Effects of Turmeric, and Black Cumin on Induced Colitis in Rabbits

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Abstract

Back ground: The failure of current treatment strategies to control many cases of IBD makes a strong stimulus to find out new modalities of treatment.

Objective: to study the effects of oral curcumin, and black cumin on induced colitis in rabbits.

Materials and Methods: Colitis was induced in rabbits by rectal acetic acid-ethanol (model 1), or acetic acid (model 2). The effects of tested agents (curcumin, and black cumin) were compared to distilled water (control), and prednisolone regarding changes in body weight, colon segment weight, and gross and microscopical scores.

Result: In model 1, severe gross and microscopical damage observed in colon. Gross and microscopical scores of curcumin group were not significantly different from that of control and of prednisolone groups.

In model 2, a less severe inflammation occurred; yet, an evident gross and microscopical damage were observed.

Black cumin and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control. The gross and microscopical damages were apparently lowered when black cumin, curcumin and prednisolone were used, but these changes were significant for prednisolone, and black cumin (grossly), and for prednisolone (microscopically).

The gross and microscopical effects of curcumin, and black cumin were comparable to those of prednisolone.

Conclusion: Acetic acid-induced colitis in rabbits (model I) is preferred for testing the anti-inflammatory effectiveness of new therapeutic modalities.

Black cumin oil and curcumin have an anti-inflammatory activity in this model.

Keyword: Inflammatory bowel disease, free oxygen radicals, induced colitis, acetic acid, curcumin, black cumin.

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Introduction

Idiopathic inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic or relapsing immune activation and inflammation within the gastrointestinal tract [(1)]. Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of idiopathic IBD [(2)].

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Ulcerative colitis and CD pursue a protracted, relapsing and remitting course, usually extending over years [(3)].

Recent studies pointed to the important role of free oxygen radicals in the pathogenesis of IBD both in animal models of induced colitis and in human beings.

One of the more commonly used models of Induced Colitis in Rabbits is acetic acid induced colitis [(4)].This experimentally induced colitis is similar to the human condition in certain aspects {e.g., acute inflammation with neutrophil infiltration [(5)], increased concentrations of LT B4, and PG E2 [(6)], superoxide dismutase [(7,8)]. and increased

production of inflammatory mediators, such as hydrogen peroxide (H2O2), nitric oxide (NO), myeloperoxidase activity (MPO), and tumor necrosis factor (TNF-alpha)} [8].

Black cumin contains about 0.5-1.5% volatile oils including Nigellone and thymoquinone, which are responsible for Black cumin's anti-histamine, anti-oxidant, and anti-infective effect. Both are effective in their own standards, however, black cumin oil is more concentrated than the herb itself [9]. Antioxidant activity of Nigella sativa essential oil is documented in many studies [10].

Many studies showed that curcumin, exhibits antiinflammatory، antitumor, and antioxidative properties [11, 12]. and it has been found to have an acceptable safety in human. A phase 1 human trial with 25 subjects using up to 8000 mg of curcumin per day for 3 months found no toxicity from curcumin. Five other human trials using 1125-2500 mg of curcumin per day have also found it to be safe. These human studies have found some evidence of anti-inflammatory activity of curcumin [13].

Materials and Methods

Colitis was induced in male rabbits by rectal administration of %٥acetic acid-30% ethanol (model 1) [14], or 2% acetic acid (model 2)[15]. The animals were allowed to have free access to food and water all over the period of study except for 24 hours before induction of colitis (fasting). Animals in different groups were orally administered 10 ml of distilled water (control), prednisolone (2 mg/kg/day dissolved in 10 ml distilled water), or curcumin (50 mg/kg/day dissolved in 10 ml of distilled water) (model I), black cumin oil (0.2 ml/kg/day, each dose was followed by 5 ml of distilled water orally in addition to distilled water, prednisolone, and curcumin (model II).

Each agent (including distilled water) was administered orally two days prior to induction of colitis, the day of induction, and a dose ٢٢hours post-induction (i.e., 2 hours prior to killing of the animal). Twenty-four hours after induction, the animals were sacrificed and the abdomen was opened longitudinally, and a segment of colon 8 cm [16]. proximal to anus was removed for assessment of colonic inflammation.

The effects were observed as changes in body weight, colon segment weight and gross histological score (using a dissecting microscope) (Table-1) [17].

