Effects of Oral Zinc Sulfate 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.

Aims: to study the effects of zinc sulfate 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 zinc sulfate were compared to distilled water (control), and prednisolone regarding changes in body weight, colon segment weight, and gross and microscopical scores. Plasma zinc and copper concentrations were measured in control and zinc sulfate groups in both models.

Results: In model 1, severe gross and microscopical damage observed in colon. Gross and microscopical scores of zinc sulfate 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.

Zinc sulfate and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control. The gross and microscopical damages were significantly lowered in zinc sulfate and prednisolone groups.

In both models (1 and 2), a significant decrement in post induction mean plasma zinc level was detected ($p < 0.05$); however, such decrement could be corrected by zinc sulfate therapy.

Conclusions: Acetic acid -induced colitis in rabbits (model II) is preferred for testing the anti-inflammatory effectiveness of new therapeutic modalities. Zinc sulfate has a valid prophylactic activity in this model.

Keywords: Inflammatory bowel disease, induced colitis, acetic acid, zinc sulfate, prednisolone.

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

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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 (H_2O_2), nitric oxide (NO), myeloperoxidase

activity (MPO), and tumor necrosis factor (TNF-alpha) [8].

Zinc has both antioxidant and anti-inflammatory actions. Zinc consideration as an antioxidant stems from its presence in Cu/Zn-Superoxide dismutase, an enzyme with a major role as scavenger of free radicals in the cytoplasm of many types of cells and in the extracellular space.

Hence according we try to explore the possible beneficial prophylactic and therapeutic effect of oral zinc sulfate for induced colitis in rabbits.

Materials and Methods

Colitis was induced in male rabbits by rectal administration of 5% acetic acid-30% ethanol (model 1) [9], or 2% acetic acid (model 2) [10]. 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 zinc sulfate (50 mg/kg/ day dissolved in 10 ml distilled water). Each agent (including distilled water) was administered orally two days prior to induction of colitis, the day of induction, and a dose 22 hours 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 [11], 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 (Table 1) [12].

Colonic samples (0.5 cm of length) were taken from the 8 cm segment, fixed in 10% formaldehyde and the routine 5 micrometer sections were prepared. Tissues were routinely stained with haematoxylin and eosin, coded, and evaluated blindly by light microscopy (with 40x high power objective lens) [13]. Each slide was scored according to Christian, et al., [14] 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.

Scores ranged from 0 to 40, four criteria were depended: 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; Percent involvement from 0-4 as 0%1–25%, 26–50%, 51–75%, 76–100%, respectively. The score (total score) represents the sum of the products of each criterion by the score of the percentage involvement [15].

Plasma zinc and copper concentrations were measured in control and zinc sulfate groups in both models before the first dose of treatment and just before killing with the aid of atomic absorption spectrophotometry.

Results are expressed in tables as means $\pm$ standard error of mean (SEM), or drown 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 $0.01 < p$

≤ 0.05 was considered as significant [16].

Results

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

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

Zinc sulfate treatment and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control group (Table 2).

The mean (±SEM) post-induction rectal temperature for control group (38.78 ± 0.2Cº) showed a statistically insignificant ($p > 0.05$) increment from the mean pre-induction readings (38.55 ± 0.14Cº). On the other hand, post-induction readings for zinc sulfate group (38.73 ± 0.17Cº) and post-induction readings for prednisolone group (37.84 ± 0.38Cº) decreased insignificantly ($p > 0.05$) from mean pre-induction readings (38.94 ± 0.21Cº), and (38.61 ± 0.15Cº) respectively.

When comparing mean post-induction rectal temperature of zinc sulfate group and prednisolone group to the corresponding readings of control group, the differences were insignificant ($p > 0.05$), while when comparing corresponding readings of prednisolone and zinc sulfate group, there was a significant decrease ($0.01 < p < 0.05$) in mean post-induction rectal temperature of prednisolone group, (Figure 5) .

The mean (±SEM) colon segment weight of zinc sulfate group (2.02 ± 0.14 mg) and prednisolone group (1.87 ± 0.16 mg) were insignificantly ($p > 0.05$) more than that of the control group (1.76 ± 0.10 mg), (Figure 6).

Compared to that of prednisolone group, the mean weight of colon segment of zinc sulfate group did not differ significantly ($p > 0.05$).

