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Research Article

Antioxidant and Hepatoprotective Effects of *Eucheuma denticulatum* (N. L. Burman) F. S. Collins & Hervey in Carbon Tetrachloride-Induced Liver Injury

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ABSTRACT

Oxidative stress-related liver injury is a major global health concern and highlights the need for safe and effective hepatoprotective agents from natural sources. *Eucheuma denticulatum* (N. L. Burman) F. S. Collins & Hervey, a red seaweed of economic importance, is a primary source of ι-carrageenan with potential medicinal and therapeutic value. This study evaluated the antioxidant and hepatoprotective effects of the ethanolic extract of *E. denticulatum* (EEED). Antioxidant activity was assessed using total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, while hepatoprotective effects were investigated in Sprague-Dawley rats (n = 4 per group) with carbon tetrachloride (CCl_4)-induced liver injury. Biochemical analyses measured serum liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and hepatic antioxidants including glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and glutathione S-transferase (GST). EEED showed low TPC (9.93 ± 0.10 mg GAE/g) and weak DPPH scavenging activity (40.77 ± 0.01%) compared with ascorbic acid (98.24 ± 0.01%) at 5,000 µg/mL. In CCl_4-treated rats, ALT and AST were significantly elevated, while GSH, CAT, and GST were depleted with an increase in MDA. EEED pretreatment reduced ALT by 18–28% and AST by 8–13%, increased GSH by 65–82%, reduced MDA by 53–89%, and improved antioxidant status with increases in CAT of 15–30% and GST of 55–64%. Overall, EEED demonstrated hepatoprotective effects despite low radical scavenging activity, which indicates potential as a natural therapeutic candidate for oxidative liver injury.

Keywords: Oxidative stress; hepatoprotection; ethanolic extract; *Eucheuma denticulatum*; antioxidants; carbon tetrachloride.

INTRODUCTION

The liver plays a crucial role in detoxifying harmful substances, metabolising nutrients and drugs, synthesising essential proteins, and regulating many biochemical processes. Due to these diverse functions, the liver is highly vulnerable to damage caused by alcohol consumption, environmental toxins, infections, obesity, and various xenobiotics (Qadri et al., 2025). Liver disease remains a major global health concern that contributes significantly to morbidity and mortality and places a heavy burden on healthcare systems. One of the main mechanisms involved in liver injury is oxidative stress, a condition in which the antioxidant defence system becomes overwhelmed and results in DNA damage, lipid peroxidation (LPO), protein modification, and eventual cell death (Chaudhary et al., 2023). Although antiviral medications and corticosteroids are frequently prescribed for liver conditions, effectiveness is limited by high cost, restricted availability, and adverse effects that include gastrointestinal complications, immunosuppression, and nephrotoxicity, and these issues reduce patient adherence and compromise long-term safety (Gabrielli et al., 2025).

Natural products are increasingly used as supplementary or alternative medicines because many communities perceive such remedies as safer, more economical, and more accessible than conventional pharmaceuticals, and because their utilisation aligns with cultural and traditional practices (Aware et al., 2022). For many decades, bioactive compounds from marine organisms, plants, and fungi such as polysaccharides, alkaloids, flavonoids, polyphenols, and terpenoids have been recognised for antioxidant and hepatoprotective activities that may relieve oxidative stress and improve liver function. Natural and traditional treatments remain firmly embedded in many societies, and in several developing nations medicinal plants and marine organisms have long been incorporated into diets and healthcare practices because of affordability and cultural acceptance (Ahmad et al., 2025). Beyond therapeutic potential, natural products also offer opportunities for sustainable drug discovery through the integration of traditional knowledge with scientific validation in the identification of affordable, environmentally friendly, and socially acceptable hepatoprotective remedies. As such, the combination of contemporary pharmacology with ethnomedicine presents a realistic option to mainstream drugs while supporting broader global health resilience.

