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ORIGINAL ARTICLE

Ameliorative Effect of *Sargassum fusiforme* Polysaccharides on Oxidative Stress and Inflammation in Ethanol-induced Gastric Ulcer

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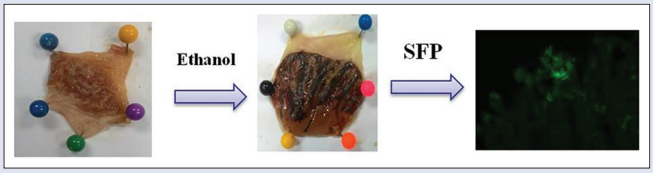
ABSTRACT

Background: Gastric ulcer is also known as stomach ulcer which is complex with multifactor-associated disease and shows high prevalent worldwide. It affects most of the people around the world, and the initiation and progression of the disease are due to the imbalance in the destructive and defensive mechanism and associated factors present in the mucosa of gastric. The current study is interested to evaluate the ethanolic extract of *Sargassum fusiforme* polysaccharides (SFPee) toward gastric ulcer induced by ethanol in rat model. **Materials and Methods:** *S. fusiforme* is a Chinese pharmacopeia and used as a medicinal ingredient. It contains polysaccharides, phlorotannins, and meroterpenoids which are the major contributors for its pharmacological properties. While the polysaccharides are the predominant ingredients, which account for 40%–60% of the algae dry weight. *S. fusiforme* polysaccharides show a potent antioxidation, anticancer, antiaging, and anticlotting function and thus promote health benefits. However, the mechanisms behind its pharmacological properties remain largely unknown. Here, we assessed the SFPee ameliorative effects on gastric ulcer induced by ethanol in male Wistar rats. We are also interested to identify the molecular machinery involvement of antigastric ulcer property of SFPee in gastric ulcer-induced rats. **Results:** Based on the investigation, the role of SFPee on gastric ulcer on various molecules, oxidative stress markers, antioxidant enzymes, the expression of nuclear factor-kappa B, IκB, nitrotyrosine, cyclooxygenase-2, and the assessment of the inflammatory response. **Conclusion:** Our results show that SFPee exhibited significant anti-inflammatory and antioxidant properties against ethanol-induced gastric ulcer by altering various molecules involved in the gastric ulcer initiation.

Key words: Cyclooxygenase-2 inhibitors, gastric ulcer, nuclear factor-kappa B, oxidative stress, *Sargassum fusiforme*

SUMMARY

- Mucosal defensive factors enable the mucosa to stay intact despite its frequent exposure to external substances and ranging pH, osmolarity, and temperature and notably to substances with detergent or cytotoxic action and bacterial products that induce local and systemic inflammatory reactions
- Ethanolic extract of *Sargassum fusiforme* polysaccharides (SFPee) showed a significant change in the molecules which is altered due to gastric cancer induction by ethanol. SFPee brings back the ulcerative changes and acts as a gastroprotective agent by playing a major role in directly or indirectly by showing its antioxidant and anti-inflammatory properties.



Abbreviations used: SFP: *Sargassum fusiforme* polysaccharides; CAT: Catalase; SOD: Superoxide dismutases; ROS: Reactive oxygen species; TBARS: Thiobarbituric acid reactive substances.

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INTRODUCTION

The foremost common clinical condition of the gastrointestinal (GI) system with multiple etiologies is gastric ulcer.[1] Many literature supports that >14 million people are diagnosed with gastric ulcer annually and about four million people die from related complications and it is highly prevalent worldwide.[2,3] Gastric ulcer represents more number of severe complications associated with it and their incidence persists a main cause for higher mortality rate and health-care burden worldwide.[4] Basically, human gastric epithelial tissue is in constant turnover by maintaining a balance between cell death and new cell formation. It has some self-repair capacity to food-oriented chemical and physical damage which affects gastric mucosa.[5] Still, when the damage beats the self-repair ability of the gastric mucosa, it drops that its capacity, the structure, and function of the gastric epithelial tissue can be compromised, leading to an imbalance between cell formation and cell death, which in turn can cause gastric mucosa inflammation which leads to ulcer. Some of the common risk factors associated with pathogenesis of gastric ulcer are frequent smoking, alcoholism, being on stress, infections due to *Helicobacter pylori*, and frequent user of nonsteroidal anti-inflammatory drugs.[6]

Literature has shown that the molecular level of ulcer initiation and progression is mainly attributed by the reactive oxygen species (ROS)-mediated increased level of lipid peroxidation, lowered level of GSH, and antioxidant systems, which leads to the pathogenesis

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of almost all forms of gastric ulcers.[7,8] Under normal physiological conditions, the cell has ability to withstand for a certain level of free radical, i.e., ROS due to its endogenous antioxidant property, which plays a key role in the intestinal homeostasis. However, an extreme oxidant load results due to increased free radical generation or decreased antioxidant reduction reactions or free radical scavenging activity, will favor and can overwhelmingly enhance membrane damage by initiating permeability of the membrane, modifying the inflammatory responses, and results in the fatty acid and protein modifications, which can progress to DNA fragmentation and damage, raise in the apoptosis, and finally, a pathologic condition.[9,10] Oxidative stress, a well-known disproportion between the pro-oxidants generated by free radicals, ROS, and antioxidant system, was observed in many pathological conditions and diseases, such as diabetes, cancer, chronic obstructive pulmonary disease, atherosclerosis and cardiovascular diseases, and ulcers too. Uncontrolled oxidative stress is critical to the GI tract, and the antioxidant defenses can counteract the effects caused by free radicals, ROS. These defense mechanisms ensure that the concentrations of ROS/reactive nitrogen species are under control and will not exert harmful effects on our system. The endogenous antioxidant system mainly consists of intracellular enzymatic antioxidants, such as glutathione peroxidase, catalase (CAT), and superoxide dismutases (SODs); intracellular nonenzymatic antioxidant glutathione; and extracellular antioxidants including vitamins, minerals, ceruloplasmin, and uric acid. These systems will scavenge the free radical and thereby decrease the oxidative stress.