The means gross histological score significantly lowered in zinc sulfate group and prednisolone group in comparison to the control group ($p < 0.05$) (Table 3) and (Figure 7).

The mean microscopical score was significantly lowered in zinc sulfate group and prednisolone group in comparison to the control group ($p < 0.05$) (Figure 8).

The effects of zinc sulfate in regards to colonic segment weight, gross histological score, and microscopical score were comparable to those of prednisolone ($p > 0.05$).

In both models, (1 and 2), a significant decrement in post induction mean plasma zinc level was detected ($p < 0.05$); however, such decrement could be corrected by zinc sulfate therapy, on the other hand, post induction mean plasma copper concentration obviously increased when compared to the pre-induction levels, (Table 4) and (Table 5) below shows the changes in plasma concentrations of zinc and

copper in response to induction of colitis and zinc sulfate treatment.

Table 1: Gross mucosal inflammation scoring index. (Modified from Brian, et al., 1997)[12]

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 (±SEM) (g)	Mean Post-induction Body Weight (±SEM) (g)
Control	**1225 ± 86.2**	**1170 ± 88.96 *****
Prednisolone	**1228.3 ± 83**	**1183.3 ± 90***
Zinc Sulfate	**1285.7 ± 90.4**	**1238.6 ± 91.2 ****

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

Table 3: Mean (±SEM) 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 ± (SEM)
Control	7	8.86 ± 0.51
Prednisolone	6	7 ± 2.45 *
Zinc Sulfate	7	6.25 ± 1.23 *

**Significant reduction (0.01< p < 0.05) in comparison with the control*

Table 4: Mean plasma zinc and copper concentrations (ppm) of control and zinc sulfate groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

Group	Plasma zinc concentration		Plasma copper concentration	
	Pre-induction	***Post-induction***	***Pre-induction***	***Post-induction***
Control	**1.35 ± 0.18**	**0.31** ± 0.10**	**0.43 ± 0.08**	**0.68* ± 0.11**
Zinc sulfate	**0.93 ± 0.11**	**0.50* ± 0.10**	**0.34 ± 0.09**	**0.50 ± 0.15**

*** Highly significant reduction (p< 0.01) in comparison with its pre induction level*
** Significant difference (p < 0.05) in comparison with its pre induction level*

Table 5: Mean (±SEM) plasma zinc and copper concentrations (ppm) of control and zinc sulfate groups in acetic acid (2 %)-induced colitis

Group	Plasma zinc concentration		Plasma copper concentration	
	Pre-induction	**Post-induction**	**Pre-induction**	**Post-induction**
Control	**1.66 ± 0.10**	**1.32 ± 0.12 ***	**0.71 ± 0.10**	**0.76 ± 0.08**
Zinc sulfate	**1.39 ± 0.11**	**1.56 ± 0.15**	**0.61 ± 0.11**	**0.81 ± 0.17 ***