Colonic samples (0.5 cm of length) were taken from the 8 cm segment, fixed in 10% formaldehyde and the routine 5 micron paraffin embedded sections were prepared. Tissues were routinely stained with haematoxylin and eosin, coded, and evaluated blindly by light microscopy with low and high power (40 xs) objective lenses [18]. Each slide was scored according to Christian, et al., [19]. to assess the extent of colonic inflammation. The score ranges from 0 to 40 (total score), which represents the sum of the products of each criterion by the score of the percentage involvement. All evaluations were performed by observers unaware of the treatment groups.

Criteria of scores divided as follows:

- Inflammation severity scored from 0-3 as None, Mild, Moderate، Severe respectively,
- Inflammation extent from 0-3 as None, Mucosa، Submucosa, Transmural, respectively,
- Crypt damage from 0-4 as None, Basal 1/3 damage, Basal 2/3 damage, Crypt lost; surface epithelium present, Crypt and surface epithelium lost respectively,

- Per cent involvement from 0-4 as 1-25%, 26-50%, 51-75%, 76-100% respectively,

The score (total score) represents the sum of the products of each of the first three criteria by the score of the percentage involvement [20].

Statistical analysis

Results are expressed in tables as means ± standard error of the mean (SE), or drowns as bar charts. Paired student's T test was applied for data from the same group, while unpaired student's T test was used for data of different groups. When P value was < 0.01, it was considered as highly significant, while $p < 0.05$ was considered as significant [21].

Results

In model one, 5% acetic acid- 30% ethanol induced a severe gross and microscopical damage in colon with marked increments in weight of colonic segment. Gross score and colon segment weight of curcumin group were not significantly different from those of the control and of the prednisolone groups ($p > 0.05$), (Fig. 1, Fig. 2, and Fig. 3).

Microscopical score of curcumin group was also not significantly different from that of the control group ($p > 0.05$), but, it was significantly lower than prednisolone group ($p < 0.05$) (Fig. 4).

In model two, 2% acetic acid induced a less severe form of inflammation in colon; yet, it had a marked effect in reducing the body weight of rabbits and with evident gross and microscopical damage in colon.

Curcumin, Black cumin and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control group (Table 2).

The mean (±SE) post-induction rectal temperature for control group (38.78 ± 0.2Cº), curcumin group (38.81 ± 0.2Cº), and black cumin group (38.88 ± 0.09Cº) showed a statistically insignificant ($p < 0.05$) increment from the mean pre-induction readings (38.55± 0.14Cº), (38.56 ± 0.25Cº), and (38.74 ± 0.21Cº) respectively. While post-induction readings for prednisolone group (37.84 ± 0.38Cº) decreased insignificantly ($p > 0.05$) from mean pre-induction reading (38.61 ± 0.15Cº).

When comparing mean post-induction rectal temperature of different treatment groups to the corresponding readings of control group, the differences were insignificant ($p > 0.05$), while when comparing corresponding readings of prednisolone and other treatment groups (curcumin, and black cumin groups) there was a significant decrease ($p < 0.05$) in mean post-induction rectal temperature of prednisolone group, (Fig. 5).

The mean colon segment weight of curcumin group and prednisolone group were insignificantly ($p > 0.05$) more than that of the control group. While that of black cumin group was insignificantly ($p > 0.05$) less than that of the control group, (Fig.6).

As shown in (Table 3), there was an obvious reduction of the mean gross histological score of all treatment groups from that of the control group and this reduction was significant ($p < 0.05$) for prednisolone group and black cumin group, while it was not significant ($p > 0.05$) for curcumin group (Fig. 7).

There was an obvious reduction of the mean microscopical histological score of all treatment groups compared to the control group and this reduction was significant ($p < 0.05$) for prednisolone group only, but not significant for black cumin and curcumin groups, (Fig. 8).

The effects of curcumin and black cumin in regards to colonic segment

weight, gross histological score, and microscopical score were comparable to those of prednisolone ($p > 0.05$).

Table 1: Gross mucosal inflammation scoring index. (Modified from Brian, et al., 1997)[17]

Score	Macroscopic Appearance
0	Normal
1	No ulcer; mild petechia/hypervascularity
2	No ulcer; moderate petechia/hypervascularity
3	Ulcer <1 cm with petechia/hypervascularity
4	Same as above at 2 or more sites
5	Ulcer ≥ 1 cm with petechia/hypervascularity
6	Ulcer ≥ 2 cm with petechia/hypervascularity
7	Ulcer ≥ 3 cm with petechia/hypervascularity
8	Ulcer ≥ 4 cm with petechia/hypervascularity
9	Ulcer ≥ 5 cm with petechia/hypervascularity
10	Ulcer ≥ 6 cm with petechia/hypervascularity

