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Gelidium spinosum red algae ameliorates oxidative /nitrosative stress and inflammation in dextran sulfate sodium-induced ulcerative colitis in mice

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Abstract

Ulcerative colitis is characterized by oxidative / nitrosative stress and leukocyte infiltration which are shown to be important factors in the etiopathogenesis of colitis. The aim of this study was to evaluate the protective mechanism of the polysaccharide (PLS) fraction extracted from the seaweed Gelidium spinosum (PEGS) in Dextran Sulfate sodium; DSS-induced ulcerative colitis in male Swiss albino mice. The in vivo study was carried out with mice (pretreated with PEGS/mesalazine orally at 60 mg/75 Kg; 7 days; then 2.5 % (DSS); 7 days). Clinical symptoms (disease activity index; DAI), oxidative, nitrosative stress and inflammatory parameters were evaluated. The results revealed that PEGS lowered the symptoms of ulcerative colitis induced by DSS by reducing malondialdehyde, nitrosative, and pro-inflammatory biomarkers (NO, S-nitrosothiol, peroxynitrite, TNF-α and IL-6). These significant protective effects of PEGS was associated with a remarkable decrease of the level of myeloperoxidase in a significant way. PEGS effectively increased the superoxide dismutase and catalase activities, and restored the glutathione metabolizing enzyme (glutathione peroxidase, glutathione S-transferase) in the colon tissue. In conclusion, these findings suggest that PEGS exerts beneficial effects in experimental colitis, and therefore we propose that it could be a potential drug in treating ulcerative colitis.

Keywords: anti-inflammatory, Dextran Sulfate sodium,Gelidium spinosum,nitrosative stress,oxidative stress, ulcerative colitis.

1. Introduction

Marine algae are known for their large production of sulfate polysaccharides, as a component of their cell walls, widely used in the food and pharmaceutical industry [1].Various studies have shown that marine algae are rich sources of various bioactive compounds among them polysaccharides which have demonstrated antioxidant and anti-inflammatory effects [2][3]. Gelidium spinosum (S. Gmelin) P. Silva is Member of the red algal family Gelidiaceae are distributed from intertidal to subtidal habitats and occur in most oceans, including tropical, warm, and cool temperate waters [4]. Although they are absent from polar waters. Several Gelidium are distributed worldwide, and are even found in cold temperate areas as far apart as the Falklands and south-western Norway [5]. Gelidium is harvested in large quantities in subtropical waters such as in Japan, Korea, Spain, Portugal and Morocco [6]. Gelidium is the most speciose genus in the family that currently includes 134 species [7] [8]. Although the diverse uses of Gelidium as food and in the production of agar and paper pulp have increased research interest in this genus [9].Other studies showed that Gelidium spinosum possess antimicrobial, antiviral, antifungal, cytotoxic activities [10], and antioxidant activity [11].Inflammatory bowel diseases (IBDs) is hypothesized to be related to exposure to environmental risk factors leading to inappropriate immune responses to enteric commensal microbes in genetically susceptible individuals [12].Ulcerative colitis (UC) is one of the two main forms of IBDs which is characterized by inadequate activation of the intestinal and systemic immune system which results in deregulation of mucosal immunology[13]. Recent studies have exhibited that reactive oxygen species (ROS), nitrogenous species (RNS), and inflammatory pathway disorders may be involved in the pathogenesis of UC, and they are emerging as new therapeutic targets for the treatment of UC [14].The

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DSS colitis mouse model is widely used to study the etiology of UC due to the fact that many pathological symptoms cited with this model are commonly noted from UC in humans [15]. Experimental DSS-induced colitis in mice aims to design a model of acute intestinal injury that mimics multiple intestinal lesions consistent with IBDs [16]. Current therapeutic strategies for management of IBDs encompass pharmacological interventions, including anti-inflammatory drugs, immunosuppressives, antibiotics, and biological therapies. The aminosalicylates and/or glucocorticoids remain the main treatment for IBD [17]. However, current therapies of IBD employing this approach has had limited success and are linked to unwanted side effects[18].Accordingly, the use of natural products are fast becoming an attractive approach for the treatment of inflammatory bowel disease and new types of therapies are desired which can prevent disease or relapse. Algeria is characterized by algal diversity due to its geographical location and its rich topography. However, the characterization of polysaccharides extracted from Algerian seaweed has not yet been fully determined except for some research in this area.