** Significant difference (p < 0.05) in comparison with the pre-induction levels*

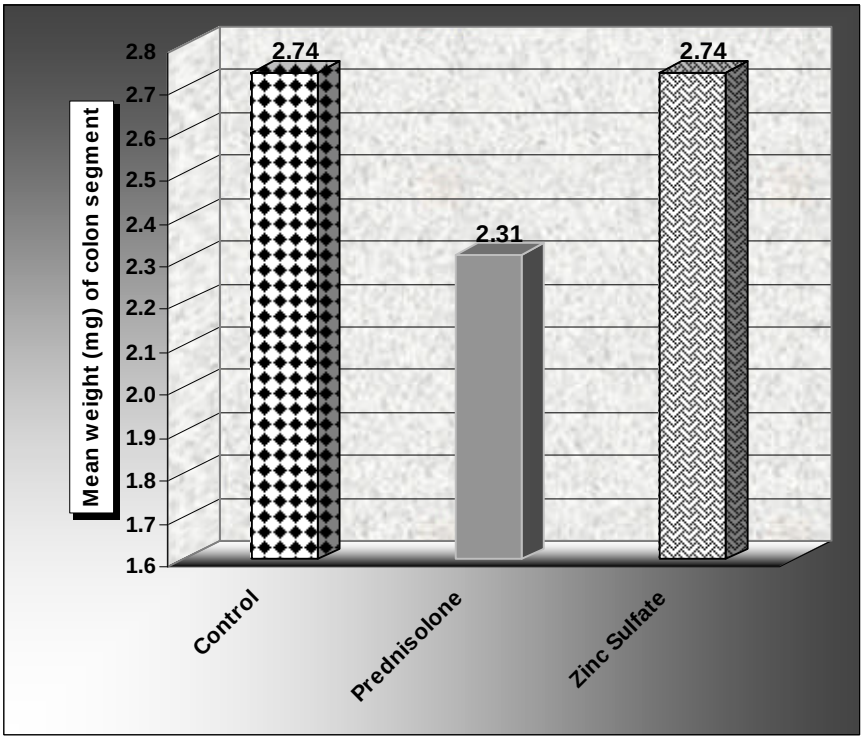


Figure 1: Mean weight of colon segment (mg) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

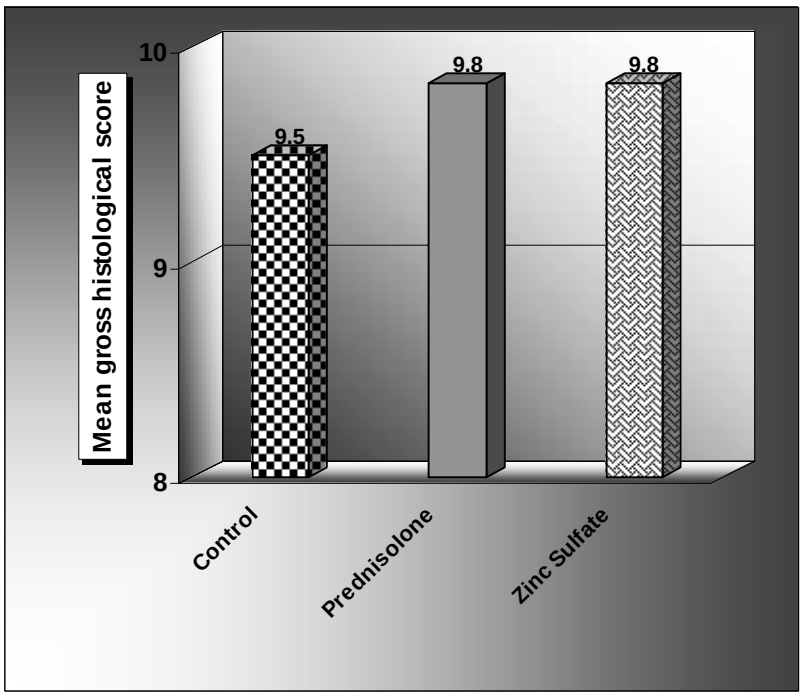
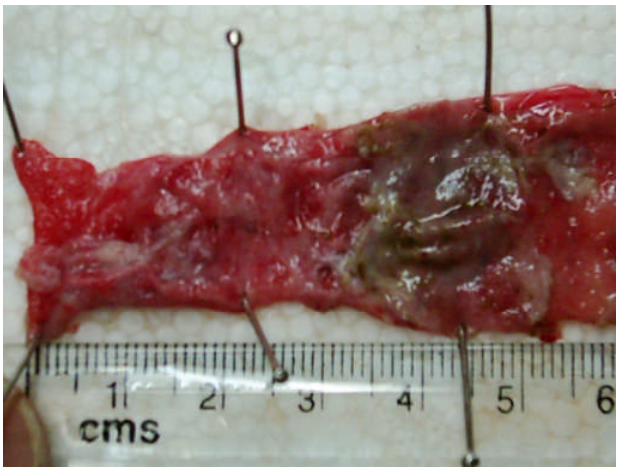


Figure 2: Mean gross histological score (0-10) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis.



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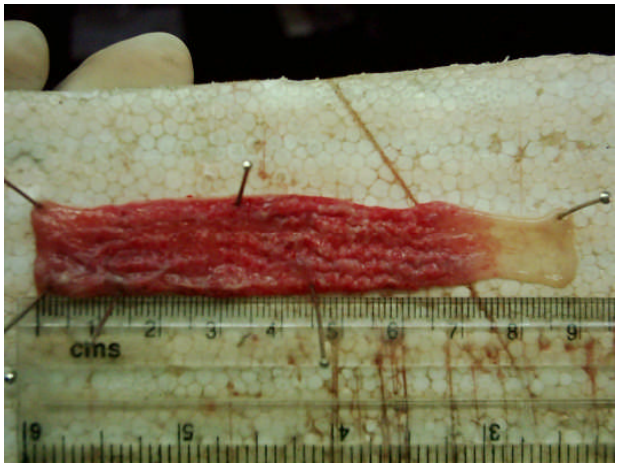