Table 2: Mean Initial and Post-induction Body Weight (g) of control and treatment groups in Acetic acid (2 %)-induced colitis

Groups	Mean Initial Body Weight (±SE) (g)	Mean Post-induction Body Weight (±SE) (g)
Control	1225 ± 86.2	1170 ± 88.96 ***
Prednisolone	1228.3 ± 83	1183.3 ± 90*
Curcumin	1168.3 ± 55	1046 ± 29**
Black cumin	1266.67 ± 117.7	1216.67 ± 107.3*

**** Highly significant ($p< 0.005$) in comparison with the initial B.Wt*
*** Highly significant ($p < 0.01$) in comparison with the initial B.Wt*
** Significant $p < 0.05$ in comparison with the initial B.Wt*

Table 3: Mean (±SE) gross histological score (0-10) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

Groups	No. of Rabbits	Mean gross score ± (SE)
Control	7	8.86 ± 0.51
Prednisolone	6	7 ± 2.45 *
Curcumin	6	7.17 ± 1.49
Black cumin	6	5 ± 1.67*

**Significant reduction ($p < 0.05$) in comparison with the control*

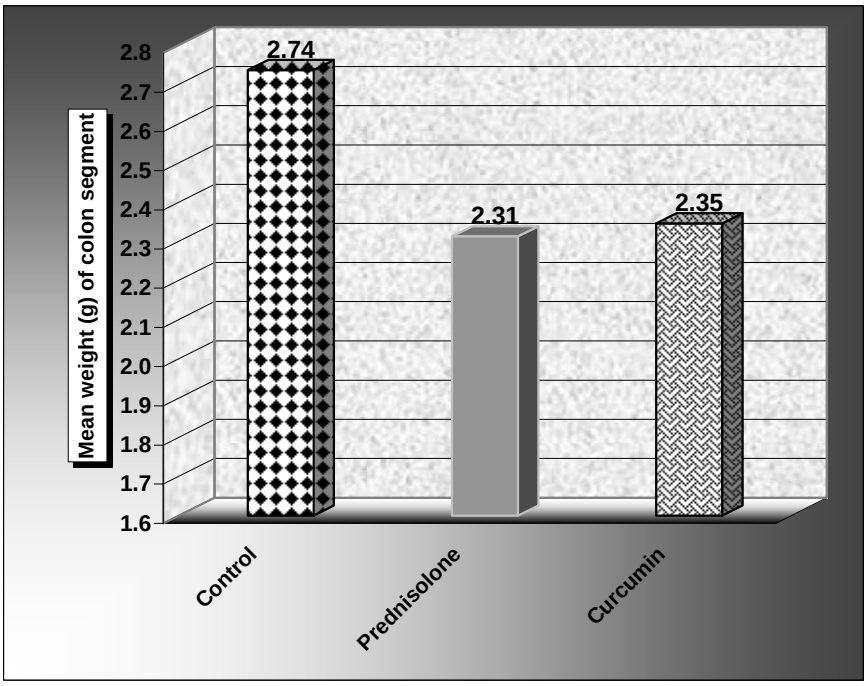


Figure 1: Mean weight of colon segment (g) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

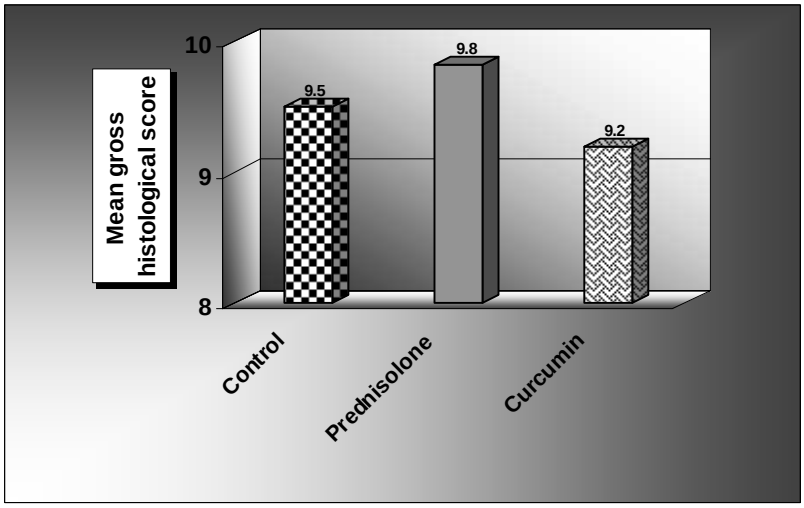
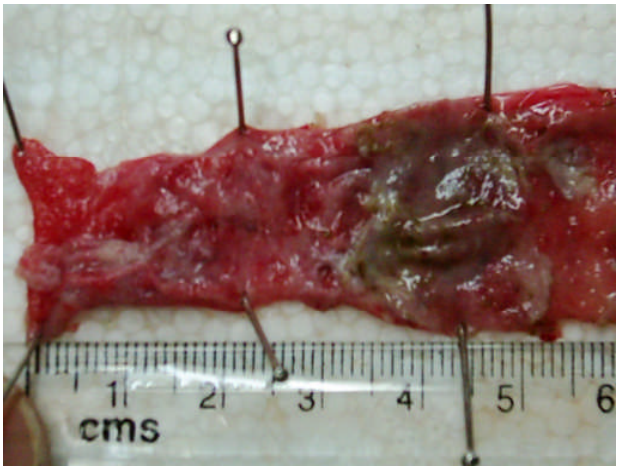


