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# Characterization of Garden Cress Mucilage and its Prophylactic Effect Against Indomethacin-Induced Enter-Colitis in Rats

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Abstract: The utilization of industrial waste such as oil industry waste in the production of natural nutraceuticals is a very beneficial issue. So, the current study was established to assess the characteristics of the mucilage extracted from garden cress seeds meal and evaluate its efficacy in the protection against enter-colitis. A defatted powder meal of garden cress seeds was dissolved in distilled water to extract the mucilage either by heating at 80°C or by ultrasonication. Functional and chemical characteristics of the two types of mucilage were assessed. Rats received oral administration of the mucilage extracted by ultrasonication, which afforded the most promising antioxidant activity, for two weeks before and during the 7 days of oral administration by indomethacin (6 mg/kg/day). Compared with the rats injected with indomethacin without the pre-treatment with the mucilage, rats administrated with the mucilage showed a decrease in the erythrocyte sedimentation rate (ESR), intestinal tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and plasma lactate dehydrogenase (LDH) activity. Also, rats administered with the mucilage recorded lower intestinal malodealdehyde (MDA) and higher intestinal reduced glutathione (GSH) compared to those of rats injected with indomethacin without the pre-treatment with the mucilage. It can be concluded that the mucilage extracted from garden cress seeds meal can be considered as a natural nutraceutical with potent antioxidant activity exhibiting protective effect against enter-colitis.

#### Keywords: garden cress; mucilage; inflammatory bowel disease; indomethacin.

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## 1. Introduction

Inflammatory bowel disease (IBD) is described by discontinuous inflammation in the gastrointestinal tract. The prevalence of IBD, including ulcerative colitis (UC) and Crohn's disease (CD), has increased over the past few decades [1]. Weight loss, diarrhea, hematochezia, vomiting, and pain are among the most common IBD clinical symptoms. Amino-salicylates, immunosuppressive agents, and corticosteroids are the most common medical therapies of IBD, but they exert several side effects and lead to mood disturbance, type 2 diabetes, obesity, acne, insomnia, avascular necrosis, and skin atrophy [2]. Non-steroidal anti-inflammatory drugs (NSAIDs), which are extensively used without medical guidance, are considered one of the main inducers of IBD [3].

Nie *et al.* [4] declared that non-starch polysaccharides (NSPs), including gum, may be promising agents for protection against IBD via several mechanisms such as anti-inflammation, stimulating the immune system, alteration of the gut microbiota, generation of the short-chain fatty acids (SCFAs), anti-oxidative stress go together by inflammation and the diminution of the absorption of toxins.

Garden cress (*Lepidium sativum*) seeds are seeds that are when soaked in water and produce a viscous gel due to its high content of colloidal substances [5]. L-arabinose, D-xylose, D-galactose, L-rhamnose, D-galacturonic acid, and 4-O-methyl-D-glucuronic acid are the compounds with the highest percentage of the *Lepidium sativum* gel composition, in addition to the presence of other compounds with a lower percentage that includes D-glucose and mannose [6]. Ahmad *et al.* [7] reported that polysaccharides extracted from garden cress reduced the level of tumor necrosis factor-alpha (TNF- $\alpha$ ) in mice' plasma. Therefore, in the present study, garden cress mucilage was extracted with two different methods and assessed for its functional and chemical properties in addition to its efficacy in the protection against enter-colitis induced by indomethacin in rats.

# 2. Materials and Methods

# 2.1. Materials and chemicals.

Garden cress seeds were brought from a local market in Egypt. Chemicals and pure reagents were purchased from Sigma Chemical Companies (Sigma-Aldrich, St. Louis, MO, USA). Indomethacin was obtained from a local pharmacy in Egypt.

