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Evaluation of therapeutic role of zinc oxide nanoparticles on dextran sulfate sodium salt-induced ulcerative colitis in rats via modulation of COX-2, IL-6 TNF-α, oxidant and antioxidant defense system on colon

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ABSTRACT

This study designed to investigate the anti –inflammatory effect of zinc oxide nanoparticles (ZnONPs) on inflammatory mediators in dextran sulfate sodium salt induced ulcerative colitis(UC) in rats .twinty -four albino rats divided into 4 equal of eight rats each. Group 1: (normal control) received no drugs , group 2: (ulcerative colitis) rats received dextran sulfate sodium salt 3% in drinking water, group 3: (ZnONPs) rats administered ZnONPs (5mg/kg body weight) orally for 3weeks, group 4: (ZnONPs +UC) rats with ulcerative colitis orally treated with ZnONPs (5mg/kg body weight) daily for 3 weeks . The obtained results revealed that administration of ZnONPs to rats with ulcerative colitis have no change on serum total cholesterol and TG concentrations and markedly increased the reduced HDL-C level . On the other hand, elevated level of COX-2, IL-6, MDA and TNF-α in UC rats were significantly reduced, with significant increase of the reduced level of GSH. Results suggest that ZnONPs modulates UC.

Keywords: zinc oxide nanoparticles, ulcerative colitis, inflammatory mediators, histopathology.

1. INTRODUCTION

Chronic inflammation is involved in pathogenesis of many chronic diseases, including inflammatory bowel disease. Therefore, the suppressing the production of pro-inflammatory molecules could be an important target for the prevention or treatment of various diseases [1]. Ulcerative colitis is an inflammatory bowel disease (IBD) that causes long lasting inflammation and ulcers (sores) in the digestive tract. Ulcerative colitis affects the most inner lining of large intestine (colon) and rectum. In fact , the exact cause of ulcerative colitis is unknown. Researchers believe that factors such as over active intestinal immune system, genes, and environment may play role in causing ulcerative colitis [2].

Induced intestinal inflammation are one of the most commonly used models because they are simple to induce, the onset, duration, and severity of inflammation are immediate and controllable. Dextran sulfate sodium salt (DSS) induced colitis are well-established animal models of mucosal inflammation that have been used for over 2 decades in the study of IBD pathogenesis and preclinical studies. The DSS-induced colitis model has some advantages when compared to other animal models of colitis. For example, an acute, chronic, or relapsing model can be produced easily by changing the concentration of administration of DSS. Moreover, dysplasia that resembles the clinical course of human UC occurs

frequently in the chronic phase of DSS-induced colitis [3-5].

Zinc oxide (ZnO) has optical, magnetic, antibacterial and semiconducting properties. Its nanostructures exhibit interesting properties: high catalytic efficiency and strong adsorption capacity. This is extensively used in many applications such as cosmetics [6, 7].

The electrostatic properties of zinc oxide determine that it can have different charges on its surface under acid and base conditions. This can be used in the conjugation of therapeutic agents and also to internalize NPs within cancer cells , as they are high in phospholipids with negative charges on their surface [8]. The ZnONPs behave as genotoxic drugs, since they induce micronucleus formation in cells. These results could be helpful in designing more potent anticancer or anti-inflammatory agents for therapeutic uses [9].

Reduced zinc may exacerbate the oxidative stress mediated complications and proved that ZnONPs have the ability to modulates MDA [10].

2. MATERIALS AND METHODS

2.1- Chemicals:

Dextran sulfate sodium salt: (DSS) extracted from *Leuconostoc spp*. with average molecular weight of 500,000 and ZnO nanogard (purity ~99%) was manufactured by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through Alfa Acer , Egypt .

2.2- Experimental design:

Twenty-four white albino rats of 5-7 weeks old and weighting 100-120gm were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. Induction of ulcerative colitis: ulcerative colitis has been induced in rats with dextran sulfate 3% orally administrated in drinking water for 7 days [11].

Dosage of Zno nanoparticles: rats orally administered by gavag with 1ml of zinc oxide nanoparticles at dose 5mg/kg body weight [12].

