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The Chemical Structure and Gastroprotective Effect of *Pseudomonas*-Exopolysaccharide in Rats

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ABSTRACT

Radiotherapy is one of the key factors in gastric ulcer; therefore, the necessity of an efficient radio protective agent is apparent. The aim study is divided into two parts, the first part was to isolate and purify exopolysaccharide (PSEPS) from marine Pseudomonas sp. RD2SR3 and the chemical analysis and functional groups were detected by HPLC and IR spectrophotometry. After that the antioxidant activity was investigated by using DPPH radical scavenging hydroxyl radical in vitro. The results revealed that PSEPS consisted of glucose, mannose, galactose and glucouronic acid with molar ratio 2.1:0.1:0.1: 3.2 and had MW of 3.75×10^4 g/mole and it had scavenging activities on DPPH and increased antioxidant activity with an increase in its concentration. The second part investigated the mechanism of the potential of polysaccharides in the remedy and protection of gastric ulcer produced by gamma radiation. Its workings in the treatment and obstructive of gastric ulcer remain unclear. Methods: In this study, thirty two male rats were separated into four equal groups. The First group (Control) and 2nd group was exposed to whole-body gamma- radiation (γ-rays) (3Gy). The Third group (PSEPS) and 4^{th} group (γ-rays + PSEPS) were administered 100 mg/kg of PSEPS 24 h after irradiation. Rats were exposed to gamma-radiation (3 Gy) treated with PSEPS (100 mg/kg) once daily for 30 days post irradiation. Prospect ulceration impediment of PSEPS was assessed quantitative of gastric injures, gastric juice acidity, mucus production, which generated by gamma rays. Oral administration of PSEPS 24 h after irradiation produced a significant protection which was demonstrated by a significant reduction in the activity of the myelo-peroxidase (MPO), anti- inflammatory interleukin-10 (IL-10), pro-inflammatory cytokine interleukin-12 (IL-12), and thiobarbituric acid reactive substances (TBARS) assay accompanied with a significant increase in the antioxidant enzymes in stomach such as superoxide dismutase (SOD), glutathione reductase (GR), and glutathione-s-transferase (GST). Moreover, PSEPS significantly increased vascular endothelial growth factor (VEGF) and prostaglandin (PGE2) connected with a significant depletion in the gastric index compared to the irradiated group. Membrane damage is quite apparent in histological studies undertaken in the stomach tissue, which is susceptible to radiation damage. Medication of PSEPS prevented the radiation-induced exploit to an appreciable extent. This study suggests that PSEPS may serve as a prospect protective agent against gamma-irradiation-induced gastric damage in the experimental model via enhancing the antioxidant activity and inhibition of endothelial dysfunction.

Keywords: Chemical Structure, Gastroprotective, *Pseudomonas* sp. RD2SR3, Gastric ulcers, PSEPS, Radiotherapy

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INTRODUCTION

Radiation therapy is one of the most substantial methods of cancer curing, depends on the generation and uses of reactive oxygen species (ROS) to enucleate tumors (Dayal et al., 2014), and in the process, non-target tissues are also destroyed. Furthermore, there is general acquaintance that radiation, as chemotherapy, destroys the hematopoietic stem and progenitor cells, resulting in rapid loss of peripheral blood cells the damage caused by this loss applies particularly to leukocytes needed as a host defense against microbial invasion leukocyte [1]. Lehy et al. [2] reported an increase in gastric acid productivity, and plasma gastrin levels in rats exposed to 2-Gy or 6-Gy

whole-body γ-radiation. The increase in H^+ output was most marked in those irradiated with 3 Gy of gamma irradiation and this percentage was greater than in which a 6-Gy dose (47.5 and 18.2%, respectively, compared with controls) [3]. Furthermore, and in a more recent study, Plett et al. [4] and Bogomazova et al. [5] found that by decreasing the peripheral lymphocyte count over the first two or three days of treatment that this practice is a reliable indicator of exposure to humans. In a Similar way, it was a 50% decrease of a peripheral lymphocyte with a radiation dose as low as 0.5 Gy. Over the past three decades, and in conjunction with this research, many exopolysaccharides (EPS) and a complex of polysaccharide protein have been secluded from numerous plants such as mushrooms, fungi, yeasts, algae, lichens and others. Due to their immune modulator and antitumor effects, the biological activities of EPS have attracted awareness in the medical and biochemical pathways [6]. The polysaccharide (PS) fractions from several medicinal herbs have been notified to have anti-ulcer effects against experiential ulcers in the rat [7]. Certain PS products have attracted increasing scientific interest in their capacity to extend remarkable effects on immune system function, inflammation and cancers on rodents and human subjects [8]. Peptic ulcer exists due to imbalance between defensive factors of gastric mucosa and aggressive (acid, pepsin) such as mucus gastric mucosal barrier. Local mechanisms concerned with membrane defense are mucus-alkaline secretion, mucosal hydrophobicity, rapid epithelial cell regeneration and rich mucosal blood influx [9]. Prostaglandins are prevailing prostaglandins synthesized by the gastric mucosa and are known to block the secretion of viscus acid and induce the secretion of mucus and bicarbonate [10]. The treatment of ulcer is directed against either reduction of aggressive factors or the rise of mucosal defense of stomach and small intestine with cytoprotective agents. Scavenging of free radicals and inhibition of lipid peroxidation has been steered to be the effective role in promoting eminent radioprotection strategies [11]. Recently, marine microbial exopolysaccharides have attracted EPS more attention, especially those who grew up from marine bacteria [12]. Many of the new EPSs marine microbes with extraordinary structures, chemical compositions, and properties have been established to be suitable possible implementations like natural antioxidants and anti-cancer drugs [13,14]. Natural antioxidants play a serious role by ceaselessly inactivating ROS to conserve only a little quantity necessary to preserve of cell function [7]. *Pseudomonas* is one of the bacteria distributed widely in nature specific marine [15]. EPSs were assumed to protect bacterial cells; EPSs matrix provides an efficient barrier that restricted penetration with chemicals bioacids, antibiotics, and antimicrobial agents; for that, these EPSs showed the importance in bacterial resistance by simulating diffusion of antibiotics to cells. Also, Shigeta et al. [16] they protect bacterial cells from dehydration, heavy metals, organic compounds, or other environmental stresses [17]. EPSs generated by certain important bacteria such as *P. aeruginosa, P. fluorescens*, *P. stutzeri*, and *P. putida* had a potential benefit in biotechnological applications [18]. Presently, the search supplementary efficacious radio protectors have been concentrated due to enhanced use of ionizing radiation in radiotherapy for the treating of malignant tumors. The present study *Pseudomonas* sp. was isolated and identified by morphological, biochemical properties and 16S rRNA. *Pseudomonas* sp. RD2SR3 could produce huge amounts of viscous polysaccharide through liquid media. PSEPS was purified via ethanol precipitation, fractionation, dialysis, and then freeze drying. The chemical characteristic of the PSEPS were determined, and functional groups were unveiled by IR spectrophotometer beside, antioxidant activity was tested, and then investigates the action of PSEPS, towards gastro protective anti-apoptotic, anti-inflammatory and antioxidant effects of pose against γ-rays-stimulated gastric ulcers in rats.

MATERIALS AND METHODS

Bacterial strain

Strain RD2SR3 was isolated from a sample of soil around from Mangrove tree (Egypt). The methods of sampling and isolating strain have been described Asker et al. [19].

