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Hepatoprotective effect of polyphenols of marine brown algae *Sargassum graminifolium* through modulation of Nrf2/HO-1

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Bioactive compounds from the marine brown algae *Sargassum graminifolium* protect against toxin-induced liver injury. In this study, we assessed the hepatoprotective effects of *Sargassum graminifolium* against CCl_4 toxicity in rats via the activation of the Nrf2/HO-1 pathway. Marine polyphenols were fractionated and quantified using the DMBA assay and characterized using LC-MS and FT-IR. The experimental groups were as follows: Group I (normal saline), Group II (CCl_4 1mL/kg in olive oil), Group III (silymarin 50 mg/kg+ CCl_4), and Groups IV and V (polyphenol extract of *Sargassum graminifolium* 200 and 400 mg/kg+CCl_4). LC-MS analysis identified 7-hydroxyeckol (*m/z* 387.2921) and hydroxytrifuhalol A (*m/z* 405.3619), along with other unknown biologically active compounds exhibiting antioxidant activity. The extract significantly ($p \leq 0.05$) reduced liver biomarkers, lipid profiles, and inflammatory cytokines(IL-1β, IL-6, TNF-α, and NF-κB), while enhancing tissue antioxidants and reducing MDA levels to restore CCl_4 toxicity. Furthermore, the probable mechanism of action for the hepatoprotective effect of *Sargassum graminifolium* was observed through upregulation of the Nrf2/HO-1 signaling pathway and rebuilding of liver histopathological alterations. In conclusion, the present study demonstrated that the polyphenol fraction of *S. graminifolium* prevents CCl_4-induced liver injury by upregulating the Nrf2/HO-1 pathway and exhibits potential antioxidant and anti-inflammatory effects.

Keywords: Liver toxicity, Polyphenols, Anti-inflammation, Antioxidant

The liver plays a crucial role in detoxification of both endogenous and exogenous toxic compounds[1]. Globally, liver disorders represent a major health issue, resulting in approximately two million fatalities annually and these are often underestimated and rank eleventh among fatal illnesses[2]. Carbon tetrachloride (CCl_4) is a potent toxic liver agent that produces copious amounts of oxygen free radicals. It is metabolized by the liver cytochrome P_{-450} enzyme system to produce highly reactive trichloromethyl (-CCl_3) and trichloromethyl peroxy radicals (-$OOCCl_3$), which directly break the fatty acid chain of the cell membrane result in hepatocyte damage[3]. CCl_4 induces oxidative stress, leading to the dissociation of Nrf2 from Keap1, which enables Nrf2 to move into the nucleus, where it binds to the antioxidant response element (ARE) located in the promoter regions of cytoprotective genes such as Nrf2/HO-1[4].Quantification of these cytoprotective antioxidant response elements helps to understand the hepatoprotective mechanism of CCl_4-induced toxicity in experimental models.

Among the various food resources found in the oceans, brown algae are considered a future food source and play a revolutionizing role in the global food system. They contain approximately 36% dry mass, with low fat and high protein content, and serve as balanced food sources. East Asian countries, such as China, South Korea, and Japan, have been consumed as food sources because of their potential health benefits and nutritional value[5]. *Sargassum graminifolium* (Sargassaceae), a marine brown alga found throughout tropical areas of the world, is commonly used as a source of sodium alginate, animal feed, fertilizer, and medicine. Recently, there has been increasing interest in the purification of active compounds from marine algae, particularly *Sargassum* sp[6]. Marine polyphenols (phlorotannins) are phloroglucinol polymers that are primarily restricted to marine algae. These compounds have up to eight interconnected phloroglucinol rings, and are therefore more potent free-radical scavengers than

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polyphenols derived from terrestrial plants, including green tea catechins, which have only three to four rings. They have potential biological activities, including antioxidant and anti-inflammatory activities, and are considered relatively safe for consumption, with few side effects[7].The background of the present study was that the free radicals released by CCl_4 disrupted the liver antioxidant system and aggravated liver failure. Supplementation with a polyphenol-rich extract of *Sargassum graminifolium* reverses CCl_4 induced hepatotoxicity in experimental rats. The present study was designed to evaluate the hepatoprotective effect of a polyphenol-rich extract of *Sargassum graminifolium* by modulating inflammatory markers and Nrf2/HO-1 genes under controlled conditions.

Materials and Methods

Fresh *Sargassum graminifolium*was collected in August, 2024 from the Mandapam region (09 17.417 0N; 079 08.558 0E), located on the southeast coast of India. After collection, the algae (seaweed) were washed with water to remove epiphytes. The brown algae were matched with the herbarium voucher specimen of *Sargassum graminifolium*(Voucher number: 1249). The seaweed was dried in the shade, ground into a powder, and stored at -20°C for future use.

Isolation and fractionation of marine polyphenols from *Sargassum graminifolium*

Dried and ground seaweed (500 g) was extracted with 4L 95% ethanol using the maceration method at ambient temperature for 72 h, followed by filtration through Whatman No. 1 filter paper. The resulting extract was concentrated under vacuum at 40°C using a rotary evaporator under low pressure to obtain a dark-green semi-solid. The concentrated crude extract was successively fractionated using water and n-hexane (3×400 mL). After removing the n-hexane fraction, the aqueous portion was extracted with dichloromethane (3×800 mL) and ethyl acetate (3×400 mL). Each solvent fraction was individually concentrated to dryness, and the yield was calculated[8].

Quantification of total phenolic content

The total phenolic content (TPC) in the ethanolic crude extract and ethyl acetate fraction was quantified using a modified Folin-Ciocalteu method, with phloroglucinol as the standard. A series of test drug concentrations were combined with 50% Folin-Ciocalteu reagent (0.5 mL) and water (0.5 mL) followed by the addition of 20% Na2CO3 (2.0 mL), and the mixture was incubated for 1 h and 45 min in the dark at room temperature (25±2°C). After incubation, the samples were centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was measured at 730 nm using a spectrophotometer. A standard graph was used to calculate the total polyphenol content.

Analysis of marine polyphenol abundance using DMBA assay

The 2,4-dimethoxybenzaldehyde (DMBA) assay is specifically employed to identify phenolic structures with one, three, or one, three, and five hydroxyl groups, which are characteristically found in seaweeds[9]. The procedure was conducted as described by Poole et al. and involved the preparation of two solutions. Solution A was prepared by dissolving DMBA (0.5 g) in 25 mL of glacial acetic acid, whereas Solution B consisted of 4 mL of hydrochloric acid mixed with 21 mL of glacial acetic acid. These solutions were combined immediately before use to create the working reagents. The assay involved adding 2.5 mL of the working reagent to 0.1 mL of the test sample (1 mg/mL), followed by 0.1 mL of N-N dimethylformamide. The mixture was incubated at 30 °C for 1 h in the dark. A UV-visible spectrophotometer was used to measure the absorbance at 515 nm. The results were quantified using a phloroglucinol standard curve and expressed as milligrams of phloroglucinol equivalents per gram of the test sample. Since the ethyl acetate fraction contains the maximum amount of polyphenols, it is called the polyphenol-rich fraction of *S. graminifolium* (PSG).

Liquid chromatography-quadrupole time-of-flight high-resolution mass spectrometry (LC-QTOF-HRMS) analysis

The molecular masses of the compounds in the ethyl acetate fraction of *S. graminifolium* were tentatively identified by liquid chromatography-quadrupole time-of-flight high-resolution mass spectrometry (LC-QTOF-HRMS) using a sophisticated analytical instrument facility (SAIF), IIT Madras, Tamil Nadu, India (Waters ACQUITY H-CLASS + UPLC/XevoG2 XS QTOF via LC-HRMS equipped with an electrospray ionization (ESI) source). Liquid chromatography separation was performed by injecting 10µL of the sample into an ACQUITY UPLC BEH C18 column maintained at 40°C, with a 20-minute run time with a graded

solvent system consisting of methanol and water. Following detection of the compounds through the flow cell of the diode array detector, the column eluate was channelled to an electrospray interface-equipped Q-TOF HRMS. The ESI Source was operated in positive ion mode, scanning a mass range of 100–1200 Da at a rate of 1.014 s. Mass detection was performed under the following specific conditions: gas temperature of 30 °C, gas flow of 13.34/min, nebulizer pressure of 40 psi, 3500 VCap, 175 fragmentor, 65.0 skimmer 1, and 20 Quadrupole Peak Width. The resulting mass and peak area data were then cross-referenced with the Comprehensive Marine Natural Products Database and SciFinder for identification purposes[10].