2.3- Animal groups:

Rats were randomly divided into four equal groups , 8 animals each , placed in individual cages and classified as following : group1(control group) **,** feed standard pellet diet and clean drinking tab water . Group2 (UC induced group) : Rats were orally received dextran sulfate3% in drinking water . Group3 (ZnONPs administered group) : rats orally administered by gavage with 1ml of ZnONPs (5mg/kg body weight) for 3weeks.Group4 (ZnONPs +UC group): rats with ulcerative colitis orally administered by gavage with 1ml of ZnONPs (5mg/kg body weight), daily for 3weeks.

2.4- Sampling:

Blood samples and colon tissues were collected from all animal groups at the end of the experiment.

2.4.1- Blood samples

Blood samples were collected after overnight fasting in dry, clean and screw-capped tubes. Serum was separated by centrifugation at 4000 r.p.m for 15 min. the clear serum was received in dry, sterile sample tubes and kept in a deep feeze at -20° C until used for subsequent biochemical analysis. All sera analyzed for the following parameters: total cholesterol, HDL-C, TG, TNF-α and IL-6.

2.4.2- Tissue samples (colon tissue):

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The abdomen was opened and the colon specimen was quickly removed and opened gently using a scrapper , cleaned by rinsing with ice-cold isotonic saline to remove any blood cells , clots and scraps of food , then blotted between 2 filter papers and quickly stored in a deep freezer at (-20°C) for subsequent biochemical analysis. Briefly, colon tissues were divided into appropriate portions, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant were used for the determination of the following parameters: GSH , MDA, COX-2

2.5- Histopathological examination:

Washing colon tissues was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for examination through the light electric microscope [13].

2.6- Biochemical analysis

Serum total cholesterol, HDL-C, TG, were determined according to the methods described by [14-17] and TNF-α and IL-6 estimated by ELISA kit supplied by R &D system Quantitative, USA. Also, colon tissue Malondialdehyde (MDA), COX-2 and reduced glutation (GSH) were determined according to the methods described by [18].

2.7- Statistical analysis

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA). All analysis performed using the statistical package for social science (SPSS,2009). The Values were considered statistically significant when $p \leq 0.5$.

Table 1: Effect of ZnONps treatment on serum total cholesterol, Triglyceride and HDL-C levels in DSS induced ulcerative colitis in rats and their control.

Animal groups	Cholesterol(mmol/dl)	TG (mgl/dl)	HDL (mg/dl)
Control	138.4 ± 12.3	108.9±6.6	57.1±4.0[b]
Ulcerative colitis(uc)	142.7 ± 11.5	109.8±13.2	21.9±2.5[a]
ZnONPs	128.8 ±10.2	95.6±3.6	59.3±3.9[b]
UC+ZnONPs	145.2 ± 14.4	109.6 ± 9.0	42.3± 3.0[ab]

Data are preaented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (p≤0.5)

Table 2: Effect of ZnoNps treatment on serum TNF-α, IL6 and COX-2 gene expression levels in DSS induced ulcerative colitis in rats and their control.

Animal groups	COX2 (u/g)	IL6 (pg/mL)	TNFa (pg/ml)
Control	1.1±0.1[b]	34.7±2.4[b]	33.2±4.7[b]
Ulcerative colitis (uc)	13.1±1.7[a]	149.0±12.1[a]	122.9±3.9[a]
ZnONPs	1.6±0.6 [b]	31.1±5.5[b]	37.1±3.4[b]
UC+ ZnONPs	6.1± 1.9[ab]	71.2± 7.7[ab]	68.6± 12.2[ab]

Data are preaented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (p≤0.5)

Table 3: Effect of ZnoNps treatment on MDA level and GSH level in DSS induced ulcerative colitis in rats and their control.

Animal groups	MDA (mmol/mg)	GSH (mmol/mg)
Control	1.1±0.1[b]	56.4±2.5[b]
Ulcerative colitis (uc)	21.1±8.1[a]	17.8±2.2[a]
ZnONPs	1.3±0.7[b]	59.2±3.4[b]
UC+ZnOPs	6.2± 0.2[ab]	44.8± 2.9[ab]

Data are preaented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (p≤0.5)

3. RESULTS AND DISCUSSION

3.1- Effect of ZnoNps treatment on some serum and colon tissue parameters of DSS-induced ulcerative colitis in rats.

The obtained results in table (1) revealed that ,administration of DSS induced UC in rats exhibited no change in serum total cholesterol and TG concentrations and significantly decreased HDL-C level when compared with normal control groups. Treatment with ZnoNps to DSS induced ulcerative colitis in rats have no change on serum total cholesterol and TG concentrations, and increase markedly HDL-C level when compared with ulcerative colitis non-treated group.