# 2.2. Extraction of the garden cress mucilage.

Garden cress seeds were grounded then subjected to defatting using a soxhlet apparatus and n-hexane as a defatting solvent. The defatted meal was spread on a tray to dry than the ground to obtain a fine powder. The polysaccharide extracted by two methods: First, by soaking in hot water (MHW), 300 g garden cress meal was stirred well by electric stirrer in 9 liters distilled water then soaked for 24 hours at 80°C. Second, by the aid of ultrasonic bath (MUS), 300 g garden cress meal was stirred well by electric stirrer in 9 liters distilled water then soaked in an ultrasonic bath for two hours at room temperature. Both extracts were centrifuged for 30min at 3000 xg then the supernatant collected and concentrated by rotary evaporator at 60°C. The polysaccharide precipitated with ethanol's addition to the final extract then freeze-dried by (Crest Alpha 1-4 LSC plus Germany). The freeze-dried mucilage was sieved by 140 meshes then kept in a refrigerator until used.

# 2.3. Functional properties of the two types of extracted mucilage.

# 2.3.1. Bulk density.

Bulk density was estimated according to Taha and Ibrahim [8].

2.3.2. Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC).

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) were determined as described by [9] and expressed as percentage g of water or oil held/g sample.

2.3.3. Emulsifying capacity (EC).

30ml of 1:100 and 2ml corn oil were added according to Shahidi *et al.* [10]. The emulsifying capacity was calculated as a ratio of emulsified versus total volume.

2.3.4. Foaming properties.

Foaming properties, including foaming capacity (FC) and foam stability (FS), were determined by the method of Shahidi *et al.* [10] with the minor modifications made by Wang *et al.* [11]. Foaming capacity was calculated using the following equation: FC %= (V-V<sub>0</sub>/V<sub>0</sub>) × 100

Where V and  $V_0$  are the volumes immediately measured after whipping and before whipping, respectively.

2.3.5. Apparent viscosity.

Apparent viscosity was measured at different temperatures using a Brookfield digital viscometer (Model DV-II, Canada) fitted with spindle 3 at 200 rpm with 1% (w/v), and apparent viscosity was expressed as cp and different rpm fitted with spindle 0.

# 2.4. Chemical composition of the two types of extracted mucilage.

Moisture, Protein, and Ash contents were estimated according to AOAC [12]. The functional groups of the extracted garden cress mucilage were determined using Fourier transform infrared (FTIR) spectra in the range of 500-4000 cm<sup>-1</sup> on Shimadzu 8400S FTIR spectrophotometer using the KBr disk technique. Minerals contents were determined using a flame atomic absorption spectrometer (Agilent Technologies); one g of each sample was placed in a clean crucible and placed in a furnace. The content was ashed at 650 °C for 10 h until a whitish-grey matter was obtained then cooled. 2 M HCl was added, then deionized water (completed to 20 mL) was added and filtered then the filtrate was used for the metal assay. The amount of individual element was calculated by the obtained values following their standard curves, and the measurements were made at 589.6 nm (Na), 766.5 nm (K), 239.9 nm (Ca), 202.6 nm (Mg), 248.3 nm (Fe), 279.5 nm (Mn), 324.7 nm (Cu) and 213.9 nm (Zn).

2.5. Cytotoxic effect on human normal fibroplast cell line (BJ1).

Mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide) to purple formazan was used to assess the cell viability [13].

In a sterile zone, using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA), cells were suspended in DMEM-F12 medium, 1% mixture of antibiotic-antimycotic (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate, and 25µg/ml Amphotericin B) and L-glutamine (1%) at 37 °C under 5% CO<sub>2</sub>

Cells were cultured for 10 days in batches. They were seeded  $(10x10^3 \text{ cells/well})$  in a wells-microtiter plastic plate, contained fresh complete growth medium, at 37 °C for 24 h under 5% CO2 through a water-jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). The medium was removed, and a fresh medium (without serum) was inserted. Cells were incubated for 48 h alone as a negative control and with different concentrations of a sample (100-50-25-12.5-6.25-3.125- 1.56 and 0.78 µg/ml), then the medium was removed and 40 ul MTT salt (2.5µg/ml) were added to each well and incubated for 4 h at 37°C under 5%

CO2. Solution (200  $\mu$ L) of sodium dodecyl sulfate (10%) was added to each well to stop the reaction, then wells were incubated overnight at 37°C. DOX (100  $\mu$ g/ml), which gives 100% lethality under the same conditions [14], was used as a positive control.

A microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) was used to measure the absorbance at 595 nm. The percentage of change in viability was calculated using the following equation:

((Reading of extract/Reading of negative control) -1) x 100

2.6. Evaluation of the prophylactic effect against indomethacin-induced enter-colitis.

2.6.1. Animals.

Male Wistar rats (14 weeks old) of  $234.44 \pm 14.2g$  as mean  $\pm$  SD were taken from the animal house of National Research Centre, Cairo, Egypt. Each rat was housed in a stainless steel cage under standard laboratory conditions (23–25 °C, 12 h light/dark cycle) and free access to diet and water. According to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, this study has been carried out and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.6.2. Animals' diet.

Balanced diet (12% casein as a source of protein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% salt mixture, and 1% vitamin mixture), salt, and vitamin mixtures were prepared according to AIN-93 [15].

2.6.3. Design of the animals' experiment.

Eighteen rats were divided after one-week acclimatization into three groups (n=6) as follows:

G1: The normal control group where rats were daily orally given distilled water.

G2: Indomethacin group (INDO) where the rats were treated orally with distilled water for two weeks then administered with indomethacin (6 mg/kg/day) dissolved in 5% sodium bicarbonate for 7 days [3].

G3: Garden cress mucilage extracted by the aid of ultrasonic bath + indomethacin group (MUE+INDO) where rats were daily treated orally with garden cress mucilage (100 mg/Kg b.w.) for two weeks before and during the 7 days administration of indomethacin (6 mg/kg/day) dissolved in 5% sodium bicarbonate [3].

All rats were fed on a balanced di*et al*l over the study period (three weeks). At the end of every week, total food intake and body weight gain were calculated. Total food intake and body weight gain were estimated after the end of the treatment period. Additionally, the feed efficiency ratio was calculated.

2.6.4. Blood analysis.

Twenty-four hours after the last administration of indomethacin, blood was collected from each rat under slight anesthesia. A portion of the whole blood was analyzed for erythrocyte sedimentation rate (ESR) and hemoglobin (Hb) according to Drabkin [16], and the remaining blood was centrifugated for 10 min at 1000g and serum of each rat was kept under -20 °C until used. Serum iron, total iron-binding capacity (TIBC), and lactate dehydrogenase (LDH) activity were determined according to Stookey [17], Betts and Stuart [18], and Zimmerman *et al.* [19], respectively.

2.6.5. Hemoglobin of the intestinal flush.

Hb concentration was assessed in the intestinal flush according to Crosby and Furth [20] and Schuck-Phan *et al.* [21]. A benzidine solution (1 mL) containing 1% (w/w) of benzidine dihydrochloride in 90% (v/v) of glacial acetic acid was added to a test tube. The intestinal flush (0.02 mL) was added, then 1 mL of 1% of hydrogen peroxide was added to the tube with mixing, and then a diluent containing 10% (v/v) glacial acetic acid was placed. After 10 min, the absorbance at 515 nm was measured by a spectrophotometer, and Hb concentration (mg/mL) was calculated using a standard curve.

## 2.6.6. Small intestine homogenate analysis.

Segments of each rat' small intestine were taken after the sacrificing and measured for their length. The small intestine homogenate was immediately analyzed for malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) according to Ohkawa *et al.* [22], Montgomery and Dymock [23], and Sedlak and Lindsay [24], respectively. Also, the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and superoxide dismutase (SOD) were determined using Eliza kits (SinoGeneclon Biotech Co., Ltd).

## 2.7. Statistical analysis.

Statistical analysis was done using SPSS version 21. The results were expressed as mean  $\pm$  standard error (SE) and analyzed statistically using a one-way analysis of variance (ANOVA) followed by Duncan test. The statistical significance of difference was taken as  $P \leq 0.05$ . Results of the chemical composition, function properties, and mineral contents of the two types of mucilage were analyzed statistically using a T-test and expressed as mean  $\pm$  standard deviation (SD).