In view of these considerations, this study aims to appraise and to evaluate for the first time the mechanisms by which polysaccharide fraction extracted from red algae Gelidium spinosum (PEGS) influences intestinal recovery, thus, the main purpose of this study was first to investigate whether PEGS is able to reduce oxidative and nitrosative stress-induced by DSS in the Colonial mucosa of mice through the activation of key enzymes associated with the maintenance of glutathione ,on the other hand, the inflammatory modulation.

2. Experimental

2.1. Algae material collection

The marine algae Gelidium spisosum class of rhodophyceae (red algae) was identified by associate professors Nina Lundholm and Ruth Nielsen from the Natural History Museum of Denmark, University of Copenhagen. G. spisosum was collected in March 2016 from the northeast coast (Altitude: N 36°53'20.74"; Longitude: E 006°07'47.52") of Algeria (Jijel, "Beni Belaid") 20 meters of depth. After collection, algae were rinsed directly with seawater to remove the macroscopic epiphytes and other extraneous matter, and then rinsed in tap water and dried at room temperature and darkness.

2.2. Extraction of crude polysaccharide

40 g of algae powder was dipped in 1: 20 volumes (800 mL) of distilled water at 90 °C for 3 h. The mixture was filtered using a cheesecloth; further, the supernatant was separated from the algae residue by centrifugation at 8000 rpm for 15 min. The residue was re-extracted in a similar way, the supernatants were combined and dialyzed in cellulose membrane (molecular weight cut of 12.000 KDa) extensively (24h) against distilled water. The polysaccharide was precipitated with the twice volume of 95 % alcohol (ethanol) overnight, then centrifuged at 3500 rpm for 30 min; the precipitate was washed with alcohol (100 °C) followed by drying under hot airflow at 60 ºC[19].This procedure was repeated according to the experimental needs. The polysaccharide extracted from Gelidium spinosum (PEGS) was performed for the in vivo studies.

The polysaccharide extraction yield (Y) was calculated as follows:

$$Y\,(\%) = \frac{(100 \times \mathrm{WPEGS})}{\mathrm{Wsample}}$$

Where W-PEGS is the weight of the PEGS and W-sample is the weight of the sample.

2.3. Acute toxicity evaluation of PEGS

Five mice were used which received a single oral dose of 2000 mg/kg of PEGS. The animals were kept overnight fasting prior to administration of PEGS by oral gavage. The animals were observed individually at least once during the first 30 min after dosing, then periodically during the first 24 h (with special attention for the first 4 hours), and daily the rafter for a period of 14 days. General behavioural changes, common side effects such as mild diarrhea, weight loss needed to be detected [20]

2.4. Induction of Ulcerative colitis in mice

Animals and experimental design

Forty adult male Swiss albino mice, weighing (28±2 g) were used as experimental animals. All the animals were kept under standard laboratory conditions (temperature 25±2 °C and 12 h light/12 h dark cycle). They were fed with a standard rodent pellet diet and water ad libitum. The in vivo experimental protocol was approved by the Institutional Project Committee (PRFU, D01N01UN250120190002). The experimental procedures adopted in this study was in strict compliance with the Guidelines for Reporting Animal Research [21].

Animals was divided into four equal groups (n = 10 per group).

Group I (control - mice group), received orally normal drinking water for 14 days.

Group II (DSS - mice group the animals, after successive oral administration of normal drinking

water for 7 days, had free access to water containing DSS (2.5%, w/v) for seven days[22].

Group III (PEGS -mice group), the animals received orally 60 mg/Kg [23] [24] PEGS for 7 days, the colitis was induced in the same way as in group II.

Group IV (Mesalazine-mice group) the animals received mesalazine at 75 mg/Kg as a reference drug[25] for7 days, acute colitis was induced in the same manner as in groupII. During the experimental period, the mice were checked daily for colitis as the disease activity index (DAI) based on monitoring body weight, gross rectal bleeding, and stool consistency.

At the end of the experiment on day 15, the mice was killed by cervical dislocation, and the colon was excised, and the length of each colon was measured, the colon tissues were dissected for further analysis. . The remainder of each sample was stored at -80 ° C for biochemical analyzes.

