Original Article

The Possible Modulatory Effect of Curcumin on a Rat Model of Crohn's Disease

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A B S T R A C T

Background: Crhon's disease (CD) is a multifactorial chronic inflammatory disease that affect the gastrointestinal tract from mouth to anus. The effect of various drugs used to treat CD was investigated. **Aim**: The aim of the present study is to evaluate the possible therapeutic effect of curcumin on the severity of CD induced by intra-colonic instillation of NaOH in rats. **Methods**: Adult male Wister rats were assigned into five groups. Group I was kept as normal animals without treatment. Group II, III, IV and V were subjected to the induction of Crhon's disease by intra-colonic injection of 2ml NaOH (6.25%). GroupII received 0.9% saline, group III received dexamethasone at dose of (1mg/kg), group IV and V received intraperitoneal curcumin (40,100mg/kg) once daily starting 2 days before NaOH infusion until the end of the experiment at day 15 post induction. Assessment of the inflammatory response was done by histology and measurement of interleukin-1β (IL1), tumor necrosis factor (TNF-α), myeloperoxidase activity (MPO) and reduced glutathione (GSH) levels in colon mucosa. **Results**: High dose of curcumin significantly decreased colonic IL-1β, TNF-α, and MPO activity and increased GSH concentration. Moreover, curcumin attenuated the macroscopic and histopathological changes induced by NaOH. **Conclusion**: These results suggest that curcumin may be effective in the treatment of CD through its scavenging properties on the oxygen-derived free radicals.

Key Words: Crhon's disease, Curcumin, oxidative stress markers, reactive oxygen species.

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1. INTRODUCTION

Crhon's disease is a complex and debilitating chronic inflammatory bowel disease, its incidence has increased since the mid-1970s in Western countries as well as in developing world. It is clinically characterized by abdominal pain and bloody diarrhea. Distinguishing features include mucosal ulceration and transmural inflammation which may occur anywhere along the gastrointestinal tract but most commonly affects the distal small intestine. It takes the whole thickness of the bowel wall and associated with lymphoid aggregates and granuloma (Hovde and Moum, 2001 & Boyapati et al., 2015).

The etiology of CD is complex, the current literature suggest genetic, immune and environmental hypothesis. Oxidative stress is also involved, as it alters inflammatory response, cause apoptosis and DNA damage (Baliga et al., 2012).

Most of the current therapies for CD involve treatment with anti-inflammatory compounds like (5ASA andcorticosteroids), immune modulators (methotrexate), biological agents (anti-TNF-α, PPAR-ligand), probiotics, antibiotics and surgery. Currently available options are effective in only approximately 50% of patients. So, additional therapeutic approaches are being tried (Piechota-Polanczykand Fichna, 2014).

Curcumin, the active ingredient of Indian spice turmeric, is a natural antioxidant compound. It shows promise in treating many diseases with excellent safety profile even at high doses (Taylor and Leonard, 2011).

The aim of our study is to evaluate the possible curative effect of curcumin in an experimental model of Crhon's disease in rats, and to elucidate its antioxidant tmechanism underlying this curative effect.

2. MATERIALS AND METHODS

Animals:

Adult male Wister rats (200-250g, n=50) aged 4 months old, were purchased from Animal House of Faculty of Medicine Assiut University. The animals were kept under controlled conditions and fed with normal rat chow and water *ad libitum*. All animal experiments were

approved by the Institutional Animal Ethics Committee, Assiut University.

Chemicals

NaOH was purchased from El Nasr Pharm Chem Co., Egypt; Curcumin was purchased from Columbus Chemical Industries, Inc. and dexamethasone were purchased from Amriya for pharmaceutical industries, Alexandria-Egypt. Rat ELISA kit for determination of TNF-α and IL1-β was obtained from Koma Bioteck Inc. Assessment of colonic tissue content of GSH and MPO were performed by spectrophotometer (Biodiagnostic, Egypt). All levels were quantified according to the instructions supplied with the kits.