According to the FAO (2020), global seaweed production increased markedly from 16,100 tonnes in 2000 to 174,100 tonnes in 2018. Seaweeds are generally classified into three main groups based on chemical composition, nutritional value, and pigment content, namely Chlorophyta (green seaweeds), Rhodophyta (red seaweeds), and Phaeophyta (brown seaweeds) (Hakim & Patel, 2020). In 2019, five genera dominated worldwide cultivation and accounted for more than 95% of total production. These were *Laminaria* and *Saccharina* (35.4%), *Eucheuma* and *Kappaphycus* (33.5%), *Gracilaria* (10.5%), *Porphyra* (8.6%), and *Undaria* (7.4%) (Sultana et al., 2023). Wu et al. (2023) reported that some microalgal species contain protein levels of up to 50%, while red and green seaweeds typically provide around 25% with amino acid profiles comparable to conventional protein sources. Red seaweeds such as *Eucheuma*, *Kappaphycus*, and *Gracilaria* are also widely used in aquaculture for human consumption and for the extraction of gelling agents (Ismail et al., 2024).

Belonging to the Solieriaceae family and occurring in tropical and subtropical marine waters, *Eucheuma denticulatum* (N. L. Burman) F. S. Collins & Hervey is distributed mainly across Southeast Asia and the Pacific Islands and holds substantial commercial value due to its high content of ι-carrageenan, a polysaccharide with gelling and thickening properties widely applied in food, pharmaceutical, and cosmetic industries (Necas & Bartosikova, 2013).

Phytochemical investigations have identified diverse bioactive constituents in *E. denticulatum*, including alkaloids, flavonoids, phenolics, saponins, steroids, tannins, and terpenoids, and these constituents have been associated with antimicrobial activity (Mayore et al., 2018). The potential of *E. denticulatum* as a functional food has also been explored through studies on carrageenophyte lectin recognition aimed at elucidating the biological activities and functional relevance of lectins derived from this seaweed (Hung et al., 2015). Furthermore, ι-carrageenan from *E. denticulatum* has demonstrated antiviral activity against herpes simplex and dengue viruses, thereby supporting its therapeutic potential as a red seaweed with valuable biofunctional properties.

Although several studies have been conducted, none have specifically examined the antioxidant activity of *E. denticulatum* in relation to hepatoprotective effects against oxidative stress and hepatic enzyme activity. More research, particularly on the liver, is required to bridge the gap between the identification of these attributes and the determination of their therapeutic usefulness. Ethanol was selected as the extraction solvent because previous studies demonstrated its effectiveness in recovering medium-polarity bioactive compounds such as phenolics, flavonoids, tannins, and terpenoids from *E. denticulatum*, and these compounds are closely associated with antioxidant and hepatoprotective activities (Bitwell et al., 2023). The polarity of these constituents aligns well with ethanolic extraction and provides further justification for its use in the present work. Hence, this study assessed the antioxidant and hepatoprotective properties of an ethanolic extract of *E. denticulatum* (EEED) in a rat model of CCl_4-induced liver injury. The study aims to address current knowledge gaps through a detailed evaluation of the potential therapeutic benefits of EEED in reducing CCl_4-induced oxidative stress and liver injury.

We posit that EEED exerts antioxidant and hepatoprotective effects against CCl_4-induced liver injury in rats. The null hypothesis states that EEED has no effect on oxidative stress markers or hepatic enzyme activity in CCl_4-treated rats. The alternative hypothesis states that EEED leads to significant improvement in oxidative stress markers and hepatic enzyme activity compared with the CCl_4 control group.

METHODOLOGY

Sample preparation

Whole *E. denticulatum* samples (Fig. 1) were collected by local farmers from seaweed farms located 3.6 km off Omadal Island, Semporna, Sabah, Malaysia (coordinates 4°23'58.8" N, 118°43'39.5" E). Identification and authentication were carried out by Assoc. Prof. Dr. Wilson Thau Lym Yong of the Biotechnology Research Institute, Universiti Malaysia Sabah. The samples (voucher no. GS004) were stored in the plant tissue culture laboratory at the same institute. The seaweed was dried in an oven for 48 h until a constant weight was achieved. The dried material was then ground into a coarse powder using a heavy-duty blender.