2.5. Sample preparation

The distal portion of the colon (8 cm; 190–200 mg)was excised, and were diluted 20 % w/v in 20 mM ice-cold Tris-HCl, pH 7.4, and homogenized using a Teflon homogenizer, the homogenates were centrifuged at 10,000 × g for 15 min at 4 °C to separate cytosolic fraction that was stored at -80 °C until use[26].

2.6. Lactate dehydrogenase (LDH) activity

The LDH activity in the colonic tissue was estimated according to Kornberg [27].The substrate used was sodium pyruvate; the pH of the buffer was maintained at 7.5 at 37 °C. In the presence of an enzyme, pyruvic acid is converted into lactic acid. Further, pyruvic acid reacts with 2.4-dinitrophenyl hydrazine and forms an intensely colored brown hydrazone, which has a high absorbance at 400-500 nm. The LDH activity was expressed as U/g tissue. The protein content of each sample was estimated by the method ofLowry [28], using the bovine serum albumin as a standard.

2.7. Oxidative stress studies

Measurement of malondialdehyde (MDA) level, superoxide dismutase (SOD) and catalase (CAT) activities.

The amount of MDA was assessed as the generation of thiobarbituric acid-reactive substances (TBARS) which were determined by measuring the optical density of the supernatant at 535 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 x 105 M-1cm-1; the results were expressed as nmol/mg protein [29]. The SOD activity was assayed using the method of Marklund and Marklund [30] using the inhibition of pyrogallol autoxidation, the enzyme activity was expressed as units/mg protein at 420 nm, one unit of SOD activity was defined that inhibits the auto-oxidation of pyrogallol by 50 %. The CAT activity was determined from the rate of decomposition of H2O2, monitored by a decrease of 240 nm [31], the activity was expressed as units/mg protein (µmol H2O2/min/mg protein).

Measurement of glutathione-metabolizing enzymes glutathione peroxidase (GPx), glutathione S-transferase (GST) activities.

Reduced glutathione (GSH) levels were estimated by the method of Ellman [32] and Sedlak [33].The estimation of GSH levels was based on the reaction of GSH with DTNB producing a yellow complex estimated at 412 nm. The GSH levels were calculated using an extinction coefficient of 13.600 mol/cm, the values were expressed as nmol/mg protein. The GST activity was assayed by quantifying the conjugate glutathione resulting from the conjugation of CDNB with GSH following the method of Habig [34] The GST activity was monitored at 340 nm for 3 min. The enzyme activity was expressed as units/mg protein, one unit of GST activity was defined as µmoles CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 x 103 M-1cm-1. The GPx activity was determined by the method of Rotrucket [35] based on the degradation of H2O2 in the presence of GSH, the enzyme activity was expressed as units/mg protein, one unit of GPx activity was defined as nmol GSH/mg protein.

2.8. Nitrosative stress markers

Measurement of nitric oxide, s-nitrosothiol and peroxynitrite

Sample preparation may be one of the most important steps in quantifying any and all NO metabolites regardless of the method employed. All steps of tissue preparation should occur under reduced ambient lighting conditions: to minimize photolytic decomposition of tissue NO products, tissue homogenates should be kept on ice in the dark and immediately analyzed within 2 min. It is best to analyze samples immediately on harvesting. Tissues should be perfused free of blood with NEM/EDTA PBS and whole tissues immediately snap-frozen in an aliquot of the NEM/EDTA perfusion buffer; The tissues can then be thawed and homogenized immediately before analysis[36][37].

Production of NO was evaluated by measuring the level of nitrite (an indicator of NO) in the colonic mucosa supernatants using Griess reagent as described byCortas and Wakid[38].The concentration of nitrite in the sample was determined from a NaNO2 standard

curve and was expressed as nmol/mg protein. S-nitrosothiol measurement was performed by using the colorimetric method described by Wink [39], based on the reaction of the Griess reagent with Hg2+ ions. The absorbance of the resulting complex was measured at 490 nm. The results were expressed as nmol/mg protein. The level of peroxynitrite was assayed according to the method described byBeckman [40].The basis of the assay was peroxynitrite-mediated nitration of phenol resulting in nitrophenol formation. The absorbance of the obtained complex were measured at a 320-nm wavelength, the results were expressed as µmol /mg protein.

