.8, 8.5 and 9.0 respectively for three acidic protons. It is insoluble in water under acidic or neutral conditions but dissolves in alkaline conditions. Curcumin is unstable undergoing rapid hydrolytic degradation in neutral or alkaline conditions to feruloyl methane and ferulic acid. It is insoluble in aqueous medium and has poor stability towards oxidation, light, alkalinity, enzymes and heat. Exposure of curcumin pigment to alkaline foods or ingredients is difficult to avoid. Hence, it is essential to protect it before its industrial application. Complexation of curcumin with transition metals has attracted much interest over the past years as one of the useful requirements for the treatment of Alzheimers ‘s disease and in vitro antioxidant activity. All these metallocomplexes of curcumin have been prepared under relatively high temperature synthesis conditions and in the presence of organic solvent.

The rhizome of turmeric (Curcuma longa L., Zingiberaceae) has been used in indigenous medicine for the treatment of inflammatory disorders since ancient times. Studies of turmeric have revealed numerous pharmacological activities including antioxidant, anti-inflammatory, antiparasitic, antimutagenic, anticancer, chemoprotective, hepatoprotective, antimicrobial and antiviral properties. Inflammation plays a major role in most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. The anti-inflammatory activity of curcumin has been known for centuries, and is now known to be due to the component curcuminoids. These compounds have been reported to regulate a number of inflammatory mediators and enzymes such as transcription factors, cytokines, protein kinases and cyclooxygenases.

**MATERIALS AND METHODS**

**Plant material**

The rhizomes of Curcuma longa was purchased from local market and authenticated by Dr.A.S. Upadhye, Scientist, Agarkar research institute, Pune and a voucher no. R-123 was allotted.

**Extraction, Isolation and Analysis of Curcumin**

A 95% alcoholic extract of turmeric powder was used to extract curcuminoids. The curcuminoids in alcoholic extract was purified using column chromatography in the solvent chloroform: methanol. Curcumin was recovered from the fractions and was recrystallized with ethanol. The isolated curcumin was characterized by its m.p, UV, FTIR and HPLC studies. Analysis of curcumin was studied by HPLC on silica C18 column using the mobile phase acetonitrile: water (90:10) and detection at 425nm. A standard curve was prepared in the range of 20-100ppm.

**ABSTRACT**

The purpose of present study was to stabilize curcumin by its complexation with divalent ions like Fe²⁺ and evaluate its stability in vitro compared to curcumin alone. The curcumin complex was prepared by mechanical mixture of curcumin and ferrous sulfate (metal: curcumin- 1:1mol) into unconventional and nontoxic propylene glycol/water solvent. On thermogravimetric analysis, Fe-curcumin complex was thermally stable up to 65°C. Then, the sample weight of complex decreased up to 205°C which is due to the elimination of coordinated water molecule from curcumin. On evaluation of in vitro stability, the complex was found to provide a higher stability than curcumin alone. As an indication, at buffer pH 7.0, curcumin was totally degraded after 1 hour, while; less than 5% of complex was degraded. The anti-inflammatory activity of Fe-curcumin complex was evaluated in a carrageenan-induced rat paw edema test and compared with that of indomethacin. Rats treated with Fe-curcumin (equivalent to 200mg of curcumin/kg b.w.) showed a reduction in carrageenan-induced paw edema with significant inhibitory effects of 22.46-59.76% (p<0.05).

**Keywords:** Curcumin, Fe-curcumin complex, stability, anti-inflammatory
Figure 1: UV spectrum of curcumin and Fe-curcumin complex.

Figure 2a: IR spectrum of Curcumin

Figure 2b: IR spectrum of Fe-curcumin complex

Figure 3a: Thermal analysis of Curcumin

Figure 3b: Thermal analysis of Fe-Curcumin complex
Preparation of ferrous-curcumin complex

Ferrous sulphate (FeSO₄) was mechanically mixed in mortar with curcumin (Fe²⁺: Curcumin 1/1 mol) until homogenous powder mixture was obtained. Then propylene glycol/water (1:1 v/v) solution was added to mixture followed by mechanical shaking at 25°C until pasty combination, which was dried at 50°C to obtain powdered complex of Fe²⁺-curcumin.

Analysis of test samples (curcumin and Fe²⁺-curcumin complex)

An UV spectral scan in the wave number range of 350–600 nm was recorded for curcumin and Fe²⁺-curcumin complex in DMSO solvent using Elico spectrophotometer (SL 159). A standard curve for curcumin in DMSO was prepared in a concentration range of 1–5 mg/ml and absorption recorded at λ max 425 nm. Also absorbance of a 0.05% solution of the complex in DMSO was measured at 425 nm. The concentration of curcumin in complex was calculated using standard curve. The FTIR spectrum of test samples was recorded on a MAGNA 550 (Nicolet Instrument Corporation USA) FTIR using KBr pellets and the spectrum was recorded in the range of 400 to 4000 cm⁻¹. The thermograms of the test samples were recorded on Perkin Elmer Thermal Analysis. A sample quantity of 20–40 mg and temperature condition from 25°C to 600°C at a speed of 10°C/min in air atmosphere was maintained. For the study of in vitro stability, the kinetic degradation reaction of curcumin and curcumin complex was monitored spectrophotometrically at buffer pH 7.

