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RESEARCH ARTICLE

EFFECTS OF A METAL-CURCUMIN COMPLEX ON ACETIC ACID INDUCED ULCERATIVE COLITIS IN RATS: ROLE OF OXIDATIVE STRESS AND TNF-α

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ABSTRACT

Ulcerative colitis, often known as UC, is an inflammatory bowel disease (IBD) that affects the lining of the colon and rectum and can lead to irritation, inflammation, and ulceration in these areas. Curcumin forms complexes with metal ions. Previous research has shown that combining a metal with curcumin improves its pharmacological effect. In the current study, a metal curcumin complex (MCC) was synthesized using sodium. Its efficacy in treating an ulcerative colitis model caused by acetic acid in Sprague Dawley rats was assessed.Sprague Dawley rats were given an intracolonic injection of 4% v/v acetic acid to induce colitis. Following induction, the rats were given either saline (0.9% v/v), MCC (30, 50, and 100 mg/kg), or curcumin (100 mg/kg) once a day for six days. Weight, stool consistency, and rectal bleeding were observed throughout the six days. On the seventh day, using cervical dislocation and diethyl ether, animals were sacrificed.Each animal's blood was collected, and the serum was separated for biochemical assessment. The colon of each animal was excised, and observed was macroscopical damage. The serum that was isolated following blood collection was tested for cytokine levels. The colon tissue homogenate was used for the biochemical study of oxidative stress parameters. Treatment with MCC demonstrates considerable protection against UC, proven by a significant decrease in the DAI and colonic damage compared to acetic acid-induced colitis rats. In the MCC and curcumin-treated groups, the depleted levels of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were significantly restored. At the same time, the enhanced lipid peroxidation (LPO) and myeloperoxidase (MPO) were significantly decreased. MCC dramatically reduced the activation of the TNF-alpha pathway in colon tissues, as seen by lower TNF-α levels in MCC-treated rats.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD)are two chronically recurrent inflammatory disorders of the gastrointestinal tract (GI) tract. They are both referred to as inflammatory bowel disease (IBD). UC usually affects the colon, but CD can affect any part of the GI tract. Ulcerative colitis is a relapsing condition that causes irritation, inflammation, and ulceration of the colon and rectum lining. It is identified by mucosal injury and ulcerations(1).Various variables may influence ulcerative colitis, including intestinal dysbiosis, genetic disorders, immune system disruptions, environmental factors, and lifestyle habits(2). Severe illnesses like colorectal cancer can develop due to chronic infection(3). UC was previously reported to be more widespread in developed nations; however, in recent years, it has been discovered that the frequency seems higher in Asian countries but has not yet acquired the exact prevalence as in Western hemisphere countries (4). Because the symptoms of experimentally produced UC in animals closely resemble those that occur in people, these animal models are being used in scientific research to understand the disease's numerous pathophysiological pathways better and look for potential treatments (5). But in addition to the different in vivo experimental animal models, several in vitro and ex vivo systems have also been developed that closely imitate ulcerative colitis and resemble the human gut. These are superior to ulcerative colitis animal models (6). The increased production of prostaglandins and leukotrienes is the most prevalent pathological consequence linked with UC(7).The involvement of oxidative stress in the onset of ulcerative colitis has also been supported by earlier research (8–10).An altered colonic environment mainly influences the pathophysiology of IBD, atypical mucosal glycosaminoglycan, impaired oxidation

of short-chain fatty acids, increased intestinal permeability, and sulfide generation (11). Anti-TNF-α antibodies (infliximab), sulphasalazine, corticosteroids, azathioprine, and methotrexate are extensively used medications for the treatment of UC. These aim to control the mucosa's complications, disease relapse, and inflammation(12,13). The drug is selected based on the disease's consequences, extent, and severity, as well as the patient's reaction to current or previous treatment(14). Unfortunately, the current treatments are ineffective because they need prolonged usage and might cause drug intolerance, adverse drug responses, and allergic reactions. As a result, plant medications may be an alternative for treating UC. Due to their low cost, few side effects, and continued widespread acceptance, plant-based medications and their natural products may be an alternative for treating ulcerative colitis (15). There have been a significant number of studies published so far that demonstrate the impact of various plants and the chemical components of those plants on ulcerative colitis(1,16–20).

Curcumin, an Indian spice, has been used extensively in Ayurvedic medicine for ages due to its non-toxicity and multiple therapeutic characteristics, such as anti-inflammatory, antioxidant, analgesic, and antiseptic capabilities(21). Numerous studies have indicated that curcumin has anti-cancer, hypocholesterolemic, wound-healing, anticoagulant, hepatoprotective, antibacterial, and antispasmodic properties (22–26). Curcumin is a yellow-colored chemical component derived from the rhizomes of the *Curcuma longa* plant. Curcumin has a notable role in ulcerative colitis because it modifies the inflammatory response by suppressing the activity of the enzymes inducible nitric oxide synthase (iNOS), lipoxygenase, and cyclooxygenase(27–29). Even though there is a lot of evidence that curcumin has pharmacological effects, its low solubility and bioavailability make it hard to know how to get the best therapeutic results(30). Because of its low solubility and poor oral bioavailability, curcumin's application in the prevention and treatment of human diseases has been severely restricted. Curcumin is unstable at neutral and basic pH values, which is also the cause of the negative pharmacokinetics of this molecule, therefore in addition to solubility and bioavailability issues, this molecule also suffers from instability in the gastrointestinal tract (31–33). Researchers have developed potential delivery systems and approach in recent years to increase curcumin's stability, bioavailability, and water solubility(30).Through chelation, curcumin has a remarkable capacity to form complexes with metal ions. Metal-curcumin complexes have attracted much attention in recent years due to their success in addressing the abovementioned issues. Compared to curcumin alone, metal curcumin complexes have a more profound impact on diseases (34–38).In previous studies, the sodium metal-curcumin complex has anti-inflammatory and anti-diarrheal properties (39–41). In the current research, we have synthesized a metal curcumin complex and investigated its ameliorative effect on acetic acid-induced ulcerative colitis in Sprague Dawley rats.

MATERIALS AND METHODS

Chemicals and Reagents: Curcumin 95% (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) was purchased from NatBiome Speciality Healthcare Pvt Ltd (Gandhinagar, India). Sodium chloride and sodium hydroxide was purchased from Research-Lab Fine Chem (Mumbai, India). Ellman's reagent (5,5-dithio-bis-(2-nitrobenzoic acid) was purchased from Research-Lab Fine Chem (Mumbai, India). Hexadecyl trimethyl ammonium bromide (hexadecyl (trimethyl) azanium; chloride) was purchased from Yarrowchem Products (Mumbai, India). o-Dianisidine (4-(4-amino-3-methoxyphenyl)-2-methoxyaniline) was procured fromRamagundam Fertilizers and Chemicals Limited (New Delhi, India). All other compounds used in the study were of the analytical grade and were procured from Sigma-Aldrich Company (St. Louis, MO, USA).