Figure 2: Mean gross histological score (0-10) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis



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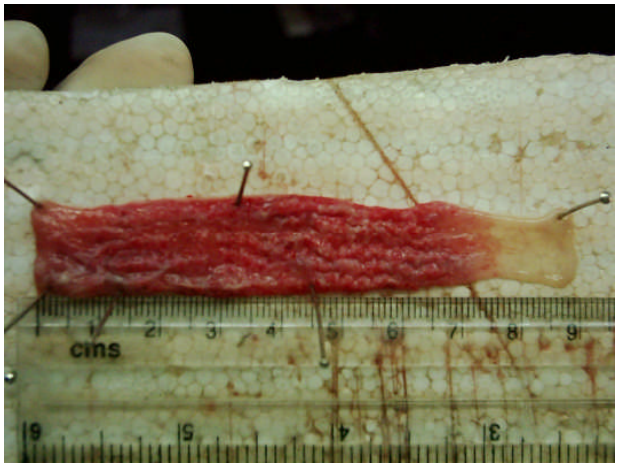

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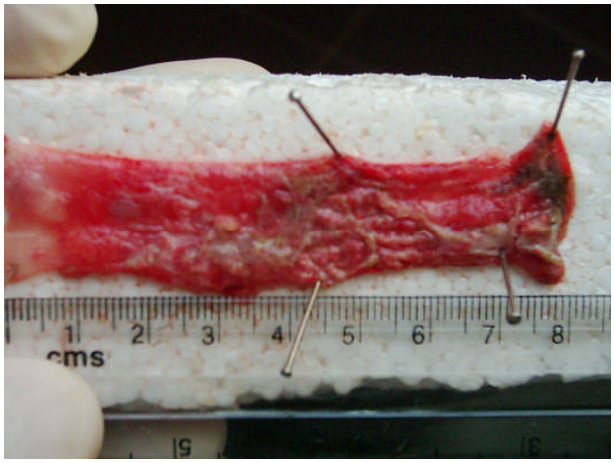

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Figure 3: the gross appearance of colon segments of control and treatment groups in acetic acid (5 %) - ethanol (30%)-induced colitis, A: control, B: prednisolone, C: curcumin.