Fourier transform infrared (FT-IR) Spectral analysis

Approximately two milligrams of the ethyl acetate fraction of *S. graminifolium* were powdered with potassium bromide, and pellets were prepared and placed in an FT-IR sample holder. The molecular functional vibrations of the chemical groups in the samples were recorded using a FT-IR spectrophotometer (INFRA-300 FT-IR, Analytical Technologies Limited. India) operated at a resolution of 4 cm-1 ranging from 4000 to 400 cm-1.

***In vitro* studies**

2, 2-Dipheny l-1-picrylhydrazyl (DPPH) scavenging assay

The DPPH radical scavenging potential of PSG was assessed based on the methodology outlined by Chitikela *et al*. 2021. 50µL of 0.1 mM DPPH in methanol was incubated for 30 min in the dark with PSG at various concentrations in triplicates, and ascorbic acid was used as a reference compound. The absorbance was measured at 517 nm against a blank (methanol) using a UV-Vis spectrophotometer (Shimadzu UV-2401PC). Control was performed using a 5 mL sample of methanolic DPPH solution without antioxidant. The half-maximal inhibitory concentration (IC_{50}) was calculated[11].

In vitro ABTS+ (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical ion scavenging assay

The $ABTS^+$ radical ion scavenging assay was performed as previously described, with some modifications[12]. 7 mM 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) was prepared using double-distilled water. The ABTS stock solution was combined with 2.45 mM potassium persulfate to create an ABTS radical cation ($ABTS^+$) solution and incubated for 16 h at room temperature in the dark. The reaction mixture contained 3 mL $ABTS^+$ and 2 µL of the extract, and the absorbance was measured at 734 nm using a spectrophotometer after incubation for 6 min. The reaction mixture containing the test sample and standard was used as a control. The concentration of the sample required to scavenge 50% of the $ABTS^+$ radical ions was calculated to produce IC_{50} values from the graph and the values were expressed as mg gallic acid required/g of extract was used to express $ABTS^+$ ion scavenging capacity.

Ferric reducing antioxidant power (FRAP) assay

The capacity of *S. graminifolium* extracts to reduce ferric ions ($Fe3^+$) to ferrous ions ($Fe2^+$) was determined using the FRAP method described by Skroza *et al.* with a few modifications in triplicate[13]. The FRAP reagent was mixed with the ethyl acetate fraction of *S. graminifolium* and incubated for 30 min, and the absorbance was measured at 593 nm using a UV-Vis spectrometer. The results were expressed as milligrams of gallic acid equivalent per gram of dry extract.

Cell culture

HepG2 liver cells were cultured in RPMI medium containing 10% inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 until they reached confluency. To detach the cells, 0.05% trypsin was applied, followed by centrifugation at 1000 rpm for five minutes. The supernatant was removed and the cell pellet was resuspended in 2 mL of fresh RPMI medium. After evaluating cell viability, a single cell suspension with a concentration of 5.0x105 cells/mL was prepared.

Cell viability (MTT) assay

HepG2 liver cells were grown in microtiter plates at a density of approximately 1 × 104 cells/well. Cell viability was assessed after exposure to various PSG concentrations (0, 5, 10, 20, 40, and 100 µg/mL). Subsequently, 20µL of MTT was added to each well, and the cells were incubated at 37°C for 4 h. After removing the medium, 150 µL of DMSO was added to each well to solubilize the formazan crystals. Optical density was measured at 570 nm using a Spectramax i3X microplate reader (Molecular Devices, USA)[11]. The percentage inhibition was calculated as follows.

% inhibition= [(OD of test − OD of control)/OD of test] × 100

***In vivo* studies**

The experimental animals were maintained in ventilated polysulfonate cages with sterilized corncob bedding (Sparconn Life Sciences Pvt. Ltd., Karnataka). Rats were kept in a climate-controlled setting at 23 ± 2˚C and 55-65% relative humidity with a pelleted diet (VRK Industries Pvt. Ltd., Maharashtra, India), and unrestricted access to water was provided *ad libitum*. The study maintained a consistent 12-hour light-dark cycle. Prior to each experiment, the animals were acclimated to laboratory conditions. This research adhered to the ARRIVE (Animals Research Reporting *In Vivo* Experiments (ARRIVE) guidelines. The study design and execution strictly followed the ethical standards endorsed by the Committee for Control and Supervision of Experiments on Animals (CCSEA) and the Institutional Animal Ethical Committee (IAEC No. VFSTR 2046/IAEC/VI/2024-1) of School of Biotechnology and Pharmaceutical Sciences, Guntur, Andhra Pradesh.

Acute toxicity study

Acute toxicity was determined according to OECD 425 guidelines for female Wistar rats. The control group animals received the vehicle 0.5% carboxymethyl cellulose (CMC), whereas the test group received 2000 mg/kg PSG orally as a single dose. All behavioral, motor, and autonomic parameters were assessed according to guidelines. The animals were monitored twice daily for moribund or mortality, and their body weights were recorded weekly. After 14 days, surviving animals were euthanized using carbon dioxide (CO_2), dissected, and subjected to a thorough gross necropsy examination.

Group	Treatment	Purpose
Group 1	0.9% NaCl	Served as normal control
Group 2	1mL/kg CCl_4 in olive oil (1:3 ratio)	Served as disease control
Group 3	Silymarin 50mg/kg+1mL/kg CCl_4 in olive oil	Served as standard treatment control
Group 4	PSG 200 mg/kg+1mL/kg CCl_4 in olive oil	Served as test control at low dose
Group 5	PSG 400 mg/kg+1mL/kg CCl_4 in olive oil	Served as test control at high dose

Treatment schedule

Thirty male Wistar albino rats were randomly divided into five groups with six animals in each group. The treatment schedule was as follows: all oral administrations were administered daily, preferably simultaneously, for 14 consecutive days.

At the end of the study, all rats were euthanized under isoflurane anesthesia using a small animal anesthesia system (Orchid Scientifics Pvt. Ltd.). Blood samples were extracted from the retro-orbital plexus of rats in each group using heparinized capillary tubes and centrifuged at 3000 rpm for 10 min to collect serum for subsequent biochemical analysis.

Estimation of serum biochemical and inflammatory markers

The biochemical parameters of liver function, including ALT, AST, ALP, total bilirubin, direct bilirubin, total protein, and the lipid profile markers TG, total TC, HDL-c, LDL-c, and VLDL-c, were estimated to assess the hepatoprotective potential of PSG using standard protocols of commercial kits and an automated biochemical analyzer (ERBA EM360). Serum levels of inflammatory markers such as tumor necrosis factor-alpha (TNF-α), IL6, IL-1β, and NF-κB were estimated using enzyme-linked immunosorbent assay (ELISA) (Abbkine, USA)[14].

Estimation of tissue antioxidant enzymes

Liver tissues were carefully excised from the surrounding tissues, rinsed with ice-cold phosphate-buffered saline, and weighed. The liver samples were then homogenized with phosphate-buffered saline (25 mM, pH 7.4) and centrifuged at 2500 rpm for 10 min, and the resulting supernatant was collected and stored at −20 °C for further use. Oxidative stress markers were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Abbkine, USA) following the manufacturer's protocol. The assessed parameters included catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPx)[11].

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) Analysis

To study the gene expression of Nrf2 and HO-1, RNA was extracted from liver tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized using the Script™ cDNA synthesis kit

(Bio-Rad, California, CA, USA). A Bio-Rad CFX 96 instrument was used for quantitative real-time PCR (qRT-PCR) with SYBR® Green (Invitrogen). The amplification protocol consisted of 2 min at 95 °C, followed by 40 cycles of 60 s at 94 _C and 90 s at 60 °C. Each assay was conducted in duplicate to determine the delta CT. IDT supplied the specific primers. β-actin served as the housekeeping gene. The following primer sequences were used: Nrf2 and HO-1[15].