2.9. Inflammation markers assessment

Cytokines assessment in colon

For the determination of cytokines in the colonic mucosa, protein extracts were obtained by homogenization of the colonic mucosa (0.5 mg tissue/mL) in 50 mM Tris HCl, pH 7.4, 0.5 mM dithiothreitol, and 10 µg/mL cocktail of proteinase inhibitors containing phenylmethylsulfonyl fluoride, pepstatin, and leupeptin. Samples were centrifuged at 10,000 x g for 20 min at 4 °C, and the supernatants were stored at -80 °C until assay. Levels of the cytokines, interleukin-6 (IL-6),tumor necrosis factor-α (TNF-α) were determined by a specific sandwich ELISA using capture/biotinylated detection. Antibodies from BD Biosciences were used according to the manufacturer's recommendations [41].

2.10. Myeloperoxidase (MPO) activity

Colonic MPO is a marker for neutrophils, was evaluated according to the method of Bradley [42] Colonic tissue samples were homogenized by suspending the colon sections in HTAB buffer (0.5 % HTAB in 50 mM potassium phosphate buffer, pH 6). 0.50 mg of tissue/mL was freeze-thawed three times using Ultra Turrax. The homogenates were centrifuged (15 min, 13200 × g, 4 °C) and the supernatants were transferred to new test tubes. 100 µL of supernatant were added on test tubes, followed by 2.9 mL 50 mM potassium phosphate buffer (pH 6), containing 0.167 mg/mL of O-dianisidine hydrochloride and 0.0005 % H2O2. Absorbance was measured at 400 nm after 5, 10, and 15 minutes. The results were expressed as MPO U/mg protein, one unit of MPO activity was defined as the amount that degraded 1 mmol peroxidase per minute.

2.11. Statistical analysis

Results were expressed as the mean ± standard deviation (SD). Data were analyzed using a one -way analysis of the variance test (One-way ANOVA) followed by Honest significant difference test (HSD) of Tukey used as post hoc test to compare significance between groups at $p<0.05$ and $p<0.01$, using the Open stat 2014 program. All in vitro assay reactions were performed in triplicate; n=10 for in vivo studies. Values of $p<0.05$ was regarded as significant.

3. Results and Discussion

During the extraction of polysaccharides from Gelidium spinosum, Polysaccharide gelation occurred rapidly and the total yield was 4.2 %. Regarding other research the yield can be varied seasonally and also it's depend on the method of extraction (water extraction, ethanol extraction, acetic acid extraction …), in addition to other different conditions of extraction like time and temperature.

According to Khatulistiani [43], 2.52 % is the yield percentage obtained from Gelidium sp. That was extracted by maceration method. Under different conditions , Croce [44] revealed that the yield of extraction of polysaccharides extracted with water extraction+boiled method from Gelidium was 8.62%, Mollion [45] reported that Gelidium madagascariense showed a yield of 1.6% when extracted with a Cold extract and 1.5% with an Ethanol extract method.

Table 1. Extraction of crude polysaccharide

Algae	Extraction	Yield
Gelidium spinosum	Polysaccharides	4.2 %

3.1. Evaluation of acute toxicity of PEGS

Acute oral administration of PEGS was found to be safe up to a dose of 2000 mg/kg, p.o. During the following 14 days after, administration of PEGS produced no signs of acute toxicity in treated animals; no mortality and no abnormal behavior were noted. The dose of 60 mg/kg of PEGS was selected for the evaluation of colon protective activities.

3.2. Effect of PEGS on the disease activity index (DAI) and colon length

After 7 days of exposure to 2.5 % DSS in drinking-water, the DSS group developed the clinical symptoms of acute colitis, as indicated by the high significance ($p < 0.05$.) DAI (weight loss, diarrhea, and rectal bleeding) scoring (5.05±0.19) when compared to the control group (0.68±0.08) (Figure 1). In addition, the colon length was found to be 5.3±0.32 cm, significantly ($P<0.01$) shorter in the DSS group than that of control mice (6.85±0.56 cm). Pretreatment with 60 mg/kg of PEGS reversed (59.71 %) the DAI score and prevented colon shortening (60 %) significantly ($P<0.01$) as compared with those of mesalazine group (83.95 %; 70.32 %) (Fig. 1).