Sample extraction

The sample was extracted using a Soxhlet extractor (Bionics Scientific, Delhi, India) following the method described by Jinoni et al. (2024) with slight modifications and employing ethanol as the solvent. A 50 g blended sample was extracted with 300 mL of ethanol at 70 °C for 72 h or until the solvent became clear. The extract was then concentrated at 50 °C using a rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) at 35 rpm to obtain a dry residue, which was subsequently freeze-dried for 72 h to yield a powdered extract.

Figure 1: Collection and preparation of *E. denticulatum* sample.

Chemical antioxidant assays

Using the method described by Mohd Rosdan et al. (2024), the total phenolic content (TPC) of EEED was determined with gallic acid as the reference standard. Optical density was measured at 725 nm using a spectrophotometer (PerkinElmer, Waltham, MA, USA), and results were expressed as mg GAE/g of extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of EEED was evaluated according to Awang et al. (2023), with optical density measured at 517 nm. Ascorbic acid was used as the reference antioxidant, and results were expressed as percentage inhibition. All analyses were performed in triplicate (n = 3).

Experimental procedure

Female Sprague Dawley rats (n = 16, 8–12 weeks old, 150–250 g) were used in the study and acclimatised to laboratory conditions at the Biotechnology Research Institute. Ethical approval was obtained from the Animal Ethics Committee under protocol number UMS/IP7.5/M3/4-2012. The rats were housed in polypropylene cages with free access to tap water and standard rodent chow. To evaluate the effects of EEED on CCl_4-induced hepatotoxicity, rats were divided randomly into four experimental groups (n = 4 per group), as shown in Fig. 2.

The normal group received saline only and served as the baseline reference for comparison with healthy liver function. The CCl_4 control group also received saline but was administered CCl_4 to induce hepatotoxicity, enabling assessment of liver injury without extract treatment. The experimental groups consisted of rats pretreated with EEED at 150 mg/kg b.wt. or 300 mg/kg b.wt., followed by CCl_4 exposure. All rats received treatment by oral gavage for 14 days. On days 13 and 14, CCl_4 (Sigma-Aldrich, St. Louis, MO, USA) in corn oil (1:1) was administered via gavage at 1 mL/kg b.wt. to all groups except the normal group, following the method of Iqbal et al. (2025) to induce hepatotoxicity.

All animals were euthanised 24 h after the final administration of CCl_4. Anaesthesia was induced using light ether, after which blood samples were obtained by cardiac puncture and collected into lithium heparin-coated tubes for plasma separation. The livers were excised, cleared of connective tissue, rinsed, and perfused with 0.85% w/v sodium chloride (ice-cold saline) to remove residual blood. The samples were then stored at −80 °C for subsequent biochemical analyses of liver function and hepatic antioxidant enzyme activity.

Determination of serum ALT and AST levels

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured according to the method of Zakaria et al. (2020).