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
Nrf2	TTGTAGATGACCATG AGTCGC	TGTCCTGCTGTATGC TGCTT
HO-1	GTAAATGCAGTGTTG GCCCC	ATGTGCCAGGCATC TCCTTC
β-actin	AGGAGTACGATGAG TCCGGC	CGCAGCTCAGTAAC AGTCCG

Western blot analysis of Nrf2 and HO-1 protein expression

Liver tissues were cleaned and homogenized in radioimmunoprecipitation buffer. The mixture was heated to 70°C for 5min and used for protein separation on a 4% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). Each well was filled with 15µL of the test sample and 6µL of Tris-HCl loading gel buffer, and the gel was run for 1h. Proteins were subsequently transferred onto a polyvinylidene fluoride membrane, and the quality of the transfer was assessed using Ponceau staining. Blocking was performed in a solution of 3% skim milk in 20mM Tris buffer containing 150mM saline and 0.1% Tween 20 (TBST) in 1XPBS for 1 h. The membrane was washed thrice with TBST for 5 min at a constant temperature. Primary antibodies (1:1000 dilution of Nrf2 and HO-1) were incubated with the membrane in 1XPBS overnight at 4°C with continuous shaking. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for approximately 2h and washed three times with 1XPBST for 5 min each. The membrane was visualized using the chromomeric substrate 3, 3'-diaminobenzidine. The visible bands were photographed, and quantitative densitometry analysis was performed using ImageJ software. β-actin antibody served as a loading control[33].

Histopathological examination of CCl_4- intoxicated liver tissue

The collected liver tissues were cleaned with ice-cold saline and fixed in 10% buffered formalin solution. Liver samples were processed using an automatic tissue processor and embedded in paraffin wax using Leica EG 1160. Using a microtome, each tissue was cut into sections that 5-6 µm thickness. The processed tissues were stained with hematoxylin and eosin to observe histological alterations in the liver under a microscope. A light microscope (Olympus CX 31; Olympus, Japan) was used for microscopic observation.

Statistical analysis

The obtained data are represented as the mean ± standard error of the mean (SEM) using the statistical program Graph Pad Prism 5. The data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test as a post-parametric test. Statistical significance was set at $P< 0.05$.

Results

Percentage yield

The percentage yield of the crude ethanolic extract of *S. graminifolium*was found to be 8.2% whereas n-hexane 1.82%, dichloromethane 0.16% and ethyl acetate fraction was 0.15%.

Total phenolic content and marine polyphenol abundance of *S. graminifolium*

The total phenolics quantity of crude extract of *S. graminifolium* was 13.54 ± 0.06 mg, and the ethyl acetate fraction was 6.34 ± 0.02 mg of phloroglucinol equivalent per gram. A significant difference was found in the total phenolic sum of ethyl acetate fraction than ethanolic crude extract. The total marine polyphenols (phlorotannins) present in the ethyl acetate fraction and crude ethanolic extract of *S. graminifolium* were quantified using the DMBA assay. PSG contained more than double the quantity (11.35± 0.05 mg of phloroglucinol/g) extract of phlorotannin compared to the crude extract (4.59± 0.01 mg of phloroglucinol/g extract).

LC-QTOF-HRMS analysis of marine polyphenols of *S. graminifolium*

Data obtained from HRMS analysis were processed by eliminating ion peaks with more than 50% missing values within each group. Missing values were imputed by replacing half of the minimum detected value with the remainder. The positive ion data were analyzed using the amalgamation database as the input. These data reveal the presence of biologically active polyphenols and other compounds with independent molecular weights. The mass spectrometry results were collected in positive mode $[M + H]^+$ (Fig. 1 and Table 1) from *m/z* 100 to 1500. The data were then analyzed by searching for theoretical monoisotopic masses equivalent to those

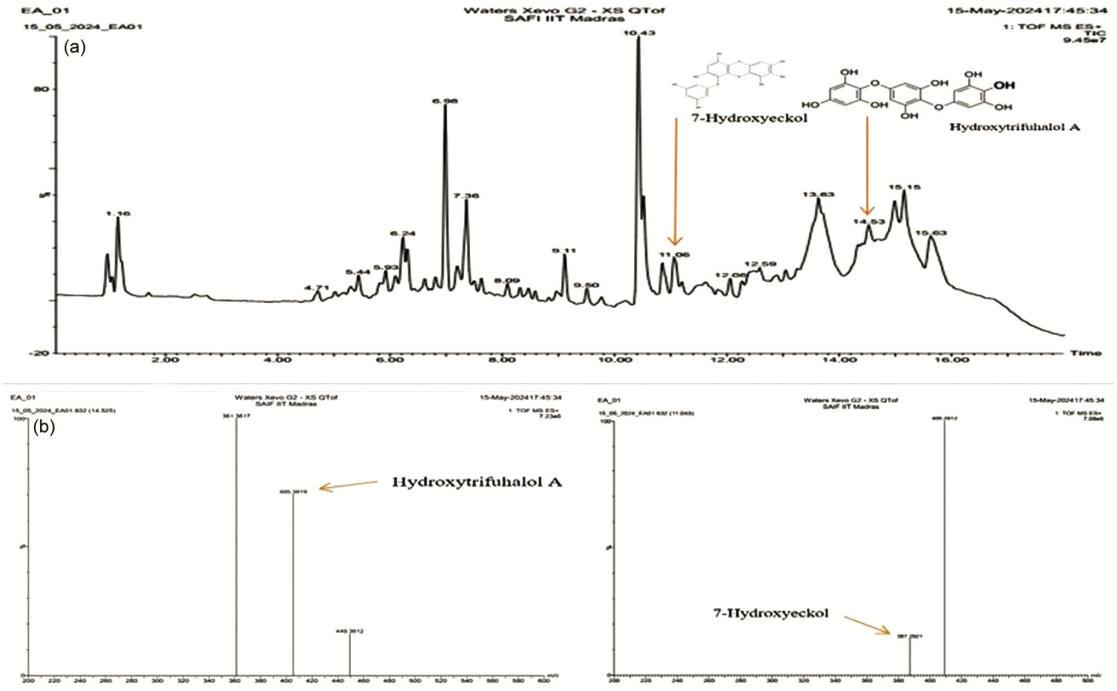

Fig. 1 — A) Total chromatogram of ethyl acetate fraction of *S. graminifolium* B) Mass spectrums of tentatively identified marine polyphenols.

of identified compounds, including polyphenols. The compounds identified by LC-MS from the ethyl acetate fraction of *S. graminifolium* correlated with the previously identified compounds, with m/z values similar to those of various marine brown algae. 7-hydroxyeckol was identified with *an m/z* value of 387.2921 and retention time (RT) of 11.043. Hydroxytrifuhalol A, with RT 14.525 and *m/z* 405.3619[32], along with unknown polyphenols and other compounds, were identified based on their individual *m/z* values (Table 1).

Table 1 — Identification of marine polyphenols from ethyl acetate fraction of *S. graminifolium.*

RT	*m/z* value	Compound	Tentative name
11.043	409.1631		Phlorotannin derivative
0.969	244	$C_{13}H_{20}O_3$	derived by oxidation of a carotenoid pigment
7.358	453.9169	$C_{18}H_{15}Br_2NO_3$	
11.043	387.2921	$C_{18}H_{12}O_{10}$	7-Hydroxyeckol
14.525	405.3619		Hydroxytrifuhalol A
10.414	318.4525	C11H12NSO8	Unknown polyphenol
10.414	230.4336	C8H7O6S	Phenolic acid sulphate

FT-IR spectral analysis

FT-IR spectral analysis of PSG revealed significant peaks within the range of 3855-540 cm-1. Peaks at stretching bands around 3650.59 based on correspond to the –OH stretching, 3019.01 and 2985.27 cm−1 are for aromatic C–H stretching, 1734.66 cm−1 for C═C stretching,1375.00 cm−1 indicates the presence of carbonate group. Similarly, 1246.75 cm−1 denotes C–N═O dimer, 1043.30, 847.56, and 765.60 cm−1 are responsible for the aromatic rings with C–H vibrations 607.47 to 254.54 are responsible for halogen alkyl groups (Fig. 2).The spectra obtained for the ethyl acetate fraction of *S. graminifolium*were compared and interpreted with those of phloroglucinol. Similar functional groups were found, confirming the presence of multiple phloroglucinol molecules in different marine polyphenols.

Free radical scavenging potential of PSG

The polyphenol-rich fraction of *S. graminifolium* showed robust radical scavenging activity against DPPH radical scavenging assay at various concentrations (20, 40, 60, 80 and 100µg/mL). The 50% inhibitory concentration (IC_{50}) was found to be 52.50 µg/mL whereas that of ascorbic acid was 47.34 µg/mL. The highest radical scavenging activity was observed at 100 µg/mL of the ethyl acetate fraction of *S. graminifolium* compared to the control (Fig.3).