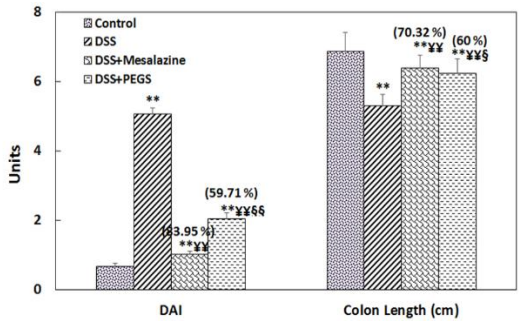

Fig 1. Effect of PEGS and mesalazine on disease activity Index (DAI) and colon length.Values are mean ± SD, (n = 10). ($^{**}p$<0.01): DSS-group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine; ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples /(values of DSS)-values of control.

3.3. Effect of PEGS on oxidative stress markers in the colonic mucosa of mice TBARS and LDH Levels

Fig. 2 showed that the level of TBARS was significantly (P<0. 01) increased in DSS colonic mucosa by about 2.17 fold. However, the activity of LDH was decreased by about 1.62 fold, as compared to the healthy colonic mucosa. Pretreatment of mice with the PEGS (60 mg/kg) or mesalazine (75 mg/Kg) orally decreased significantly (P<0. 01) the level of MDA up to 75.54 % and restored (60.97 %) the LDH activity as compared to mesalazine (84.78 %; 80.48%) respectively.

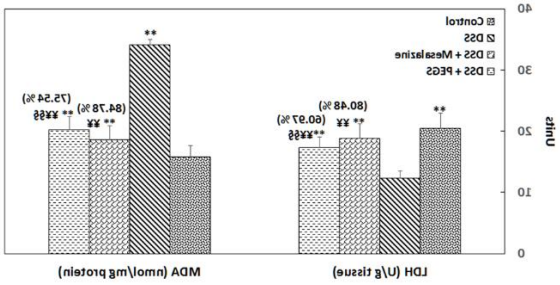

Fig 2.The effect of PEGS (60 mg/Kg) on MDA and LDH levels in DSS-group. Values are mean ± SD, (n = 10). ($^{**}p$<0.01): DSS-group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine; ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples / (values of DSS)-values of control.

3.4. SOD and CAT activities

Fig. 3a showed that the exposure of the mice to DSS 2.5 % caused a significant (P<0.01) reduction in SOD and CAT activities of colonic mucosa (1.75 and 2.3 folds respectively). However, pretreatment with PEGS restored the SOD and CAT activities (55.22 %, 69.14 %; P<0.01) respectively as compared to mesalazine (82.08 %; 85.71 %; P<0.01 %) respectively.

3.5. Glutathione and Glutathione-metabolizing enzymes.

DSS treatment clearly depleted the colonic mucosa GSH level and reduced the GSH-related enzymes as evidenced by the decline in the GST and GPx levels [Figure 3b]. Pretreatment of rats with 60 mg/kg of PEGS efficacy restored the GSH level and GSH-system enzymes towards normal levels. A marked response (61.97 %) of GSH was observed in the colonic mucosa pretreated with PEGS, as compared to mesalazine pretreatment (82.85 %). Moreover, our results clearly showed that the pretreatment with PEGS extract significantly (P<0.01) regulated the changes in glutathione metabolizing enzyme levels in the colonic mucosa by reversing (61.94 %) GPx and GST (59.17 %) levels, the efficacy of PEGS was comparable to that of mesalazine in restoring GPx (72.67 %) and GST (79.28 %) (Fig. 3b).

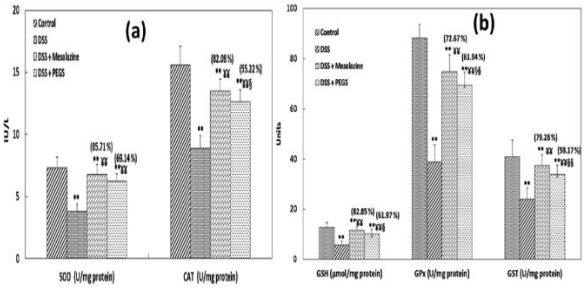

Fig 3. (a, b).The effect of PEGS on oxidative stress markers colonical mucosa ,SOD and CAT activities (a).Glutathione and Glutathione-metabolizing enzymes (b).Values are mean ± SD, (n = 10). ($^{**}p$<0.01): DSS- group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine; ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples/ (values of DSS)-values of control.