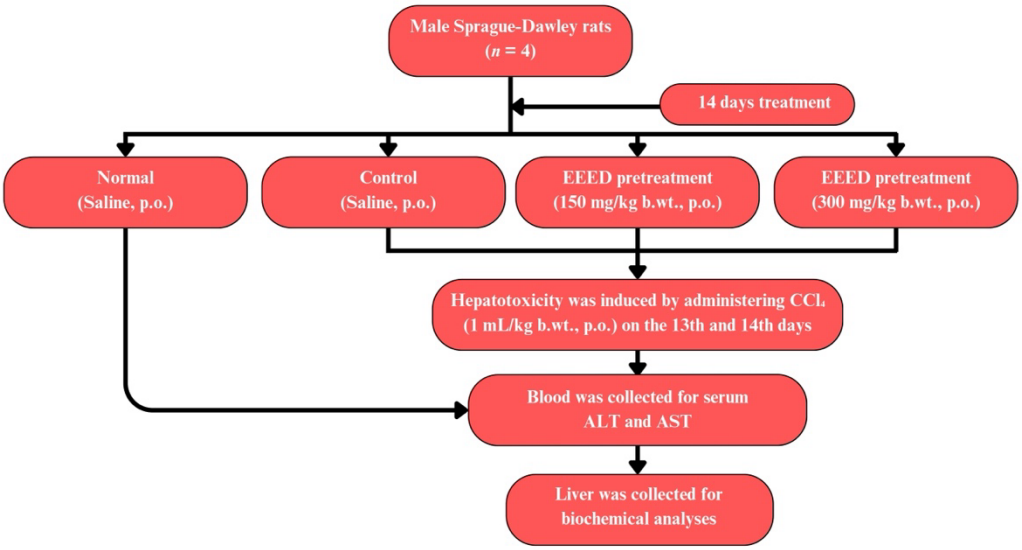

Figure 2: Summary of experimental design.

For both assays, serum was incubated with the respective substrates, namely α-ketoglutarate with L-alanine for ALT and α-ketoglutarate with L-aspartate for AST, in phosphate buffer at pH 7.4 and 37 °C for 15 min. The reactions were terminated with DNPH, and optical density was recorded at 510 nm after a 20 min reaction period followed by an additional 30 min incubation.

Preparation of post-mitochondrial supernatant

Tissue fractionation of liver samples for biochemical analysis was performed following the method of Iqbal et al. (1999). The samples were homogenised at a ratio of 1 g to 10 mL in chilled phosphate buffer at 0.1 M and pH 7.4 containing potassium chloride at 1.17% w/v using a homogeniser (Kinematica AG, Malters, Switzerland). The post-mitochondrial supernatant was prepared from the homogenate for the evaluation of endogenous antioxidant enzymes. Protein content was determined beforehand using the Bradford assay with bovine serum albumin as the reference standard.

Determination of hepatic enzyme levels

Reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and glutathione S-transferase (GST) were assessed as hepatic antioxidant markers. GSH activity was measured according to the method of Jollow et al. (1974). Optical density was measured at 412 nm, and results were expressed as µmol reduced GSH per gram of tissue. MDA, a marker of LPO, was quantified using the thiobarbituric acid reactive substances (TBARS) assay described by Buege and Aust (1978). Optical density of the supernatant was measured at 535 nm, and MDA activity was expressed as nmol MDA formed per gram of tissue. CAT activity was determined following the methods of Thanebal et al. (2021) and Claiborne (1985), while GST activity was determined following the methods of Habig et al. (1974) and Athar and Iqbal (1998). Optical density for CAT was measured at 240 nm and reported as nmol H_2O_2 consumed per minute per milligram of protein, while for GST, optical density was measured at 340 nm and reported as nmol CDNB conjugate formed per minute per milligram of protein.

Statistical analysis
Results were presented as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism (Version 10), and differences in the measured parameters were considered significant at $p < 0.05$. Group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test to determine specific differences between groups.

RESULTS

Effect of EEED on TPC and DPPH radical scavenging activity
The limited antioxidant capacity of EEED was evident from the TPC analysis, which showed a low value of 9.93 ± 0.10 mg GAE/g. The DPPH assay further demonstrated that EEED exhibited free radical scavenging activity in a concentration-dependent manner. As the concentration of EEED increased, the reduction of DPPH also increased, as illustrated in Fig. 3. The scavenging activity reached a maximum of 40.77 ± 0.01% at 5,000 µg/mL, whereas ascorbic acid achieved 98.24 ± 0.01% at the same concentration.

Effects of EEED on serum ALT and AST
ALT and AST are primary biomarkers of hepatic injury. In the control group, ALT and AST levels were significantly elevated ($p < 0.05$) by 61% and 40%, respectively, compared with the normal group (Fig. 4). Treatment with EEED at both doses significantly ($p < 0.05$) reduced these levels compared with the control. ALT decreased by 18% and 28%, while AST decreased by 8% and 13% following EEED treatment at 150 and 300 mg/kg b.wt., respectively.

Effects of EEED on GSH activity
As shown in Fig. 5, the control group exhibited a significant ($p < 0.05$) reduction in GSH activity, with a 76% decrease compared with the normal group. In contrast, GSH activity was significantly ($p < 0.05$) restored in a dose-dependent manner following EEED pretreatment. Increases of 65% and 82% were recorded at 150 and 300 mg/kg b.wt., respectively, compared with the control.