## 3. Results and Discussion

It was evident from previous studies that the remnants of squeezing garden cress seeds can be used to produce products of high health value [25]. The current study indicated that the products of squeezing garden cress seeds could be exploited and converted from waste that requires disposal to products of biological value such as mucilage. Mucilage could be extracted from the whole mucilaginous seeds or from the meal with different methods, including hot water, microwaves, magnetic stirring, and ultrasounds [26, 27]. In the current study, mucilage of garden cress seeds was extracted by hot water and ultrasound aid. The characteristics of the two differently extracted types of mucilage were studied.

Figure 1 illustrates the soluble garden cress seeds in distilled water and the soluble mucilage of garden cress meal in water. The freeze-dried powders of the two extracted mucilage types were brownish-white in color, as shown in Figure 2. Both types of the extracted mucilage (either with hot water or with the aid of an ultrasonic bath) were approximately the same in color.



A. Soluble Seeds

**B.** Soluble Mucilage

Figure 1. Soluble garden cress seeds (A) and soluble mucilage (B).



A. MHW B. MUS Figure 2. Powder of mucilage extracted by hot water (MHW) (A) and Powder mucilage extracted with the aid of ultrasonic bath (MUS) (B).

The chemical compositions and functional properties of both types of the extracted mucilage, either (MHW) or (MUS), are presented in Table 1. The extracted mucilage, with the aid of an ultrasonic bath, recorded protein and ash contents higher than those of the extracted MHW. This may be attributed to the fact that ultrasonic waves help disrupt plant cell walls, which improves the solvent penetration enhanced mass transfer across the cell membrane [28]. The ash value is an important parameter for detecting purity and the presence of other substances. The mucilage's lower ash value indicates its purity from the accompanying materials [29]. The MUS recorded higher values of the functional properties than MHW. These functional properties of the gel extracted from plant waste have great importance in determining the aspects of this gel's use in the industry. Naji *et al.* [30] determined the emulsifying and foaming characteristics of garden cress mucilage and observed that the garden cress mucilage possessed great emulsifying and foaming ability (92% and 25.4%, respectively) at 25oC. Also, it was revealed that cress seed mucilage was a superior emulsifier than gum acacia [31]. Moniri *et al.* [32] reported that freeze-dried garden cress mucilage possessed high emulsifying capacity and stability (> 80%).

Composition	MHW	MUS
Moisture %	14.9±0.46	12.9±0.45
Protein %	31.2±0.75	34.1±0.79
Oil %	0.0	0.0
Ash %	11.34±0.65	13.46±0.5
Functional properties		
Water holding capacity%	1153±10	1210±15
Oil holding capacity%	144 ±5	180±6
Foaming capacity%	16±0.29	26±0.69

Table 1. Chemical composition and function properties of the two types of mucilage.

Composition	MHW	MUS
Foaming stability after 2hours%	10±0.5	21±0.6
Bulk density g/cm	0.61±0.1	0.56±0.1
Emulsion stability%	85±0.5	82±0.5
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The values are expressed as the means  $\pm$  SD.

As illustrated in Table 2, both types of the extracted mucilage (MUS and MHW) had approximately the same contents of minerals. Singh and Paswan [33] and Prajapati and Dave [34] showed that garden cress seed contains considerable amounts of potassium and calcium.

Minerals	MHW (mg/g)	MUS (mg/g)
Copper	$0.032 \pm 0.001$	0.033±0.001
Iron	$0.079 \pm 0.001$	0.132±0.001
Zinc	$0.047 \pm 0.001$	0.049±0.001
Manganese	$0.026 \pm 0.001$	0.041±0.001
Sodium	$1.576 \pm 0.02$	1.229±0.01
Magnesium	8.300±0.1	7.980±0.1
Potassium	18.395±0.2	17.580±0.2
Calcium	5.710±0.2	6.140±0.1

Table 2. The mineral content of the two types of mucilage expressed as mg/g dry weight.

The values are expressed as the means  $\pm$  SD.