Screening and identification of strain RD2SR3

Pure strain was then inoculated into 50 mL of screening marine nutrient medium in 250-mL Erlenmeyer flask, incubated at 37°C in a rotary shaker at 100 rpm for 72 h. After centrifugation at 5000 rpm for 30 min, the supernatant was mixed with four volumes of cold absolute alcohol. Then the precipitate was collected and also the pellets were dried at 50°C under vacuum. EPS production was determined by quantifying the carbohydrate content of the pellets as glucose equivalents using the phenol-sulfuric acid methodology [20]. Strain RD2SR3 was known supported biochemical, morphological, and physiological characteristics of the potential producer resolve by adopting standard methods [21, 22]. Phylogenetic analysis based on the 16S rRNA gene sequence was made as described Asker et al. [20] and Tamur et al. [23]. Briefly, the 16S rRNA sequence of strain RD2SR3 was compared to reference 16S rRNA gene sequence available in the GenBank and EMBL database obtained from the National Centre of Biotechnology Information database using BLAST search (http://ncbi.nlm.nih.gov/ BLAST/).

Culture conditions

Inoculum was performed by transmitted one loop choked with culture from marine nutrient slant to an 250-mL Erlenmeyer flask containing 50 mL (glucose 20, yeast extract 0.1, $CaCO_3$ 1, $NH4NO_3$ 0.8, K_2HPSO_4 0.6, KH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.05, $MnSO_4.4H_2O$ 0.1 and dissolved in 50% brine pH 7 [24]. The seed cultures were grown at 37°C on a rotary shaker incubator at 100 rpm for 24 h. After incubation, 5 mL of the seed culture was transferred into an 250-mL Erlenmeyer flask containing 50 mL of production medium containing (g/L) sucrose 20; $CaCO_3$ 0.01; $MgSO_4.7H_2O$ 0.5;$MnSO_4.4H_2O$ 0.05; $FeSO_4.7H_2O$ 0.01 and dissolved in 50% brine or seawater pH 7 [25]. The fermentation cultures were then incubated at 37°C with shaking at 100 rpm for 5 days.

Production and purification of exopolysaccharide

The EPS sample was prepared from strain RD2SR3 culture in the production medium. The fermented broth was collected and centrifuged at 5000 rpm at 4°C for 30 min. The clear solution was collected and mixed with four volumes of absolute ethanol, and left overnight at 4°C for EPS isolation. The precipitate in the centrifuging tube was rinsed carefully with water, filtered and then dried at 50 °C in an oven to get the biomass dry weight [26]. The EPS, which was in supernatant, was purified again by absolute ethanol and left overnight. The precipitate was re-dissolved in distilled water and dialyzed three times (1 L×3) against flowing tap-water using dialysis tubing (MWCO 2000) for 48 h [27]. The EPS solution was sited at −20°C overnight thawed rapidly and centrifuged (15000 rpm, 20 min) to examine the precipitation. The yield EPS was fractionated by ethanol precipitation method into three fractions followed by dialysis against deionized water for 48 h [28]. The yield major fraction finally lyophilized to desire purified EPS coded PSEPS. The UV absorption spectrum was recorded using a UV-Vis Spectrophotometer 2401PC (Shimadzu, Japan) between 200 and 500 nm, in order to examine the existence of proteins and nucleic acids [29]. The yield of PSEPS was determined by phenol colorimetric method [20].

Analysis of monosaccharide composition

Twenty milligrams of PSEPS was hydrolyzed with 6 N HCl at 100°C in an exceedingly sealed tube for 5 h. Excess acid was taken away by flash evaporation on a water bath at a temperature of 40°C and co-distilled with water (1 mL×3) [30]. The contents of monosaccharides were quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm × 30 cm), using deionized water as a moving medium with flow rate 0.5 mL/min, as described by El-Sayed et al. [31]. Sugar identification was done by comparison with authentic sugars. Uronic acid contents were determined by measuring the absorbance at 525 nm using the m-hydroxybiphenyl colorimetric procedure and with glucuronic acid as the standard [32]. Sulfate was measured using the turbidimetric method (Dogson and Price, 1962) [33] together with sodium sulfate as standard. N-acetyl glucose amine was estimated by the Elson and Morgan reaction (Morgan and Elson, 1934) [34]..

Determination of the molecular mass of PSEPS

The molecular weight of PSEPS was resolve on associate Agilent 1100 HPLC system equipped with a refractive index detector (RID) and FPl gel particle size (5µm), 3 columns of pore type (100, 104, 105 A°) on series, length 7.5 × 300 mm (1000 and 5000000 mwt) for DMF solvent Styrogel HR-DMF, 3 µm (7.8 × 300 mm), manufactured by Water Company Ireland. One column (5000-600000 mwt) for water solvent polyethylene oxide/glycol standard (PL aquagel-OH) 7.5 mm and 30µm pore type 8um particle size. PL aquagel-OH 7.5 mm, 50 um pore type, 8um particle size, in series Mw from 100-1250000 g/mol. The sample 0.01 g was dissolved in 2 mL of solvent, and then it filtrated by siring filter 0.45 then the sample but in GPC device [35]. The polydispersity index (PI) calculated from the Mw/Mn magnitude relation [36].

Fourier-transform infrared spectrometric analysis (FTIR)

The FTIR spectrum of the PSEPS was measured on a Bucker scientific 500-IR Spectrophotometer. The PSEPS was mixed with KBr powder, ground and pressed into a 1 mm pellets for FTIR measurements in the range of 4000-500 cm^{-1} [37].

Radical-scavenging activity (RSA) of PSEPS fraction toward DPPH radical

The free radical-scavenging activity of PSEPS was measured by 1,1-diphenyl- 2 picrylhydrazyl (DPPH) radicals. Five milliliters of DPPH ethanol solution (freshly prepared at a concentration of 0.1 mM) were added to 1 mL of PSEPS solution of different concentrations (25–200 µg/mL) in water. After 90 min, absorbance was measured at 517 nm by utilizing a UV–visible photometer (2401PC; Shimadzu, Japan). Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity that was analyzed from the graph [38]. The experiment was

administered in triplicate and averaged. The ability to scavenge the DPPH radical was calculated using the subsequent equation:

Scavenging ability (%) = $[(\nabla A_{517 \text{ of control}} - \nabla A_{517 \text{ of sample}}) / \nabla A_{517 \text{ of control}}] \times 100$.

The EC_{50} value is the effective concentration of PSEPS at which the DPPH radicals were scavenged by 50%.

Biological studies
Sprague-Dawley albino rats weighing (180-200 g) were used in the present investigation. The rats were obtained from the laboratory animal colony at the Institute of Ophthalmology, Cairo University, Egypt. The animals were provided with food and water *ad libitum* and all rats were fed on throughout the experimental period. The experiment was executed in accordance with the guidelines of the experimental animal ethics. Rats were randomly assigned to four groups after 7 days of acclimatization (8 per group): Animals in the 1st group were given daily with saline for 4 weeks and served as a normal group. Those of the 2nd group were given with saline, daily and served as control irradiated group. Group 3 treated with PSEPS (100 mg/kg^{-1}) by gavage. Group 4 irradiated rats were treated daily with PSEPS extract for four weeks.

Gamma-radiation
Whole body gamma-irradiation was carried out using a Cesium (137CS) source, Gamma Cell-40 biological irradiator, at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The animals were exposed to a single dose of (3 Gy) gamma ray with a dose rate of 0.47 Gy/min.

Tissue Collection and Processing
Preparation of gastric tissue homogenate
A sample of the gastric wall from each animal was homogenized (10%) in cold of 0.1 mol/L phosphate-buffered saline pH 7.4. The pure supernatant which was obtained by centrifuging of the homogenates at 10,000 rpm for 15 min at 4°C, then it was used to quantify the gastric tissue contents of GST, TBARS.