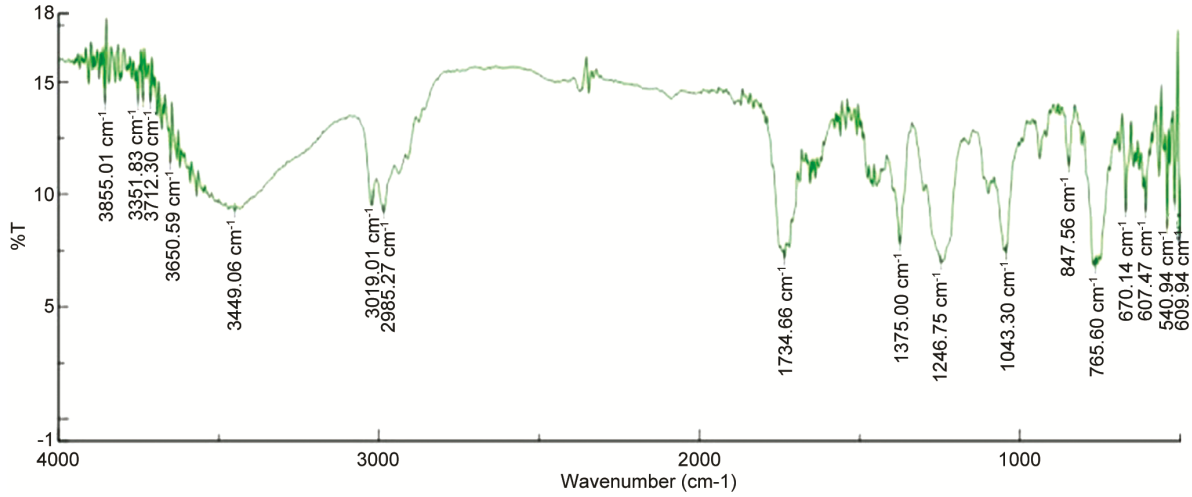

Fig. 2 — FTIR spectral analysis of polyphenol rich fraction of *S. graminifolium.*

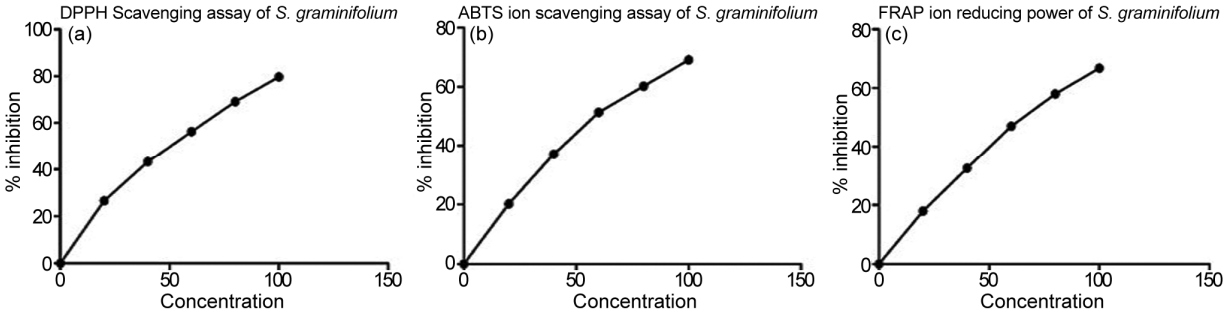

Fig. 3 — *In vitro* antioxidant potential of polyphenol rich fraction of *S. graminifolium*, (a) DPPH radical scavenging assay,(b) $ABTS^+$ scavenging assay, (c) FRAP assay.

$ABTS^+$ and FRAP reducing power of *S. graminifolium*

The *in vitro* antioxidant potential of *S. graminifolium* was evaluated using the $ABTS^+$ scavenging assay and ferric (Fe^{3+}) ion reducing power using ascorbic acid as a standard. The ethyl acetate fraction of *S. graminifolium* showing a significant antioxidant effect through scavenging $ABTS^+$ ions and the IC_{50} value was found to be 63.9 µg/mL while ascorbic acid was 48.65 µg/mL. Similarly, *S. graminifolium* significantly reduced ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions with an IC_{50} value of 69.03 µg/mL while ascorbic acid was 48.09 µg/mL (Fig. 3).

Effect of PSG on HepG-2 cell viability

The effect of *PSG* on HepG-2 cell viability was confirmed using the MTT assay at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. Cell viability declined as the concentration of PSG increased, and the IC_{50} value was >100 µg/mL with no significant cytotoxicity (Fig. 4).

Effect of PSG on acute oral toxicity

Acute toxicity studies revealed no signs of toxicity, fatalities, or severe illness within 24 h of administering the test drug at a 2000 mg/kg body weight. Similar outcomes were observed during the 14-day observation period. No statistically significant differences in body weight were observed between the control and drug-treated groups. Consequently, to assess the hepatoprotective activity, 200 mg/kg was chosen as the lower therapeutic dose, whereas 400 mg/kg was selected as the higher dose.

Effect of PSG on liver function biomarkers

The disease control group, which received CCl_4 alone, showed a significant (P<0.05) increase in AST, ALT, total bilirubin, direct bilirubin, and ALP levels and a significant (P<0.05) reduction in total protein levels compared to the normal control, indicating severe liver damage. PSG treatment exhibited a dose-dependent hepatoprotective effect by significantly (P<0.05) decreasing AST, ALT, total bilirubin, direct bilirubin,

and ALP levels, while increasing total protein levels, compared with the disease control (Fig. 5).

Effect of PSG on lipid profile in CCL_4 treated rats

The study observed a significant (P<0.05) elevation in serum lipid parameters, while a significant (P<0.05) reduction in HDL-C levels was observed in the disease control group compared with the normal group. Pre-treatment with PSG at doses of 200 and 400 mg/kg body weight significantly (P<0.05) reduced total cholesterol, triglycerides, LDL-C, and VLDL-C levels, and significantly (P<0.05) increased HDL-C levels compared to the disease control group in a dose-dependent manner (Table 2).

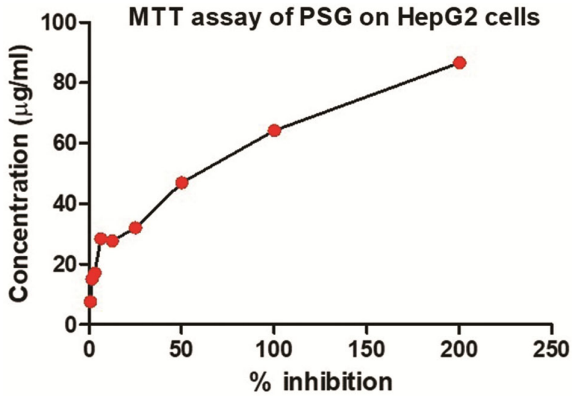

Fig. 4 — *In vitro* cytotoxicity assay of polyphenol rich fraction of *S. graminifolium.*

Effect of PSG on inflammatory markers in CCL_4 treated rats

The results demonstrated that CCl_4 administration cause as significant (P<0.05) increase of inflammatory cytokines (IL-1β, IL-6, TNF-α) and NF-κB activation in liver tissue compared to the normal control group, indicating the induction of hepatic inflammation. Treatment with PSG at doses of 200 and 400 mg/kg produced a significant (P<0.05)