Currently, there are more number of treatments available for gastric ulcer, which are proton-pump inhibitors that help as a H2-receptor antagonists, various drugs and antacids or alginate, that protect the lining of stomach, etc. It has been shown that there are several side effects, and incidences of relapse are major drawbacks of the available treatment.[11] Several research studies and clinical trials show that screening and development of novel gastroprotective agents from natural source are associated with effective treatment option with lesser side effect, which might be toxic less and cost-effective.[12] Currently, more number of studies have risen and carried out to identify the effectiveness of herbal medicines in the treatment of gastric ulcers. Polysaccharides from several herbs and compounds, including *Sargassum fusiforme*, have paying attention of researchers due to their multiple biological properties, such as immune stimulatory, anti-inflammatory, antioxidant, antifungal, hypoglycemic, cardioprotective, and free radical scavenging activities.[11]

S. fusiforme is bitter, cold, with a soft solid loose knot and used in purging heat, reducing phlegm and water swelling.[13,14] *Sargassum* possesses various biological properties, including it regulates pacify blood coagulation, thyroid function, diminish blood pressure, decrease fat, maintains sugar, improves body's immunity and cellular antioxidant activity.[15] Polysaccharide is not only a nutrient but also can be used as a drug, due to its widely recognized safety and effectiveness. Polysaccharides partake very clear pharmacological properties in help to immunity and tumor treating without side effects; therefore, it helps to one of the possibilities for the treatment of some of the common ailments.[16-18] Several researches have pointed out that polysaccharides, like those found in *S. fusiforme*, can act as powerful antioxidant and anti-inflammatory agent.[19] Therefore, the search of biologically and structurally novel drug-like polysaccharides from plant or *S. fusiforme* has great importance to treat gastric ulcer. Hence, the present study designates the leading exploration of therapeutic role of ethanolic extract of *Sargassum fusiforme* polysaccharides (SFPee) and their potential mechanisms of action against gastric ulcer-induced rats by ethanol.

MATERIALS AND METHODS

Experimental design

Animals

Wistar albino adult male rats were used for this study, and rats weighing 150–200 g were segregated into three groups in a cage of six animals each. Entire experiments were carried in the controlled laboratory conditioning, where food and water were given *ad libitum*. The ethical clearance was approved for this study according to the guidelines for the Care and Use of Laboratory Animals (published 1996 by National Academy Press) and the experimental protocol approved by the Institutional Research Ethics Committee.

Work design

Rats were randomly segregated into three groups, and every group consists of six animals (n = 6); Group I (control): Rats were served as normal controls (received vehicles orally by intragastrical gavage), Group II (gastric ulcer induced): rats were received ethanol (5 ml/kg, intragastrically), Group III (gastric ulcer induced + SFPee treated): rats were received ethanol (5 ml/kg, intragastrically) along with ethanolic extract of *S. fusiforme* (SFPee: 300 mg/kg b. wt).

Isolation and purification of the polysaccharide

S. fusiforme were air-dried and soaked in 95% ethanol to remove the pigments and lipophilic molecules. The obtained residues were then extracted with a 10-time volume of distilled water at 90°C for 3 h, and the process was repeated thrice. The extracts were pooled, filtered, concentrated, and precipitated with the help of 95% ethanol (v/v^{-1}:4) at 4°C for overnight. The precipitates were collected by centrifugation, and proteins were removed by Sevag method. Finally, the deproteinated supernatant was lyophilized to form a polysaccharide crude. The polysaccharide crude was then purified by DEAE-52 cellulose and Sephadex G-200 column, and the main polysaccharide fraction (SFP) was collected and lyophilized. Collected SFP was used for further study.

Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) analyses of SFP were analyzed by FTIR spectrophotometer IRAffinity-1S (Shimadzu, Japan) in the wavelength range of 400–4000 cm^{-1}. This instrument offers 30,000:1, 1-min accumulation, neighborhood of 2100 cm^{-1}, and a maximum resolution of 0.5 cm^{-1}. AuNPs were spun and reconstituted in sterile water for purification before FTIR analysis [Figure 1].

Gastric ulcer induction

Gastric mucosal injury was induced by a single intragastric dose of 5 ml/kg absolute ethanol which is administered by orogastric intubation. The control group was treated with instead of ethanol the same volume of saline.[20]

Preparation and tissue collection

On the last day of treatment, the animals were prepared for anesthetic condition using ether; during anesthetic condition through retro-orbital puncture, the blood was collected for biochemical estimation 4-h postethanol treatment. After blood collection, all the animals were sacrificed by cervical dislocation, and the animal stomach was removed and opened along the greater curvature and then rinsed gently using phosphate-buffered saline to remove the gastric mucosal contents and blood clots. The stomachs and serum were subjected to further investigations.