Stomach was cut along the greater curvature and was kept in a Petri-dish containing normal saline after that it was dried with a blotting paper. Thereafter, scanned images were performed by using the two transparency sheets then they saved and evaluated for ulcer index with the help of Image software. The ulcer area in mm^2 was determined as the total of gastric lesions for each stomach in the group. The ulcer index and the protection percentage were calculated according to the following equation: Ulcer index= 10/×

Where ×= Total mucosal area/Total ulcerated area

Protection (%) = (Uc – Ut)/Uc × 100

Where Uc, Ulcer index of negative control group, Ut, Ulcer index of test group

Determination of ulcer index in gastric tissues
The stomach was removed carefully, and opened the greater curvature and washed it slowly under the running tap water [39]. It placed on the glass slide and observed under the microscope (10×) for ulcers. Mean ulcer score for each animal is expressed as an ulcer index. It was measured by method of Ganguly and Bhatnagar [40]. It was calculated from an arbitrary scale by considering, the ratio of the total area of the stomach mucosa and area of ulceration. The volume of the supernatant was detected as ml/100 gm body weight, and the centrifuged samples were decanted and analyzed for gastric volume, pH and total acidity. Afterwards, the mucosa was flushed with saline and Ulcer index was scored, and determined.

Estimation of Total and Free Acidity
It was measured by the Method of Hawk et al. [41] 1 ml of supernatant liquid was pipette out and diluted to 10 ml with distilled water, pH of this solution was noted using pH meter. The solution was titrated against 0.01N sodium hydroxide using topfer's reagent as an indicator. The end point was titrated when the solution turned to orange color.

Gastro protective Assessments
Elongated bands of trauma lesions parallel to the long axis of the stomach were determined within the gastric of animals that received γ-rays. The length (mm) and dimension (mm) of the ulceration on the gastric mucosa were measured using a planimeter [(10 ×10 mm^2 = ulcer area) under dissecting microscope (1.8×)]. The realm of ulceration was measured by investigating the quantity of small squares, 2 mm × 2 mm, covering the length and width of each ulcer band. The total of the areas of all lesions for each stomach was applied within the calculation of the ulcer area (UA) whereby the total of small squares × 4 × 1.8 = UA mm^2. The inhibition percentage (I %) was calculated as described in Njar et al. [42] as the following formula:

Inhibition percentage (I %) = $[(UA_{control} - UA_{treated}) / UA_{control}] \times 100$

Biochemical Analysis
Lipid peroxidation
The low supernatant fraction of stomach was used for thiobarbituric acid-reactive species (TBARS) assay in line with Ohkawa et al. [43]. Therefore the concentration of TBARS was observed at 532nm using a standard curve of MDA and the results were expressed as nmol MDA/mg protein.

Assays of antioxidant activity
Determination of gluthatione S-transferase (GST) activity
GST activity was measured using the method of Habig et al. [44].

Glutathione reductase activity (GR)
The GR activity was measured by monitoring the decrease in absorbance of NADPH in phosphate buffer, pH 7.8 at 340 nm elicited by oxidized glutathione (Sigma-Aldrich, St. Louis, MO, USA). The absorbance was read every minute for 10 min [45].

Superoxide dismutase activity (SOD)
The SOD activity was analyzed by the reduction of nitroblue tetrazolium utilizing a hypoxanthine-xanthine oxidase system (Sigma-Aldrich, St. Louis, USA). The absorbance was read every minute for 10 min at 560 nm [46].

Mediators of inflammation
Determination of the gastric mucosal levels of TNF-α, IL-10 and IL-12
the cytokines were detected in the supernatants of the tissue homogenate, tumor necrosis factor alpha (TNF-α), interleukin (IL-10) and IL-12 levels using rat cytokine ELISA kits from R&D Systems (Minneapolis, MN), according to the manufacturer's instructions. The absorbance for all cytokines studied was measured using a micro plate reader at 450 and 550 nm.

Histological examination
A small piece of the gastric wall from each animal was fastened in 10% buffered formol solution then the fixed tissues were dehydrated with alcohol and xylene. Then, each sample was embedded in paraffin wax, and sectioned at 5 µm in slides prior for staining with Hematoxylin and eosin (H & E) stain for light microscopy [47]. Moreover, some slides were also stained by periodic acid Schiff Base (PAS) (Sigma Periodic Acid-Schiff Kit), to evaluate mucus production, to observe mucus production and to evaluate modifications of acidic and essential glycoprotein [48].

Immunohistochemistry study
For cyclooxygenase-2 (COX-2) staining using mouse monoclonal antibody against rat monocytes (Kyoto, Japan) and were stained with mouse monoclonal antibody (mouse anti-rat ED-1; Serotec, Oxford, England) and goat polyclonal antibodies against rat COX-2 (Santa Cruz Biotechnology, Inc.) in PBS. COX-2 staining was performed by a streptavidin-biotin peroxidase method using a LSAB2 kit (DAKO Japan) for monocytes/ macrophages and goat ImmunoCruz Staining System (Santa Cruz, Biotechnology) for COX-2. Diaminobenzidine (Dojin, Kumamoto, Japan) and tetramethylbenzidine were used for the first and the second round of detection respectively.

Statistical analysis
Statistical analysis Results were expressed as mean ± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was considered at $p < 0.05$.

RESULTS

Screening for the exopolysaccharides producing bacteria
Numerous of microorganisms can produce EPSs out of the cell as soluble polymers. These applications can be applied in many vital technological applications, such as food, the pharmaceutical, cosmetics, mining minerals, and extraction of oil [49]. The marine environment, which acts 70% of the earth's surface and 90% of the size of its crust, provides a wide origin of natural products [49]. The polysaccharide are beneficial for protecting versus gastrointentestinal problems, wound healing, anti-tumor, and anti-atherosclerotic agents these results are thought to include components of the innate immune system such as the complement system, nitric oxide (NO), and the release of the reactive oxygen species (ROS), and cytokines by dendritic cells, macrophages, and granulocytes [50,51]. In this study, different bacterial isolates were used for the production of EPS. Among these, *Pseudomonas* sp. RD2SR3 produced the maximum amount of EPS (7.3 g/L). Hence this strain was selected for further studies.

Identification of strain RD2SR3
Identification of strain RD2SR3 was carried out according to a great variety of morphological, physiological and biochemical features. The strain MA3 was found to be gram-negative cells, non-spore forming, rod shaped, nitrate negative and positive of citrate, catalase, oxidase, indole. The strain RD2SR3 utilized many carbohydrates as a sole carbon source including sucrose, lactose, glucose, and fructose. 16S rRNA gene sequencing was carried out for selected strain (RD2SR3) and their identity was determined performing a sequence similarity search using NCBI. The nucleotide succession of the marine bacterial isolate has been submitted to the NCBI Database and the name of *Pseudomonas* sp. RD 2SR3. Recently it was found that most bacteria produce the highest quantity of EPS in the stationary phase of growth, this result might be related to the competition occurring during the growth phase between EPS and cell-wall polymer biosynthesis [52], while there are microorganisms release the maximum amount of EPS through the exponential phase [53].

Composition and characterization of PSEPS
The purified PSEPS, a creamy powder, was used for subsequent analysis. It had a positive response to absorption at 280 nm in the UV spectrum and the Bradford test, referencing to protein (0.15%) and amino sugars (5.29%). As specified by *m*-hydroxydiphenyl colorimetric method, the PSEPS was contained uronic acid (24%) and sulfate (15.27%). These indicate that the PSEPS is an acidic exopolysaccharide. The molecular weight (Mw), number-average of molecular weights (Mn) and polydispersity (Mw/Mn) of the PSEPS was analyzed by GPC. The PSEPS in the GPC chromatogram was widely dispersed molecules polydispersity index (Mw/Mn = 1.29) and had an overall molecular weight (Mw) of 3.75×10^4 g/mole and number molecular weight (Mn) of 2.89×10^4 g/mole.