3.6. Effect of PEGS on in vivo nitrosative stress markers in the colonic mucosa of mice

As illustrated in Figure 4, levels of NO, S-nitrosothiol and peroxynitrite were found to be significantly (P<0.01)) higher in the colonic mucosa of DSS - group, by about 8.65 fold for the NO and by about 9.01 fold for S-nitrosothiol and by about 2.02 for peroxynitrite when compared with normal mucosa. However, pretreatment with PEGS restored (69.18 %; 52.87 %; and 73.02 %) the NO, S-nitrosothiol and peroxynitrite levels respectively as compared to mesalazine (82.2 %; 70.89 % and 85.69 %,) respectively (Fig. 4).

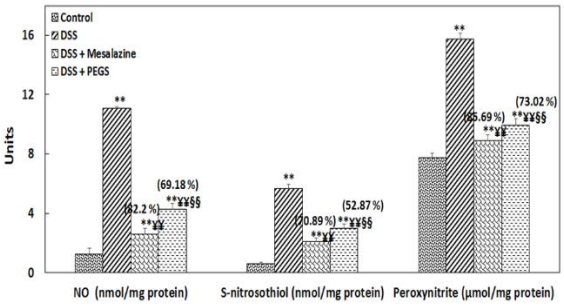

Fig 4. Effect of PEGS on nitrosative stress markers:NO, PON and RSNO levels in colonical mucosa. Values are mean ± SD, (n = 10). ($^{**}p$<0.01): DSS-group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine; ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples/ (values of DSS)-values of control.

3.7. Anti-inflammatory studies of PEGS

Pro-inflammatory cytokines in the colonic mucosa of mice

DSS significantly increases significantly (P<0.01) the production of the pro-inflammatory cytokines in the distal colon such as TNF-α (3.17 fold) IL-6 (6.05 fold), However PEGS pretreatment significantly (P<0.01) attenuated the levels of TNF-*α* (71.77 %) and IL-6 (79.79 %) as compared to mesalazine (88.04 %; 84.46 %) respectively (Fig. 5 a,b).

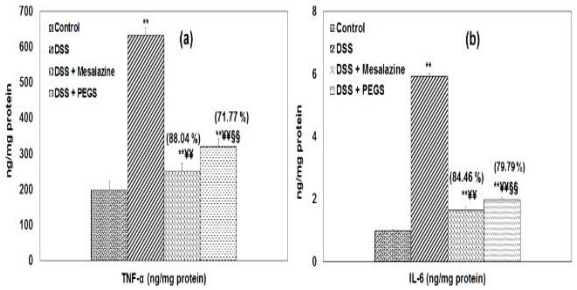

Fig 5 (a, b). Effect of PEGS Pro-inflammatory cytokines in the colonic mucosa of mice TNF-α (a), IL-6 (b).Values are mean ± SD, (n = 10). ($^{**}p$<0.01): DSS-group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine. ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples/ (values of DSS)-values of control.

MPO activity in colonic tissues

Massive PMN recruitment into the colonic tissue was further confirmed by increased MPO activity [Figure 6]. A significant (P<0.01) increase was observed in MPO activity in colonic tissues of DSS-group (8.99±0.3 U/mg protein) with respect to control tissues (3.6±0.08 U/mg protein) [Figure 6].Additionally, administration of PEGS significantly (P<0.01) normalized (67.9 %) the MPO level, in comparison to mesalazine (84.46 %) (Fig. 6).

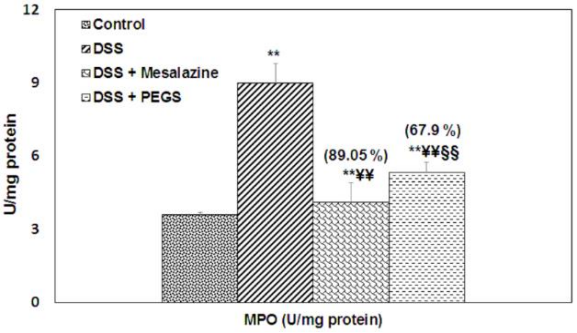

Figure 6. Effect of PEGS on MPO activity in colonic tissues. Values are mean ± SD, (n = 10), ($^{**}p$<0.01): DSS-group vs control groups; ($^{¥¥}p$<0.01): DSS group vs DSS + mesalazine; ($^{§§}p$<0.01): DSS group vs DSS + PEGS).Values in parentheses indicate percent protection. The % of protection is calculated as: 100 x (values of DSS)-values of samples/ (values of DSS)-values of control.