Synthesis of a metal-curcumin complex: The metal-curcumin complex (MCC) was synthesized using a method proposed by Peni *et al*. (42). 250mg of curcumin was dissolved in 30mL of ethanol, previously mixed with 25mg of solid NaOH (sodium hydroxide), which was then reacted with 20mg of NaCl (sodium chloride). The resultant mixture was then subjected to reflux for 2-3 hours until the color changed to deep red. The schematic prediction of the reaction of sodium metal precursor with curcumin is shown in Figure 1.

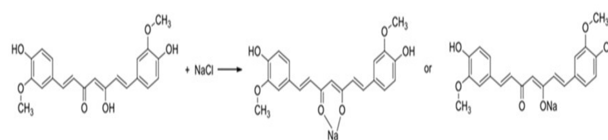


Fig.1. Schematic prediction of the reaction of sodium metal precursor with curcumin

Characterization of MCC

Thin Layer Chromatography: The metal curcumin complex was analysed using thin layer chromatography with precoated silica gel 60 F254 aluminium plates (2 x 4 cm) serving as the stationary phase. The mobile phase used was a mixture of chloroform: ethanol: glacial acetic acid (9.5: 0.4: 0.1). After air drying, the developed chromatographic plate was examined with UV light (254 and 365 nm). Calculated R_f values were compared to curcumin Rf values (43).

UV-visible Spectroscopy: UV-visible spectroscopy of MCC and curcumin was performed on a UV-vis spectrophotometer (Agilent Cary 60). Samples were scanned in the range of 200-800 nm and absorbance v/s concentration was performed.

Solubility studies: Equilibrium solubility method was utilized to perform the solubility studies of MCC.In conical flasks, excess MCC was added to 25 mL of 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8), and phosphate buffer (pH 7.4). These flasks were placed in solubility shaker at 37°C. After 12 h, all solutions were filtered and analysed using a UV spectrophotometer at 466 nm against 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8), and (pH 7.4). The solubilized amount of drug was then calculated.

Animal care: Female Sprague-Dawley rats weighing 100-150 g were procured from the animal house facility of Zydus Lifesciences Ltd, Ahmedabad, India. Individual propylene cages were used to house all the animals. The animals were fed with commercial NPD (regular protein diet) pellets and were given free access to water. Animals were maintained under controlled environmental conditions (20-25°c, 50-55%

humidity, and 12hr light/dark cycle). Before beginning the study, animals were acclimatized for seven days. Experiments on animals were carried out following protocols approved by the SSR College of Pharmacy, Institutional Animal Ethics Committee (IAEC) and found to be in accordance with the provision of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility.

Acute toxicity study: The OECD (Organization for Economic Co-operation and Development) guideline 420 for the testing of chemicals was followed for conducting an acute oral toxicity study(44). Four groups of six rats each were formed by randomly assigned the animals. The first group, the control group, was given unlimited access to food and purified water. The three other groups received a single oral dosage of 500, 1000, or 2000 mg/kg b.w. MCC. All rats were closely monitored for any unusual behavior during the first four hours, and the number of rats perished was noted after 24 hours. Each animal was then monitored for 14 days to look for toxicity, behavioral abnormalities, and symptoms of impending death. All the animals were anesthetized with diethyl ether before the experiment and then euthanized through cervical dislocation. The removal of internal organs was done, and they were examined for toxicity.

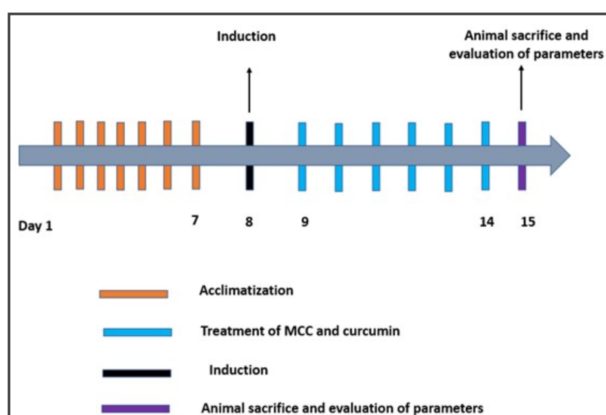


Fig. 2. Experimental Design

Experimental Design: The experimental design is shown in the figure 2. Thirty-six healthy female rats were randomly divided into six groups containing six rats in each group (n=6). They were then subjected to acclimatization for seven days.

Group 1 (Control)- Intracolonic administration of saline (0.9% v/v NaCl)
Group 2 (A.A control)- Intracolonic administration of 1-2 ml 4%v/v acetic acid

Group 3 (Cur 100 + A.A)- 100 mg/kgb.w curcuminorally
Group 4 (MCC 30 + A.A)- 30 mg/kg b.w MCC orally
Group 5 (MCC 50 + A.A)- 50 mg/kg b.w MCC orally
Group 6 (MCC 100 + A.A)- 100 mg/kg b.w MCC orally

For six days, Group 1 received saline daily. Acetic acid (1-2 ml, 4% v/v) was intrarectally administered to the animals in groups 2 to 6. After induction, a treatment regimen using curcumin and MCC was initiated and followed for six days. Weight, stool consistency, and rectal bleeding were observed throughout the six days. On the seventh day, using cervical dislocation and diethyl ether, animals were sacrificed. Each animal's blood was collected, and the serum was separated for biochemical assessment. The colon of each animal was excised, and observed was macroscopical damage.

Induction of Colitis: Ulcerative colitis may be readily and reproducibly caused by administering 1-2 mL of 4 percent v/v acetic acid intrarectally for 30 seconds (45). Pentobarbital sodium (30 mg/kg i.p.) was used to anesthetize the rats. A polyethylene catheter was placed approximately 8 cm into the anus for rectal administration. Each rat was maintained in the Trendelenburg posture for 30 seconds before being rinsed with saline to neutralize the acid's harshness.

Assessment of Disease Activity Index (DAI): The clinical severity of colitis was evaluated by noting changes in growth rate, stool consistency, and the presence of bleeding and occult blood in stools. Each day, these were graded on a scale of 0 to 4 (Table 1). The DAI was measured using the Cooper *et al.* grading system (46).