Effects of EEED on MDA activity
As illustrated in Fig. 6, the TBARS assay showed that MDA activity in the normal group was significantly ($p < 0.05$) lower by 16% compared with the control. EEED pretreatment significantly ($p < 0.05$) suppressed CCl_4-induced hepatic MDA activity in a dose-dependent manner, with reductions of 53% and 89% at 150 and 300 mg/kg b.wt., respectively.

Effects of EEED on CAT activity
Fig. 7 shows that CCl_4 exposure caused a significant ($p < 0.05$) two-fold decrease in CAT activity compared with the normal group. Pretreatment with EEED at 150 and 300 mg/kg b.wt. substantially ($p < 0.05$) increased CAT activity by 15% and 30%, respectively, compared with the control. The restoration of antioxidant capacity impaired by CCl_4 was reflected in the CAT activity observed in the EEED-treated groups.

Effects of EEED on GST activity
GST activity in liver tissue homogenates across all groups is presented in Fig. 8. The control group showed a significant ($p < 0.05$) 3.5-fold decrease in GST activity compared with the normal group, indicating oxidative stress and reduced antioxidant capacity. In contrast,

pretreatment with EEED at 150 and 300 mg/kg b.wt. significantly ($p < 0.05$) enhanced GST activity by 55% and 64%, respectively, compared with the control group.

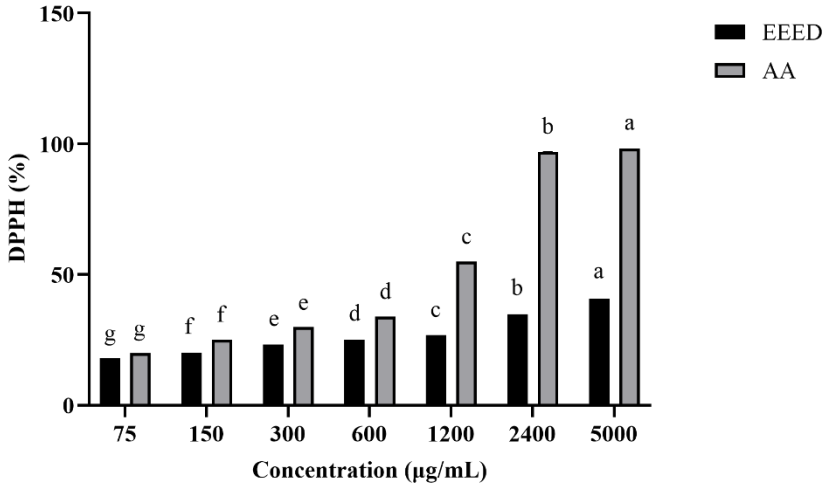

Figure 3: DPPH radical scavenging activity of EEED at concentrations ranging from 75 to 5,000 µg/mL compared with AA. Values are presented as mean ± standard deviation ($n = 3$). Letter annotations indicate significant differences between groups ($p < 0.05$).