The results presented in table (2) showed that administration of DSS induced in rats exhibited a significant increase in serum level of TNF-α , IL-6 , and COX-2 gene expression when compared with normal groups. Treatment with ZnONps to DSS induced ulcerative colitis in rats significantly reduced elevated level of TNF-α, IL6 and COX-2.

The obtained results in table (3) revealed that administration of DSS induced UC in rats exhibited a significant increase in colon tissue MDA, and significantly decreased GSH concentration when compared with normal group. Treatment with ZnoNps to DSS induced ulcerative colitis in rats significantly reduced the elevated level of MDA, and markedly increase the reduced GSH level in colon tissue.

3.2- Histopathological findings:

Histopathological studies on colon tissue sections of control group showed no histological alteration were observed, while rats group which treated with DSS showed massive numbers of inflammatory cells infiltration in the colon mucosal and submucosal layers. Treatment of DSS-induced UC rats by ZnoNps show focal inflammatory cells in the base of the mucosa.

Inflammatory bowel disease (IBD) is a complex multifactorial disease [19and 20]. It commonly refers to ulcerative colitis (UC) and Crohn's disease (CD), the two chronic conditions that involve inflammation of the intestine. Despite recent advances in treatment , there remains a need for a well-tolerated therapy with a rapid onset, and increased capacity for maintaining long-term remission [21] .

Nanotechnology represents a new and enabling platform that promises to provide a broad range of novel uses and improved technologies for biological and biomedical applications. Treatment with ZnoNps to DSS induced ulcerative colitis in rats showed non-significant changes in serum total cholesterol and TG concentrations, with increase the HDL-C. These results are nearly similar to those reported by [22], who reported that no significant changes in the total

cholesterol when supplemented with zinc on feed mixture. Also, [23] reported that, ZnONPs administration caused non-significant change in TG serum, and [24] found that serum HDL level increased when broiler supplemented with nano zinc.

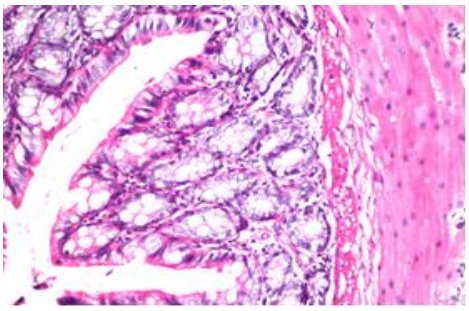

Figure 1: Control group: There was no histopathological alteration in the colon.

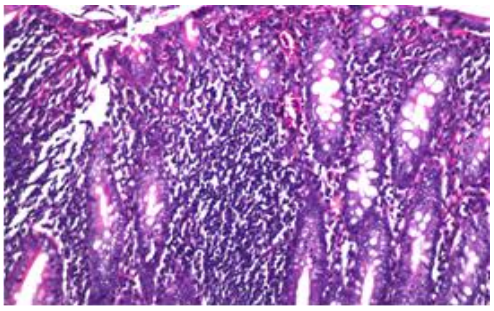

Figure 2: UC group: The mucosal and submucosal layers showed massive numbers of inflammatory cells infiltration in the colon.

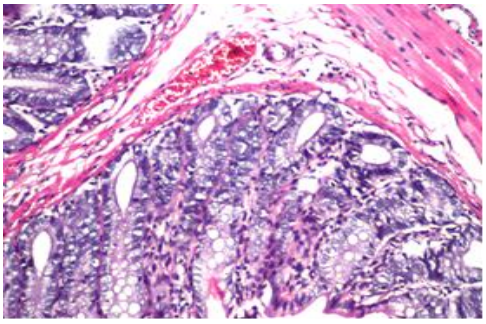

Figure 3: Zinc oxide nanoparticles group: There was no histopathological alteration

Figure 4: Group of experimentally inducted and treated by zinc oxide nano particles:
Focal inflammatory cells infiltration was observed in the lamina propria of the mucosa associated with lymphoid hyperplasia in the submucosal layer.