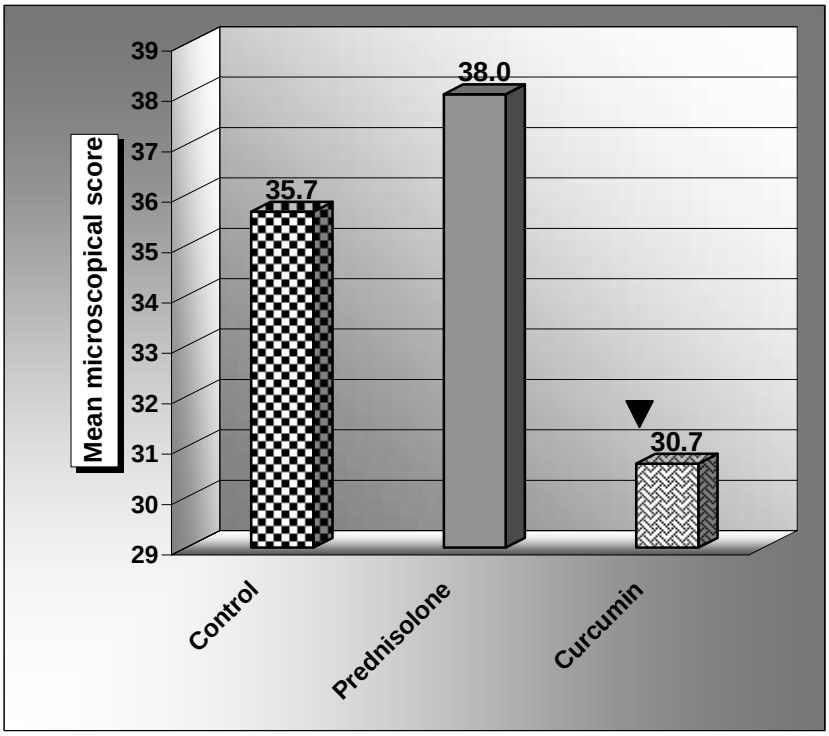

Figure 4: Mean microscopical histological score of the colon (0-40), of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis
▼ ***Significant difference (p < 0.05) in comparison with prednisolone group***

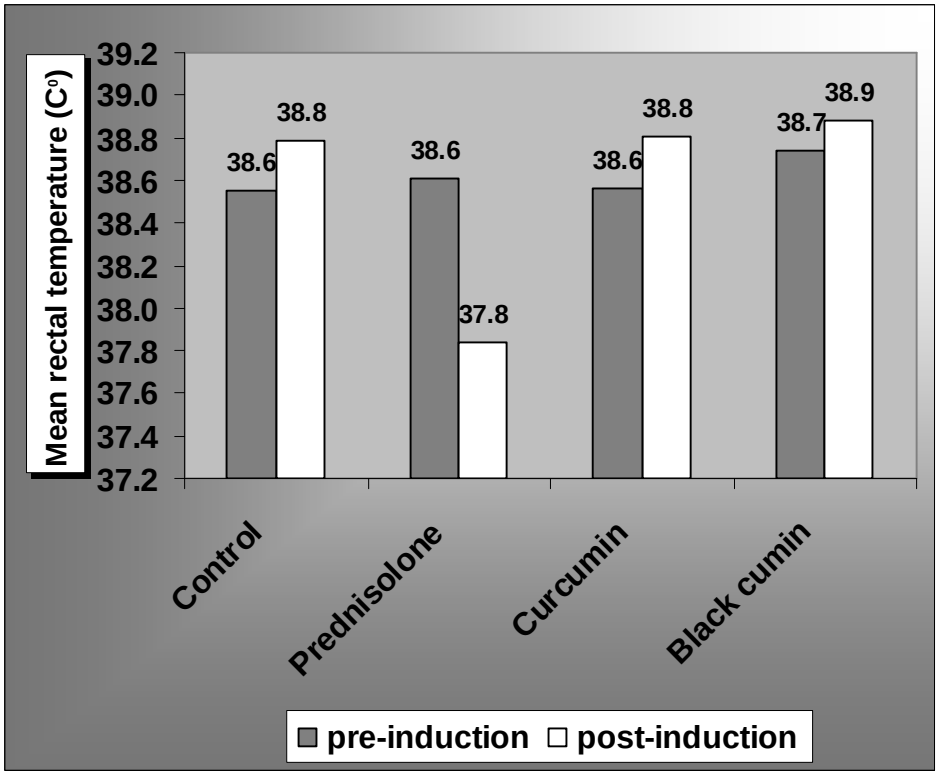

Figure 5: The mean pre-induction and post-induction rectal temperature of rabbits in control and treatment groups in acetic acid (2%) - induced colitis
▼*Significant reduction (p < 0.05) in comparison with post-induction rectal temperature of curcumin and black cumin groups*