Stomachs were blotted dry, and macroscopical examination was done to measure and investigate the gross gastric injury (expressed as ulcer index) and pictured for future determination of gastric lesions area. Then for immunohistochemical and histopathological examination, the stomachs were dichotomized; among that, one moiety was immersed in 10% formalin saline buffer, while the glandular gastric tissue of the

Table 1: Yield and chemical composition (percentage, dry weight) of *Sargassum fusiforme* polysaccharides

Composition	Dry weight
Yield (%)	1.93
Total sugar (%)	42.69
Sulfate (%)	25.69
Fucose (%)	19.45
MW (kDa)	229

other moiety was separated into three parts and stored at −80°C for the other investigations. To this end, one part was homogenized in 10 volumes of ice-cold phosphate buffer (100 mM, pH 7.4) used for determination of oxidative stress markers, antioxidants, and cellular defenses (malondialdehyde [MDA], SOD, and CAT).

Measurement of gastric juice acid content (pH)

The stomach gastric contents of each rat were collected and centrifuged at 4000 rpm for 10 min. The supernatant pH for each sample was measured using with pH meter. Total and free acidity was measured by titrating against sodium hydroxide (0.01 N) using Topfer's reagent (indicator) to find the total and free acidity expressed as mEq/L.[21]

Oxidative stress markers

Determination of malondialdehyde level

Lipid peroxidation was assessed by the measurement of the MDA concentration in the homogenate from each sample according to Draper and Hadley. This method is based on an enzymatic reaction with thiobarbituric acid. Gastric samples were prepared with 1 mL of 10% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The reaction was heated in a boiling water bath for 15 min and then 2:1 v/v of n-butanol was added. After centrifugation at 800 g, for 5 min, thiobarbituric acid reactive substance contents were determined at 535 nm. MDA concentration was expressed as mol/g of tissue.

Superoxide dismutase

The activity of SOD was assayed by Misra and Fridovich[22] method by monitoring the rate of nitroblue tetrazolium (NBT) reduction inhibition. One unit is defined as the amount of enzyme, which caused half-maximal inhibition of NBT reduction.

Catalase

The stomach was homogenized using 50 mM Tris–HCl, pH 7.5 (1/10, w/v) and centrifuged for 15 min at 2400 g. Spectrophotometrically (U-2001 Hitachi– Japan), the supernatant was assayed by Aebi[23] method, which involves checking the degradation of H_2O_2 in the existence of cell homogenate at 240 nm.

p65, IκB-α, and cyclooxygenase-2 protein expression

The gastric tissues were homogenized using cold RIPA buffer along with protease inhibitor, and the protein was assayed using Western blot analysis described previously. Protein from the lysate was estimated, and 50 μg of protein was electrophoresed and allowed to separate, in SDS-PAGE, and transferred to PVDF membrane. The membrane was blocked using milk powder and incubated with nuclear factor-kappa B (NF-κB)-p65, IκB, and cyclooxygenase-2 (COX-2) (Abcam, Cambridge, MA, USA) and β-actin (Sigma Chemical Co., MO, USA) primary antibodies. After incubation, the membranes were washed with TBS and TTBS and were incubated with enzyme-conjugated secondary antibody and it was identified using ECL chemiluminescence (Amersham Biosciences, Buckinghamshire, UK). Immune-reactivity intensity was quantified.

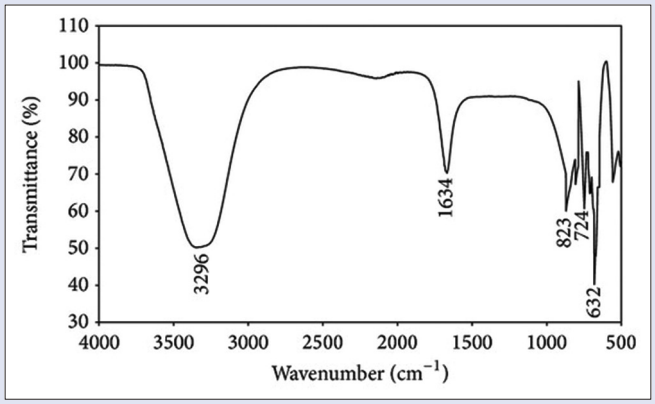

Figure 1: Fourier-transform infrared spectroscopy analysis of SEP

Immunohistochemistry

Immunohistochemical examination was done according to Mannick *et al.*[24] method. Fixed tissues were deparaffinized and rehydrated. Pepsin (Biomeda, Foster City, CA, USA) was applied to a section at room temperature for 10 min. The endogenous peroxidase was quantified using 3% H_2O_2 for 10 min in methanol, followed by rinsing with PBS. Nonspecific binding was blocked using PBS containing normal 10% goat serum for 20 min. The sections were rinsed with Tris buffer and incubated with rabbit polyclonal immunoglobulin G (IgG) for rat COX-2 (1:500) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or a rabbit polyclonal IgG for nitrotyrosine (1:250) (Upstate Biotechnology, Lake Placid, NY, USA) to overnight in a humidity chamber at 4°C. Antimouse and antirabbit IgG conjugated to Alexa 488 and 568, respectively, at room temperature for 45 min (both 1:200). Sections were counterstained with Hoechst 33342 (Invitrogen) and visualized under a Nikon Eclipse 80imicroscope.

Histological (hematoxylin and eosin) staining

Histopathological assessment of gastric tissue was fixed in buffered 10% formalin and further processed for histopathological investigation as described before.[24] Briefly, four-micrometer thickness paraffin sections were prepared and stained by hematoxylin and eosin used for light microscope investigation (magnification at ×20).