As shown in Table 3, both types of the extracted mucilage (either with hot water or with the aid of an ultrasonic bath) had potent antioxidant activity. However, The MUS exhibited higher radical scavenging activity than that of the MHW and even higher than the antioxidant butylated hydroxytoluene (BHT). The superiority of the extracted mucilage with the aid of ultrasonic bath as an antioxidant may be due to ultrasonic wave effects on the cell's hydrolysis, facilitating the disruption of cell wall liberating protein and sugar, which may cause considerable interference in these antioxidant activity assays. Alkahtani *et al.* [35] found that garden cress mucilage exhibited radical scavenging activity. Omer *et al.* [36] and Singh and Paswan [33] reported that garden cress seed fractions exposed scavenging activity versus 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and good Ferric Reducing Antioxidant Power (FRAP). So, it acts as in vivo and in vitro antioxidant.

Table 3. Antioxidant activity of 1% the two types of mucilage compared to BHT.

Samples	DPPH %	FRAP mg/g
MHW	61.3 <sup>a</sup> ±0.49	15.6 <sup>b</sup> ±0.29
MUS	88.9 <sup>b</sup> ±0.88	18.2°±0.48
BHT	63.7 <sup>a</sup> ±0.79	7.5 <sup>a</sup> ±0.25

Dissimilar superscript letters in the same column denote significant differences at p < 0.05. The values are expressed as the means  $\pm$  SD



Figure 3. Effect of temperature on viscosity of the two types of mucilage.

As shown in Figure 3, with the increasing temperature, the viscosity also decreases with the increasing rpm, the viscosity decreases as illustrated in Figure 4. It was observed that the extracted MUS was more viscous than the extracted MHW, which was also confirmed through the high value of water holding capacity of MUS as declared before in Table 1.



Figure 4. Effect of rpm on the viscosity of 1:100 (w/v) of MHW and MUS.

The FTIR spectrum (Figure 5) shows no difference in composition between both types of the extracted mucilage (either with hot water or with the aid of an ultrasonic bath). The FTIR spectrum shows the same functional groups related to constitutes of the mucilage, such as protein and carbohydrates. The O-H stretching and N-H group appears at 3430, 3427, and 2926 cm<sup>-1</sup>, respectively. Further, the C-H aliphatic appears at 2885, 2857 cm<sup>-1</sup>. The peak is shown at the wavenumbers 1741 and 1738 cm<sup>-1</sup> corresponding to the -C=O group. Similar findings were obtained by Moniri *et al.* [37]. The peaks at 1123 and 1033 cm<sup>-1</sup> denote the monosaccharides such as glucose and mannose, as confirmed by a previous study [38]. The fingerprint district for carbohydrates is between wavenumbers 800 and 1200 cm<sup>-1</sup>. Amid (N-H) group indicates the presence of protein [37].



Figure 5. FTIR spectra of both MHW and MUS.

The garden cress mucilage extracted by ultrasonic waves was non-toxic for normal cell lines and didn't produce a change in cells' viability within the studied concentration (0.78- $100\mu$ g/ml).

Non-steroidal anti-inflammatory drugs, such as indomethacin, are well known to cause gastrointestinal damage either in rats or humans. The long-term NSAIDs treatment is known to cause mucosal injuries of the small intestine, among them bleeding, erosion, and ulcers [39].

In the current results, there were non-significant changes in the weight gain and total food intake of the three studied groups after the first week of the study (Figure 6 and 7). The 7 days treatment with indomethacin produced a significant reduction in weight gain and total food intake. Previous studies found that indomethacin administration caused a significant reduction in weight gain and total food intake [3, 40]. Rats pre-treated with the garden cress mucilage recorded total food intake and body weight gain significantly higher than rats treated with indomethacin only.

As evident from Table 4, a decrease in the final body weight gain, although not statistically significant, was recorded by the rats treated with indomethacin when compared with the normal rats. It was reported in previous studies that the treatment with indomethacin resulted in shortening of the small intestine length [3, 41]. So, the small intestine length was measured in the current study. It was found that rats treated with indomethacin recorded less small intestine length. The small intestine length of the rats pre-treated with the garden cress mucilage was approximately near that of the normal rats.