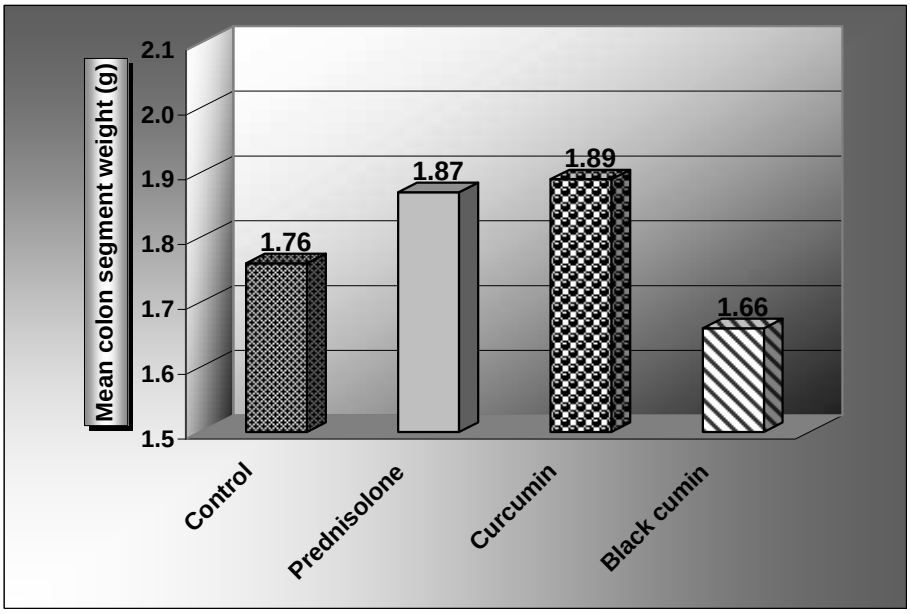

Figure 6: Mean colon segment weight (g) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

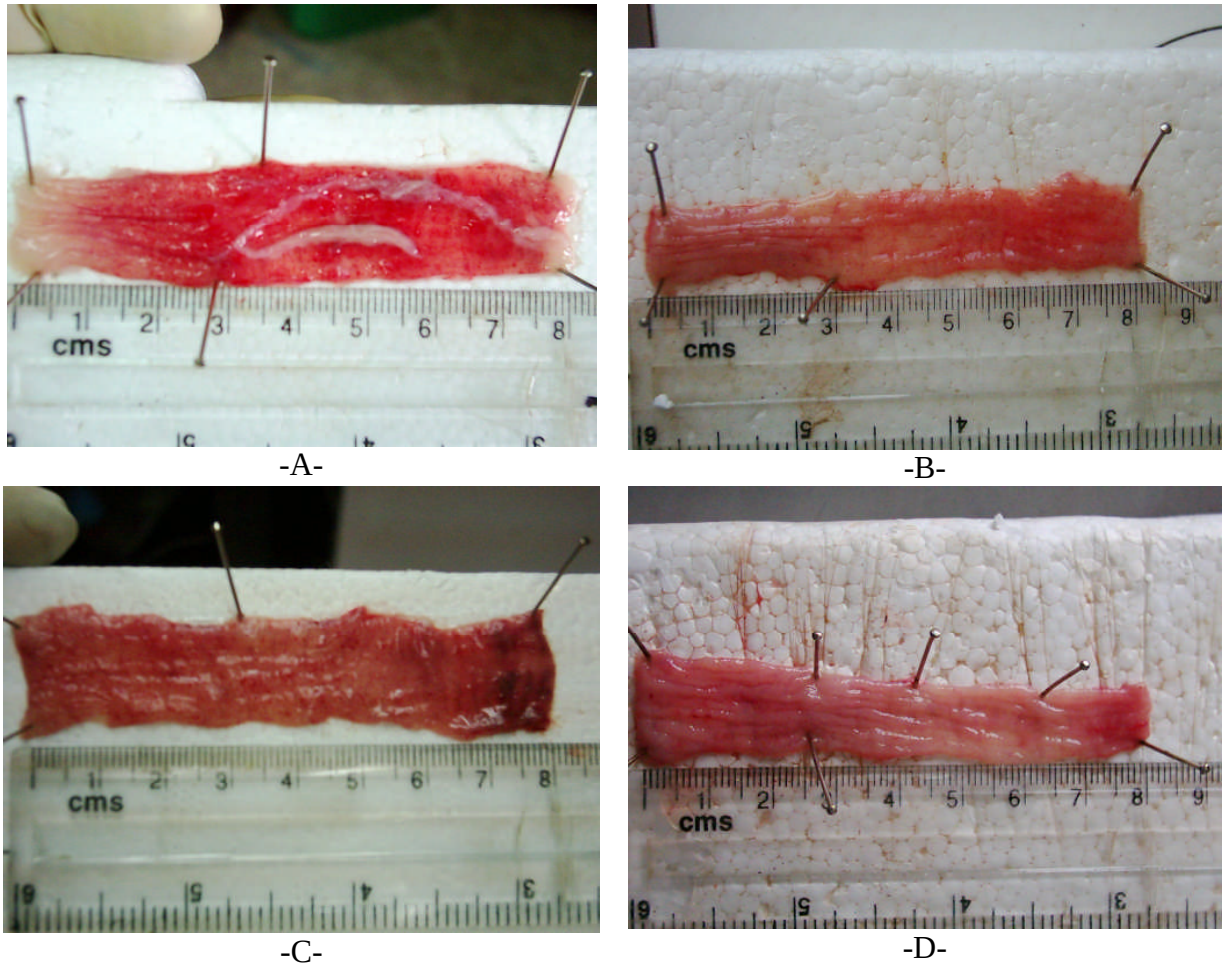

Figure 7: the gross appearance of colon segments of control and treatment groups in acetic acid (2%)-induced colitis, A: control, B: prednisolone, C: curcumin, D: black cumin