Experimental design:

Rats were divided into 5 groups (n=10 per group).Group I was kept as normal animals without treatment. Group II, III, IV, and V were subjected to the induction of Crhon's disease by intra-colonic injection of 2ml NaOH (6.25%) (Koçak et al., 2011). Group II served as Crhon's control group and received 0.9%saline alone. Group III, and IV were treated with intraperitoneal dexamethasone (1mg/kg) and curcumin (40 and 100mg/kg) respectively once daily starting 2 days before NaOH infusion until the end of the experiment at day 15 post induction.

Induction of colonic inflammation

All animals (except group I) were fasted for 12h prior to study, with access to water *ad libitum*. On the day of induction, all rats were anesthetized by an intraperitoneal injection of 1%sodium pentobarbital in a dose of 50mg/kg. Initially each rat received 1ml saline (0.9%) to remove any feces. Rats were maintained in a supine Trendelenburg position. 2ml NaOH (6.25%) was infused for 30s using a soft pediatric catheter size of 6F 2mm in diameter, inserted rectally until the tip was 5cm proximal to anus. In normal controlled experiments, rats received 0.9% saline alone using the same technique. On the 8th day after operation, all rats were weighed and euthanized by cervical dislocation. Total colectomy was performed and segments of distal colon were dissected out, cut along their longitudinal axis .The colonic mucosa was irrigated with physiological saline to remove fecal residue and preserved for macroscopic and histological studies (Koçak et al., 2011).

Biochemical study

The colonic tissue were homogenized by motor-driven Teflon pestlein 10mmol Tris-HCl buffer (pH7.4) and the homogenate were used for the measurement of IL-1β, myeloperoxidase (MPO), reduced glutathione (GSH), and tumor necrosis factor-α (TNF-α).

Assessment of colonic mucosal lesions

The colon was washed with ice-cold saline, flattened on a piece of glossy photographic paper with mucosal surface up, examined with a magnifier and scored for gross mucosal lesions. Scores were given according to the method described by Koçak et al., 2011.The macroscopic damage was scored on a scale of 0-5 (0: no damage, 1: localized hyperemia with no ulcers or erosions, 2: ulcers or erosions with no significant inflammation, 3: ulcers or erosions with inflammation at one side, 4: two or more sites of ulceration and/or inflammation, 5: two or more major sites of ulceration and inflammation or one major site of inflammation and ulceration extending > 1cm along the length of the colon). All sections were evaluated by light microscopy and scored on a scale for ulcer (0: none, 1: > 3mm, 2: < 3mm), inflammation (0: none, 1: mild, 2: severe), granuloma (0: none, 1: present), depth of the disease (0: none, 1: submucosal, 2: muscular, 3: serosal) and fibrosis (0: none, 1: mild, 2: severe).

Histopathological examination of colonic tissue:

Tissue samples representing obvious lesions were taken and fixed in 10% neutral buffered formalin solution for routine histopathological preparation, embedded in paraffin, sectioned at 4μ thickness and stained with hematoxylin and eosin for histological examination by light microscopy then photographed.

Statistical Analysis

Data are provided as mean ± SE. Student t test was used to examine the difference between two groups with a significance value at $P \leq 0.05$. The multiple-way ANOVA test was used to examine the difference between groups of the enrolled animals, followed by Newman-Keuls for multiple comparison. Statistical analysis was performed using Graph Pad Prism® Software, Inc version 5.

3. RESULTS

Histopathological results

The NaOH treatment produced severe focal necrotic and ulcerative lesions with thickening in the distal colonic wall as assessed by the colonic damage score. There was no significant distinction between the gross pathological lesions of the induction group and the group treated by low dose of curcumin.Treatment with high dose of curcumin reduced the severity of the gross lesion score. Dexamethasone also, significantly reduced the intensity of inflammation.

Microscopically, the colons of rats administered NaOH showed massive coagulative necrosis involving the whole thickness of the mucosa leaving remnant of karryorhetic nuclei and heavy inflammatory cellular infiltration. Submucosal layers revealed hyperemia, severe edema, as well as heavy inflammatory cellular

aggregates with obvious transmural inflammation (fig 1). While no significant difference was observed between the group received NaOH and the group treated by low dose of curcumin (fig 2), treatment of rats with high dose of curcumin (fig 3) or dexamethasone (fig 4) reduced the histological signs of inflammation.