Table 1. Scoring of DAI

Score	Decrease in growth%	Stool Consistency	Rectal Bleeding
0	0	Normal	Normal
1	1-5	Normal	+
2	5-10	Loose stools	++
3	10-15	Loose stools	+++
4	>15	Diarrhea	Gross bleeding

The disease activity index can be calculated according to the following formula:

$$DAI=\frac{\text{\% Body weight loss score+Diarrohea score+Rectal score}}{3} \quad (1)$$

Assessment of colon damage by macroscopic scoring: Using the grading system outlined by Wallace *et al.*(47), colonic ulceration and inflammation were evaluated. From the proximal end to the anus, a colonic segment of approximately 8 cm was separated. Following that, a longitudinal incision was made in each separated colon tissue. The colon tissue lesion was graded using the scale in Table 2.

Table 2 Criteria for scoring of ulceration and inflammation

Score	Appearance
0	Normal
1	Localized hyperaemia, no ulcers
2	Ulceration without hyperaemia or bowel wall thickening
3	Ulceration with inflammation at one site
4	Two or more sites of ulceration
5	Major sites of damage extending >1 cm along length of colon
6-10	Area of damage extended >2 cm along length of colon, score was increased by 1 for each additional cm of involvement.

Assessment of cytokine level (serum TNF-α): Blood was drawn from each animal and allowed to clot for 1 hour before being centrifuged for 20 minutes at 1000xg at 2-8^{0}C. The separated serum was utilized to test the cytokine (TNF-α). The serum was tested for colonic TNF-α using an enzyme-linked immunosorbent assay (ELISA) kit, as directed by the manufacturer (Elabscience Biotechnology Inc., United States).

Preparation of Tissue Homogenates: After cervical dislocation, colon tissues were removed from each animal group and washed in cold saline.

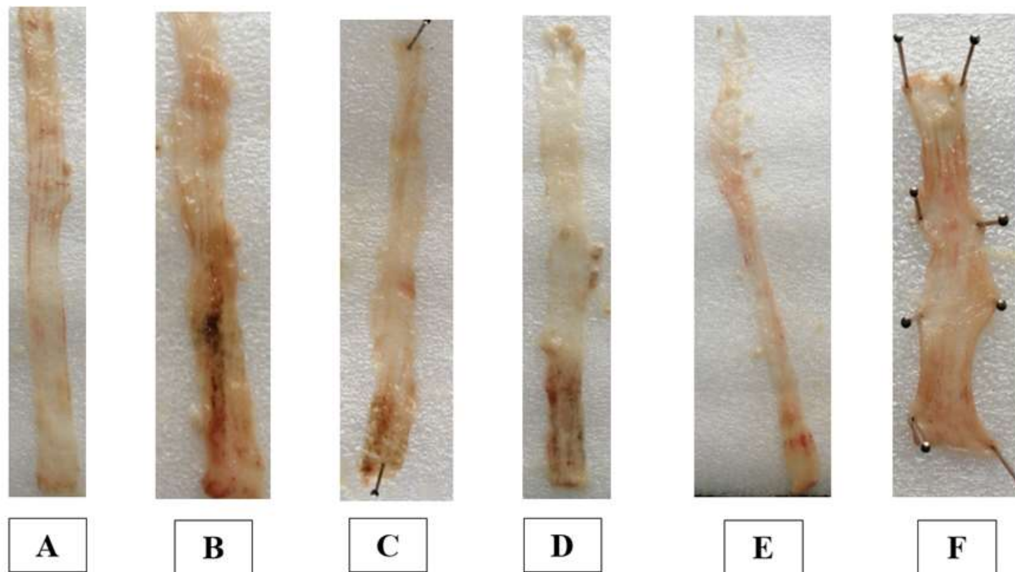
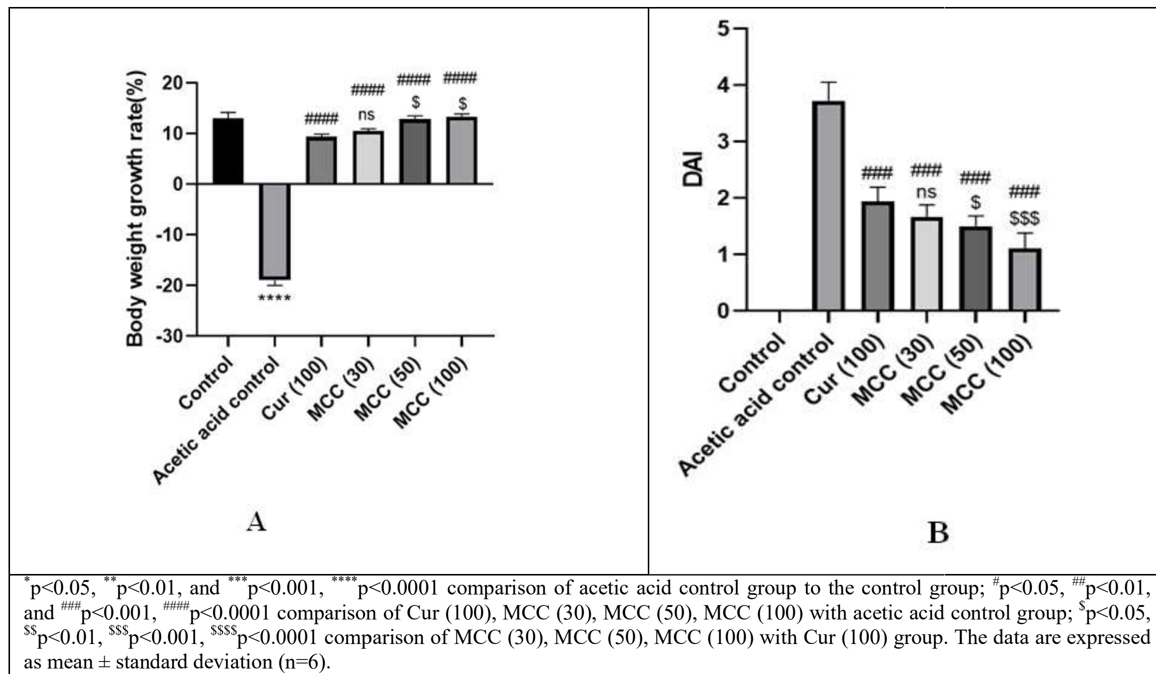
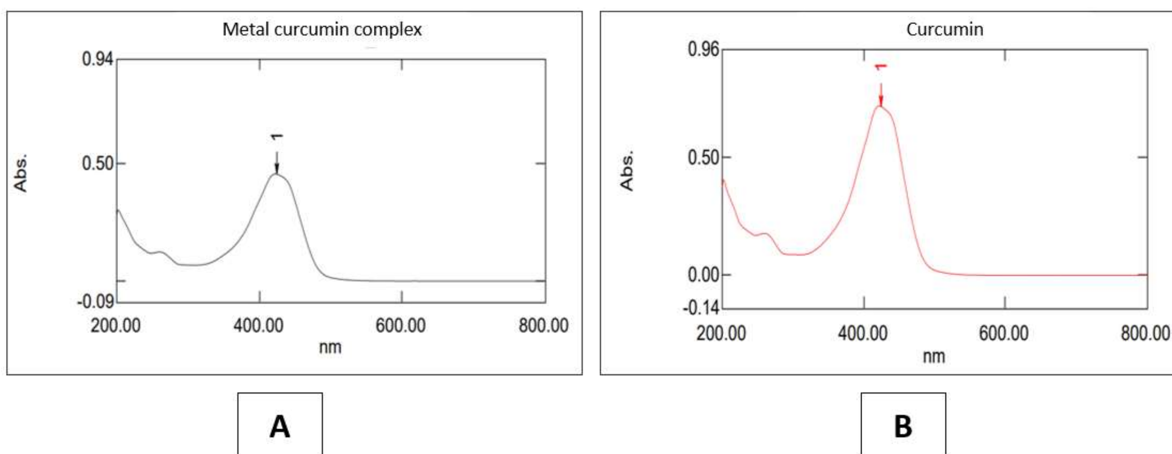