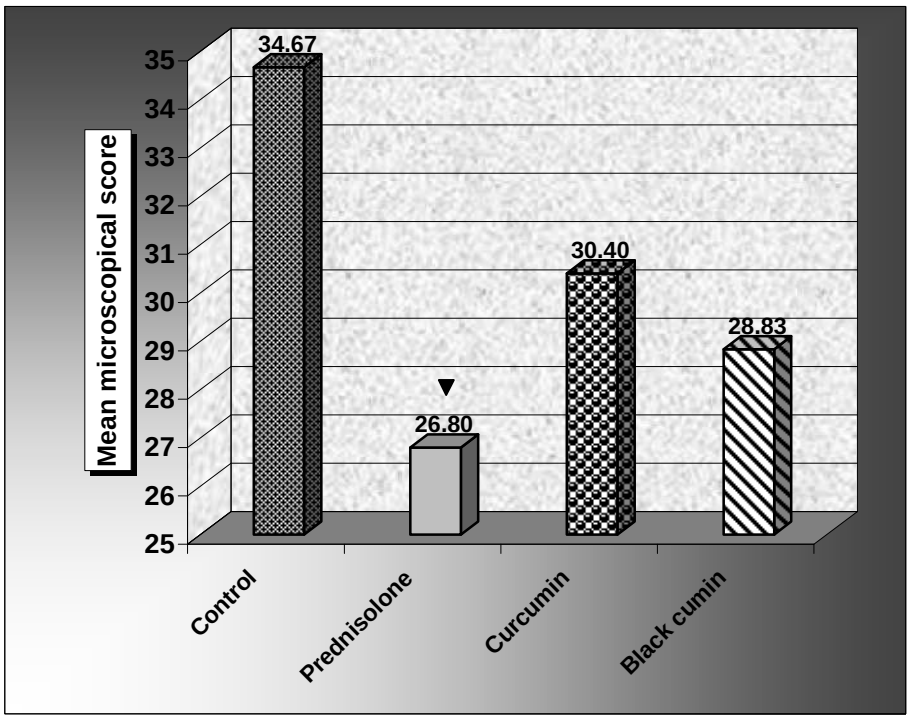

Figure (8): Mean microscopical histological score (0-40) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

▼ ***Significant reduction ($p < 0.05$) in comparison with the control group***

Discussion

Various animal models have provided a foundation for future investigation into the mechanisms responsible for IBD, which will hopefully result in the development and testing of novel therapeutic regimens [(22)].

Acetic acid-induced colitis is used widely because of its reproducibility (with lesions occurring in 100% of animals). In addition, it provides an inexpensive model useful in comparing the effectiveness of novel therapeutic agents. [(23)]. Its similarity with human IBD in many aspects make researchers still use it as one of the models of induced colitis.

Pilot studies done prior to the present work governed the selection of the two models of colitis induction particularly the second model, i.e., acetic acid (2%) that was used in rabbits for the first time.

In the first model of the present study, ethanol was used in combination with acetic acid in order to decrease the mucosal barrier [(24)]. so that the damaging effect of the acid was found to be more severe and deeper than that induced by acetic acid alone.

Finding an orally effective anti-inflammatory agent is of a major importance since the advantages of oral route are well known.

Moreover, selection of such route of administration in the present in vivo study could give a chance for the tested agent to act systemically and / or locally at the colon.

Prednisolone, the oral corticosteroid used commonly as a standard therapy to control acute attacks of IBD [(3)]. was used in the present study as what is called a positive control [(25, 26)].

It was found that non-steroidal antiinflammatory drugs exacerbate experimental colitis in rats [27]. For that, these agents were not used in this study.

Turmeric (the dried rhizome of the perennial herb *Curmcua Loga;* a major constituent of which is curcumin) and black cumin oil are relatively safe and used orally as food additives, and are available and relatively cheep; besides, they are known to have anti-inflammatory and antioxidant properties، which may be the starting point for an effective drug therapy in IBD.

The schedule of therapy (2 days before and 1 day after induction of colitis) was dependent in this study to evaluate mainly the possible prophylactic role of the tested agents in addition to their effectiveness in initial therapy for acute attacks of colitis, which is the major problem of the relapsing and remitting IBD.