The monosaccharide composition of PSEPS was analyzed using HCl hydrolysis and the HPLC analysis methods. The results indicate PSEPS was composed of glucose, mannose, galactose and glucouronic acid with molar ratio 2.1:0.1:0.1: 3.2, respectively. As shown in the FTIR spectrum in the **Figure (1)** PSEPS exhibited a significant, broad characteristic peak at around at 3423.03 cm^{-1} region was attributed to the expansion vibration of O–H in the ingredient sugar residues [54]. The band at 2940.91 cm^{-1} was correlated with the stretching vibration of C–H in the sugar ring. The PSEPS also appears to have a particular band between 1200 and 1000 cm^{-1}, which is dominated by circle vibrations, interfered with stretching vibration of C-O glycosidic bond vibration [55-58]. The prominent absorption observed at 1654.62 cm^{-1} was referred to the stretching vibration of C=O and C–N. The absorptions around 1426.10 cm^{-1} represented CH2 and OH bonding. The strong absorption at 1076.08 cm^{-1} was controlled by glycosidic linkage ν (C–O–C) -stretching vibration [59]. The absorption at 1130.08 cm^{-1} could be imputed to the existence of sulfate groups as S=O and C–O–S [60]. Moreover, the band at 922.77 cm^{-1} indicated the β-pyranose form of the glucosyl residue, and the strap at 815.74 cm^{-1} suggested the β-pyranose form [61, 62]. *Pseudoalteromonas* sp. SM9913 is a γ-proteo bacterium isolated from the sediments of Yellow Sea of China. EPS producing in the laboratory good conditions and demonstrated that the yield of the EPS increased at reducing culture temperatures within 30–10°C, and it reached to yield 5.25 g/L at 15°C for 52 h [63]. Its structure is a linear arranging of α-(1→6) linkage of glucose with a high grade of acetylation and with a molecular weight of 4×10^4 Da. Furthermore, this EPS has been tested for its flocculation attitude and biosorption capacity, supplying insight into its

ecol target rule [64]. Most microbes in the marine are encompassed by EPSs, which may help microbial communities to tolerate extremes of salinity, temperature, and nutrient availability [65]. Because of the charming chemical and rheological properties of the EPSs generated by microorganisms, the studies performed to test their potential applications in biotechnology and environmental defense [66, 67].

The product and quality of microbial EPSs are highly influenced by the environmental and nutritional status [68]. Most EPSs produced by marine bacteria are hetero-polysaccharides containing different unit of monosaccharides coordinated in a range of about ten to compose repeating units [69]. Most EPSs are linear, with molecular weight ranging from 1×10^5 to 3×10^5 Da [70]. Several EPSs are neutral molecules; however the majorities of them are polyanionic for the existence of sulfate, pyruvate, phosphate and uronic acids. Furthermore, the linkages between monosaccharide's that have been most generally found are β-(1--4)- or β-(1--3)-linkages in the backbone characterized by strong hardness and α-(1--2)- or α-(1--6)-linkages in the more malleable ones.

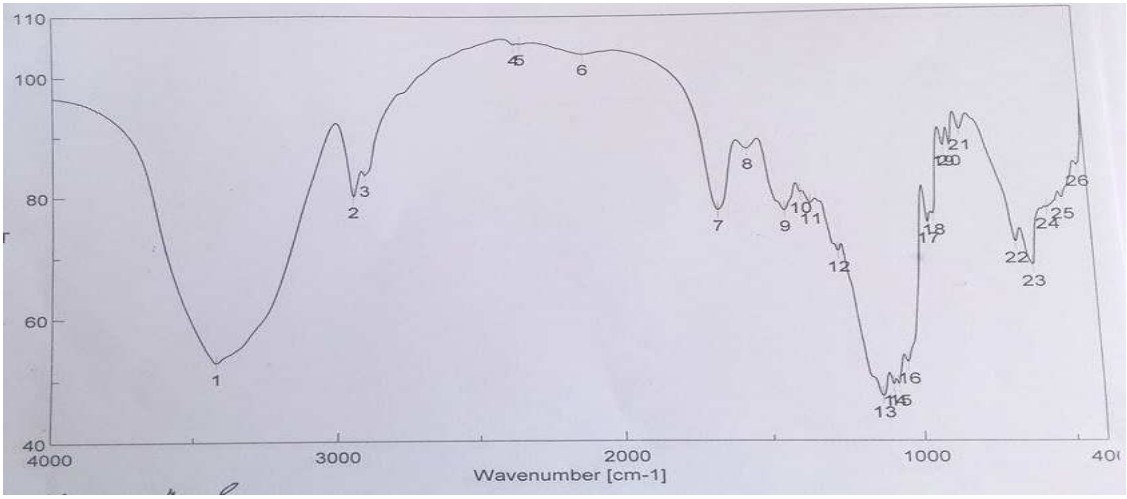


Figure1: Fourier-transform infrared spectrum of PSEPS from *Pseudomonas* sp. RD2SR3B

The physical properties of EPSs are mightily affected by the way of the monosaccharides are arranged jointly and the aggregation of the one polymer chains [71]. The biological activities of the EPSs based on the chemical structure and the molecular weight. Various sorts of PS have shown anti-ulcer activities [72,73]and microbial polysaccharides have unusual structures and having immunomodulating properties for anti-ulcer activities specific prevalence of gastric ulcer in male 4.2% and 2.4% of women. On the other hand, the quantities of polysaccharide extracted from the bark and leaves from *C. cordifolia* vary in both galactose and galactouronic acid and its potential anti-ulcer impact [74]. Furthermore, Teixeira et al. [75] reported that the experimental model used, caused aphthous ulcers and the cellulosic polysaccharide sponge film can be used as assistance in the symptomatic curing and healing of the ulcer active lesions of the oral mucosa.

Radical-scavenging activity (RSA) of PSEPS

The antioxidants were capable to minimize the constant DPPH radical to the yellow-coloured diphenylpicryl hydrazine. The DPPH radical-scavenging activities of PSEPS and the data are plotted in **Figure (2).** As illustrated, PSEPS exhibited scavenging efficiency through DPPH radicals in a concentration-contingent method, at an IC_{50} value of 100 µg/ml. These results indicated that PSEPS had a palpable effectiveness on the scavenging free radical, especially at high concentration. It is recognized that ROS, like hydroxyl radicals, superoxide anion and hydrogen peroxide, are attached to the pathogenesis of various diseases [76, 77].

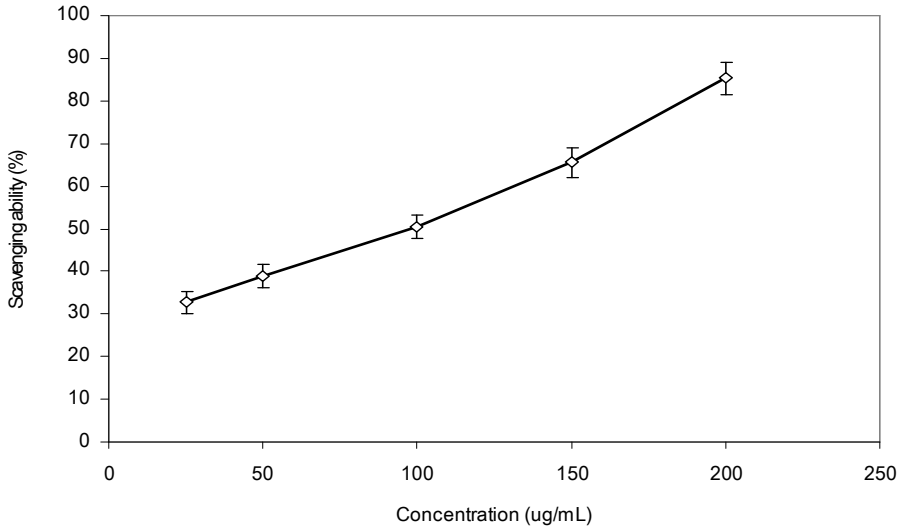


Figure 2: Scavenging effects of PSEPS during DPPH test and measured by changes in absorbance at 517 nm

Biological activities of PSEPS

The **Table (I)** showed the gastric volume, pH, acidity and ulcer index of experimental groups. All these parameters were found to be significantly increased in the untreated γ- irradiation rats compared to the control group. Treatment with PSEPS (100 mg/Kg^{-1}) for four weeks followed to γ irradiation caused a significant reduction ($P < 0.05$) in the above parameters. In this study, extracts of PSEPS have been demonstrated to possess anti- ulcer activity against the experimentally stimulated ulcer model (γ-irradiation) **Table (2) and Figure (3).** Also illustrates that γ-rays induced increased significantly of TBRA, decreased SOD, GST and GR activities in their stomach tissues as compared to normal rats. Whereas, the treatment with PSEPS showed significantly a decrease in THBRA and increased of GST and GR approached to the control range except SOD activity was higher than control values (282.35±14.28 and 225.72±11.75, respectively).