DSS as a model for studying colitis-associated carcinogenesis [25], [26] investigated that the validated DSS model by using different therapeutic agents for human IBD and showed that DSS-induced colitis can be used as a relevant model for the translation of mice data to human disease [27]. Intestinal microflora and their products have been implicated in the pathogenesis of human IBD and in several animal models [20 and 28]. The importance of the intestinal flora is directly supported by studies of somewhere colitis is not observed when they are reconstituted with bacteria that are considered normal constituents of luminal flora. It has been demonstrated that intestinal flora is implicated in the pathogenesis of DSS colitis in mice. First who suggested contribution of colonic bacteria or their products in the development of colitis in this model were Okayasu. They observed increased numbers of Enterobacteriaceae, Bacteroidaceae and Clostridium spp. in the colons of mice affected by DSS colitis [29].

DSS-induced breakdown of mucosal epithelial barrier function allows the entry of luminal antigens and microorganisms into the mucosa resulting in overwhelming inflammatory response Numerous inflammatory mediators have been implicated in the pathogenesis of human IBD .Changes in production of inflammatory mediators in DSS-treated mice were investigated during different phases of colitis, in the serum and/or colon and by different methods. Increased expression of different inflammatory mediators (TNF-α) was observed as early as the first days of DSS treatment [30]. The production of these inflammatory mediators increased progressively during DSS treatment. Different profile of inflammatory mediators in acute and chronic phase of DSS colitis was demonstrated as recorded by elevated levels of IL-6 [31]. Progressive upregulation was observed with increasing dosage of DSS [32] . These inflammatory mediators not only play a role in the pathogenesis of DSS-induced colitis but are important as intervention targets against colitis as excellently described by [33], Cytokine profile in DSS colitis correlates with clinical and histological parameters as well as barrier properties. Human and animal studies support the idea that TNF-α and interleukins are important pathological

mediators of IBD [34]. In humans with IBD , approximately two-thirds of patients respond to anti-TNF-α treatments [35], and intestinal inflammation is attenuated significantly by anti-interleukins and/or anti-TNF-α monoclonal antibodies in mice [36 and 37]. TNF-α and interleukins as IL-6 mRNA expressions in the colon of DSS-exposed rats are dramatically increased compared to non-colitic rats , suggesting that immune cells are attracted to the site of inflammation. This results are similar also to those which reported by [38] who found that, the levels of pro-inflammatory cytokines (IL-1β , IL-6 , TNF-α) were downregulated in ZnONPs -treated mice.

COX-2 can be activated to produce excessive PGE2, an important inflammatory mediator in IBD [39]. COX-2 is proinflammatory protein that play a pivotal role in mediating inflammation and contribute to chemical-induced inflammation in mice [40]. In the present study ZnoNps was found to be significantly down regulate COX-2 gene expression in colon. These results are nearly similar to those reported by [41] who suggested that, ZnONPs treatment revealed excellent anti-inflammatory activity by suppressing mRNA expression of COX-2. COX-2 enzyms ,which catalyze prostaglandin biosynthesis, has become an important target for the discovery and development of new anti-inflammatory agents [42].

MDA level, is a marker of oxidative stress, was significantly higher in the DSS group. Meanwhile in treatment group the MDA levels in the colonic tissue markedly decreased compared with the DSS group. This similarly, agreement [43] which proved that serum MDA level was significantly decreased in diabetic rats treated with ZnONPs. Zinc is a necessary factor in the variety of antioxidant enzymes e.g. Zn super oxide dismutase, Zn-metallothionein etc, [44] . Other investigators have suggested that, the Zn-metallothionein complex in the islets cells provides protection against free radicals produced in the cell from any cause. The more depleted the intracellular Zn stores, the less able the cell is to defend itself against this oxidative load , It has been proposed that an imbalance between pro-oxidant and antioxidant mechanisms may play an important role in the development of intestinal inflammation and mucosal tissue injury in colitis [45]. GSH plays a common role in cellular resistance to oxidative damage as a free radical scavenger as protein-bound GSH and by generation of ascorbate and/or tocopherol in liver [46]. Treatment causes a significant increase in GSH level. These results were nearly similar to those reported by [47] who investigated that, ZnONPs are known to be able to prevent the loss of GSH during oxidative damage induced by infection.

4. CONCLUSION

Administration of ZnONPs to rats with ulcerative colitis show non-significant change on serum total cholesterol and TG concentrations, and markedly increased the reduced HDL-C level. On the other hand, elevated level of COX-2, IL-6, MDA and TNF-α in UC rats were significantly reduced, with significant increase of the reduced level of GSH. Results suggest that ZnONPs modulates ulcerative colitis.

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