In model 1 in this study, the insignificant difference in the means of weight of colonic segment of curcumin, and prednisolone groups from that of the control group may be due to the severe form of inflammation and edema induced by the 5% Acetic acid-30% Ethanol in all groups. For the same reason there was no significant correlation between the gross histological score and the segment weight in this model correlation coefficient (r) = 0.185, $p > 0.05$.

In comparison with model 1, although there were no significant differences ($p > 0.05$) in model 2 in colon segment weight, but there was a positive correlation between the gross histological scores and the corresponding segment weight ($r = 0.37$, $p < 0.05$). Rachmilewitz، et al., (1995) [28]. showed that the weight of colon segment involved by inflammation is increased and could be decreased by an inhibitor of nitric oxide synthase.

Regarding the mean gross histological score in model 1, its insignificant difference for all treatment groups from that for the control group, could be explained by severity of inflammation induced by acetic acid (5%)–ethanol (30%) that even could abolish the expected prednisolone effect (mean gross score = 9.8 ± 0.16). In model 2, the obvious reductions in mean gross histological score for all treatment groups pointed to the effectiveness of the tested agents، particularly prednisolone, and black cumin oil, to reduce inflammatory process in acetic acid (2%) model.

Moreover, compared to effect of prednisolone on mean gross histological score, curcumin (models 1 and 2), and black cumin oil (model 2) had comparable effects; this could indicate their possible potent initial anti-inflammatory effects.

In model 1, the insignificant differences (obtained by all tested agents) in mean microscopical histological scores which simulated what was found in regard to mean gross score (see above), enforced the idea of unsuitability of acetic acid (5%) –ethanol (30%) model to evaluate the effectiveness of tested agents in initial treatment of induced colitis in rabbits.

In model 2, the reductions in microscopical scores induced by curcumin and black cumin oil emphasized the effective anti-inflammatory role of these agents particularly when these findings conjugated with their anti-inflammatory effects detected grossly (see above). However, for black cumin group, p value = 0.056 (i.e., so near to the significance level).

In model 2, compared to control group، prednisolone and black cumin oil exerted a better apparent protective

role than curcumin regarding the induced reduction in mean body weight.

The significant reduction of mean body temperature of prednisolone group from the corresponding readings for the other treatment groups may point to its effect on the body temperature in this model (unknown mechanism). Although , all of the means of body temperature were within the normal range (37.8. - 39.4. C).

Results of the present study revealed that curcumin in a daily oral dose of 50 mg/kg had a comparable effect with that of prednisolone (given orally in a dose of 2 mg/kg/day) against acetic acid –induced colitis in rabbits.

Salh B., et al., (2003) [29]. concluded that curcumin was able to attenuate experimental colitis through a mechanism correlated with the inhibition of the activation of NFkappaB. Other studies showed that intestinal mucosal CD4 (+) T cells and B cells increase in animals treated with curcumin, suggesting that curcumin modulates lymphocyte- mediated immune functions [30].

In the present study, results of black cumin oil administered orally in a daily dose of 0.2 ml/kg/day revealed its potential efficacy in attenuation of experimentally-induced colitis in rabbits; such efficacy was comparable to that of prednisolone given orally in a dose of 2 mg/kg/day.

The anti-inflammatory and analgesic effects of black cumin are documented by Al-Ghamdi (2001) [31]. Mansour, et al., (2001) [32] and Badary, et al., (2003) [33]. found that thymoquinone (a volatile oil of black cumin) to be a potent superoxide anion scavenger of different free radicals thus may play an important role as antioxidant. El- Abhar, et al., (2003) [34]. found that black cumin oil or thymoquinone administered orally have a protective activity against gastric mucosal injury in rats which were subjected to ischemia/reperfusion protocols. Thus, the present study supports the protective role of black cumin oil administered orally against induced injury in gastrointestinal mucosa.

Conclusions

This study showed that:

1. Acetic acid (2%) administered rectally is preferred in induction of colitis in rabbits for testing the anti-inflammatory effectiveness of new therapeutic modalities.

2. Oral black cumin oil (in dose of 0.2 ml/kg/day) had a potential efficacy in attenuation of acetic acid -induced colitis in rabbits.

3. Oral curcumin (in dose of 50 mg/kg/day) had an accepted intiinflammatory activity against acetic acid –induced colitis in rabbits.

Recommendations

- Further in vivo studies are required to elaborate the preferred doses, safety, and exact mechanism of action of black cumin oil، and curcumin in prophylaxis and treatment of experimentally induced colitis.
- Subsequently, clinical studies are recommended to explore the potential anti-inflammatory effect and safety of black cumin oil either as monotherapy or as adjunct to the routinely used treatment of patients with inflammatory bowel diseases.

Acknowledgment

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