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Biochemical and Therapeutic Effect of *Boswellia carterii* extract against dextran sulfate sodium-induced acute Colitis

A El-Sayed [a] ***, MY Abdelaal*** [a] ***Hussam A. El-Gayar*** [b] ***,Omali Y.El-Khawaga*** [a*]

[a] *Biochemistry division, Chemistry department, Faculty of Science, Mansoura University, Egypt.*

[b] *Urology and Nephrology Centre, Faculty of Medicine, Mansoura University, Mansura, Egypt*

****Corresponding author:** Omali Y. El-Khawaga, Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt. Email: elkhawaga70s@mans.edu.eg, +201028464738*

https://orcid.org/0000-0002-9049-7537

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Abstract Inflammatory bowel disease (IBD) including chron's disease and ulcerative colitis is a public health challenge. *Boswellia carterii* extract is known for its ability to inhibit the synthesis of inflammatory mediators, such as leukotrienes, and with its antioxidant properties. During the development of colitis, there is an excessive production of reactive oxygen species (ROS), therefore in this study, we aimed to study the therapeutic effect of *Boswellia carterii* oleoresin extract in a DSS-induced colitis model through estimation of SOD activity as an antioxidant enzyme and the level of protein carbonyl as an oxidative stress marker. The extract considerably increased SOD activity and reduced the protein carbonyl levels, which led to a reduction in the changes induced in the colon by DSS, based on the histological results. These results indicated the therapeutic effect of the extract that may be due to improving the oxidative state.

keywords: DSS, Colitis, Boswellia carterii, oxidative stress, protein carbonyl

1.Introduction

Ulcerative colitis and Crohn's disease are the major two forms of inflammatory bowel disease (IBD) that's associated with the gastrointestinal tract (GIT) acute and chronic inflammation [1]. Inflammation, that impairs the GIT function, has symptoms such as weight loss, severe diarrhea, rectal and abdominal pain, and rectal bleeding [2]. The pathogenesis of IBD may be due to genetic or environmental factors, epithelial barrier defects, immune system or gut microbiota [3],[4]. IBD is a public health challenge with rapid increase incidence in newly industrialized countries as well as westernized ones [5].

In ulcerative colitis, there is a continuous pattern of superficial mucosal inflammation lasting from the rectum to the proximal colon in varying extents, there is also an immune infiltration in the superficial ulcers and mucosa [6]. This occurs because of the colonic barrier rupture with a subsequent invasion of bacteria and antigenic stimuli that leads to inflammatory mediators release such as arachidonic acid metabolites, and cytokines as well as the oxygen free radicals release that cause oxidative damage [7]. In patients with colitis, there is an overproduction of ROS and reactive nitrogen species (RNS) causing membrane lipid peroxidation and attack on DNA tissue proteins [8]. Antioxidants are the defense systems against ROS, they may be enzymatic antioxidant such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), or non-enzymatic such as ascorbic acid, reduced glutathione (GSH), flavonoids and others. They act to prevent the formation of ROS that maintaining the levels of ROS at low concentrations [9]. It is important to find substances with the ability to prevent, inhibit or ameliorate the damage caused by those ROS found in patients with colitis [10].

For at least 3000 years. *Boswellia* tree resin,

that is known as olibanum or frankincense, had been important trade material for the North Africa and Arabian Peninsula civilizations [11]. The natural oleo-gum resin is obtained through incisions made in the trunks of trees of the genus *Boswellia.* The genus *Boswellia* comprises 25 species. *Boswellia carterii* is one of the widely distributed species in North Africa and Somalia. The frankincense of *Boswellia carterii* is regarded as a widely used crude drug in traditional Chinese medicine. It has been used for the treatment of chest obstruction, rheumatic arthralgia, and amenorrhea. It also has, anti-inflammatory, analgesic, sedative, antihyperlipidemic and antibacterial activities and has been used to treat ulcers, osteoarthritis and rheumatoid arthritis [12]. Therefore, our study aimed to investigate its efficacy in the treatment of colitis in the selected animal model.

2. Material and method

Chemicals

Absolute ethanol (Thermo Fisher), Dextran sulfate sodium MW ca 40000 (Alfa Aesar), Nitro blue tetrazolium (NBT), NADH, Phenazine methosulfate (ACROS ORGANICS), Dinitro phenyl hydrazine(BDH chemicals England) , Pierce™ BCA protein Kit (Thermo Scientific™, Cat. No. 23227). The oleo-gum resin of *B. carterii* was purchased from a local market in Mansoura, Postal Code 35516, Egypt. All used chemical was analytical grade.

Preparation of *Boswellia carterii* ethanolic extract

50 g of the plant was crushed to powder, soaked in 400 ml absolute ethanol and gently agitated. After that, the extract was filtered, and the solvent was removed in air. The residues, oleoresin fraction, was then stored at -20 °C until use.