RESULTS

Fourier-transform infrared spectroscopy and chemical analysis

FTIR analyses of SFP formed from SFP were performed in the wave number range of 400–4000 cm^{-1}. It shows the presence of various chemical groups and FTIR signals of SFP correspond to CH stretching of alkanes (2931 cm^{-1}), 2097 cm$^{-1,}$ C = O stretching vibration of carbonyl (1750 cm^{-1}), (CH or = CH-H) 1407 cm$^{-1,}$ (C-O stretching) 1014 cm^{-1}, and aromatic CH bending (817 cm^{-1}). The final yield of SFP 1.93% which contains total sugar (42.69), sulfate (25.69), fucose (19.45), and the MW (kDa) around 229 [Table 1].

Effect of ethanolic extract of *Sargassum fusiforme* polysaccharides on gastroprotective parameter content in gastric ulcer induced by ethanol

Table 2 shows gastroprotective action of SFPee on various parameters on gastric content in gastric ulcer induced by ethanol. Gastric ulcer-induced rats showed secreted volume of. 47 ± 0.19 mL, total acidity of 84.32 ± 2.57 Meq/L, and pH of 1.62 ± 0.14 when compared with

control. However, the SFPee treated intraduodenally in gastric-induced rats did not change any of the factors of gastric secretion [Table 2]. SFPee reduced the volume ($P < 0.05$) of total acidity ($P < 0.01$) and increased the pH ($P < 0.01$) which was induced by ethanol and completely brings back near to control.

Morphological investigation

Figure 2 shows the antiulcer activity of SFPee on gastric ulcer-induced rats compared with control and gastric ulcer. (a) Control rats show normal mucosa, (b) hemorrhagic erosion was seen in ethanol-induced rats (5 m1/kg), and (c) rats that were induced with ethanol and treated with SFPee (300 mg/kg) show nearly normal mucosa.

Histopathological findings of gastric mucosa and scoring

Histopathological investigation shows [Figure 3], the gastric mucosa from the control rats showed normal mucosa, submucosa, muscularis, and serosa. Ethanol-induced rats showed formation of ulcer with damaged mucosal epithelium and distorted gastric glands and cell debris; SFPee (300 mg/kg) co-administered with ethanol protected against these changes. SFP treatment showed protective effects on gastric mucosa as evidenced by epithelial cell loss reduction.

Effect of *Sargassum fusiforme* polysaccharides on the expressions of nuclear factor-kappa B pathway-related proteins

The anti-inflammatory activity of SFPee supports the gastroprotection by altering NF-kB pathways, which plays a major role in inflammation; here, we analyzed the expressions of NF-kB and IkB-alpha proteins using Western blot method. As shown in Figure 4, the phosphorylation patterns of IκB-α reveals that SFPee is significantly increased and the level of phosphorylation of IKβα and GU induced by ethanol is statistically decreased when compared with control, and NF-κB was increased during the induction, and SFPee reduced the same as mere to control. β-actin was used as an internal control to normalize the level.

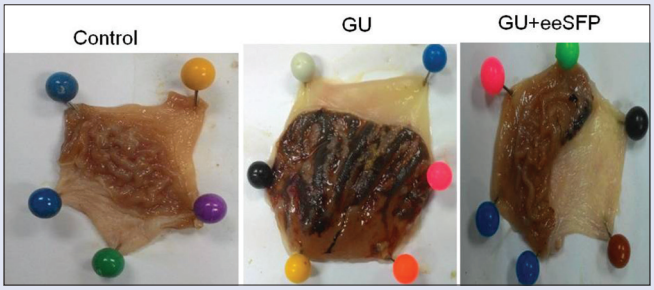

Figure 2: Effect of SFPee on gastric ulcer index, gastric morphology in experimental rats

Effect of *Sargassum fusiforme* polysaccharides on nitrotyrosine and cyclooxygenase-2 protein expression in gastric ulcer-induced ethanol in rats

Immunohistochemical staining analysis reveals the COX-2 and nitrotyrosine expression in the gastric mucosal epithelial cells [Figures 5 and 6]. As observed, COX-2 and nitrotyrosine were expressed in gastric ulcer-induced rats, when compared with GU + SFPee. SFPee decreased the COX-2 and nitrotyrosine expression of gastric mucosal epithelial cells, which was typically observed in the gastric mucosal inflammatory area [Figure 6]. Figure 6a shows the COX-2 protein expression, the ethanol-induced rats show increased COX-2 expression and the treatment effectively decreased the expression. Thus, SFPee considerably arrested the COX-2 activation in the gastric mucosal inflammatory area and activity.

Effect of ethanolic extract of *Sargassum fusiforme* polysaccharides on gastric mucosal lipid peroxidation and antioxidant system

The gastric lesion-induced ethanol maybe intermediated in the course of increased ROS generation. The two ways of defense mechanism against ROS mainly reveal in augmentation of endogenous antioxidant enzymes. As for lipid peroxidation, the ethanol-stimulated gastric tissues level of MDA was considerably decreased in SFPee-treated rats when compared to the control group and ulcer-induced group. In gastric SOD and CAT levels induced group significantly decreases the level of SOD and CAT and SFPee-treated group bring back to normal level when compared with the control group and induced group. The statically significant results were observed in the antioxidant role of SFPee against gastric ulcer-induced rats [Figure 7].