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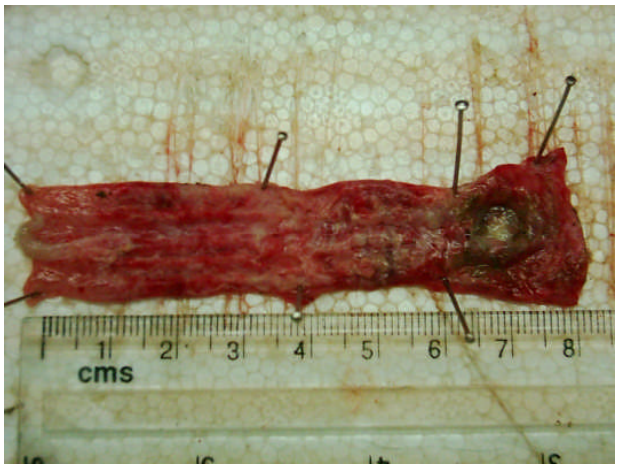

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Figure 3**: The gross appearance of colon segments of control and treatment groups in acetic acid (5 %) - ethanol (30%)-induced colitis, A: control, B: prednisolone, C: zinc sulfate.**

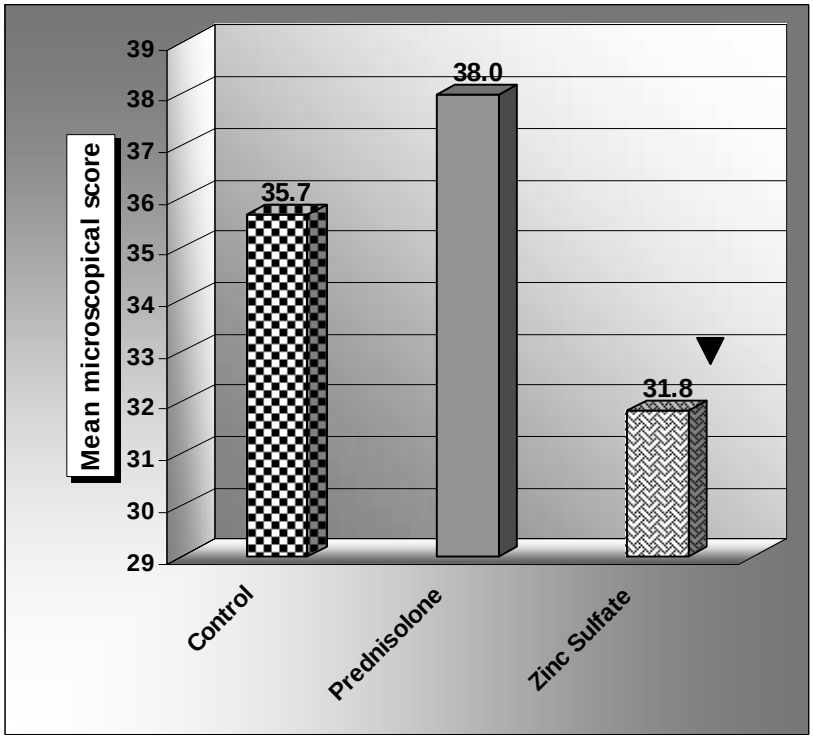


Figure (4): Mean microscopical histological score of the colon (0-40), of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

▼ ***Significant difference (0.01< p < 0.05) in comparison with prednisolone group***