Table 1: Effect of PSEPS, irradiation (IR, 3 Gy) and their combination on the levels of VEGF and PGE2 activities in rat stomach tissue

Groups	Normal	PSEPS	IR	Treatment	F
				IR+ PSEPS	
Gastric PH	3.02±0.52^{b}	4.85±0.44^{a}	0.983±0.081^{c}	5.571±0.23^{a}	26.63*
Ulcer area(mm)	0.00	0.00	798.88±15.33^{a}	65.95±4.98^{b}	26.63*
Protection	-	-		91.75%	

*a, b, ab & c: Statistically significant from control or radiation group, respectively at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. Data expressed as mean ±SE. *Significant ly different at P ≤ 0.05.*

Table 2: Effect of PSEPS, irradiation (IR, 3 Gy) and their combination on the levels of TBARS, SOD, GST and GR activities in rat stomach tissue

Groups	Normal	PSEPS	IR	Treatment	F
				IR+ PSEPS	
TBARS (nmol/g)	84.17±5.76^{b}	89.75±6.61^{ab}	184.25±5.30^{a}	92.0±5.79^{b}	66.33 *
SOD (U/mg)	225.72±21.75^{b}	264.75±18.55^{ab}	112.18±4.67^{c}	282.35±14.28^{a}	22.42*
GST (nmol/mg)	3.25±0.62^{a}	3.55±0.23^{a}	1.69±0.27^{b}	3.0±0.37^{a}	33.60*
GR (nmol/mg)	37.67±2.33^{a}	40.35±3.0^{a}	27.75±2.39^{b}	35.67±2.9^{ab}	4.31*

*a, b, ab & c: Statistically significant from control or radiation group, respectively at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. Data expressed as mean ±SE. *Significant ly different at P ≤ 0.05.*

Effects of PSEPS on rats; radiation reduced VEGF and PGE2

As illustrated in **Table (3)**, four weeks following radiation the VEGF was (139.75 ± 5.27) and in turn was significantly lower than in the non-irradiated control group (186.5 ± 12.45) ($p<0.05$). As well as, the PSEPS oral administration markedly protected the rats from the radiation induced injury (179.18 ± 4.40). Following the 4th week, after being irradiated the PGE2 was (2.75 ± 0.25) significantly decreased ($P< 0.05$) when compared to the

non-irradiated control group, and (3.97 ± 0.36) in the PSEPS-treated rats were higher than those found in the irradiated control group.

Table 3: Effect of PSEPS, irradiation (IR, 3 Gy) and their combination on the levels of VEGF and PGE2 activities in rat stomach tissue

Groups	Normal	PSEPS	IR	Treatment	F
				IR+ PSEPS	
VEGF (pg/ml)	186.50±12.45^{a}	188.38±11.25^{a}	139.75±5.27^{b}	179.18±4.40^{a}	6.335*
PGE2 (µg/dl)	4.64±0.47^{c}	4.02±0.44^{c}	2.75±0.25^{a}	3.97±0.36^{b}	4.624*

*a, b, ab & c: Statistically significant from control or radiation group, respectively at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. Data expressed as mean ±SE. *significantly different at P ≤ 0.05.*

The levels of IL-10, IL-12, MPO and TNF-α in the stomach tissues were significantly increased in the irradiated group compared with the normal and PSEPS control groups ($P < 0.05$) **(Table 4).** The increase in proinflammatory cytokines concentration in the gastric mucosa elicited by radiation were significantly decreased by PSEPS treatment a, b & c: Statistically significant from control or radiation group, respectively at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. Data expressed as mean ± SE. *significantly different at P ≤ 0.05.

Table 4: Effect of PS, irradiation (IR, 3 Gy) and their combination on the levels of IL6, IL12 and MPO activities in rat stomach tissue

Groups	Normal	PSEPS	IR	Treatment	F
				IR+ PSEPS	
IL6 (pg/mg)	18.45±1.44^{b}	21.55±2.71^{b}	38.92±4.43^{a}	24.61±2.01^{b}	9.901*
IL12(pg/mg)	17.21±1.30^{c}	15.47±1.67^{c}	96.14±4.36^{a}	43.75±8.72^{b}	10.97*
TNF-α(pg/ml)	6.05±0.52^{b}	8.70±0.66^{b}	29.97±1.76^{a}	11.21±0.84^{b}	21.33*
MPO(U/g)	0.28 ±0.07^{b}	0.32±0.06^{b}	6.15±0.81^{a}	1.597±0.25^{b}	42.47*

Histological and histochemical evaluation

In the histopathological examination of the control group; tissue sample had a normal appearance and no mucosal damage or glandular cell necrosis were detected. Histological mucosal damage, and massive number of focal inflammatory cell infiltration were showed in irradiated rats when compared with control groups **(Figure 4 E &F)**. Histopathological examination furthermore confirmed the radio protective effectiveness of PSEPS and its *in-vivo* influence on stomach damage induced by irradiation as recorded in **(Figure 4 G & H).** It is evident from the results **(Table I)** that the PSEPS causes a reduction in the intensity of gastric ulcerations as observed from the significant reduced ulcer index in the PSEPS treated groups. The histochemical finding of sections of stomach of control rats stained using the Periodic Acid Schiff`s technique (PAS) to highlight the mucin is focused inside the epithelium of the stomach mucosa **(Figure 3 I).** The results portrayed in **Figure (4 K)** and show that gamma irradiation application on gastric mucosa reduced the mucin. But, oral administration of PSEPS increased PAS-staining for mucin **(Figure 4 L),** when compared to the ulcerated group **(Table 4 & Table 5).** The severe immunoreactivity in the mucosa (COX2) was shown in the stomachs of ulcer model group **(Figure 4 N**). On the other hand, stomach of rat in group 4 showing mild immunoreactivity in the mucosa **(Figure 4 P & Table 5).**

Table 5: The histochemical (PAS) and immunoreactivity (COX-2) in mucosal layer of stomach of experimental groups