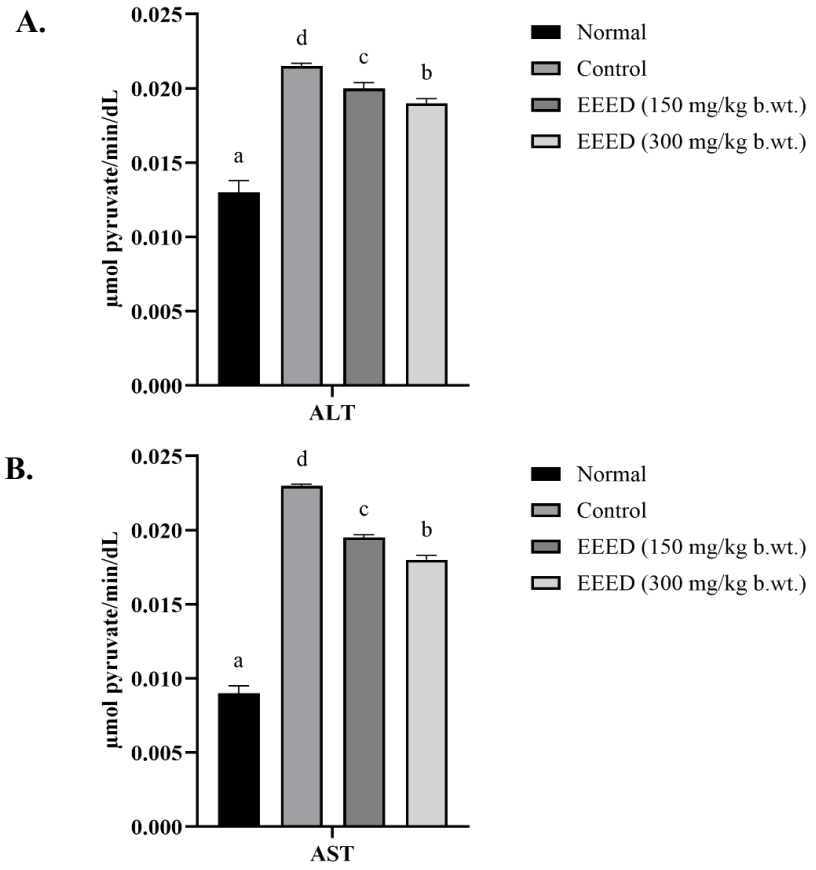

Figure 4: Effects of EEED on serum **A.** ALT and **B.** AST levels. Values are presented as mean ± standard deviation ($n = 4$). Letter annotations indicate significant differences between groups ($p < 0.05$).

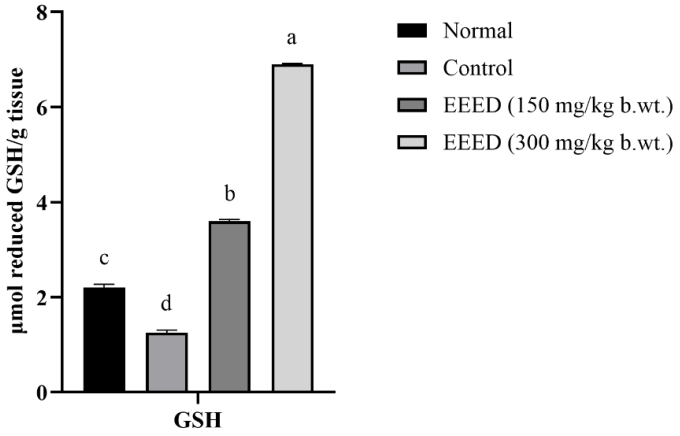

Figure 5: Effects of EEED on GSH activity. Values are presented as mean ± standard deviation ($n = 4$). Letter annotations indicate significant differences between groups ($p < 0.05$).

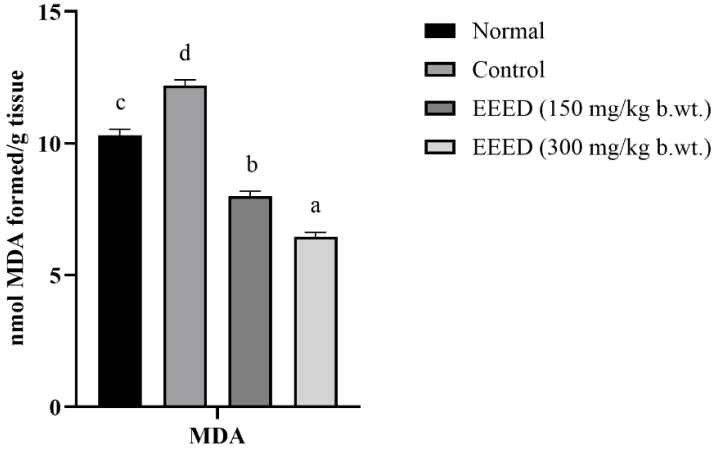

Figure 6: Effects of EEED on MDA activity. Values are presented as mean ± standard deviation ($n = 4$). Letter annotations indicate significant differences between groups ($p < 0.05$).