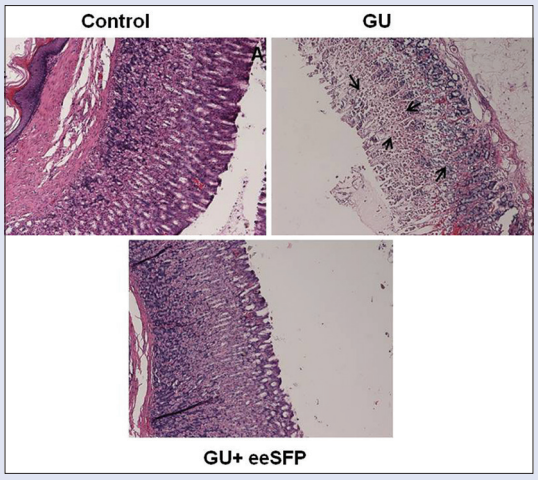

Figure 3: The histological evaluation of effect of SFPee on gastric mucosal staining with (hematoxylin and eosin) in ethanol-induced gastric ulcer in rats

Table 2: Effect of SFPee on gastroprotective parameters content in ethanol-induced gastric ulcer in rats

Treated groups	Gastric volume (ml)	Gastric pH	Gastric total acidity (Meq/L)	Observed ulcerated area (%)	Inhibition percentage (gastroprotection) (%)
Group I	3.24±0.11	2.47±0.19	58.47±1.32	0	–
Group II	6.47±0.19*	1.62±0.14*	84.32±2.57*	37.26±4.92*	-
Group III	2.61±0.14#	3.37±0.28#	48.19±2.98#	12.64±0.61#	73.14±0.17*

#,*Represents statistical significance between control and other groups at $P<0.05$, $P<0.01$ level respectively using Dunnett's test

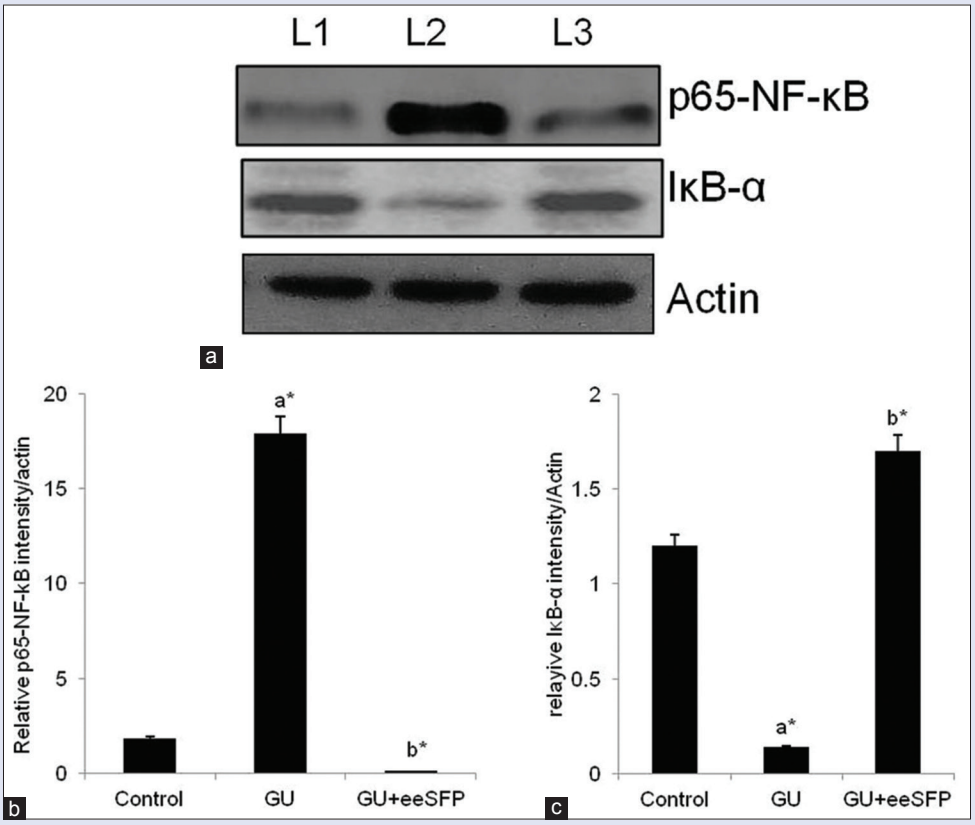

Figure 4: (a-c) Effect of SFPee on the protein expression of p65, IκB-α expression in ethanol-induced gastric ulcer in rats. *represents statistical significance between control and other groups at $P < 0.05$, $P < 0.01$ level, respectively, using Dunnett's test