Groups	Normal	PSEPS	IR	Treatment
				IR+ PSEPS
PAS	+	+	+++	+
COX-2	+	+	+++	+

+ Mild; +++ sever reaction

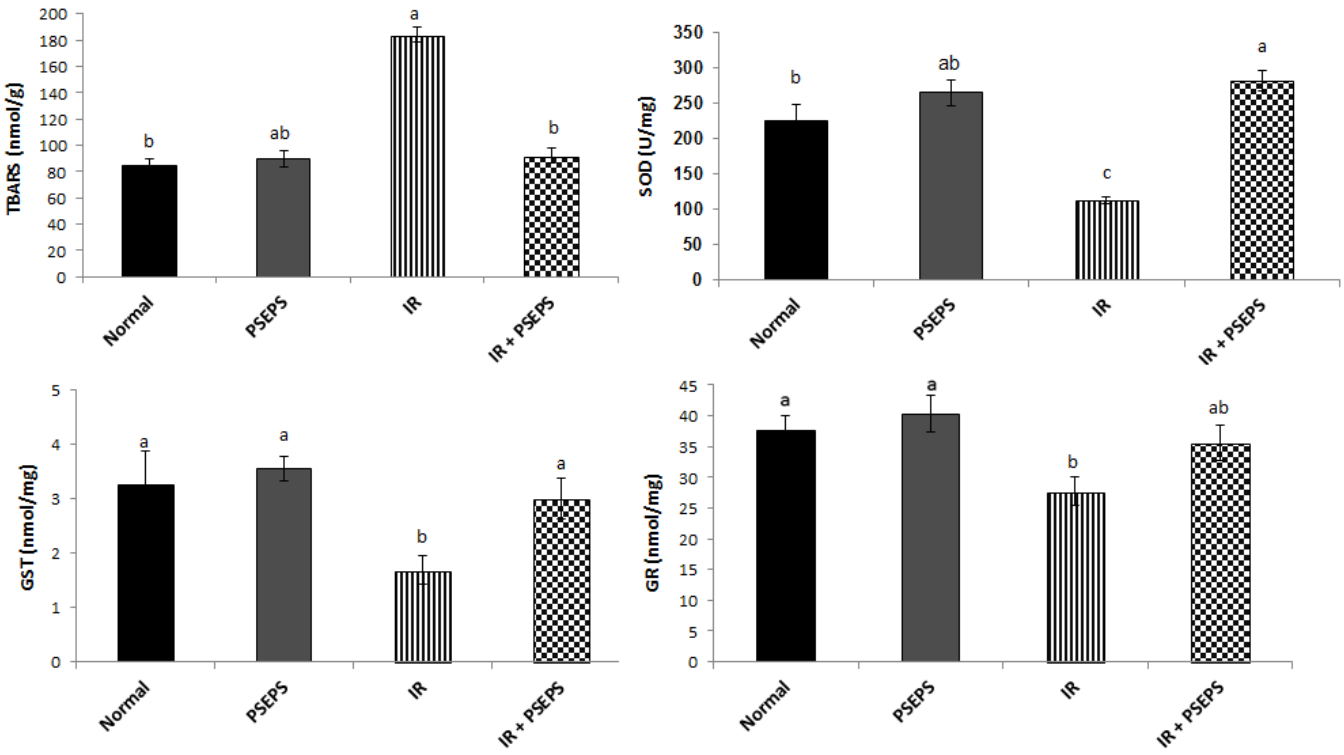
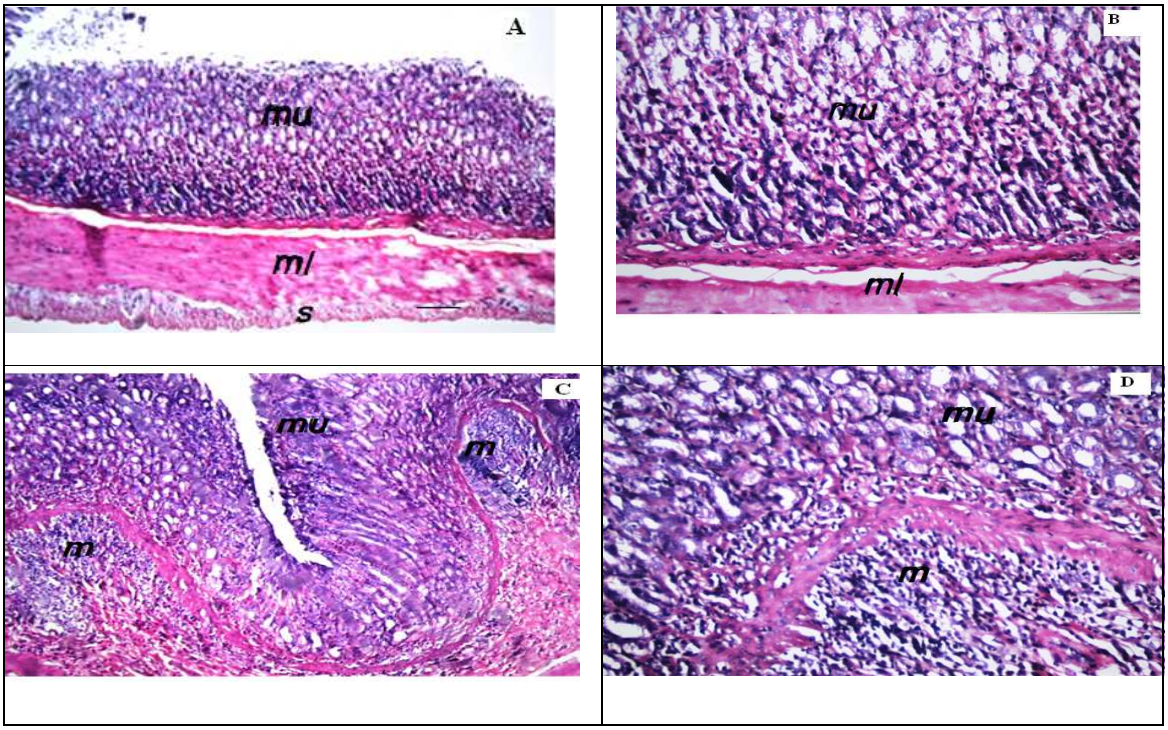
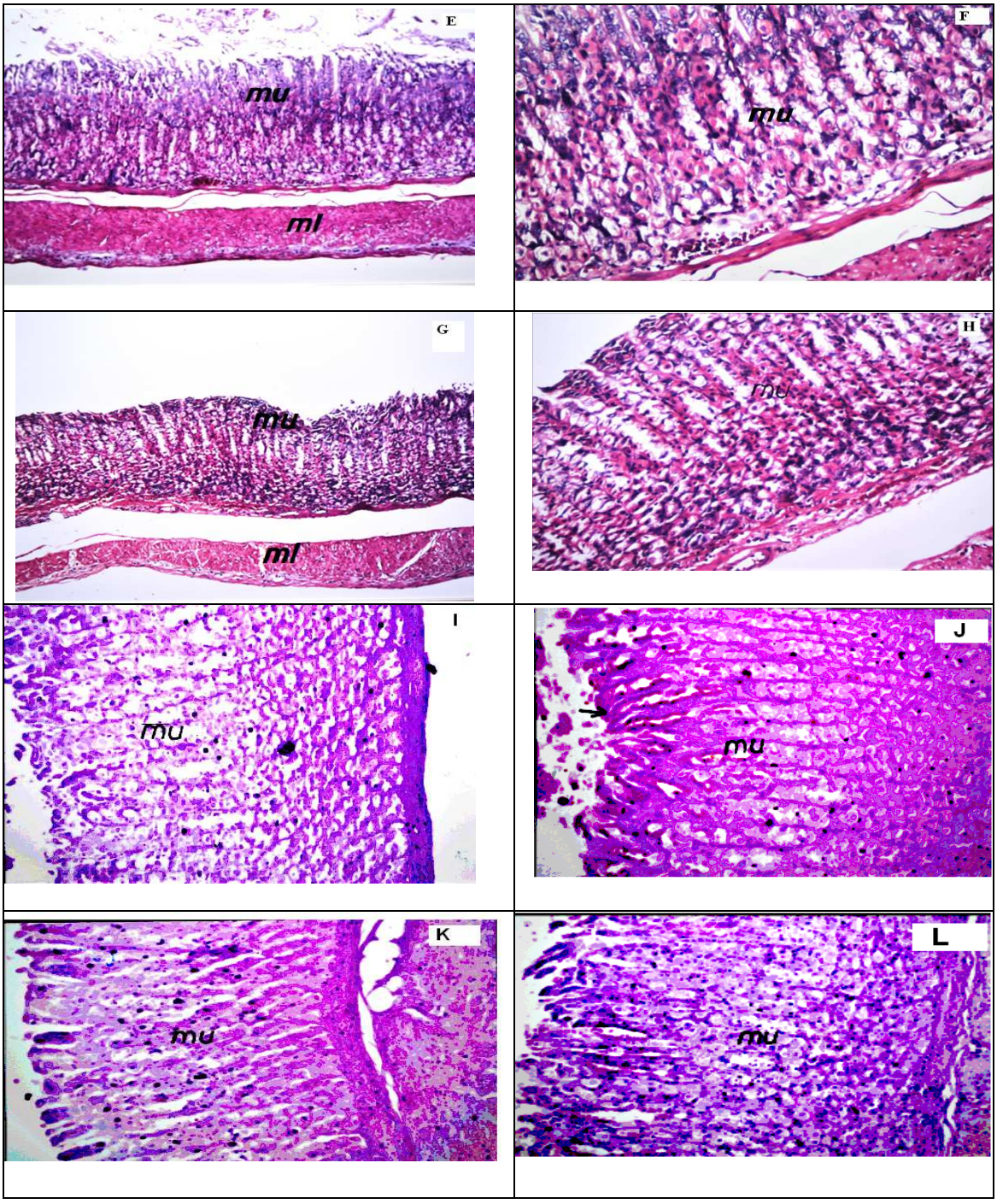