Fig. 3. UVspectra of metal- curcumin complex (A) and curcumin (B)



*p<0.05, **p<0.01, and ***p<0.001, ****p<0.0001 comparison of acetic acid control group to the control group; #p<0.05, ##p<0.01, and ###p<0.001, ####p<0.0001 comparison of Cur (100), MCC (30), MCC (50), MCC (100) with acetic acid control group; $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 comparison of MCC (30), MCC (50), MCC (100) with Cur (100) group. The data are expressed as mean ± standard deviation (n=6).

Fig. 4 Effect of MCC on % growth rate of body weight (A) and DAI (B) in acetic acid induced colitis



A: Control; B: Acetic acid colitis control; C: Curcumin (100 mg/kg) + colitis; D: MCC (30 mg/kg) + colitis; E: MCC (50 mg/kg) + colitis; F: MCC (100 mg/kg) + colitis

Fig. 5. Effect of MCC on gross anatomy of colon in acetic acid induced colitis

Table 3. Comparison of solubility of MCC and curcumin

Sr. No.	pH	Solubility (mcg/ml)	
		MCC	Curcumin
1	7.4	169 ± 0.75	3.34 ± 0.19
2	6.8	58.4 ± 0.49	1.2 ± 0.14
3	1.2	42.4 ± 1.62	0.8 ± 0.06

Each tissue was homogenized using a tissue homogenizer (Remilabworld, India) after being manually minced. In cooled phosphate buffer, a 10 percent tissue homogenate was produced (0.1M, pH 7.4). The tissue homogenates were centrifuged for 10 minutes at 700xg and 40 degrees Celsius to remove debris. The recovered supernatant was employed for testing various parameters.

Assessment of Myeloperoxidase (MPO) activity: 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer, pH 6, containing 0.167 mg/ml O-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured with a UV-Visible spectrophotometer. Myeloperoxidase activity was described as the weight of wet colon tissue's U/g (units/gram). The procedure previously described by Janice *et al*. (48)was used to determine the MPO levels in tissue homogenates. It was calculated by using the following formula:

MPO activity (U/g)= X/weight of the piece of tissue taken. (2)

Where X= 10 x change in absorbance per min/ volume of supernatant taken in the final concentration

Assessment of Superoxide dismutase (SOD) activity: SOD activity was measured using the method previously described by Marklund *et al*. with slight modifications (49). 0.1M potassium phosphate buffer (pH 7.4) was mixed with a 0.2 ml sample. To this solution, 0.2 ml of previously prepared pyrogallol solution was added. Pyrogallol solution was prepared by dissolving 2.6 mM pyrogallol solution in 10mM HCl. Immediately after the 30s, the absorbance of the mixture was taken at 325 nm. SOD activity was reported as U/mg protein. The following formula was used to assess the activity:

Unit of SOD/mL of sample = [(A-B) x 100]/A x 50 (3)

Where, A = change in absorbance per minute of standard,
B= change in absorbance per minute of the test sample.

Assessment of Reduced glutathione (GSH) activity: Colonic reduced glutathione (GSH) levels were analyzed using the Ellman method (50). In brief, 0.02 mL DTNB (0.1 M in Phosphate buffer pH 7.0), 3 mL homogenate, phosphate buffer 2 mL, water 5 mL were added, and absorbance was taken at 412 nm after 2 minutes. The reduced GSH level was calculated using the following formula:

A = a.b.c
(a = 0.017 $mM^{-1}cm^{-1}$) (4)

Assessment of Catalase activity: Catalase was estimated by the procedure described by Hugo Aebi (51). In 2 mL of tissue homogenate, 1 mL of H2O2 (30 mM in 50 mM of Phosphate buffer pH 7.0) was added, and absorbance was taken at 240 nm with 30-sec intervals. Catalase activity was calculated using the following equation. The result was expressed as nano moles of H_2O_2 consumed/minute/mg protein.

$$\text{Catalase activity} = \frac{\frac{\Delta A}{\text{minute}} \times \text{total volume of assay}}{0.81 \times \text{sample volume} \times \text{mg of protein}} \quad (5)$$

Assessment of Lipid peroxidase (LPO) activity: To determine the degree of lipid peroxidation, the amount of malondialdehyde (MDA) in the colon homogenates was utilized (52). In 0.2 mL of homogenate, 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 mL of 0.8% aqueous solution of TBA were added. The combination was prepared to a volume of 4 mL using distilled water and heated for 60 minutes in an oil bath at 95 °C. After cooling, 5 mL of the n butanol/pyridine (15:1, v/v) mixture and 1 mL of distilled water were added and rapidly shaken. The organic layer was removed, and its absorbance at 532 nm was measured after centrifugation at 4000 rpm for 10 minutes. The results were represented in nanomoles per mg of protein. The LPO activity was calculated according to the following formula:

A= a.b.c (6)

a= 1.56×10^{-5}

Statistical Analysis: One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used to compare the different groups with the disease group and/or control group. Statistical significance was considered if $p<0.05$. The analysis was performed using GraphPad Software (version 8.0.2).