Figure 6. The weekly body weight gain of normal rats and rats treated with indomethacin. The values are expressed as the means  $\pm$  SE. (n = 6). Dissimilar superscript letters on each bar denote a significant difference at P  $\leq$  0.05. INDO, indomethacin; MUS, mucilage extracted by the ultrasonic bath.



Figure 7. The weekly total food intake of normal rats and rats treated with indomethacin. The values are expressed as the means  $\pm$  SE. (n = 6). Dissimilar superscript letters on each bar denote a significant difference at  $P \le 0.05$ . INDO, indomethacin; MUS, mucilage extracted by the ultrasonic bath.

Table 4. Effect of the garden cress mucilage on the growth performance parameters.

Parameters	Groups			
	Control normal	INDO	MUS + INDO	
Initial body weight (g)	234.50 <sup>a</sup> ±2.81	234.33 <sup>a</sup> ±7.27	234.50 <sup>a</sup> ±7.35	
Final body weight (g)	290.67 <sup>a</sup> ±3.27	276.17 <sup>a</sup> ±6.89	283.83 <sup>a</sup> ±7.39	
Feed efficiency ratio	$0.14^{a}\pm0.01$	0.13 <sup>a</sup> ±0.01	0.13 <sup>a</sup> ±0.01	
Small intestine length (cm)	83.58 <sup>b</sup> ±1.05	72.67 <sup>a</sup> ±1.05	80.75 <sup>b</sup> ±1.30	

The values are expressed as the means  $\pm$  SE. (n = 6). Dissimilar superscript letters in each row denote a significant difference at P  $\leq$  0.05. INDO, indomethacin; MUS, mucilage extracted by the ultrasonic bath.

As shown in Table 5, the concentration of hemoglobin decreased significantly in the rats treated with indomethacin. However, the intestinal hemoglobin increased significantly in the rats treated with indomethacin. In addition to the impairment of iron absorption, the blood loss, subsequent to the treatment with NSAID, leads to anemia. Also, the hemorrhage is associated with small intestine inflammation subsequent to NSAID treatment [42]. The intestinal hemoglobin was found to be decreased in rats pre-treated with garden cress mucilage, which indicated garden cress mucilage's role in preventing bleeding after the treatment with indomethacin. Also, ESR and LDH activity increased significantly in the current results is coinciding with Shu *et al.* [43], who confirmed the release of LDH due to indomethacin-induced cell damage. The pre-treatment with garden cress mucilage prevented the elevation of ESR and LDH activity significantly. There were non-significant differences in iron and TIBC values between the groups.

Donomotors	Groups		
Farameters	<b>Control normal</b>	INDO	MUS + INDO
Hemoglobin (g/dl)	12.81 <sup>b</sup> ±0.56	11.24 <sup>a</sup> ±0.22	12.04 <sup>ab</sup> ±0.21
Iron (mg/dl)	237.69 <sup>a</sup> ±11.12	235.77 <sup>a</sup> ±6.16	236.15 <sup>a</sup> ±8.28
TIBC (µg/dl)	66.26 <sup>a</sup> ±1.39	69.82 <sup>a</sup> ±0.88	66.71 <sup>a</sup> ±1.43
ESR (mm/h)	2.83 <sup>a</sup> ±0.31	7.67 <sup>b</sup> ±0.80	3.00 <sup>a</sup> ±0.26
LDH (U/I)	268.03 <sup>a</sup> ±8.28	322.89 <sup>b</sup> ±7.12	286.55 <sup>a</sup> ±4.53
Intestinal Hemoglobin (mg/100ml)	4.05 <sup>a</sup> ±0.22	87.59 <sup>c</sup> ±1.05	6.74 <sup>b</sup> ±0.56

Table 5. Effect of the garden cress mucilage on the blood biochemical parameters and intestinal hemoglobin.