Antiinflammatory activity by Carrageenan-Induced Paw Edema Test

Animals and grouping

Wistar albino rats of either sex weighing 150-200 gm maintained under standard environmental conditions (27±2°C. Relative humidity 60 ±5 % light dark cycle of 12 hr) were used for the entire animal study. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water ad libitum. Six animals were used in each treatment group. The study protocol was approved by the Institutional Ethics committee. The healthy albino rats were divided into four groups of six rats each. Group I served as a control, receiving 0.9% w/v NaCl, group II served as standard receiving indomethacin (10 mg/kg b.w.p.o.) while the group III served as test1 and received standard curcumin (200 mg/kg b.w.i.p) and Group IV served as test
2 and received ferrous-curcumin complex (equivalent to 200mg of curcumin/kg b.w.i.p).

Experimental procedure
After 30 minutes of administration of the drug samples, 0.1ml of 1%w/v suspension of carrageenan was injected subcutaneously onto the plantar surface of right hind paw to all the four groups. The volumes of right hind paw of each rat were measured using a Plethysmometer at every half-hourly interval until a period of four hours after the injection of the carrageenan. The Hind paw swelling was calculated as oedema percentage according to the formula

\[ i = \frac{\Delta V_{\text{Treated}}}{\Delta V_{\text{Untreated}}} \times 100 \]

Where, \( i \) = % inhibition of paw oedema, \( \Delta V_{\text{Treated}} = \) Mean change in paw volume of treated rat, \( \Delta V_{\text{Untreated}} = \) Mean change in paw volume of untreated rat

Analysis of Data
The results were expressed as mean ± S.E.M. Differences in mean values between groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. Statistical significance was assessed as \( p < 0.05 \).

RESULTS AND DISCUSSION

Extraction, Isolation and Analysis of Curcumin
Alcoholic extraction of turmeric powder gave a yield of 1.5%w/w of crude curcumin. The isolated curcumin had a m.p of 182°C and IR and UV bands corresponded with standard data. HPLC analysis gave a single symmetrical peak at Rt 6.9 with 99% purity. A linearity curve (20-100ppm) gave a regression coefficient of 0.991 and a slope value of 211.1 with intercept of 131.6. The method of preparation of curcumin complex was found to be reproducible yielding 98% of product. On assaying the complex, it was found to contain 50% of curcumin.

Analysis of test samples
The UV-visible spectrum of curcumin in DMSO showed absorption maximum at 425 nm. Compared with curcumin, the Fe-curcumin complex in DMSO showed a maximum absorption at 418 nm and a shoulder peak at 450 nm which can be attributed to the curcumin → Fe²⁺ charge transfer (Figure 1). The peaks assigned to IR spectrum of curcumin and Fe-curcumin complex (Figure 2) is presented in Table 1. A great decrease in the intensity of (C=O) carbonyl band, accompanied by a shift (\( \Delta \nu = 70 \) cm⁻¹) to high wave values and disappearance of OH peak indicated the possibility of interaction at these sites and its involvement in complexation by new created link between Fe²⁺ and curcumin compound. On TG-DTA analysis (Figure 3), curcumin was thermally stable up to 160°C. Above this temperature an endothermic peak at 174°C (weight loss: found 3.3%, calc. 3.1%) related to the deshydroxylation of OH groups by elimination of two water molecules was observed. After 400°C curcumin was totally decomposed. The Fe-curcumin complex was thermally stable up to 65°C. At 102°C one molecule of crystalline water was eliminated (weight loss: found 3.2%, calc. 3.0%). The existence of an anhydrous complex [Fe(LIGAND)(H₂O)] was apparent from the plateau between 90 and 150°C. Further the sample weight of complex decreased up to 205°C which was due to the elimination of coordinated water molecule, leading to the formation of [Fe(L)] species (weight loss: found 11.2%, calc. 11.4%). After 250°C, a chemical decomposition of curcumin was observed. On the basis of IR and TGA studies a tentative structure of 1: 1 Fe-curcumin complex was deduced (Figure 4). The invitro stability studies at buffer pH 7.0, showed complete degradation of curcumin at the end of 1 hour, while in the same conditions; less than 5% of complex was degraded (Figure 5)

Anti-inflammatory activity by Carrageenan-Induced Paw Edema Test
Significant anti-inflammatory activity was observed for curcumin and Ferrous-curcumin complex in carrageenan induced rat paw oedema model. Curcumin could reduce the rat paw oedema by 51.32% (p< 0.05) at a dose of 200mg/kg b.w.i.p and Ferrous-curcumin complex could reduce the rat paw oedema by 59.76% (p< 0.05) at a dose of 200mg/kg b.w. (equivalent to curcumin) intra peritonially whereas, standard drug, indomethacin showed 61.76 % inhibition (Table 2). The carrageenan induced inflammation in the rat involves three phases: an initial, second and the third phase caused, by the release of histamine and serotonin, bradykinin and prostaglandins respectively. The doses of Curcumin and Ferrous-curcumin complex used in this study showed significant reduction of paw edema at 2 hr or more after carrageenan injection, suggesting that curcumin produces an anti-inflammatory effect during the second phase, similarly to indomethacin. Data from preclinical models suggested that mechanism of anti-inflammatory activity of curcumin involves reduction of prostaglandin through inhibition of cyclooxygenase⁹,¹⁰. The anti-inflammatory effect of curcumin had a delayed onset with a longer duration of action. This phenomenon may partly be due to the low systemic bioavailability of curcumin following oral dosing, due to efficient first-pass metabolism and some degree of intestinal metabolism¹⁰ whereas anti-inflammatory effect of Ferrous-curcumin complex had a faster onset. In addition, the efficacy of Ferrous-curcumin complex was comparable to that of indomethacin which may be attributed to inhibition of activation of transcription factors and enhanced bioavailability.

CONCLUSION
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