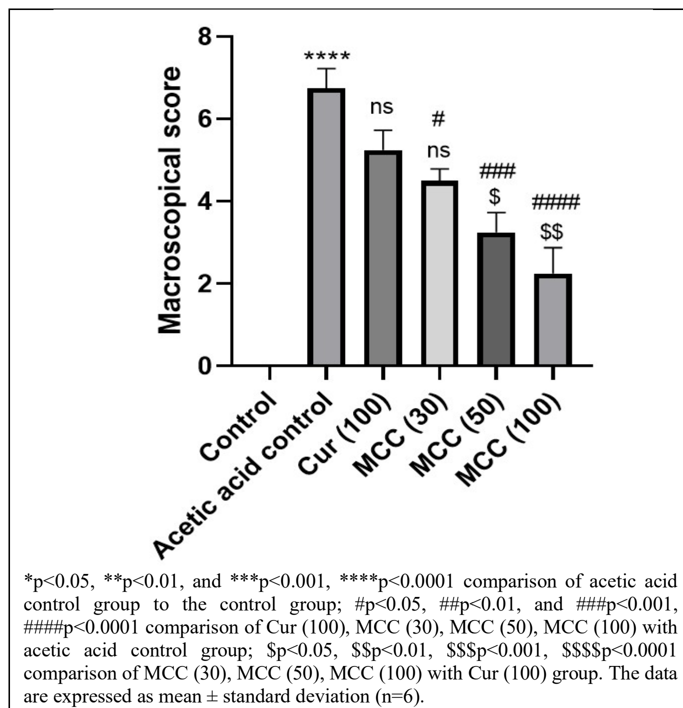
RESULTS

Characterization of MCC: Thin layer chromatography of MCC and curcumin was performed using a mixture of chloroform : ethanol : glacial acetic acid (9.5 : 0.4 : 0.1). The R_f value of MCC was found to be 0.92 ± 0.01, whereas that of curcumin was 0.96 ± 0.01. UV spectra of curcumin and MCC are depicted in the figure3. It shows that both have λ_{max}value equal to 425 nm.

Solubility studies: Solubility studies of MCC and curcumin were performed using equilibrium solubility method and was found that MCC was more soluble than curcumin alone (Table 3).

Acute toxicity study: MCC was shown to be safe at all three of the tested dosages. Throughout the study, no indicators of death or toxicity were recorded. So, for further in vivo research, dosages of 30, 50, and 100 mg/kg b.w were chosen following the toxicity test conducted on MCC.

Effect of MCC on Disease Activity Index (DAI): The extent and severity of the colitis induced by acetic acid administration were evaluated in all of the animals. Acetic acid-induced colitis in rats exhibited a marked decrease ($p<0.001$) in body weight, as shown in figure 4(A).



*p<0.05, **p<0.01, and ***p<0.001, ****p<0.0001 comparison of acetic acid control group to the control group; #p<0.05, ##p<0.01, and ###p<0.001, ####p<0.0001 comparison of Cur (100), MCC (30), MCC (50), MCC (100) with acetic acid control group; \$p<0.05, \$\$p<0.01, \$\$\$p<0.001, \$\$\$\$p<0.0001 comparison of MCC (30), MCC (50), MCC (100) with Cur (100) group. The data are expressed as mean ± standard deviation (n=6).

Fig.6 Effect of MCC on macroscopical ulcer score

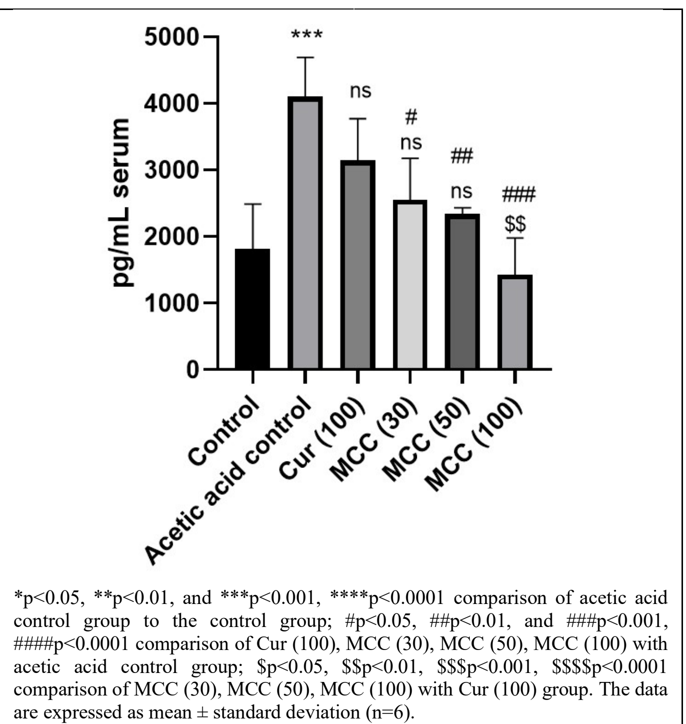


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Fig.7 Effect of MCC on serum TNF-α levels in acetic acid induced colitis

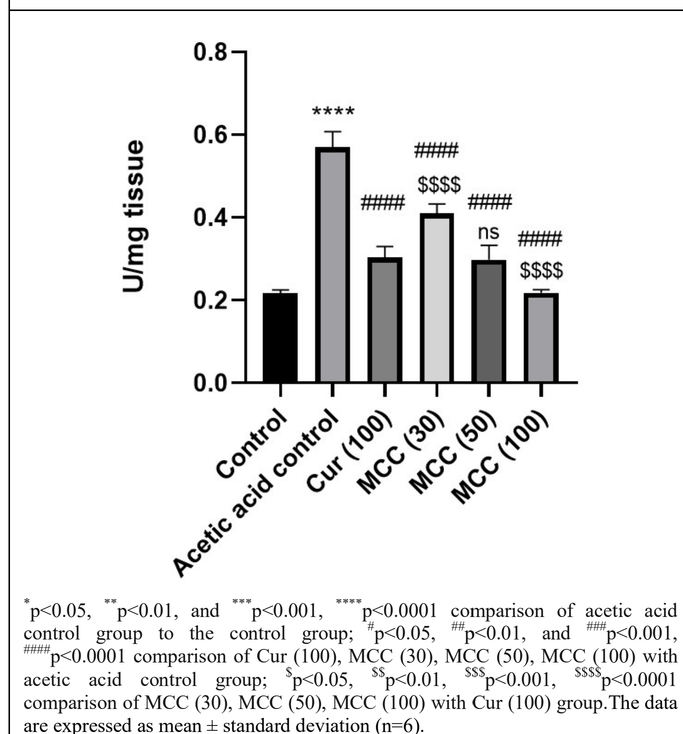

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Fig. 8 . Effect of MCC on myeloperoxidase activity in acetic acid induced colitis