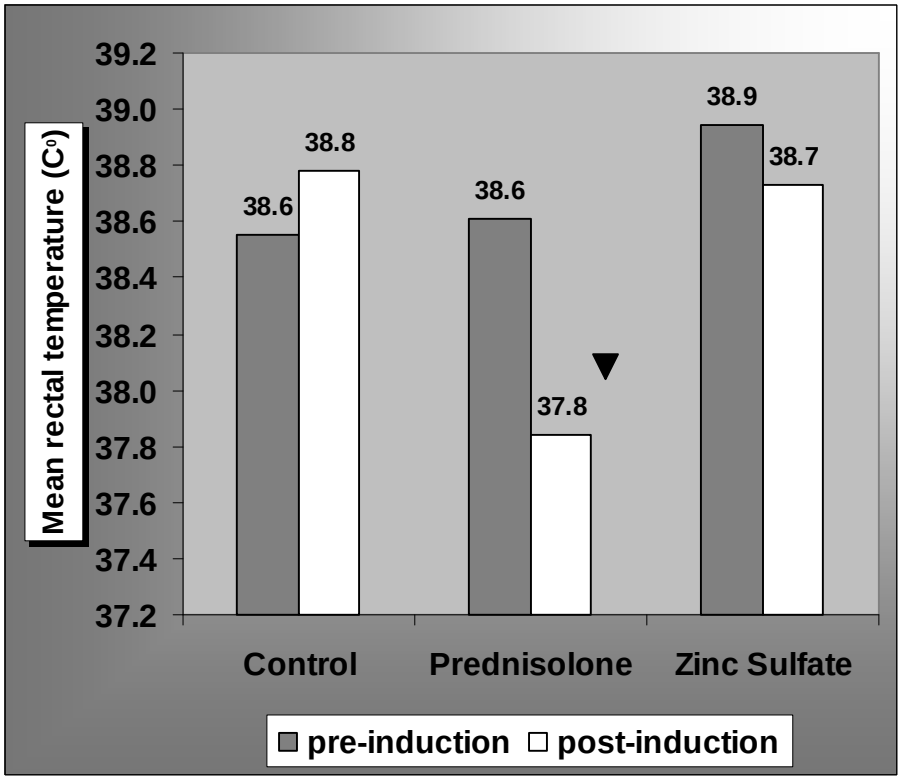


Figure (5): The mean pre-induction and post-induction rectal temperature of rabbits in control and treatment groups in acetic acid (2%) - induced colitis
▼*Significant reduction ($p < 0.05$) in comparison with pos-induction rectal temperature of zinc sulfate group*

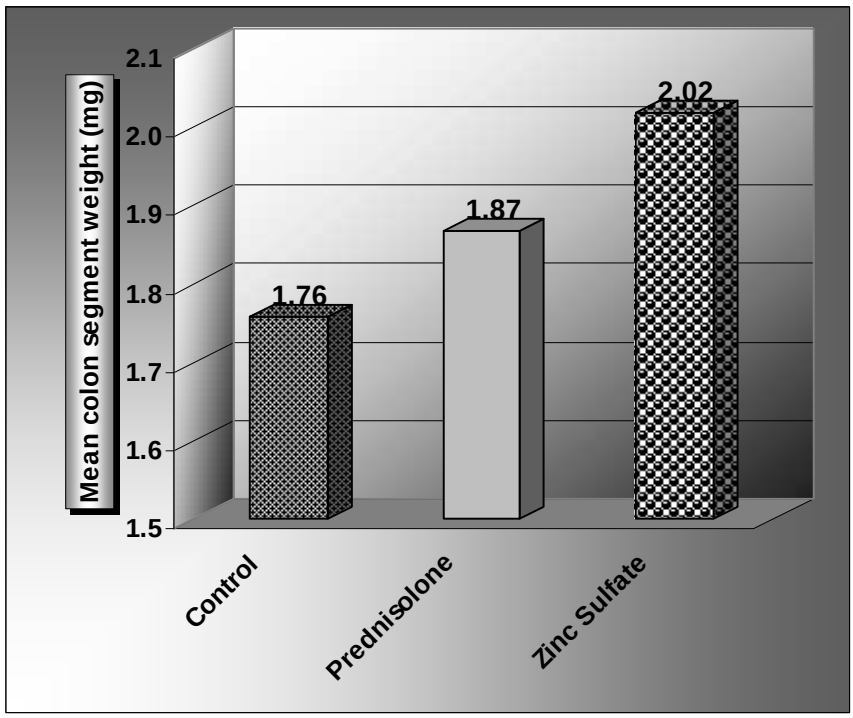
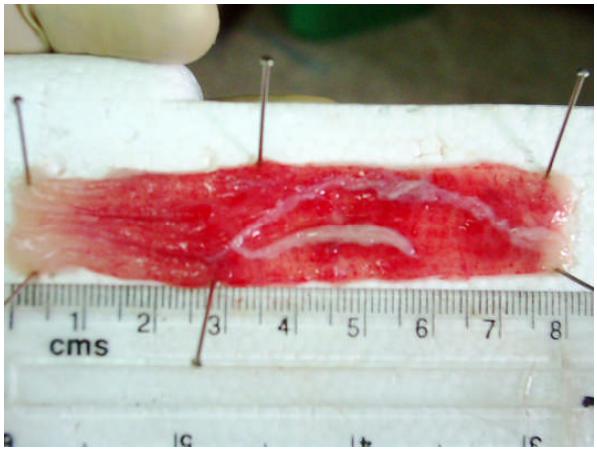