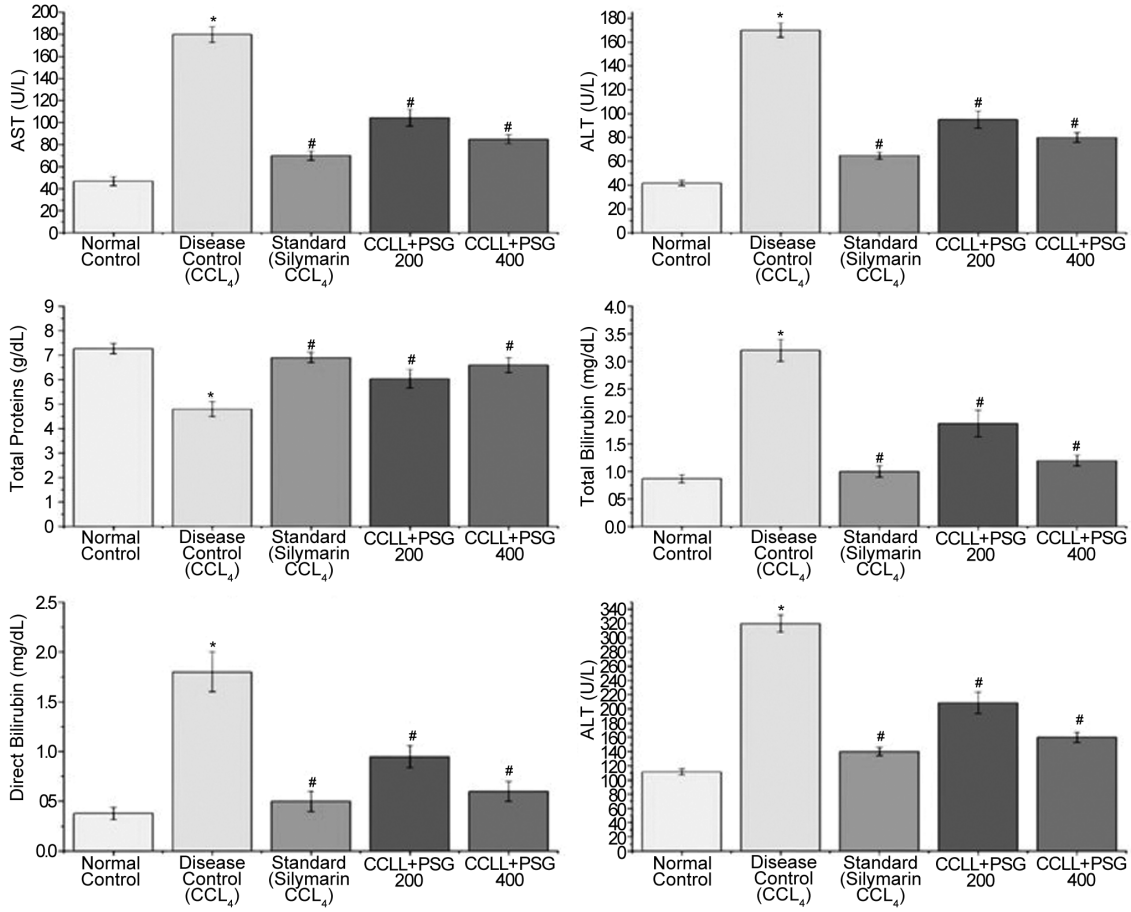

Fig. 5 — Effect of *S. graminifolium* on liver function biomarkers. All data are presented as the mean ± SEM; n=6; the data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. *P<0.05, compared with normal control; #p<0.05, compared with disease control.

reduction in these inflammatory markers, with a significant reduction in NF-κB activation, compared to the disease control group (Fig. 6).

Effect of PSG on oxidative markers in CCL_4 intoxicated rats

Changes in the levels of tissue-based antioxidant enzymes, such as SOD, GSH, catalase, and MDA, in experimental rats intoxicated with CCL_4 were estimated (Table 3). A significant ($P< 0.05$) decline in the levels of SOD, GSH, and catalase, and a significant ($P< 0.05$) increase in MDA levels were observed in the liver tissues of the disease control rats. However, these levels were significantly ($P< 0.05$) restored near normal pretreatment with PSG at doses of 200 and 400 mg/kg and the standard drug silymarin when compared with the disease control rats.

Effect of PSG on antioxidant pathway in CCL_4 treated rats

This study assessed the mRNA expression levels of Nrf2 and HO-1, which are key regulators of the antioxidant defense system. The mRNA expression levels of Nrf2 and HO-1were significantly ($P<0.05$) reduced in CCl_4-induced hepatotoxic rats compared with those in the normal control group (Fig. 7). Treatment with silymarin and PSG at 200 and 400 mg/kg significantly ($P<0.05$) upregulated Nrf2 and HO-1 mRNA expression compared with that in the disease control, suggesting an enhanced antioxidant response.

Effect of PSG on Nrf2/HO-1 signaling pathway in CCL_4 intoxicated rats

The mRNA expression levels of Nrf2 and HO-1 and the protein levels (Fig. 8) determined through western blot analysis showed a significant ($P<0.05$)reduction in both the levels in the CCl_4-induced hepatotoxic rats compared with the normal control group. PSG treatment significantly ($P<0.05$) enhanced the expression levels of Nrf2/HO-1 compared to the disease control group.

Table 2 — Effect of poly phenol rich fraction of *S. graminifolium* on lipid profile in CCL_4 intoxicated rats.

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal Control	135.21±5.02	105.11±4.05	55.16±3.2	50.21±3.01	30.51±1.04
Disease Control (CCL_4)	200.31±8.05*	180.01±7.11*	25.14±2.07*	140.14±6.07*	45.12±2.01*
Standard (Silymarin + CCL_4)	130.12±5.01$^\#$	100.23±4.15$^\#$	48.11±3.03$^\#$	60.22±3.1$^\#$	22.41±1.01$^\#$
CCL_4 + PSG 200	156.45±7.05$^\#$	134.5±7.53$^\#$	38.02±4.2$^\#$	94.23±7.73$^\#$	33.14±2.77$^\#$
CCL_4 + PSG 400	140.41±6.03$^\#$	110.06±5.11$^\#$	45.05±3.01$^\#$	70.06±4.95$^\#$	25.12±1.1$^\#$

All data are presented as mean ± SEM; n=6. The data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. $^*P<0.05$, compared with the normal control; $^\#P<0.05$, compared with the disease control.

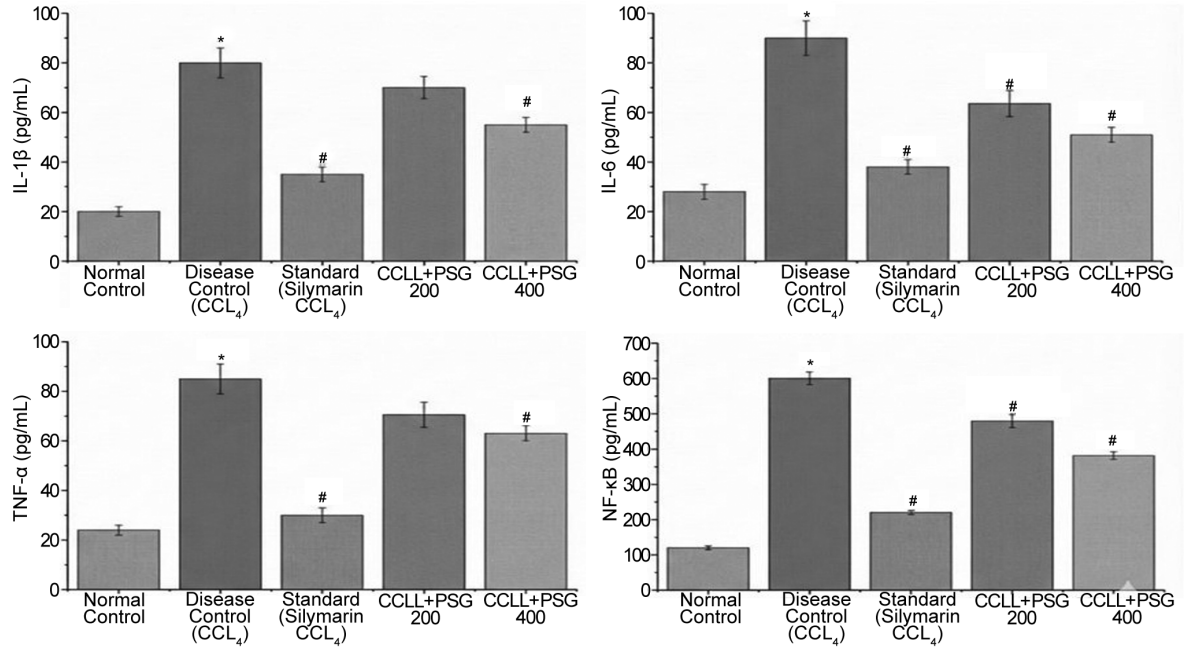

Fig. 6 — Effect of *S. graminifolium* on inflammatory cytokines. All data are presented as the mean ± SEM; n=6; the data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. *$P<0.001$ compared to normal control; #$P<0.05$, compared to disease control.