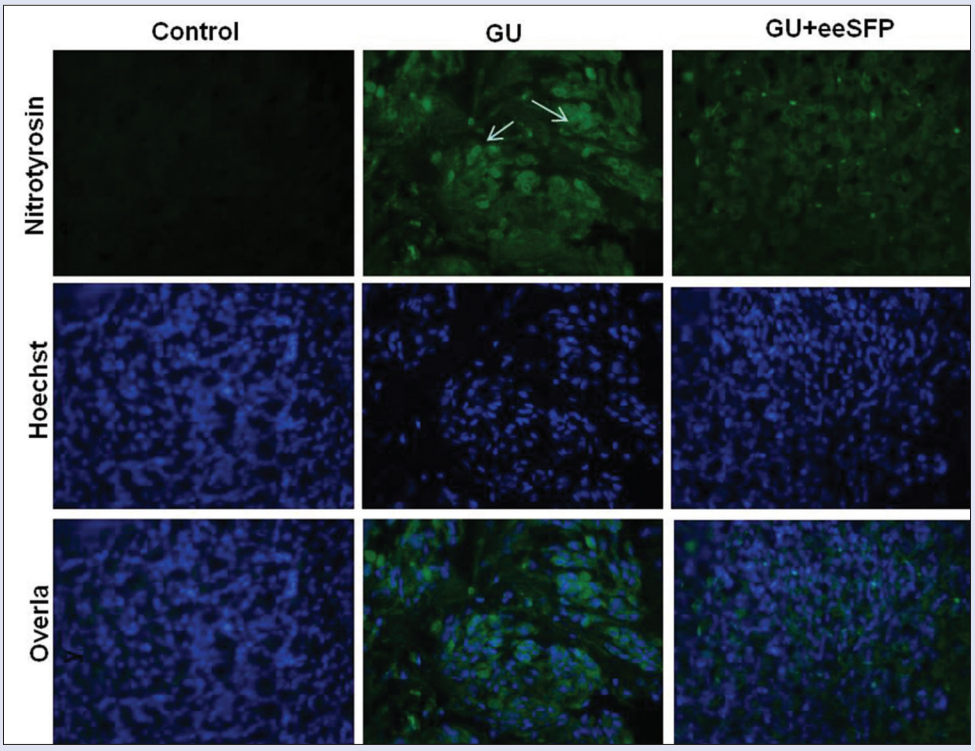

Figure 5: Effect of SFPee on nitrotyrosine protein expression in ethanol-induced gastric ulcer in rats

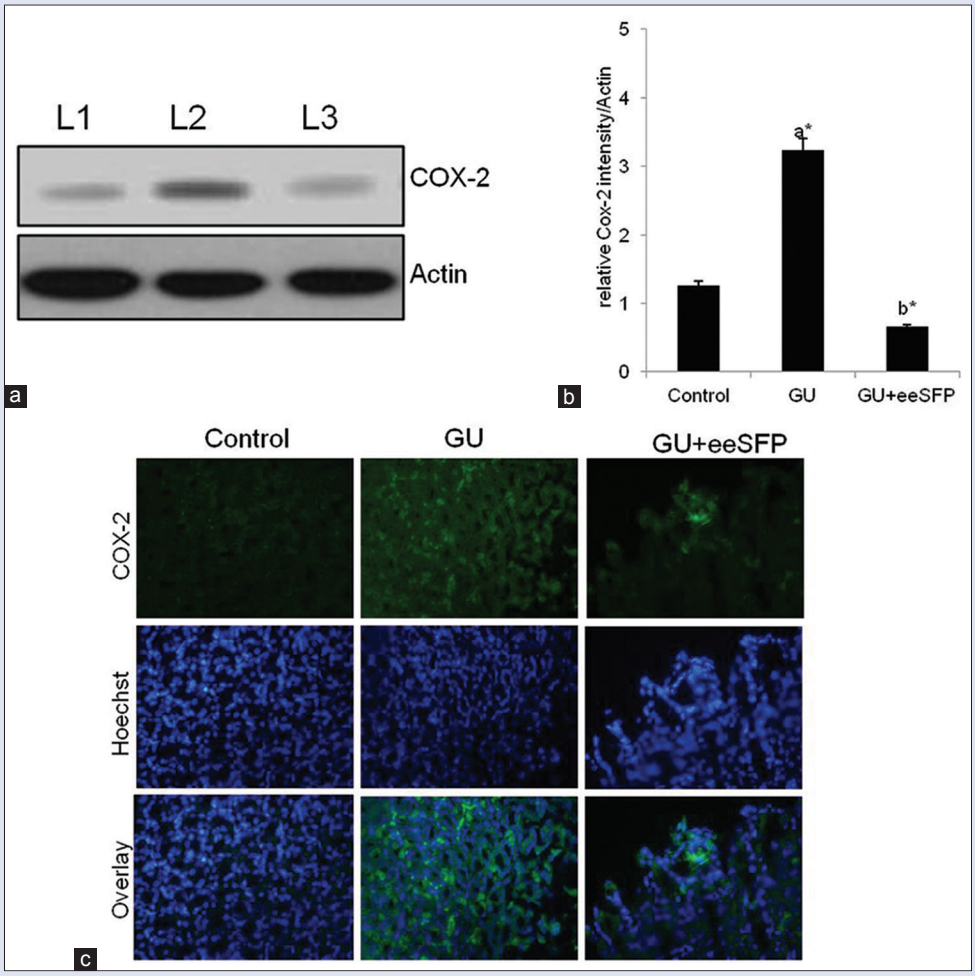

Figure 6: (a-c) Effect of SFPee on cyclooxygenase-2 protein expression in ethanol-induced gastric ulcer in rats. L1-control, L2-GU, L3-GU + eeSFP. *represents statistical significance between control and other groups at $P < 0.05$, $P < 0.01$ level, respectively, using Dunnett's test