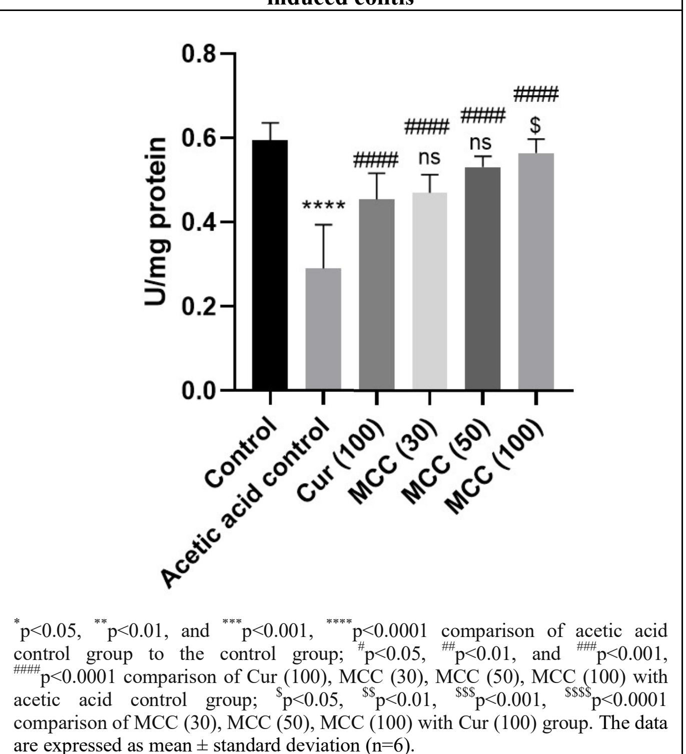

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Fig. 9. Effect of MCC on superoxide dismutase activity in acetic acid induced colitis

However, MCC treatment in colitis rats resulted in a significant increase in the % growth rate of body weights compared to the control and curcumin-treated rats. From figure4(A), it is also clear that 100 mg/kg b.w MCC was able to significantly increase the % growth rate of the body compared to the same dose of curcumin (100 mg/kg b.w).

The DAI score is a significant indicator to assess the colonic injury, from signs such as decreased bodyweight, diarrhea, and rectal bleeding. The lower DAI score is considered as the animal is closer to the normal physiological state.

In the present study, acetic acid-induced colitis rats exhibited symptoms of UC, as indicated by diarrhea and bloody stools. DAI scores of the disease control groups were higher than those of the other groups (figure4B). A decrease (p<0.0001, p<0.0001) in the DAI was observed by MCC at doses 50 and 100 mg/kg as compared to the curcumin-treated group. But the marked decrease in the DAI score was observed at the dose 100 mg/kg b.w of MCC.

Effect of MCC on colon damage (macroscopical scoring): Colon tissues of the control group exhibited typical intact