Myeloperoxidase activity

Tissue MPO activity showed a statistically significant (p<0.01) difference between the group received NaOH and the control non-treated rats.Treatment with high dose of curcumin or dexamethasone significantly reduced the MPO activity (fig 6)

IL1-β concentration

As compared with the normal control group, colonic inflammation induced by NaOH caused significant increase of IL1-β level. Administration of high dose of curcumin or dexamethasone resulted in a significant

reduction of IL1- level in comparison to the NaOH treated group (p<0.01) (fig 6).

Tumor necrosis factor-α concentration

A significant increase in the TNF-αactivity in the NaOH treated group in comparison with the normal control one was observed (fig 7). Administration of high dose curcumin or dexamethasonecaused a significant reduction of TNF-α colonic level.

Reduced glutathione concentration

Fig 7 demonstrates a significant decrease in the mucosal GSH in the inflamed colon after NaOH administration. After treatment with high dose curcumin or dexamethasone resulted in a significant increase in the GSH concentration, with no significant difference between the two groups.

Table (1): Effect of curcumin on gross lesion score of NaOH-induced Crohn's disease model in rats

Treatment	Microscopic	Macroscopic
Control	0.0±0.0	0.0±0.0
NaOH	7.1±1.2[*]	5.2± 1.3[*]
Dexamethasone	3.9±0.63[#]	2.5±0.5[#]
Curcumin (40mg/kg)	6.5±0.5	4.8±2.4
Curcumin (100mg/kg)	4.1±0.8[#]	2.6±0.12[#]

Values are expressed as mean± SE of ten rats per group.