DISCUSSION

Gastric ulcer is the most common health burdens among the human population. Damages or destruction in the gastric mucosal is caused mainly due to the imbalance between the defensive and destructive mechanisms, which play a major role in the destruction of gastric mucosa and is reflected the overall outcome of the activities of some endogenous factors and destructive exogenous factors.[25,26] The gastric mucosal integrity was mainly hinge on efficient defense of the gastric mucosal barrier, by maintaining defenses such as the gastric mucus layer, which includes preepithelial factors such as mucusbicarbonate barrier and mucosal microcirculation, which might be destructed by internal and external stimulus factors.[27] Inflammatory mediators and cytokines play a vital role in the damage caused to the mucosa by internal factors and external stimuli.[28] Gastric mucosal injury may occur mainly, when harmful factors hit the intact mucosal defense or when the mucosal protective mechanisms are impaired. The gastric mucus layer is the first line of defense that serves to protect stomach tissue from external stimuli.[29] Mucosal defensive factors enable the mucosa to stay intact despite its frequent exposure to external substances and ranging pH, osmolarity, and temperature and notably to substances with detergent or cytotoxic action and bacterial products that induce local and systemic inflammatory reactions.

Ethanol is well known to causative factor for gastric damage by modifying the balance between the protective factors and destructing factors present in the gastric mucosa, by decreasing blood circulation and mucus production. Ethanol is commonly used to induce ulcers in experimental animals and causes acute gastric mucosal damage. The molecular mechanism of the ulcer induction by ethanol, it is mainly attributed by the generation of ROS, decreased cell proliferation and an exacerbated inflammatory response.[30,31] The ROS production and associated antioxidant reduction are the key responsible factors for the cell damage and cell death. The ROS hits vital cell elements such as lipids, proteins, and nucleic acids and causes the toxic formation.[32] Additional important factor in the gastric ulcers pathogenesis is the increased gastric acid secretion. Due to gastric acid, it shows high acidity which can worsen existing damage or increases the action of a destructive agent on the gastric mucosa.[33] Therefore, inhibiting the ROS and gastric acid secretion is crucial for the treatment of gastric ulcer pathologies. Recently, many literature shows that natural compounds have tendency to control the ROS by inducing antioxidant or direct acting antioxidant in the system. Polysaccharides from various *Sargassum* species exhibit different biological activities, as well as antioxidative activity.[19]

It has been stated that the polysaccharides from SFP are effective hydroxyl radicals and superoxide anion scavengers in cells. Literature supports that SFP suppresses oxidative stress and induces antioxidant defense.[34] SFP has been shown to possess multiple functions, such as antitumor, antioxidant, vibriosis resistance, immunity, and antihyperlipidemia.[35] In this current study, we planned to explore the role of SFP on therapeutic

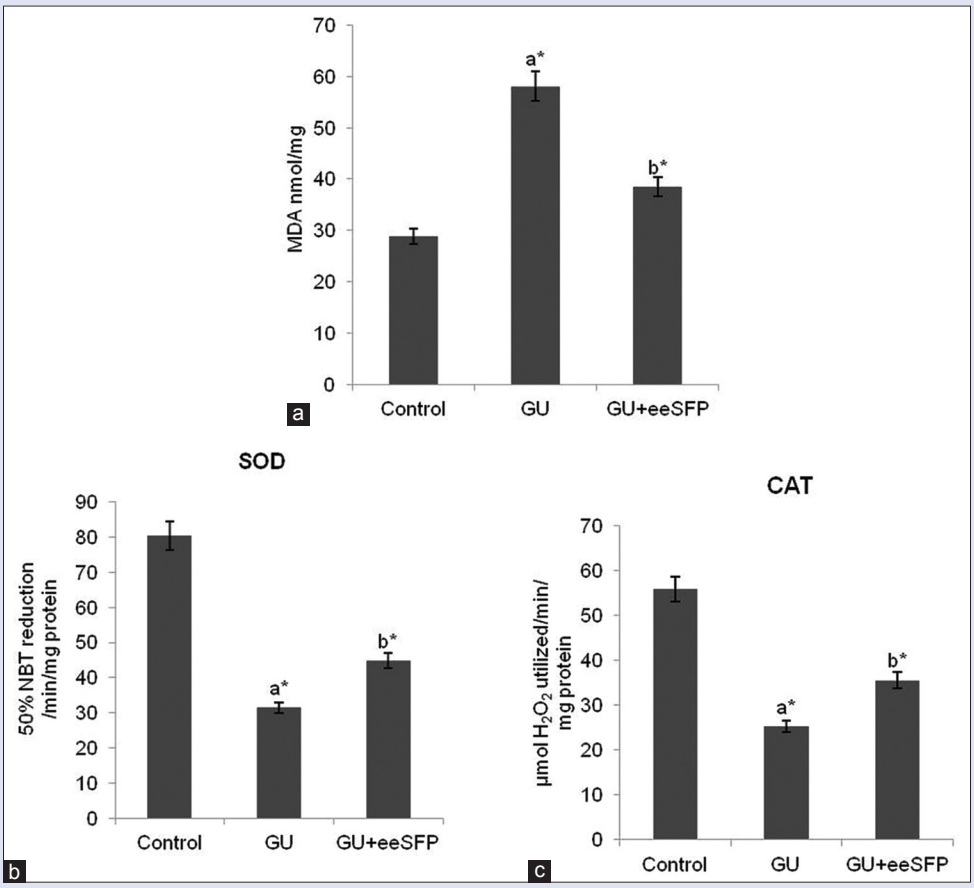

Figure 7: (a-c) Effect of SFPee on gastric mucosal oxidative stress and antioxidant enzymes in ethanol-treated rats in ethanol-induced gastric ulcer in rats. #,*represents statistical significance between control versus other groups at *$P < 0.05$ level, respectively, using Dunnett's test

and their potential mechanism of action against gastric ulcers induced by ethanol in rats. Gastric mucosal damage is enthused, both directly and indirectly, through ROS and inflammation which is observed through inflammatory markers.