In the present study an acute inflammation of colon was created during 7 days of administration of DSS administration to Swiss albino mice and followed by a slow regeneration of the colonic epithelium. This DSS-model exhibits symptoms similar to those of human ulcerative colitis [46] such as diarrhea, bloody fasces, body weight loss, mucosal ulceration, and shortening of colon length. The DSS-induced colitis model has been shown to exhibit several characteristics found in humans, including inflammation that begins in the distal colon and then affects the proximal colon [47].Many factors have been involved in the pathogenesis of UC. The overproduction of ROS and RNS through neutrophil recruitment which overwhelms the protective mechanisms of tissue antioxidants [48].The colon is more susceptible to oxidative damage due to the relatively small amount of antioxidants attainable in the mucosa [49].Evidence suggests that chronic intestinal inflammation correlates with increased production of reactive oxygen and nitrogen species (ROS/RNS) and the resulting oxidative stress and redox modulation by antioxidants [50].

In the present study, mice treated with DSS developed colonic damage that was observed as a substantial increase in the plasma LDH levels. The increased LDH activity can be attributed to cell membrane permeability dystrophy [51].Our results also revealed that the DSS-model exhibits intense oxidative stress in the intestinal tissue, which is evidenced by the high level of lipid peroxidation, as well as by the deterioration of the enzymatic and non-enzymatic antioxidant system involved in the elimination of superoxide anions and peroxide, including superoxide dismutase, catalase, and the glutathione-dependent enzymes. The reduction of endogenous antioxidant defense system may promote

the free radical formation, breakdown of the GSH-dependent antioxidant defense system, and sequential alteration of cellular redox balance [52].

Our results are in agreement with those of Tahan [53],Spitzet [54], and Brito [23] mentioned that the PLS from red algae Gracilaria birdiae reduced mucosal lesions in colon tissues and decreased the inflammatory response in the colon, which is linked to a decrease in oxidative stress . Luo [55] reported that guava leaf polysaccharides improve the enzymatic defense system. Barros-Gomes [56] showed that sulfated polysaccharides from seaweed Gracilaria birdiae, increased cytosolic enzyme activities (glutathione reductase and catalase).It has been reported that the polysaccharides from algae Gracilaria caudata improved the antioxidant system [57].According to Rocha de Souza [58] and Kim [59], the sulfated polysaccharides extracted from marine algae have prevented oxidative damage in living organisms.Another study also showed that mucosal damage was reduced by sulfated polysaccharides and by acting through the enhancement of endogenous antioxidants [24].It has been reported that PLS decreased the mucosal damage in colon tissue and reduced the inflammatory response which is apparently related to decrease oxidative stress [23].

In our investigation, PEGS elevated the levels of glutathione metabolizing enzymes. These effects could be due to the antioxidant properties of PEGS which enhance the removal of ROS from the colon tissue system. Overexpression of the colonic glutathione antioxidant system in mice pretreated with PEGS suggests that this potent antioxidant defense may be reactivated by the PEGS. Indeed reports have revealed the effects of PLS on the glutathione system [60].

The possible antioxidant mechanism of action of PEGS includes its ability to scavenge reactive oxygen species or/and enhance endogenous antioxidants levels. In the present study, we also showed that lesions of the intestinal mucosa were accompanied by elevated levels of RNS. Special attention should be paid to RNS, which can be produced and issued by immune cells and play an important role in the pathophysiology of ulcerative colitis[61].Recruitment and activation of neutrophils during acute inflammation contribute to the overproduction of ROS/RNS which overwhelms tissue antioxidant protective mechanisms, resulting in oxidative stress, which perpetuates inflammation of the colon[62][63].The concept of nitrosative stress stems from the fact that interactions between nitrosants and oxidants can produce products that are more toxic than either reagent alone [64].Under such conditions, nitrosylation can directly stimulate disadvantageous oxidative changes [65].The results of the present study also revealed that nitrosative stress, S-nitrosothiols (NO donors), peroxynitrite(oxidant formed from NO and superoxide anion (O2.-), were higher in colon tissue of the DSS-group. A highly reactive species, peroxynitrite (ONOO-), is formed as a result of the reaction of NO° with O2 .-, and can oxidize and nitrate the genome [66].Nitrosative stress can cause nitrosylation reactions which induce changes in the structure and function of proteins and stimulate lipid peroxidation [67].