[*]P<0.01 in comparison to control group
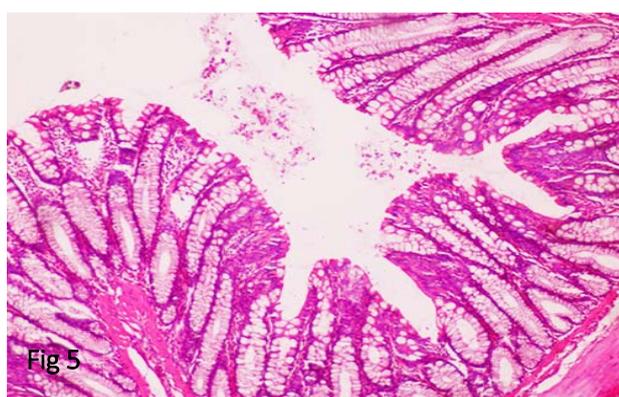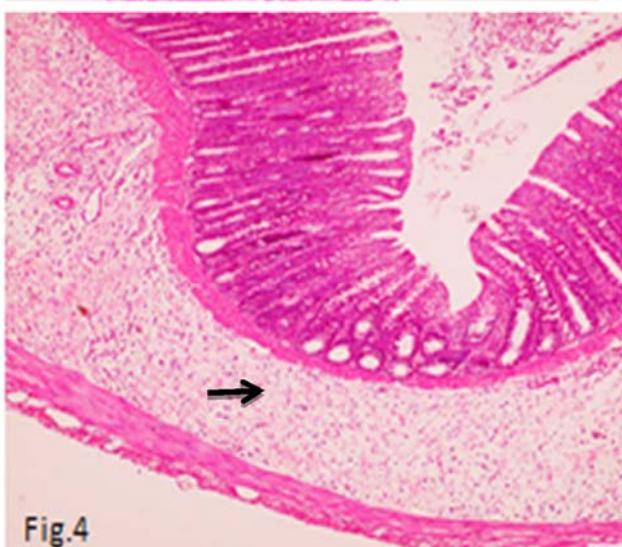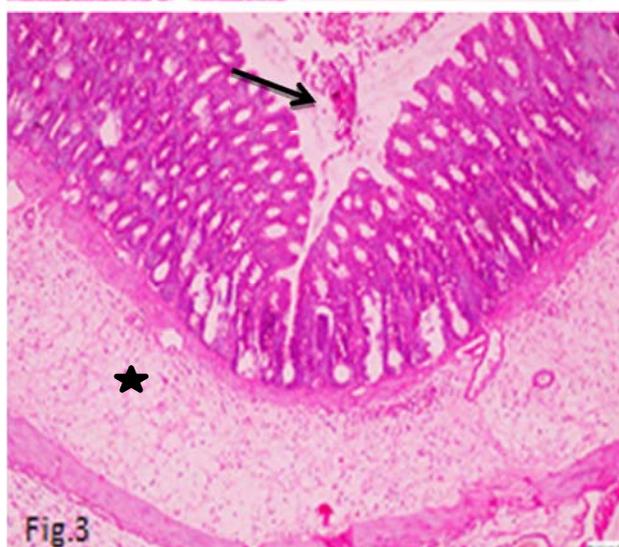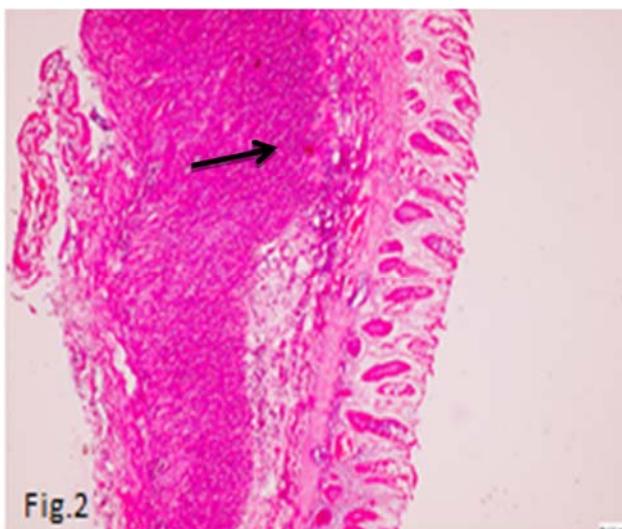
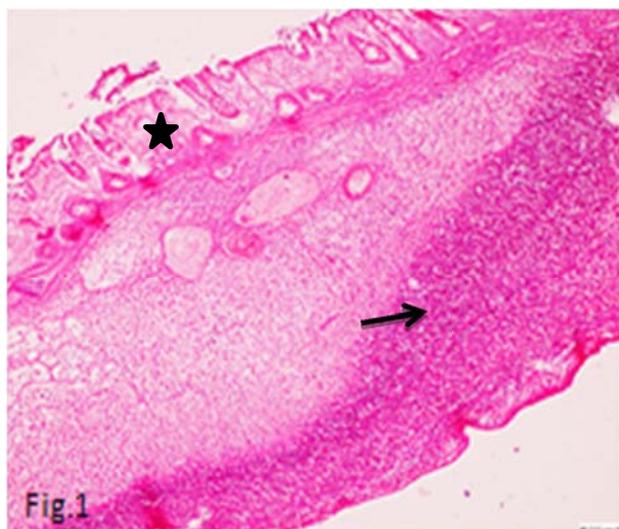[#]P<0.01 in comparison to NaOH-treated group

Fig.1: Crhon's group showing extensive diffuse necrosis in colonic mucosa (star) with heavy cellular infiltration in submucosal layer with transmural inflammation (arrow) (H&E, bar=100).
Fig.2: Group treated with low dose curcumin showing extensive necrosis in colonic mucosa with obvious transmural inflammation (arrow) (H&E, bar=100).
Fig.3: Group treated with high dose curcumin showing sloughing of superficial epithelium (arrow) with moderate submucosal oedema and inflammatory infiltrate (star) (H&E, bar=100).
Fig.4: Group treated with dexamethasone showing slight superficial sloughing of colonic epithelium with moderate submucosal edema (arrow) no transmural inflammation (H&E, bar=100).
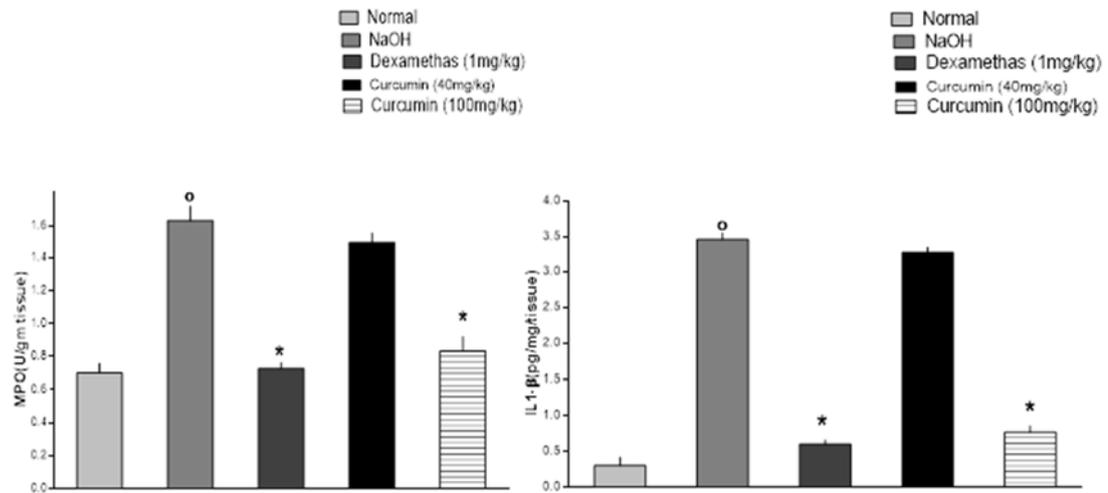**Fig 5:** Normal control group.