Table 3 — Effect of PSG on Oxidative markers in CCL_4 intoxicated rats.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/mg protein)	LPO (nmol MDA/mg protein)
Normal Control	7.5±0.3	6±0.3	10.5±0.5	3.2±0.2
Disease Control (CCL_4)	2.5±0.2*	1.5±0.2*	2.8±0.3*	9.2±0.6*
Standard (Silymarin + CCL_4)	7±0.3#	5.5±0.3#	8.8±0.4#	3.8±0.3#
CCL_4 + PSG 200	4.18±0.56#	3.36±0.48#	4.85±0.51#	7.7±0.46#
CCL_4 + PSG 400	5.2±0.3#	4.15±0.3#	6.5±0.4#	6.5±0.3#

All data are presented as the mean ± SEM; n=6; the data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. *P<0.05 compared to normal control; #P<0.05, compared to disease control.

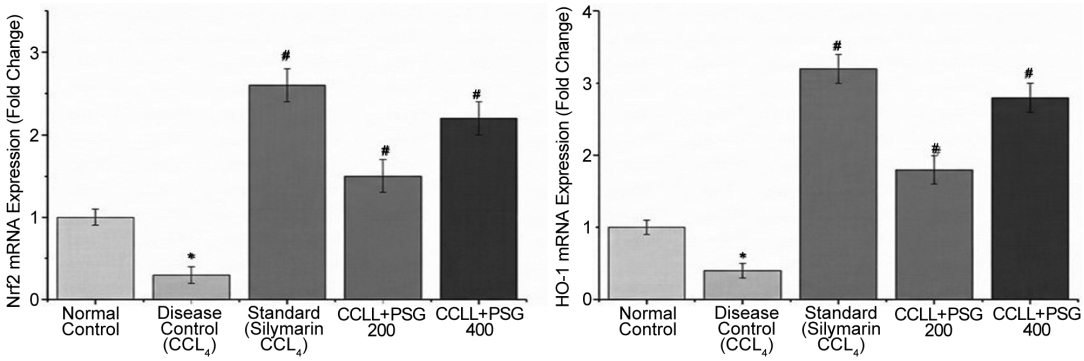

Fig. 7 — Effect of *S. graminifolium* on mRNA expression (qPCR) of Nrf2 and HO-1. All data are presented as the mean ± SEM; n=6; the data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. *P<0.001 compared to normal control; #P<0.05, compared to disease control.

Effect of PSG on histopathological changes of liver in CCl_4 intoxicated rats

Histopathological examination of a normal liver revealed a normal hepatic parenchyma with no signs of inflammation. Hepatocytes exhibit uniform cytoplasm, centrally located nuclei, and minimal sinusoidal congestion or inflammatory cell infiltration, which are indicative of normal liver function. Liver sections of rats treated with CCl_4 showed extensive hepatocellular damage with marked centrilobular necrosis, accompanied by severe hepatocyte degeneration and vacuolar changes. A Noteworthy inflammatory cell infiltration along with early signs of fibrosis and collagen deposition was observed in some areas. In rats treated with CCl_4 along with PSG, exhibited moderate improvement in hepatic structure with less extensive necrosis and inflammatory cell infiltration compared to the disease control group. Rats treated with 400 mg/kg PSG showed near-normal hepatic architecture (Fig. 9). The extent of centrilobular necrosis and inflammation was minimal, and hepatocytes appeared largely intact with restored cytoplasmic and nuclear features.

Discussion

Marine polyphenols (phlorotannins) are important biomolecules in brown algae and have gained attention because of their unique biological properties in the health and food industries. The Folin–Ciocalteu assay is a widely accepted method for quantifying polyphenols in brown algae. However, it yielded positive results, irrespective of the type of polyphenolic compounds present in the extract. In the present study, the ethyl acetate fraction of S. *graminifolium* had a higher phenolic content than the crude extract, indicating that phlorotannins are moderately polar compounds that are maximally released in ethyl acetate. Phlorotannins are conjugative compounds of phloroglucinol attached to hydroxyl groups at the 1, 3, and 5 positions in their chemical structures. DMBA interacts only with phlorotannins having 1, 3, and 1, 3, 5- hydroxyl groups and it is a unique assay to quantify the marine polyphenols of brown algae. Our study revealed that as the concentration of PSG increased, the polyphenol abundance also increased significantly, with the maximum abundance being observed in the ethyl acetate fraction[16]. Additionally, FTIR analysis confirmed the presence of –OH stretching, aromatic C–H stretching, and C═C stretching in PSG, similar to the functional groups found in phloroglucinol.

The concentration-dependent increase in antioxidant activity of PSG in DPPH, $ABTS^+$, and

FRAP reducing assays indicated that the total phenolic content and phlorotannin abundance are directly proportional to the *in vitro* antioxidant potential of PSG. Furthermore, previous studies have corroborated the potential antioxidant effects of *S.graminifolium*[17].

The present study attempted to characterize the possible polyphenols using LC-qTOF-MS based on their *m/z* value in the ethyl acetate fraction of *Sargassum graminifolium*. The data reveals the presence of biologically active polyphenols and other compounds with independent molecular weights. The identified polyphenols were correlated with previously identified compounds, with *m/z* values similar to those of various marine brown algae. 7-hydroxyeckol with an *m/z* value of 387.2921 and retention time (RT) of 11.043 was identified, and hydroxytrifuhalol A with RT 14.525 and *m/z* value 405.3619, along with unknown polyphenols and other compounds, were identified on the basis of their individual *m/z* values. Corona et al., identified 7-hydroxyeckol and hydroxytrifuhalol A as metabolites after the administration of phlorotannin rich extract and this study observed that significant attenuation of IL-8 pro-inflammatory cytokinin[18]. However, in the present study, these compounds were identified from PSG, and they might also contribute to attenuating pro-inflammatory cytokines in CCl_4 intoxicated rats.

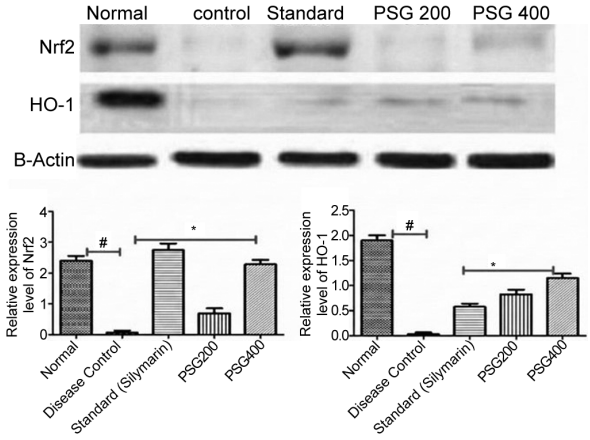

Fig. 8 — Effect *of S. graminifolium* on quantitative estimation of Nrf2 and HO-1 protein levels through western blot analysis. All data are presented as the mean ± SEM; n=6; the data were subjected to one-way Analysis of Variance (ANOVA) with Tukey's post hoc test. **P*<0.05 compared to normal control; #*P*<0.05, compared to disease control.