Higher concentrations of oxidizing species can promote the conversion of NO to higher oxide forms, such as nitrogen dioxide and peroxynitrite (ONOO-) which can lead to altered homeostasis of the redox state [68].From our results it is clear that the PEGS pretreatment, significantly neutralizes the nitrosative stress induced by DSS.In addition, the present study reports that administration of DSS to Swiss albino mice resulted in an infiltration of neutrophils which are evidenced by a high level of myeloperoxidase activity that may exacerbate the colonic tissue damage with the most generation free-radicals.

In the present study, the intestinal mucosa damage induced by DSS was also accompanied by inflammatory responses which are evidenced by the elevated levels of TNF-α and IL-6.

Several studies have shown that the immune cells and intestinal epithelial cells secrete various types of pro-inflammatory cytokines such as TNF-α IL-1ß, and IL-6 in the colonic mucosa of UC patients [69] and may amplify the inflammatory cascade resulting in intestinal tissue damage in UC induced by DSS.

Several conventional drugs have been selected as a potent antioxidant and ROS scavengers for the treatment of UC in humans, such as 5-aminosalicylate (5-ASA) and the immunosuppressant [70] [71].Unfortunately, these treatments are not devoid of potentially serious side effects, thus limiting their chronic use. It has been systematically reported in the literature that PLSs exhibit antioxidant activity, anti-inflammatory like those extracted from marine algae [72].Consequently, there is a compelling demand for safe and effective therapeutic strategies for human IBD. This could be the case for several polysaccharides exhibiting several biological activities, mainly linked to their antioxidant and anti-inflammatory properties [73][74].Interestingly, several studies suggest that blocking the cytokine overexpression pathway and reducing MPO activity may be used as an indicator of anti-inflammatory activity and may be useful in the treatment of UC [75].Many drugs have been reported to decrease the severity of UC disease, including Adalimumab, a TNF-α blocker, which has been successfully used for the treatment of patients with IBD clinically, the anti - TNF-α ,infliximab, a mouse monoclonal antibody is also believed to have an effective therapeutic effect on UC in a clinical case [76][77].Aminosalicylates

remain the cornerstone of treatment for patients with active, mild to moderate UC [78].

Corticosteroids also remain popular medications for inducing remission in UC disease. Sulfasalazine, mesalamine, and the steroids, glucocorticoids, also help reduce inflammation of the intestinal wall. However, their significant negative effect limits their long-term use[79].Recently, natural products such as flavonoids and polysaccharides have shown great potential for inflammatory inhibition [80][81]via the modulation of the expression of pro-inflammatory genes, for example, nitric oxide synthase (NOS), and the generation of pro-inflammatory mediators comprising cytokines such as TNF-α, IL-1β and IL-6 [82] .For these reasons, polysaccharides has become available for the treatment of Crohn's disease. The involvement of anti-inflammatory mechanisms of the PEGS have obviously been provided in this study. Our result also revealed that the PEGS treatment (60 mg/kg) reduced the activity of MPO as the index of inflammatory cells infiltration in colitis and modulated the inflammatory responses possibly by preserving the pro-inflammatory cytokine (TNF-α, IL-6) at values close to normal, suggesting that the protective effect of PEGS against colonic injury is also linked to the regulation of the pro-inflammatory cytokine.

4. Conclusions

This study concluded that PLS fraction extracted from the seaweed Gelidium spinosum (PEGS) has the protective effect against DSS-induced ulcerative colitis in male Swiss albino mice. The mechanisms contributing to its effectiveness may be the attenuating the clinical symptoms (disease activity index; DAI), reducing nitrosative and pro-inflammatory biomarkers (NO, S-nitrosothiol, peroxynitrite, TNF-α, IL-6, and MPO activity. These significant protective effects of PEGS were associated with a remarkable amelioration of the antioxidant defense system (SOD, CAT activities, the glutathione metabolizing enzyme glutathione) in the colon tissue. Therefore, further phytochemical and molecular investigations are needed to explain whether the PLSs a constituent may interfere with some molecular pathways of the UC protection.

5. Conflicts of interest

The authors declare that there is no conflict of interest toward the publication of this article.

6. Formatting of funding sources

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8. References

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