Figure (6): Mean colon segment weight (mg) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis



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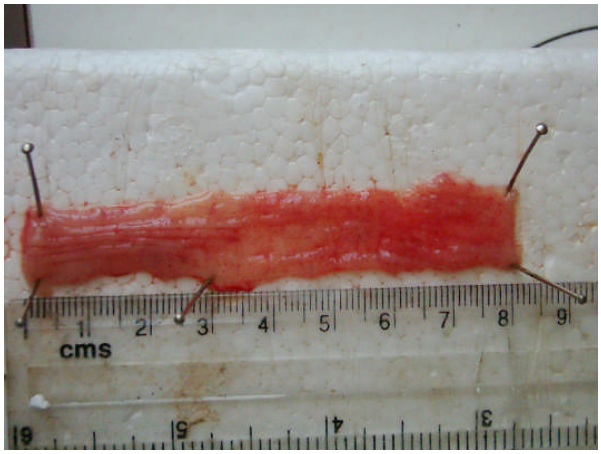

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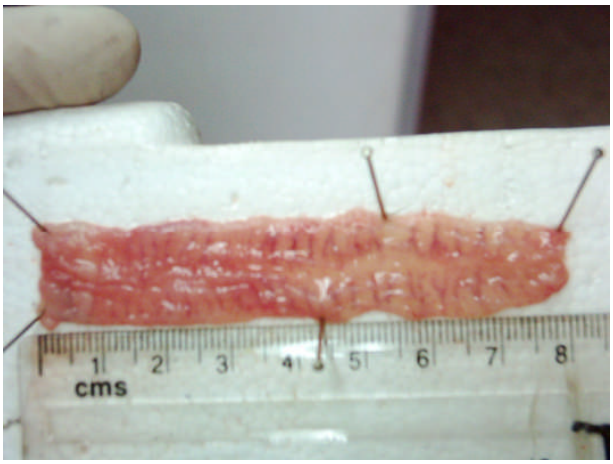

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Figure (7) **the gross appearance of colon segments of control and treatment groups in acetic acid (2%)-induced colitis, A: control, B: prednisolone, C: zinc sulfate.**

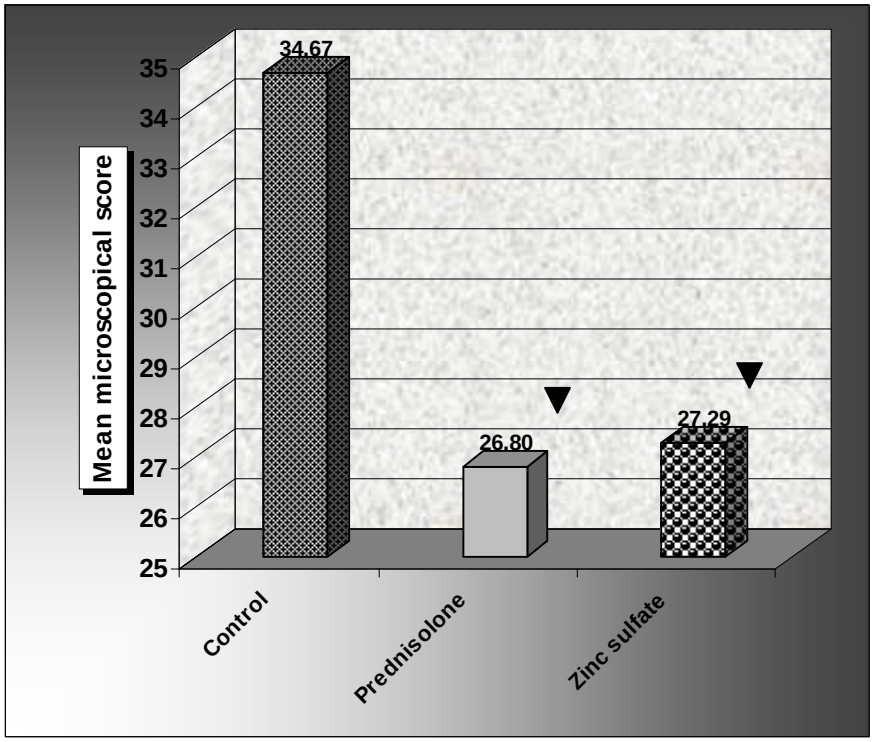

Figure (8): Mean microscopical histological score (0-40) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

▼ ***Significant reduction (0.01< 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 [17].