Figure 3. Effect of PSEPS treatment on TBARS, SOD, GST and GR in rats irradiated with 3 Gy induced gastritis. [a, b & c]Statistically significant from control or radiation group, respectively at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. Data expressed as mean ± SE

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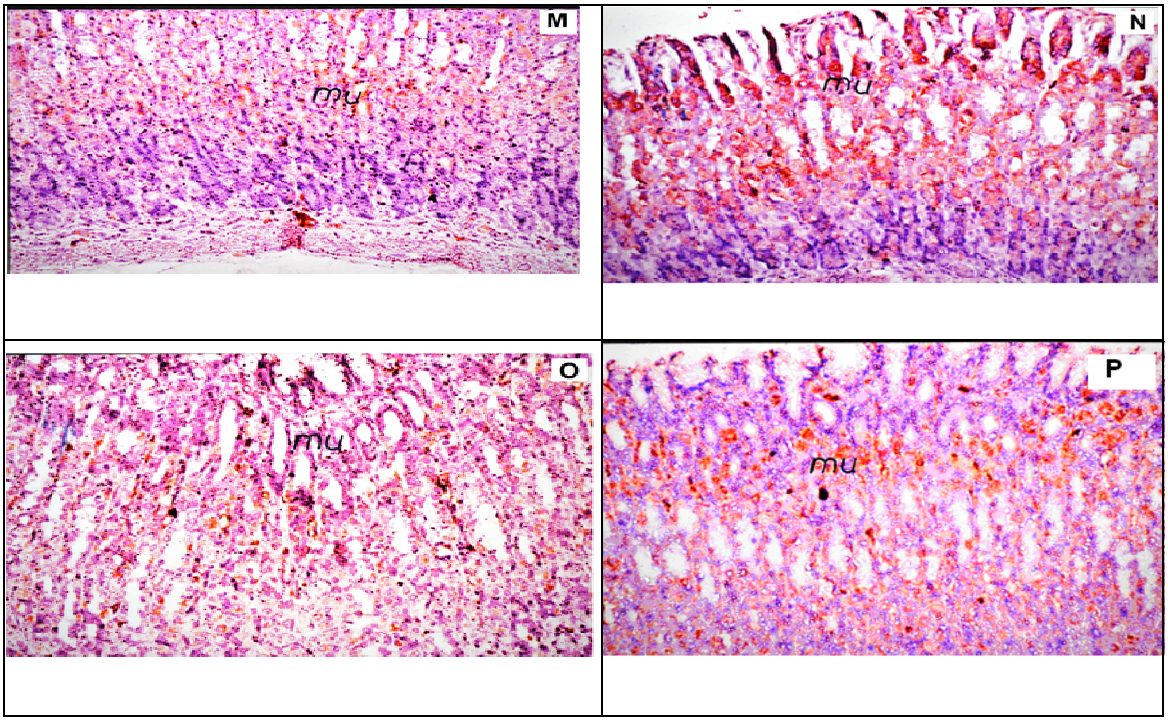

Figure 4. Photomicrographs of sections of rat stomach. A) Stomach of rat in (G1) showing normal histological structure of the mucosa (mu), muscular is (ml) and serosa(s). X16; B) the magnification of (A) X 40. C) Stomach of rat in G1I showing massive number of focal inflammatory cells infiltration (m) in submucosal layer X16. D) Stomach of rat in G1I showing massive number of focal inflammatory cells infiltration (m) insubmucosal layer X40. E) Stomach of rat in G3 showing normal histological structure X16. F) Stomach of rat in G3 showing normal histological structure X40. G) Stomach of rat in (GIV) showing normal histological structure X16 ;H) the magnification of (G).M) Stomach of rat in group 1 showing mild reaction inn mucosa (mu). N) Stomach of rat in group 2 showing severe immuno reactivity in mucosa (mu) X 40. O) Stomach of rat in group 3 sowing mild immunoreactivity in mucosa (mu) X40. P). Stomach of rat in group 4 showing mild immunoreactivity in mucosa (mu) X40. I-L (PAS); M-P(COX-2)

DISCUSSION

Radiotherapy is important in rising survival rates in cancer patients [78]. However, more and more patients suffer from the long facet effects of irradiation like stomach ulcers [79]. The exposure to radiation is thought to encourage oxidative stress during the generation of reactive oxygen species (ROS) leading to imponderables of the pro-oxidant and antioxidant activities eventually give rise to necrobiosis [80]. The foremost kinds of cellular harm stimulated by radiation are DNA injury, protein oxidation and lipid peroxidation. Results of this study elucidate enhanced in concentration of TBARS; one amongst the lipid peroxide index. The rise in TBARS level could also be referred to the overproduction of ROS. Radiation exposure prompted radiolysis of water within liquid media of the cells that drives in production of hydroxyl radicals ($OH^{\bullet}$). Hydroxyl radical reacts with the unsaturated fatty acids within the lipid fraction of biological membranes starting the lipid peroxidation and lastly damaged the cell membranes [81]. There is rising evidence that irradiation exposure raises oxidative stress via increasing the formation of ROS and lowering cellular oxidative defenses in an exceedingly method excited by neutrophil activation, inflicting a consecutive ROS-mediated inducement of protein oxidation and lipid peroxidation. The PSEPS, considerable reduced ($P < 0.05$) the acid secretary factores, i.e. total acidity similarly because the gastric ulcer index propose that acid suppression speed ulcer healing. The reduction in gastric ulceration area and simultaneous reduction in acidity may be one of the causes of ulcer healing. Additionally, ROS also negatively impacts the antioxidant defense mechanisms, reduced the intracellular concentration of glutathione (GST), and decreased the activities of SOD, and GR. Therefore, we found raise in the ROS generation in ulcerated gastric mucosa as proved by depletion of SOD and GST activity while oral administration of PSEPS showed antioxidant mechanisms returning the GST and SOD activities to basal levels, which can fast the ulceration cure practicability via scavenging of free radicals. These results are compatible with the DPPH free-radical scavenging property conferred by this polymer. In addition, in agreement with our data, arabinogalactan polysaccharide with the gastro protecting result conjointly showed DPPH scavenging [82].