The severity of liver toxicity is determined by elevated levels of liver enzymes, such as AST, ALT, ALP, and total bilirubin, irrespective of the causative agent. However, untreated liver injury can lead to acute liver toxicity and may lead to acute liver failure, a life-threatening complication of acetaminophen poisoning. CCl_4-induced liver toxicity in rats is a widely accepted experimental animal model for evaluating hepatoprotective drugs. CCl_4 produces excessive free radicals that further damage hepatocytes, as evidenced by the elevation of liver function tests. The present study shows that CCl_4 administration led to a substantial increase in liver function biomarkers such as AST, ALT, and ALP, along with elevated total bilirubin, suggesting impaired bilirubin metabolism and denoting the effective induction of CCl_4 toxicity. Rats pretreated with PSG demonstrated a dose-dependent reversal of these elevated liver function biomarkers, suggesting that the phlorotannin abundance and other biologically important compounds of PSG improved hepatocyte membrane integrity, reduced enzyme leakage, and restored liver function at multiple levels, consistent with previous studies[19]. Increased lipid levels and reduced HDL cholesterol with CCl_4 administration reflect the reduced capacity of the liver to synthesize, process, and excrete lipids, culminating in a dyslipidemic state that exacerbates hepatic damage[20]. The findings of the present study are consistent with previous research on marine polyphenolic extracts from *Sargassum* species and have also demonstrated improvements in lipid metabolism in toxin-induced liver injury models. Kim *et al.* observed that phlorotannins, such as eckol and

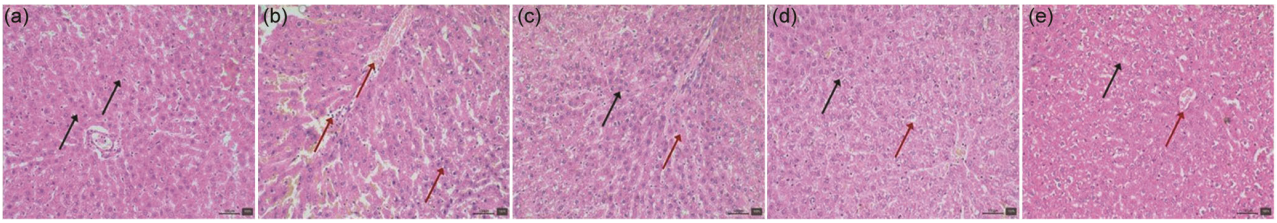

Fig. 9 — Effect of *S. graminifolium* on histopathological changes in the liver of CCl_4 intoxicated rats. (a) Normal Control, (b) Disease Control (CCl_4), (c) Standard (Silymarin + CCl_4), (d) CCl_4 + PSG 200mg/kg, (e) CCl_4 + PSG 400 mg/kg. H&E Stain; 200x Magnification; back arrows indicate normal hepatic cellular architecture with normal sinusoids. The red arrow indicates hepatic degeneration and vacuolization.

phlorofucofuroeckol A, isolated from the marine brown algae *Ecklonia stolonifera*, exhibited significant hepatoprotective activity in HepG2 cells through an antioxidant mechanism[21]. Inflammatory process is one of the key mechanisms underlying acute liver toxicity and the free radicals that are produced during CCl_4 is metabolized may disrupts the lipid membrane of hepatocytes to initiate peroxidation, inflammation followed by necrosis[22]. To assess the anti-inflammatory potential of *S. graminifolium*, pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) were measured. The results showed a noticeable increase in these pro-inflammatory cytokines in CCl_4 alone treated rats whereas pre-treatment with PSG significantly attenuated the inflammatory response by inhibiting NF-κB activation and downregulating the expression of pro-inflammatory cytokines, thereby dampening immune cell infiltration and mitigating hepatocellular apoptosis. Research has demonstrated that *Sargassum* exhibits anti-inflammatory effects by decreasing the synthesis of pro-inflammatory cytokines, including TNF-α, interleukin-6, and interleukin-1β. Additionally, it inhibits NF-κB, cyclooxygenase-2, and inducible nitric oxide synthase pathways, further contributing to its anti-inflammatory properties[23]. A recent study observed that the ethanolic extract of *Sargassum boveanum* in CCl_4 intoxicated rats effectively reduced the gene expression of TNF-α and NF-κB[24]. This finding corroborates the potential of PSG to attenuate toxin-induced inflammation.

The metabolites of CCl_4 (CCl_3 and CCl_3O_2) initiate oxidative stress and deplete antioxidant reserves, triggering lipid peroxidation. The present study showed that oxidative stress results in elevated malondialdehyde (MDA) levels, a key indicator of lipid peroxidation, and a concurrent reduction in essential antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). Treatment with PSG is effective in combating oxidative stress by replenishing antioxidant stores and restoring enzyme function, thereby reducing the accumulation of reactive oxygen species and limiting lipid peroxidation, as indicated by decreased MDA concentrations. Recent studies have revealed that *Sargassum* polyphenols effectively activate cellular antioxidant enzymes and hamper oxidative stress markers, such as malondialdehyde, and elicit their potential antioxidant effects to reduce inflammation and tissue injury[25].In the same way, PSG treatment significantly alleviated oxidative stress by reducing MDA levels and improving antioxidant defenses, such as SOD, CAT, and GSH, along with reducing inflammation, which are interconnected processes that contribute to the advancement of CCl_4-induced liver toxicity.

Additionally, this study attempted to validate the involvement of a specific mechanism (Nrf2 and HO-1 pathways) through which PSG exerts hepatoprotective effects. Under normal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, which facilitates its ubiquitination and subsequent degradation. CCl_4 metabolites and other free radicals dissociate Nrf2 from Keap1, allowing its nuclear translocation and subsequent binding to the antioxidant response element (ARE) in the promoter regions of cytoprotective genes. This activation leads to the upregulation of heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1), which enhances the ability of cells to neutralize ROS and repair oxidative damage[26]. The present study shows that PSG treatment in CCL_4 intoxicated rats upregulated Nrf2/HO-1 expression and nuclear translocation compared to control animals, indicating that PSG potentially activates the endogenous antioxidant pathway to yield hepatoprotection. Moreover, quantitative estimation of Nrf2/HO-1 proteins through western blot analysis also supports the involvement of the Nrf2/HO-1 molecular pathway to elicit PSG as a potential hepatoprotective alternative. The previous research also corroborates *Sargassum* species significantly activates Nrf2/HO-1 Han*et al.* emphasize sargachromenol a bioactive compound isolated from *Sargassum horneri* activated the Nrf2/HO-1 pathway[27]. In another study *Sargassum serratifolium* extract stimulates Nrf2/HO-1 signaling pathway against oxidative stress[28] pacifies that *Sargassum* species consumption may emerge as an effective alternative to counter toxin-induced hepatotoxicity. Therefore, oxidative stress is reduced, as indicated by lower MDA levels, stabilization of hepatocyte membranes, and improvement in overall liver function. The DMBA assay confirmed the abundance of phlorotannin, and LC-MS analysis revealed that the polyphenols, hydroxytrifuhalol-A and 7-hydroxyeckol, may be involved in eliciting the antioxidant and anti-inflammatory effects of PSG.

Histopathological analysis further validated these findings, revealing significant improvements in liver tissue architecture following PSG treatment. CCl_4 exposure resulted in severe hepatocellular

degeneration, inflammatory infiltration, vacuolization, and fibrotic changes, all of which are indicative of profound liver damage. PSG treatment notably reduced these pathological alterations in a dose-dependent manner, preserved hepatocyte integrity, and reduced fibrosis. As marine brown algae (*S. graminifolium*) have potential biological effects, including hepatoprotective effects, their effective utilization as a food source can offer innovative solutions to combat poverty and reduce economic burden.

Conclusion

In conclusion, the findings of this study indicate that *S. graminifolium* maximum has an abundance of phlorotannins, which were confirmed through the DMBA assay, and possible bioactive compounds, including phlorotannins such as hydroxytrifuhalol-A and 7-hydroxyeckol, were tentatively identified through LC-MS analysis. Furthermore, PSG potentially reversed CCl_4-induced hepatotoxicity by restoring the liver function lipid profile biomarkers to near normal levels. Furthermore, the anti-inflammatory effect was achieved by the significant reduction of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and NF-κB. *S. graminifolium* plays a crucial role in replenishing endogenous antioxidant enzymes, including SOD, CAT, and GSH. This antioxidant effect was mediated by the activation of the Nrf2/HO-1 signaling pathway which were confirmed by qPCR and western blot analysis. Additionally, the hepatoprotective effect was confirmed by the restoration of liver architecture through histopathological changes. Future research should focus on identifying the bioactive polyphenols in *S. graminifolium* and exploring their pharmacokinetics, molecular mechanisms, and clinical applications as alternatives for treating liver disorders. Confirmed

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Ethics approval

The experimental design and implementation strictly followed the ethical criteria established by the Committee for Control and Supervision of Experiments on Animals (CCSEA) and the Institutional Animal Ethics Committee (IAEC No. VFSTR 2046/IAEC/VI/2024-1)of Animal house facility, Department of Pharmaceutical Sciences, Vignan's Foundation for Science, Technology and Research, Guntur, Andhra Pradesh.

Conflict of Interest

The authors declare that they have no competing interests.

References

1 Gao S, Chen X, Yu Z, Du R, Chen B, Wang Y, Cai X, Xu J, Chen J, Duan H & Cai Y. Progress of research on the role of active ingredients of *Citri Reticulatae Pericarpium* in liver injury. *Phytomedicine,* 115 (2023)154836.