Animals

25-30 g Male BALB/c mice were obtained from Zel El Nakhil Animal Housing Facility, Cairo, Egypt. The animals were housed six per cage at 23 ± 2°C with a 12 h light/dark cycle and had free access to food and water *ad libitum*. Before starting the experiment, they were acclimated for a week. All animals were treated in accordance with the ethical approved from Faculty of Science, Mansoura University

Experimental protocol

Mice were divided into three groups (n=6 mice per group): control group, DSS-induced colitis group (DSS), and DSS-induced colitis group treated with the oleoresin extract (DSS + oleoresin). Animals received 3% DSS in drinking water for 7 days for induction of acute colitis, followed by normal drinking water. Extract (50 mg/kg) was suspended in 0.5% carboxymethyl cellulose (CMC) and given by gavage once a day for 7 days starting after colitis induction. All mice were sacrificed at the end of the experiment Colon tissues and blood were collected for subsequent analysis.

Preparation of colon tissue homogenate

Colon tissues were rinsed in ice-cold PBS to remove excess blood, chilled on ice and dissected into small pieces. The samples were weighed and homogenized in PBS, then centrifuged for 15 minutes at 10000 × g. and at 4 °C. After that, the supernatant was collected aliquoted into Eppendorf tubes and stored at -20°C for further analysis. Protein concentration was determined in all samples using Pierce BCA assay.

Measurement of SOD activity

The PMS–NADH–NBT system was employed as described by Nishikimie *et al* for determination of SOD activity [13]. This method relies on the ability of SOD to inhibit the reduction of nitro blue tetrazolium mediated by phenazine methosulphate. The SOD activity was expressed relative to the total protein concentration in each sample.

Estimation of protein carbonyl (PC) level

PC level was measured using a modified method of the one proposed by Levine *et al* [14]. Briefly, in 96-well plate, 50 µL of 2, 4-dinitrophenylhydrazine (10 mM in 0.5 M H_3PO_4) was added to 50 µL of each sample and after incubation for 10 min, 25 µL of NaOH (6 M) was added to the reaction mixture, then the plate was incubated for 10 min at room temperature and the absorbance was read after plate incubation at 450 nm (the blank was an equal volume of PBS) [15].

Histological investigation.

Distal colon was used for histological

analysis. Colon tissues samples were immersed in buffered formalin, then included in blocks of paraffin to obtain 3 □m thick cuts using a rotatory microtome. Slides were stained with hematoxylin-eosin for histological examination.

Statistical analysis

All data were presented as mean ±SE, and the statistical significance was calculated using GraphPad prism 8.0.1. Variance analysis (ANOVA) was used, adopting a significant level of 5% (P<0.05).

Results

Effect of ethanoic extract on the studied groups SOD activity

The SOD activity significantly decreased in the diseased group (DSS) compared to the control group (p= 0.0002), while its activity significantly increased in the group treated with oleoresin extract (DSS + oleoresin) compared to the diseased group (p = 0.02) (**Error! Reference source not found.**A).

Effect of ethanoic extract on protein carbonyl levels

The protein carbonyl level increased in the diseased (DSS) group compared to control group while there was a significant decrease in its level in treated group (DSS + oleoresin) compared to the diseased group (p = 0.0243) (**Error! Reference source not found.**B) .

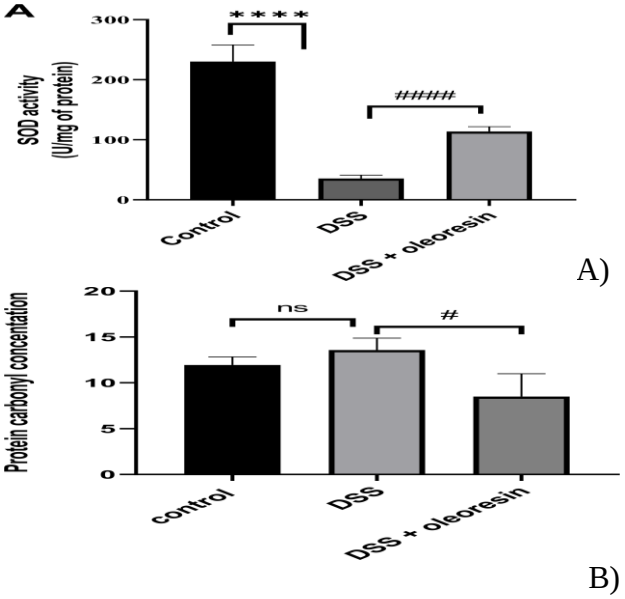

Fig 1: Effect of administration of *B. carterii* extract on the SOD activity (A), and the level of protein carbonyl (B). Each chart showed mean ± SEM (n=6). The differences among all groups have been assessed by one-way ANOVA with ($p < 0.05$) is considered significant, (*) significant when compared to control group, (#) significant when compared to oleoresin treated group.