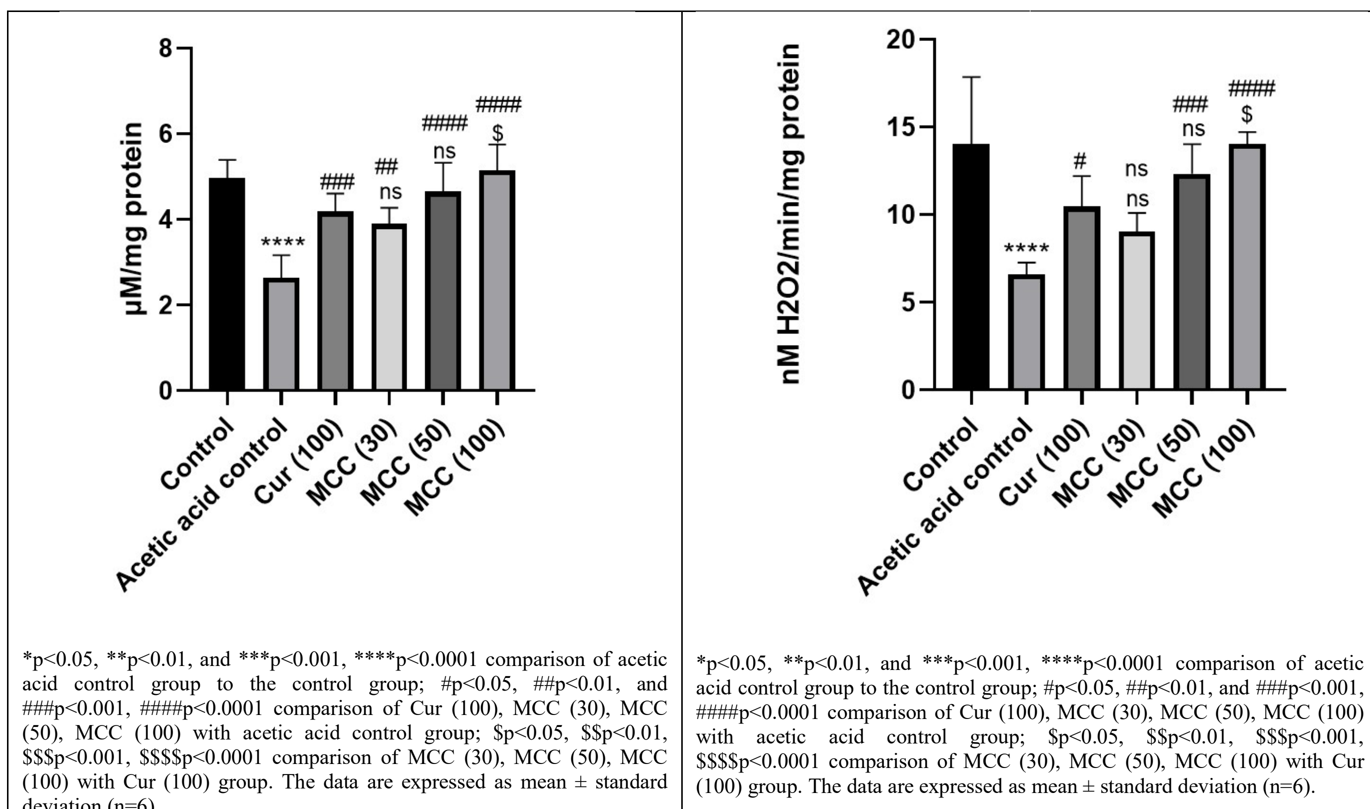

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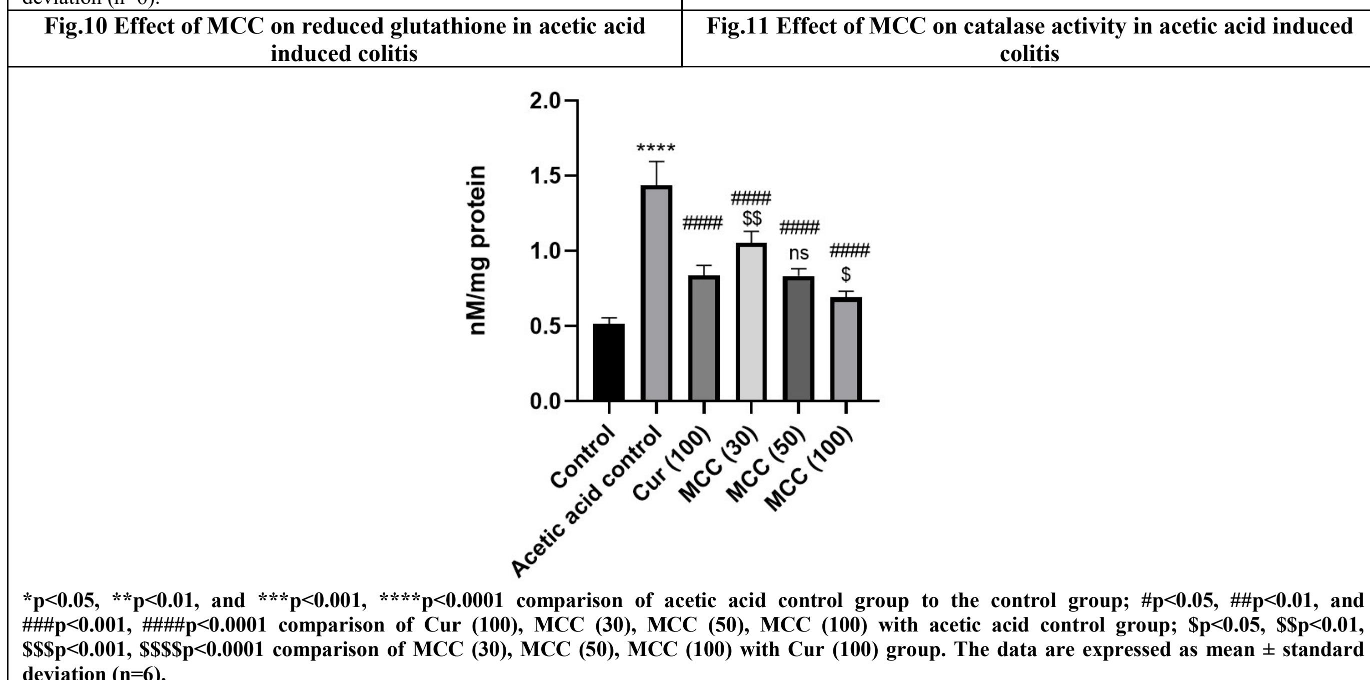
Fig.10 Effect of MCC on reduced glutathione in acetic acid induced colitis

*p<0.05, **p<0.01, and ***p<0.001, ****p<0.0001 comparison of acetic acid control group to the control group; #p<0.05, ##p<0.01, and ###p<0.001, ####p<0.0001 comparison of Cur (100), MCC (30), MCC (50), MCC (100) with acetic acid control group; $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 comparison of MCC (30), MCC (50), MCC (100) with Cur (100) group. The data are expressed as mean ± standard deviation (n=6).

Fig.11 Effect of MCC on catalase activity in acetic acid induced colitis



***p<0.05, **p<0.01, and ***p<0.001, ****p<0.0001 comparison of acetic acid control group to the control group; #p<0.05, ##p<0.01, and ###p<0.001, ####p<0.0001 comparison of Cur (100), MCC (30), MCC (50), MCC (100) with acetic acid control group; $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 comparison of MCC (30), MCC (50), MCC (100) with Cur (100) group. The data are expressed as mean ± standard deviation (n=6).**

Fig.12. Effect of MCC on lipid peroxidase activity in acetic acid induced colitis

structure with no lesions or hyperemia (figure 5 A). The colonic specimens of acetic acid control rats appeared ulcerated, edematous, and hemorrhagic (figure 5 B). Treatment of rats with curcumin (100 mg/kg) and MCC(30, 50, and 100 mg/kg) significantly restored the visible injuries produced by acetic acid (figure 5 C-F). The extent of colon damage was assessed using previously mentioned criteria (table 2). All animals were evaluated using this ulcer severity scoring. The macroscopical ulcer score was found to be significantly (p<0.0001) increased in colitis control; however, treatment of MCC 50 mg/kg and 100 mg/kg showed a significant decrease (p<0.01) in the damage to the colon as compared to curcumin-treated group (Figure 6).

Effect of MCC on cytokine level (serum TNF-α): The TNF-α levels (pg/ml) were found to be significantly (p<0.001) increased in the acetic acid control group, as depicted in figure 7.Curcumin (p<0.05) and MCC (p<0.0001) both showed a significant decrease in the levels of serum TNF-α against the acetic acid control group. Whereas MCC 30 mg/kg(p<0.01), 50 mg/kg (p<0.001), and 100 mg/kg (p<0.0001) were found to be significant in decreasing the TNF- α levels when compared to the curcumin-treated group.

Effect of MCC on Myeloperoxidase (MPO) activity: The results of the treatment of MCC on acetic acid-induced colitis are depicted in figure 8. There was a significant ($p<0.0001$) increase in the MPO activity acetic acid control compared to the control group. Curcumin-treated and MCC-treated groups both showed a significant ($p<0.0001$) decrease in the MPO activity compared to the acetic acid control group. While comparing MCC groups with curcumin, it was found that MCC (30 and 100 mg/kg) showed a significant ($p<0.0001$) decrease in the MPO activity.

Effect of MCC on Superoxide dismutase (SOD) activity: The acetic acid induction caused a significant ($p<0.0001$) decrease in the levels of superoxide dismutase enzyme compared to the control group. However, from Figure 9, it is clear that treatment of curcumin and MCC caused a significant increase ($p<0.0001$) in the activity of SOD compared to acetic acid control. It can also be depicted that a dose of 100 mg/kg MCC was able to increase the SOD activity when compared to the curcumin-treated group ($p<0.05$).

Effect of MCC on Reduced glutathione (GSH) activity: Figure 10 shows that the intracolonic administration of acetic acid significantly ($p<0.0001$) decreased the reduced glutathione levels compared to the control group. Curcumin treatment significantly ($p<0.01$) increased the reduced glutathione activity compared to the acetic acid control group. MCC 100 mg/kg ($p<0.0001$) showed marked improvement in the reduced glutathione activity compared with the acetic acid control group. When compared to curcumin-treated groups, MCC (100 mg/kg) showed a significant ($p<0.0001$) increase in the reduced GSH activity.