2 Younossi, ZM, Wong G, Anstee QM & Henry L. The global burden of liver disease. *Clin Gastroenterol Hepatol*, 21 (2023)1978.

3 Fareed MM, Khalid H, Khalid, S & Shityakov S. Deciphering molecular mechanisms of carbon tetrachloride-induced hepatotoxicity: A brief systematic review. *Curr Mol Med*, 24 (2024)1124.

4 Bardallo RG, Panisello-Rosello A, Sanchez-Nuno S, Alva N, Rosello-Catafau J & Carbonell T. Nrf2 and oxidative stress in liver ischemia/reperfusion injury. *FEBS J*, *289* (2022) 5463.

5 Saeed F, Zohra KT, Naveed K, Zia A, Khaliq M, Noor Z, Khaliq K & Ali MA. Algal proteins for sustainable nutrition and functional food innovation. *Appl Food Res*, 5 (2025) 100752.

6 Afreen AB, Rasool F & Fatima M. Bioactive properties of brown seaweed, *Sargassum wightii* and its nutritional, therapeutic potential and health benefits: A review. *J Environ Biol*, 44 (2023) 146.

7 Ersoydan S, & Rustemeyer, T. Investigating the anti-inflammatory activity of various brown algae species. *Mar Drugs*, *22* (2024) 457.

8 Hodhodi A, Babakhani A & Rostamzad H. Effect of different extraction conditions on phlorotannin content and antioxidant activity of extract from brown algae (Sargassum angustifolium). *J Food Process Preserv*, 46 (2022) 16307.

9 Poole J, Diop A, Rainville L-C and Barnabé S. Bioextracting Polyphenols from the Brown Seaweed *Ascophyllum nodosum* from Québec's North Shore Coastline. *Industrial Biotechnology*, 15 (2019) 212.

10 Obluchinskaya ED, Pozharitskaya ON, Shevyrin VA, Kovaleva EG, Flisyuk EV & Shikov AN. Optimization of extraction of phlorotannins from the arctic *Fucus vesiculosus* using natural deep eutectic solvents and their HPLC profiling with tandem high-resolution mass spectrometry. *Marine Drugs*, 21 (2023) 263.

11 Pullaiah CP, Nelson VK, Rayapu S, Narasimha Kumar GV and Kedam T. Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: in vivo and in vitro evidence. *BMC Pharmacol. Toxicol*, 22 (2021) 43.

12 Skroza D, Generalić Mekinić I, Svilović S, Šimat V and Katalinić V. Investigation of the potential synergistic effect of resveratrol with other phenolic compounds: A case of binary

phenolic mixtures. *J Food Compo. Anal,* 38 (2015) 13.

13 Zarei MH, Lorigooini Z, Khoei HA & Bijad E. Acute oral toxicity assessment of galbanic acid in albino rat according to OECD 425 TG. *Toxicology Reports*, 11 (2023) 111.

14 Vijay NKG & Vellapandian C. Ameliorative effects of phlorotannin-rich fraction of *Sargassum tenerrimum* in high-fat diet and low dose streptozotocin-induced metabolic changes and oxidative stress in diabetic rats. *J Herbmed Pharmacol*, 12 (2023) 367.

15 Ma H, Yang B, Yu L, Gao Y, Ye X, Liu Y & Li E. Sevoflurane protects the liver from ischemia-reperfusion injury by regulating Nrf2/HO-1 pathway. *Eur J Pharmacol*, 898 (2021) 173932.

16 Al-Amarat W, Abukhalil MH, Alruhaimi RS, Alqhtani HA, Aldawood N, Alfwuaires MA & Mahmoud A. Upregulation of Nrf2/HO-1 signaling and attenuation of oxidative stress, inflammation, and cell death mediate the protective effect of apigenin against cyclophosphamide-induced hepatotoxicity. *Metabolites*, *12* (2022) 648.

17 Li Y, Fu X, Duan D, Liu X, Xu J and Gao X. Extraction and Identification of Phlorotannins from the Brown Alga, *Sargassum fusiforme* (Harvey) Setchell. *Mar drugs,* 15 (2017) 49.

18 Baek SH, Cao L, Jeong SJ, Kim HR, Nam TJ & Lee SG. The Comparison of Total Phenolics, Total Antioxidant, and Anti-Tyrosinase Activities of Korean *Sargassum* Species. *J Food Qual*, 2021(2021) 7.

19 Zhang CY, Wu WH, Wang J and Lan MB. Antioxidant properties of polysaccharide from the brown seaweed *Sargassum graminifolium* (Turn.), and its effects on calcium oxalate crystallization. *Mar Drugs*, 10 (2012) 119.

20 Corona G, Ji Y, Anegboonlap P, Hotchkiss S, Gill C, Yaqoob P, Spencer JPE and Rowland I. Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *Br J Nutr,* 115 (2016) 1240.

21 Reddy GD, Mefi MB, Bharathy H, Kumar GVN, Pullaiah CP, Ganesan R. & Rajeswaran PS. Hepatoprotective activity of Aruvadha churnam a traditional siddha formulation against paracetamol induced liver damage in rats. *Res J Pharm Technol*, 11(2018) 3380.

22 Brai BIC, Falode JA, Adisa RA & Odetola AA. Effects of aqueous leaf extract of avocado (Persea americana) on total cholesterol, triacylglycerols, protein and haematological parameters in CCl4-intoxicated rats. *Clin Phytosci*, 6 (2020) 14.

23 Kim YC, Ren BA, Na YY, Taek JN and Jae SC. Hepatoprotective constituents of the edible brown alga *Ecklonia stolonifera* on tacrine-induced cytotoxicity in Hep G2 cells. *Arch Pharmacal Res*, 28 (2005) 1376.

24 Yang YM, Cho YE & Hwang S. Crosstalk between oxidative stress and inflammatory liver injury in the pathogenesis of alcoholic liver disease. *Int J Mol Sci*, *23* (2022) 774.

25 Saraswati, Giriwono PE, Iskandriati D, Tan CP & Andarwulan N, *Sargassum* Seaweed as a Source of Anti-Inflammatory Substances and the Potential Insight of the Tropical Species: A Review, *Mar Drugs*, 17 (2019) 590.

26 Janahmadi Z, Jaberie H, Esmaili A & Nabipour I. Hepatoprotective effects of brown algae *Sargassum boveanum* on bile duct-ligated cholestasis in rats are mediated by modulating NF-κB/TNF-α and Nrf2/HO-1 gene expression. *Avicenna J Phytomed*, 13 (2023) 513.

27 Selvasudha N, Ashraf A & Vasanthi HR, Antioxidant potential of bioactive molecules from marine algae in chronic diseases: a critical review of antioxidants from Indian waters, *Marine antioxidants*, (2023) 57.

28 Khan MZ, Li L, Zhan Y, Binjiang H, Liu X, Kou X, Khan A, Qadeer A, Ullah Q, Alzahrani KJ, Wang T, Wang C & Zahoor M, Targeting Nrf2/KEAP1 signaling pathway using bioactive compounds to combat mastitis, *Front Immunol*, 16 (2025) 1425901.

29 Mukherjee AG & Gopalakrishnan AV. The mechanistic insights of the antioxidant Keap1-Nrf2 pathway in oncogenesis: a deadly scenario. *Med Oncol*, *40* (2023) 248.

30 Han EJ, Zhang C, Kim HS, Kim JY, Park SM, Jung WK & Cha SH. Sargachromenol isolated from Sargassum horneri attenuates glutamate-induced neuronal cell death and oxidative stress through inhibition of MAPK/NF-κB and activation of Nrf2/HO-1 signaling pathway. *Mar Drugs*, 20 (2022), 710.

31 Park CH, Hong SH, Shin SS, Hwang DS, Han MH, Cha HJ, Kim S, Kim HS, Kim IS, Park SY, Kim Y & Choi YH, Activation of the Nrf2/HO-1 signaling pathway contributes to the protective effects of *Sargassum serratifolium* extract against oxidative stress-induced DNA damage and apoptosis in SW1353 human chondrocytes, *Int J Environ Res Public Health*, 15 (2018) 1173.

32 Uehara T, Pogribny IP & Rusyn I, The DEN and CCl4-Induced Mouse Model of Fibrosis and Inflammation-Associated Hepatocellular Carcinoma, *Curr Protoc*, 1 (2021) e211.