Microscopic Examination of Colon tissues of studied groups

Slides were stained with hematoxylin-eosin (H&E) and analyzed at low (100x) and high (400x) magnifications. Fig 2 A and B show the photomicrographs of a mice colon tissue in the control group, which shows a normal mucosa (M) containing regular crypts with simple glandular epithelium, normal submucosa (S) and normal muscular layer. Fig 2C and D are photomicrographs of colitis (DSS) group, that show a change in the architecture of the colon, multiple large areas of mucosal (M) necrosis (thick black arrow), submucosal fibrosis, dilated blood vessels (red arrows) and mononuclear cells infiltration. Sections from treated group (DSS + oleoresin) showed normal mucosa (M) and submucosa (S). Few colonic sections from this group showed small area of mucosal necrosis Fig 2E and F).

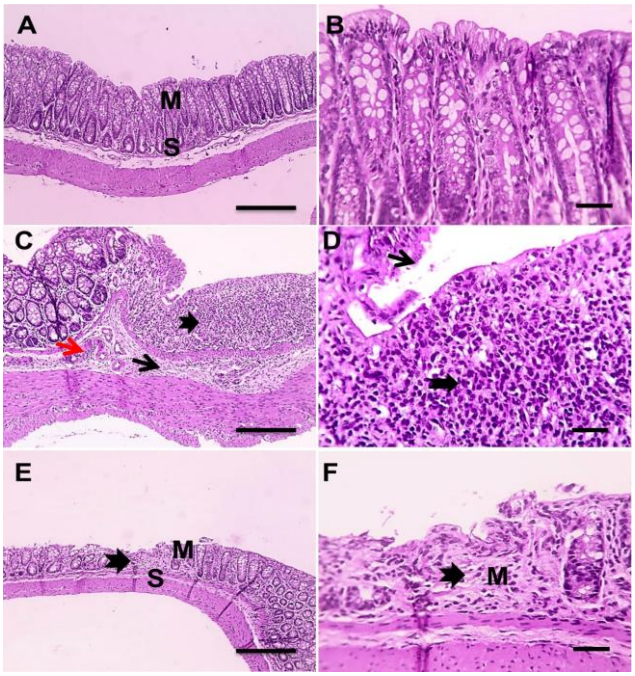

Fig 2 Photomicrograph showing H & E-stained colon tissues sections of studied groups: (A) control group (100x), (B) control group (400x), (C) colitis group (DSS) (100x), (D) colitis group (400x), (E) treated group (DSS + oleoresin) (100x), (F) treated group (400x). mucosa (M) submucosa (S). Low magnification X: 100 bar 100 and high magnification X:400 bar 50

Discusion

There are several colitis models have been utilized. Numerous studies have employed the DSS colitis model as a chemical induction of intestinal inflammation model. Symptomatically and morphologically, it resembles epithelial damage seen in human ulcerative colitis [16]. This model is characterized by rapidity, simplicity, controllability and reproducibility [17]. The proposed and most accepted DSS-induced colitis mechanism is through the disruption of intestinal epithelial monolayer lining that results in the invasion of luminal bacteria and its associated antigens into the mucosa with subsequent proinflammatory intestinal contents dissemination into the underlying tissue [18].

B. carterii extract is rich with active components that have antioxidants with anti-inflammatory action and having the ability to inhibit proinflammatory cytokines synthesis such as leukotrienes. They inhibit 5-lipoxygenase enzyme [19]. Anthoni *et al*. reported that 3-acetyl-11- keto-beta-boswellic acid (AKBA), an active component of the extract, has a protective effect on the intestinal mucosa against tissue injury as it significantly attenuates leukocyte recruitment [20].

SOD is one of the enzymatic antioxidants that is a compensatory mechanism for oxidation process. The cytosolic CuSOD and mitochondrial MnSOD form a supportive group of enzymes with catalase and peroxidases to provide a defense systems against ROS [21]. In our study, there was significant increase in SOD activity in the animals received the oleoresin extract compared to those of colitis group, where there was a decrease in its activity, indicating the antioxidant effect of the extract that help in mucosa protection

Leucocytes and activated macrophage release free radicals as a result of inflammatory reactions [22]. Protein carbonyl derivatives are produced due to proteins oxidation by the accumulated ROS [23]. In this current study, it has been observed that the levels of protein carbonyl, a biomarker of oxidative damage in the colon tissue, increased after DSS administration. However, animals received the oleoresin extract have shown a considerably decreased levels of carbonyl proteins concentrations. Protein carbonylation inhibition by the extract could prevent oxidative-induced colonic tissue damage.

The histologic evaluation of colon tissue showed the development of inflammation in the colitis group. There was a destruction of the mucosal crypt, with large area of necrosis and submucosal fibrosis. On the other hand, animals treated with oleoresin extract showed normal mucosa and submucosa in most colonic sections, only few colonic sections show small area of mucosal necrosis also there was a reduction in mucosal infiltration, that is a histologic hallmark of UC, that help in protecting mucosa against injury.

Conclusion

This study showed a therapeutic effect of Boswellia carterii oleoresin extract that targeted the colonic tissue and ameliorated the DSS-induced changes. The underlying mechanisms could be related to the anti-inflammatory and antioxidant potential of the extract.

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