The MPO is the main reason for making the neutrophil permeation in ulcerogenic activity [83]. This enzyme is present in the neutrophils to stimulate the process of oxidation of the chloride ion (Cl^-) by hydrogen peroxide (H_2O_2) to be hypochlorous acid (HClO), and the toxicity of this compound not only to microorganisms, but is it very hurtful to the host tissues [84]. This operation is responsible for the generation of free radicals, give rise to critical inflammation in the gastric tissue [85]. A previous study reported that the MPO activity in the intestinal tissue of irradiated rats was increased significantly; showing that radiation-induced oxidative stress gave rise to injury in this tissue implicates the participation of neutrophil accumulation radiotherapy [86]. The gastroprotection encouraged by PSEPS can be demonstrated by the suppression of neutrophil infiltration with subsequent MPO generation. Cells of the gastrointestinal tract have an antioxidant defense system which is able to prevent the toxicity of ROS by mechanisms that engage the work of enzymes and compounds with the potency to scavenge free radicals and block their eradicative action. The master antioxidative enzyme is SOD that stimulates the disputation of O^{2-} into minimum noxious H_2O_2, which is further degraded by catalase or GSH-Px [87].

In the present study, we observed that TNF-α level was increased after irradiation and that PSEPS was effective in reducing its level. These inflammatory sequences of events are accompanied by a high grade of lethality, which is remarkably associated with the serum concentration of TNF-*α*. Regarding the effects of PSEPS on IL-10 and IL-12 induced by irradiation, in the present study, we evidenced that PSEPS reduced the concentration of these cytokines in stomach homogenates when compared with an irradiated group. In fact, some authors have demonstrated that TNF-*α*, a powerful mediator of inflammation synthesized principally via monocots', because of T cells and macrophages, has a short-lived. It is also the stirrer of the consecutive cytokine cascade and a mighty inducer of other inflammatory cytokine [8, 89]. In our study, we also demonstrated increased levels of IL-12 post γ- irradiation. This finding may point that the anti-inflammatory cytokines are secreted as a response to the inflammatory cytokines to preserve homeostasis. Anti-inflammatory cytokines like IL-10, TNF-binding protein and transforming growth factor-*β* are produced during the normal immune response and can prevent the liberation of TNF-*α* and other inflammatory cytokines [90].

In generating ROS, the environmental stress evokes an inflammatory response that is the outcome of a complex chain of proceedings, including the immune response, which liberates a numerous of inflammatory cytokines like a tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [90]. Monocytes/macrophages at the inflammatory site, consider exciting the oxidative pathways liable for local tissue injury in gastric ulceration [91, 92]. The excretion of both cytokines promotes the influences of oxidative stress via stimulating mitochondrial ROS propagation and cytotoxicity [93]. IL-12 is a pivotal cytokine that links both immune responses the innate and adaptive. The results reported that the curing with PSEPS induced reduce in the levels of the pro-inflammatory mediators TNF-α and IL-6, IL12 and MPO. Ran et al. [94] Demonstrated that in the rat, serum levels of TNF-α and IL-6 are significantly increased after γ-radiation followed by 30% body surface burns. Similarly, Budagov et al. [95] it showed an increase in IL-6 in the blood serum at 6–24 hours after radiation, and burns' injury. PGE2 utilizes a protective effect on the stomach during the activation of prostaglandin E receptors [96]. In line with previous studies Ketuly et al. [97], this study reported that the level of PGE2 exhibited that biosynthesis of PGE2 increased within the experimental groups, suggesting that the gastric protective effect of the plant extract was interceded partially by PGE2. These results were confirmed by using PAS stain that showed gamma rays caused loss of mucous secreting cells on the surface while using PSEPS, gastric mucosa regained it's positively to PAS stain denoting a protective and a stimulating effect of the extract on gastric mucosa. The mucous has an important role in protecting the gastric epithelial tissue from acid content of the stomach [98]. Healthy gastric mucosa is always under balance between cell death and cell regenerate and mucosal injury is progressing when this balance is disturbed due to increase in apoptosis and/or suppression of cell proliferation [99]. Mucus protects formed new cells from the damage resulting from severe acidity and proteolysis from gastric secretions [100]. As expected, the treatment of animals with PSEPS could prohibit the reduction of mucin spotted with PAS, which mentioned the embroilment of gastric mucus on the regeneration activity of this polysaccharide. Promoting our observations Srikanta et al. [101], also announced an increased mucin production via a polysaccharide through the treatment of gastric ulceration.

In the present study, it was reported that gamma rays can rapidly severe immuno reactivity of COX-2 in rat gastric mucosa, in all probability as an antagonistic restraint to inhibition of gastric PG synthesis and COX-2 activity [102]. COX-2 can be encouraged by the damaging agents such as luminal acid in the gastric COX-2 in the mucosa and suppression of lessen the damage [103, 104]. It affects the preservation of gastric mucosal benignity by prohibition exogenous harm and by enhancing gastric mucosal recovering [105]. However, there are also studies reporting that COX-2 blockers exacerbate gut damage and decline the tissue's ability to respond to mild damaging agents.

Prostaglandin endoperoxides synthase/COX is that the key accelerator in gastric mucosal protection and repair [96, 106]. Interestingly, PSEPS treatment counteracted the increased of COX-2 stimulated by γ- rays' treatment, which rise in gastric mucosa. The possible mechanism is that COX-2 expression may be an effective response to excess the levels of gastric protective PG in the operation of gastric damage [107]. The data revealed that, the PSEPS stimulating anti-inflammatory and antioxidant activities in an irradiation- stimulated gastric ulcer model were investigated in rats. COX-2 is a substantial factor for epithelial cell generation, and re-epithelialization and rebuilding of the gastric glands. Gastric injury is the main side effect connected with suppression of COX-2 [108]. The action of PSEPS on COX-2 and PGE2 together the gastric mucus layer, cell proliferation and regeneration propose that it might perform a new strategy of recovering gastric ulcers. COX-2 prompts the production of various growth factors, such as VEGF, and has an important role in tissue reform [99]. This growth factor is highly specific for an endothelial cell which stimulates the angiogenesis alone is sufficient for chronic ulcer healing [109, 110]. Our results display that PSEPS treatment superfat the number of vessels at the ulcer margin Compared with other non-treated rats, referencing that PSEPS induced angiogenesis in this renovated area. As well as, angiogenesis is substantial for the curing of chronic gastric ulcers, and these factors have been specified in the gastric mucosa that plays an organ-specific function for the consistence of huge blood vessels provisioning the stomach and intestines [111]. This increase angiogenesis in the PSEPS group by raising VEGF that contributes to rise of the mucus barrier that plays substantial role in the keeping and renewal of the stomach mucosa epithelium.

CONCLUSION

In this study PSEPS obtained from marine *Pseudomonas* sp. RD2SR3 has potential as a natural antioxidant, it was a safety and effectiveness based on the chemical construction of PSEPS so, oral administration of PSEPS for 30 days hurried the curing of gastric ulcer in rats by supporting epithelial cell proliferation, increasing neutrophil number by excessing of MPO and raising mucus production. In immunohistochemical analyses, we demonstrated a large number of COX-2-expressing cells at the most in the submucosa and our results indicated that PS raises angiogenesis in healing gastric mucosa. From the above, it's concluded that the effectiveness of a novel polysaccharide on radiation-induced Gastritis in experimental rats.

Abbreviations

COX-2: Cyclooxygenase 2
H&E: Hematoxylin and eosin
PAS: Periodic acid-Schiff
VEGF: Vascular endothelial growth factor.
MPO: myeloperoxidase
IL10: interlukin 10
IL12: interlukin 12
SOD: superoxide dismutase
TBARS: thiobarbituric acid reactive substances
GR: glutathione reductase
GST: glutathione-s-transferase
PGE2: prostaglandin E2
ROS: reactive oxygen species
PBS: phosphate buffer saline
NaOH: sodium hydroxide
MDA: malondialdehyde
OH: hydroxyl radical
H_2O_2: hydrogen peroxide
HClO: hypochlorous acid
Cl^-: chloride ion
TNF-α: tumor necrosis alpha
γ- rays: gamma rays

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