Macroscopic observation of gastric mucosa shows hemorrhagic lesions in ethanol-induced gastric ulcer. The data shows SFPee-treated ulcer-induced rats shows protection to the ulcer-induced gastric mucosa by ethanol. The increased gastric ulcer index (GUI) observed in ulcer-induced rats shows the indicative of the strong ulcerogenic activity of ethanol and coincides with previous studies. The ethanol-induced GUI was significantly reduced by SFPee pretreatment which shows remarkable gastroprotective potency of SFPee. Administration of ethanol to rats produces typical characteristics of gastric ulcer, which includes the pathological condition associated with ulcers which are linear hemorrhagic lesions, extensive submucosal edema, inflammatory cell infiltration, and epithelial cell loss in gastric mucosal tissue. Ethanol induces inflammatory and necrotic lesions in the gastric mucosa through its direct toxic effect and also indirectly by reducing the secretion of bicarbonates and the production of mucus and defensive factors.

Oxidative stress is caused by imbalance between ROS generation and the endogenous antioxidant activity. Studies show that ROS generation in the ulcer-induced tissue might be due to infiltration of inflammatory cells present in the gastric mucosa. Generation of superoxide radical anions (O2−) due to induction of ulcer by ethanol may react with lipids and fatty acid which might lead to the generation of lipid peroxidation. The ulcerated tissue shows increased MDA and decreased SOD and CAT in the ethanol-induced rats; this may be due to defects in the antioxidant defense systems which were demonstrated in the present study. The reduction in the CAT and SOD might be associated with superoxide radical generation; due to superoxide radical generation, it consumes a large amount of endogenous antioxidant enzymes which lead to decreased antioxidant scavenging mechanism. The Rats treated with SFPee significantly reduced lipid peroxidation and restored the exhausted SOD and CAT activity in ulcer-induced gastric mucosa.

NF-κB, the key regulator of oxidative stress and inflammation, NF-κB belongs to Rel subfamily, which is responsible for dimerization of the molecule, recognition of binding site, ability of molecule to binding to the DNA, and interactions with proteins.[36] In the current study, ulcer-induced rats show increase in the level of NF-κB; however, SFPee-treated rats show reduced level of NF-κB. The decrease in the level of NF-κB by SFPee-treated rats might be due to increase in ROS scavenging ability of SFPee, as well as ROS activates NF-κB through IκBα phosphorylation.[37] The activity of SFPee on the downregulation of NF-κB in ulcer tissue, although it has been previously shown that antioxidants reduce NF-κB activation induced by ROS and blocked the downstream transcription of several inflammatory genes in ethanol-induced ulcerated rats.[37] The association of oxidative stress in the pathogenesis of ulcer-induced by ethanol which leads to gastric injury has been confirmed by previous studies.[11] Further, the histological findings of this study show that the results obtained support our results, as the ulcer-induced rat tissues displayed severe erosion of the gastric mucosal layer with hemorrhagic lesions which extend deeply into the gastric mucosa. The antiulcer activity of SFPee may be due to antisecretory, mucosal firming, antioxidant, and anti-inflammatory role

by NF-κB pathways. Hence, further studies are warranted to observe the gastroprotective potency of SFPee in preclinical and clinical stages. In addition, SFPee also prevented pancreatic β-cells from H2O2-induced oxidative damage, which was suggested relating to the PI3K/AKT pathway.[38] P13K/AKT all mediated NF-κB pathways. Here, in this study, SFPee increased the level of IKβ, due to the increased level in phosphorylated IKβα. SFPee might be decreased the level of NFxB SFPS directly exhibited good radical scavenging tendency by 2,2-diphenyl-1-picrylhydrazyl radical system and linoleic acid system tests. Therefore, it indicates that SFPS functions not only through its chemical property but also influencing cellular signaling transduction pathways. Further *in vivo* experiments demonstrated that the antioxidant activity of SFPS is related to antitumor, hypolipidemic, and hypoglycemic and plays a key role in restoring the immunocompetence of mice.[39,40] SFPS may enhance the expression of tumor suppressor gene p53 and activate Fas/FasL/Caspases signaling pathway,[39] thus inhibiting tumor cell cycle and inducing apoptosis. It suggested that SFPS performed antitumor activity by enhancing animal's immune activity, which is associated with NF-kB signaling pathway.[34]

CONCLUSION

A huge number of literature show that *Sargassum* species contain polysaccharide and other important biologically active compounds. These bioactive compounds and total extracts showed a significant therapeutic potential outcome and suggest that this could be taken for the novel functional ingredient preparation in the pharmaceuticals for the treatment and prevention of several pathological conditions. In this present study, SFPee showed a significant change in the molecules which is altered due to gastric cancer induction by ethanol. SFPee brings back the ulcerative changes and acts as gastroprotective agent by playing a major role in directly or indirectly by showing its antioxidant and anti-inflammatory properties. Therefore, further studies are warranted in future to bring out its maximum therapeutic potential in the field of medicinal and pharmaceutical sciences for the novel and fruitful application of *S. fusiforme* polysaccharide.

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Conflicts of interest

There are no conflicts of interest.

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