Fig 6: The effect of curcumin on MPO and IL1-β colonic activity in NaOH-induced Crohn's in rats
Values are expressed as mean± SE of ten rats.
°Significantly different from normal control rats $p < 0.05$
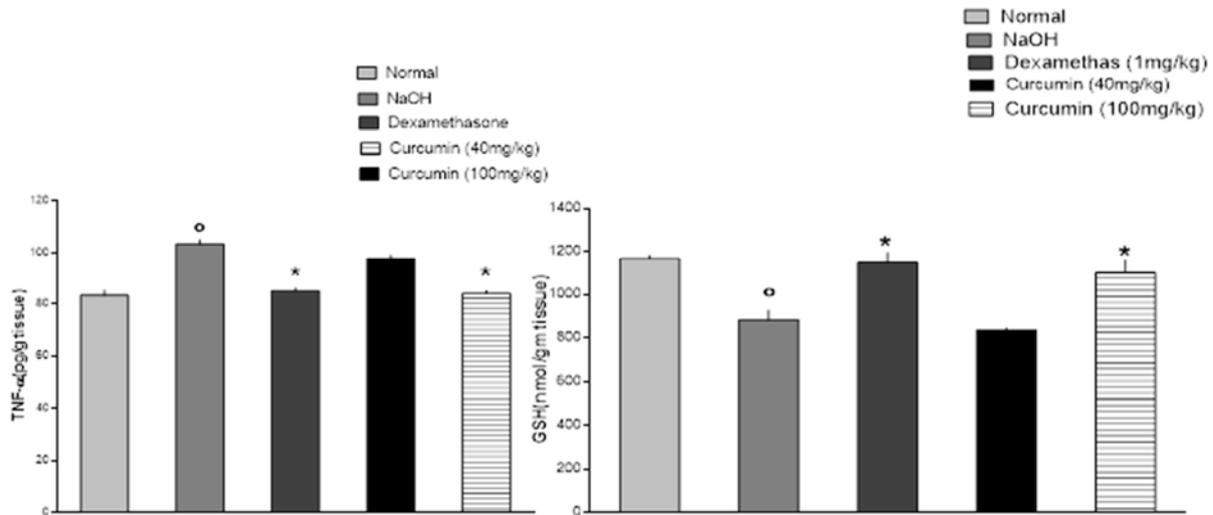*Significantly different from NaOH rats $p < 0.05$

Fig 7: The effect of curcumin on TNF-α and GSH colonic activity in NaOH-induced Crohn's in rats
Values are expressed as mean± SE of ten rats.
°Significantly different from normal control rats $p < 0.05$
*Significantly different from NaOH rats $p < 0.05$

4. DISCUSSION

Crohn's disease (also termed ileitis terminals) is achronic inflammatory bowel disease of unknown etiology. The available data concerning its pathogenesis and therapeutic strategies are insufficient (Boyapati and Satsangi, 2015). Here, we demonstrated that curcumin could attenuate the intestinal inflammation induced by NaOH as verified by macroscopic and histological data.