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 [18].

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%) which 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 [19] 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 [20, 21].

It was found that non-steroidal antiinflammatory drugs exacerbate experimental colitis in rats [22]. For that these agents were not used in this study.

Zinc sulfate is relatively safe and used orally as a drug, and it is available and relatively cheep, besides, it is 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 zinc sulfate, 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.

There were no significant differences ($p > 0.05$) in model 2 in colon segment weight. Rachmilewitz, et al., [23] showed that 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, (prednisolone and zinc sulfate), to reduce inflammatory process in acetic acid (2%) model.

Moreover, compared to effect of prednisolone on mean gross histological score, zinc sulfate (models 1 and 2) had comparable effect; this could indicate its possible potent initial anti-inflammatory effects.

In model 1, the insignificant differences induced by both 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 new tested agents in initial treatment of induced colitis in rabbits.

In model 2, prednisolone-induced and zinc sulfate-induced significant micro-scopical improvements emphasized the effective anti-inflammatory role of these agents particularly when these findings conjugated with their anti-inflammatory effects detected grossly (see above).

In model 2 in this study, compared to control group, prednisolone exerted a better apparent protective role than zinc sulfate regarding the induced reduction in mean body weight.

Although there was a significant reduction of mean body temperature of prednisolone group from the corresponding readings for the other groups, the means of body temperature were all within the normal range (37.8° - 39.4° C).

In model 1, the highly significant reduction in the mean

post-induction plasma zinc concentrations for rabbits in the control group from its mean pre-induction level and the similar significant reduction in mean post-induction zinc level in model 2 showed that acetic acid induced colitis model is associated with zinc decrement, and it simulates the results obtained in human IBD [(24)].

In the present study, zinc sulfate therapy markedly corrected the plasma zinc values in both models; this result showed that zinc sulfate (in a daily dose of 50 mg/kg taken orally for 3 successive days) was sufficiently absorbed and did not cause toxicity. Zinc homeostatic mechanisms probably could not cope with the well known redistribution of plasma zinc induced initially by inflammatory processes without an external zinc supplementation [(25)].

Plasma copper measurement was used to identify whether such a high dose of zinc sulfate therapy 50 mg/kg/day caused secondary copper deficiency; the latter causes copper deficiency associated anemia in human [(26)].

In model 1, mean plasma copper concentration of the control group was significantly increased post-induction from that of the pre-induction level, $P< 0.05$. While for the zinc sulfate group there is an insignificant increase in plasma copper caused by zinc sulfate therapy.

In model 2, the similar increment in mean post-induction plasma copper from the pre-induction level is expected because of the associated zinc deficiency, but this increment was statistically not significant which may be because of the less severe zinc decrement in comparison to that in model 1.

The significant increment in mean post-induction plasma copper from mean pre-induction level is unexplainable yet.

In the present study, oral zinc sulfate (50 mg/kg/day) appeared to have a considerable prophylactic activity that was comparable to that of oral prednisolone (2 mg/kg/day) against acetic acid –induced colitis in rabbits.

Duggan, et al., [(27)] emphasized that zinc deficiency may predispose the intestinal tract to damage by free radicals and increased NO activity. Furthermore, Mulder, et al., [(28)] documented the decrease in intestinal copper/zinc containing proteins that have antioxidant function in inflammatory bowel disease.

Di-Leo, et al., [(29)] showed that administration of zinc sulfate in a dose of 30 mg/kg/day had a little effect on the short-term course of experimental colitis; this probably in contradict to the encouraged results obtained in the present study. This probably due to difference in the applied dose of zinc sulfate in the present study (50 mg/kg/day) which was administered 2 days prior and further 1 day post induction of colitis. On the other hand, Luk, et al., [(30)] demonstrated the protective effect of zinc sulfate on dinitrobenzene-induced colitis when administered rectally; this could point to the possibility of a potential cumulative effect of zinc sulfate given in a sufficient dose when used concomitantly via both oral and rectal routes of administration.

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