The values are expressed as the means  $\pm$  SE. (n = 6). Dissimilar superscript letters in each row denote a significant difference at P  $\leq$  0.05. INDO, indomethacin; MUS, mucilage extracted by the ultrasonic bath; TIBC, total iron-binding capacity; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase.

NSAIDs interact with the intestinal phospholipids, which cause damage to the intestinal cells and increase gastrointestinal permeability. The microvascular blood flow is reduced via COX inhibition, and aggressive luminal agents alter and amplify this reaction, causing inflammation, erosions, and ulcers. Acid, bile, and bacteria in the small bowel are the main luminal aggressors [39]. It was mentioned in a previous study that mucilage could attenuate oxidative stress and inflammation, which may be attributed to the positive effect of mucilage on gut microbiota [26]. On the one hand, the treatment with indomethacin in the current study resulted in an elevation of intestinal MDA, NO and TNF-a and a reduction in intestinal GSH and SOD as shown in Table 6. On the other hand, the pre-treatment with garden cress mucilage suppressed the elevation of intestinal MDA, NO, and TNF-a. Also, the pre-treatment with garden cress mucilage reversed the reduction in intestinal GSH and SOD. Oxidative stress and inflammation subsequent to administration of indomethacin had been reported in previous studies since activation of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO) is associated with intestinal lesions subsequent to administration of NSAIDs [3, 44]. Santiago-López et al. [45] also reported that treatment with indomethacin increased TNF-a and other cytokines' values. The antioxidant activity of garden cress mucilage contributed to the suppression of elevation of intestinal MDA and reduction of intestinal GSH and SOD. Ahmad et al. [7] reported that polysaccharides extracted from garden cress reduced the level of tumor necrosis factor-alpha (TNF- $\alpha$ ) in mice's plasma. Thus, the protective effect of garden cress mucilage against indomethacin-induced enter-colitis may be attributed to its role in preventing oxidative stress and inflammation after administration of indomethacin.

Denometers	Groups		
Parameters	Control normal	INDO	MUS + INDO
MDA (nmol/g tissue)	15.59 <sup>a</sup> ±0.98	$34.62^{b}\pm 2.87$	17.25 <sup>a</sup> ±0.74
NO (umol/g tissue)	50.99 <sup>a</sup> ±1.52	81.56 <sup>b</sup> ±3.65	52.33 <sup>a</sup> ±1.58
GSH (mg/g tissue)	22.88°±0.47	$18.49^{a}\pm0.54$	20.35 <sup>b</sup> ±0.37
SOD (U/g tissue)	6.50°±0.21	4.16 <sup>a</sup> ±0.17	5.95 <sup>b</sup> ±0.15
TNF-α (pg/g tissue)	10.33 <sup>a</sup> ±0.38	21.63°±0.34	12.95 <sup>b</sup> ±0.57
1 1 01			

**Table 6.** Effect of the garden cress mucilage on the small intestine' biochemical parameters.

The values are expressed as the means  $\pm$  SE. (n = 6). Dissimilar superscript letters in each row denote a significant difference at P  $\leq$  0.05. INDO, indomethacin; MUS, mucilage extracted by the ultrasonic bath; MDA, malondialdehyde; NO, nitric oxide; GSH, reduced glutathione; SOD, superoxide dismutase; TNF-  $\alpha$ , tumor necrosis factor-alpha.

#### 4. Conclusions

The ultrasound was the highly efficient mucilage extraction method due to its positive effect on the protein content and functional properties. The mucilage extracted from garden cress seeds meal as a natural nutraceutical exhibited potent antioxidant activity. The mucilage extracted with the aid of an ultrasonic bath was higher in the antioxidant power. The mucilage extracted with an ultrasonic bath's aid prevented the elevation of the erythrocyte sedimentation rate, intestinal tumor necrosis factor- $\alpha$ , and plasma lactate dehydrogenase activity. Also, rats administrated with the mucilage recorded lower intestinal malodealdehyde and higher intestinal reduced glutathione. Therefore, the mucilage extracted with the aid of an ultrasonic bath may be useful in preventing enter-colitis.

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#### **Conflicts of Interest**

The authors declare no conflict of interest.

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