Moreover, the ameliorated immunopathology was accompanied by reduced expression of the proinflammatory cytokines such as IL1-β and TNF-α. Surprisingly, the anti-inflammatory effect of curcumin was comparable with that of dexamethasone.

Although several experimental studies concerning UC have been reported, unique animal models for CD are limited. In our study, we used the NaOH-induced CD experimental model in rat. Kocak et al., 2011 revealed that NaOH caused transmural inflammation with focal ulceration, severe fibrosis, strictures and irregular thickening of the bowel wall. This picture resembles the characteristic histopathological picture of CD. Our results showed that after instillation of NaOH in the colon of rats, an elevation in the level of TNF-α and IL1-β occurred. These cytokines can activate the mesenchymal cells when secreted in the inflamed intestine. Thereby, exaggerate the inflammatory response and contributes to fibrosis. In addition, they stimulate secretion of IL-1β, IL-6, IL-8 and PGE$_2$ from intestinal smooth muscle cells and fibroblasts (Stallmach et al., 1992). Theses cytokines produces oedema, neutrophil infiltration and epithelial cell necrosis (Ludwiczek et al., 2004). IL-1β seems to be a major stimulator to diarrhea which is a major symptom of intestinal inflammation. It liberates prostaglandins, and thus stimulates anion secretion by epithelial cells. Also, it stimulates histamine, bradykinin and hydrogen peroxide-induced epithelial chloride secretion (Eigler et al., 1997)

Our results showed a significant increase of MPO activity in the NaOH group. MPO is an index of neutrophil infiltration in the inflammed intestine. This infiltration triggers the free radicals release which exerts toxic effects on membrane lipids (van der Veen et al., 2009).

Our study showed a significant reduction of GSH level in NaOH group; this reduction may be due to liberation of oxygen derived free radicals. The increased production of reactive oxygen species, and the impaired antioxidant defense mechanisms are key contributors in inflammatory bowel diseases (Piechota-Polanczyk and Fichna , 2014).

Curcumin has been reported to exert immunopotentiator effect as it caused anti-infective and anti-tumor properties that have both protective and therapeutic anti-inflammatory and antioxidant effects (Bereswill et al., 2010). It exerts its anti-inflammatory effect in experimental colitis by suppressing NF-κβ, inhibiting COX-2 and proinflmmatory cytokines (Yan-Ting et al., 2005).We investigated whether curcumin could prevent inflammatory changes after rectal instillation of NaOH. Interestingly, this polyphenol component of the spice turmeric significantly diminished intestinal inflammation compared to untreated animals. Bereswill and coworkers reported in 2010 a protective anti-inflammatory effect of curcumin in a TH-1type driven small intestinal inflammation model. Our study showed that curcumin caused significant reduction in the levels of IL-1β, and TNF-α. Mazidi et al., 2016 has reported that curcumin regulates PPAR-γ which leads to suppression of proinflammatory cytokines and NF-κβ.

Our results showed that curcumin attenuated mucosal damage and subsequently myeloperoxidase activity. These results are in agreement with Fan and his colleagues who reported that curcumin decreased MPO activity in lung tissue after intestinal ischemia reperfusion injury by inhibiting NF-κB (Fan et al., 2015). Treatment of rats with curcumin showed a significant elevation of GSH levels in colonic tissue. Similar results were observed by Biswas et al., 2005 they reported that curcumin increased GSH levels in cultured alveolar epithelial cells. They explained this effect by the ability of curcumin to increase GSH biosynthesis by increasing glutamyl cysteine ligase catalytic subunit (GCLC expression).The anti-inflammatory effect of curcumin iscomparable to that of dexamethasone which is a well-known ant-inflammtory drug. In inflammatory bowel diseases, dexamethasone exerts its anti-inflammatory effect through reduction of activated NFκB which controls the transcription of inflammatory genes (Schreiber et al., 1998).

5. CONCLUSION

In conclusion, the present study indicates that curcumin may be useful in treatment of CD and other inflammatory bowel diseases, this curative effect may be mediated by its antioxidant and anti-inflammatory effects. However, further experimental and clinical studies are needed to verify the definite explanation of these effects.

6. REFERENCES

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