Effect of MCC on Catalase activity: Compared to the control group, a significant ($p<0.0001$) decrease in the catalase activity was observed in the acetic acid-induced colitis group. Curcumin treated group and MCC treated group (50 and 100 mg/kg) both were able to increase significantly ($p<0.05$, $p<0.001$, $p<0.0001$, respectively) the catalase activity when compared to the acetic acid control group. From figure 11, it is clear that MCC at 100 mg/kg b.w dose was able to significantly ($p<0.05$) increase the catalase activity compared to the curcumin-treated group.

Effect of MCC on Lipid peroxidase (LPO) activity: Figure 12 shows that compared to the control group, there was a significant ($p<0.0001$) increase in lipid peroxidase activity when rats were treated with intracolonic administration of acetic acid. Treatment of curcumin (100 mg/kg) and MCC (30, 50, and 100 mg/kg b.w) led to a significant ($p<0.0001$) decrease in the LPO activity compared to the acetic acid control group. MCC at the doses 30 and 100 mg/kg were able to significantly ($p<0.01$, $p<0.05$ respectively) decrease the LPO activity compared to that of the curcumin-treated group.

DISCUSSION

Due to its non-toxicity and several beneficial properties, including its antimicrobial, analgesic, antioxidant, and anti-inflammatory properties, curcumin has been utilized widely in Ayurvedic medicine for centuries (21). The pharmacological effects of curcumin are well documented, yet due to its hydrophobic nature, it has a low water solubility and bioavailability (30–33). Several researchers have developed new techniques to increase curcumin's bioavailability and solubility. Metal-curcumin complexes are one such technique that has garnered interest (30).The previous study suggests that metal-curcumin complexes have been employed successfully against various diseases in animal models (53). Still, the attempt to investigate its usage in the treatment of ulcerative colitis is unclear.The present study investigated the ameliorative effect of metal-curcumin complex (MCC) against acetic acid-induced UC in Sprague Dawley rats. By administering 4% acetic acid rectally to Sprague Dawley rats, colitis was caused. They had symptoms and indications that were similar to those of colitis patients, including bloody diarrhoea, diarrhoea, and a loss of body weight, which is consistent with earlier research (54). This investigation showed that MCC considerably reduced the degree of acetic acid-induced colonic impairment. The synthesized metal curcumin complex (MCC) was characterized by TLC and UV-visible spectroscopy. The results of TLC (R_f value) of MCC (0.92 0.01) compared to curcumin alone (0.96 0.01) showed the formation of the metal complex. A change in absorbance was observed in case of UV – visible spectra of MCC.

According to the findings of solubility tests (table 3), the complexation of curcumin with metal enhances its solubility across all pH ranges. A disease activity index (DAI) score can determine the severity of acetic acid-induced colitis. Weight loss might reflect the extent of intestinal inflammation(54).Increased cytokines like TNF-α and IL-6 have been linked to considerable weight loss in colitis due to the production of appetite-suppressing neuropeptides (55). In the present research, intrarectal administration of acetic acid resulted in a significant reduction in body weight, which may have been caused by symptoms of lack of appetite, malabsorption, and severe fluid loss due to rectal bleeding and diarrhea. However, administration of MCC (100 mg/kg b.w.) to rats with colitis significantly increased the weight gain rate. The DAI scores increased significantly in the acetic acid control groups, presumably due to severe edema and inflammatory cell infiltration (56). However, treatment of rats with colitis with MCC at doses of 50 and 100 mg/kg b.w. resulted in a substantial reduction in the DAI score compared to treatment with curcumin. This demonstrates that MCC has a better protective effect against acetic acid-induced colitis than curcumin.

TNF-α, a cytokine that promotes inflammation, is primarily thought to play a role in inflammatory diseases. According to findings, TNF-alpha is reported to be higher in the blood, stool samples, and mucosa of UC patients(57). Regulation of proinflammatory cytokines (TNF-α, IL-6, etc.) could be a realistic approach for treating UC(58,59).Acetic acid induces significant epithelial damage by releasing protons inside the epithelia's intracellular space. It raises levels of cytokines like TNF-α and IL-6 (60).Serum TNF-α levels were substantially higher in the acetic acid control group in the current study, correlating with previous findings (61). Both curcumin and MCC significantly reduced serum TNF-alpha levels compared to the acetic acid control group. However, MCC at all three doses (30, 50, and 100 mg/kg b.w.) demonstrated a substantial reduction in serum TNF-alpha compared to the curcumin-treated group.

Thus suggesting that MCC ameliorated acetic acid-induced colitis better than curcumin by significantly reducing serum TNF-α levels. One of the primary contributing causes to an inflammatory illness is oxidative stress and inhibited antioxidant defence mechanisms since these systems control the generation of reactive oxygen species (ROS)(8–10). According to the previous study, MPO and MDA levels are significantly elevated in inflammatory conditions such as ulcerative colitis (62,63). The findings are consistent with the current investigation, in which MDA and MPO levels in colon tissue homogenates were dramatically increased. Comparing the MCC and curcumin treatment groups to the acetic acid control group showed that both treatments significantly reduced MPO levels. MCC (30 and 100 mg/kg b.w.) showed a substantial reduction in the MPO levels compared to the curcumin group. Other antioxidants, such as SOD, reduced GSH, and catalase, is significantly reduced in an inflammatory state(64,65). The enzyme activities of SOD, reduced GSH, and catalase was considerably lower in the acetic acid control groups in the current study. The current study's findings corroborate the prior study's findings.Accordingly, the findings suggest that MCC was able to mitigate mucosal damage by lowering the levels of MDA and MPO and increasing levels of reduced GSH, SOD, and catalase in the colon tissues of colitis rats.

The acetic acid-induced colitis model is one of the study's limitations. We intend to test the colon protective effect of MCC on a more sophisticated model of ulcerative colitis, the dextran sodium sulfate-induced model because it will extract more information about innate immunity, and a more precise mechanism of pathogenesis can be deduced. Another limitation of the current study is the lack of precise characterization of the metal-curcumin complex (MCC), which will lead to a molecular-level approach to understanding its mechanism of disease amelioration, which will be reflected in our future research. We also aim to incorporate histological examination into our future investigations.

CONCLUSION

The current study is an initial effort to examine the metal-curcumin complex's (MCC) ameliorative impact against the acetic acid-induced model of UC. The results show that MCC reduced the severity and extent of colitis in rats via regulating TNF-α release and antioxidant levels, which had a protective impact on the mucosa. The findings also indicated that MCC had more significant potential for treating ulcerative colitis than curcumin. The results shown here consequently suggest that MCC has the potential to treat UC and will, hopefully, be explored in the future as a candidate for ulcerative colitis.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest

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