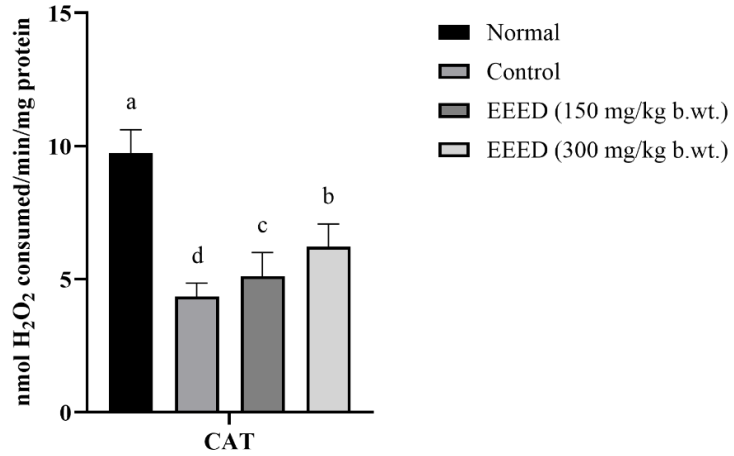

Figure 7: Effects of EEED on CAT activity. Values are presented as mean ± standard deviation ($n = 4$). Letter annotations indicate significant differences between groups ($p < 0.05$).

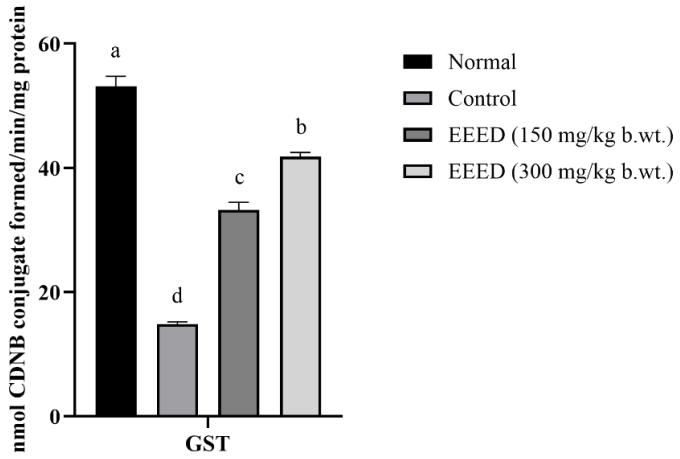

Figure 8: Effects of EEED on GST activity. Values are presented as mean ± standard deviation ($n = 4$). Letter annotations indicate significant differences between groups ($p < 0.05$).

DISCUSSION

Plant-derived compounds found in many foods act as effective antioxidants and scavengers, and the position of hydroxyl groups in their structure influences their capacity to prevent lipid oxidation, with all extracts demonstrating higher activity at increasing concentrations (Lin et al., 2016). Using the Folin–Ciocalteu method, TPC was measured based on the reduction of metal oxides by phenolic acids to form a blue solution, whereas the DPPH assay evaluated scavenging capacity by observing the colour change of DPPH from purple to yellow as it forms the non-radical DPPH-H (Gulcin, 2020; Pérez et al., 2023). Based on the findings, EEED, which showed a low TPC value of 9.93 ± 0.10 mg GAE/g, exhibited a scavenging activity of 40.77 ± 0.01% at 5,000 µg/mL, a value significantly lower than the positive control of 98.24 ± 0.01%, indicating comparatively weaker antioxidant potential. This observation is consistent with earlier work that reported 7.99 ± 0.50% using the same solvent (Balasubramaniam et al., 2020). Nonetheless, the use of the oxygen radical absorbance capacity (ORAC) assay produced different results, with a value of 36,400.00 ± 23.50 µmol TE $(100\ g)^{-1}$ DW (Balasubramaniam et al., 2016), indicating higher antioxidant potential when activity is measured through a hydrogen atom transfer-based assay rather than an electron transfer-dominant method such as DPPH. As an industrial source of ι-carrageenan, *E. denticulatum* contains a wide range of metabolites, yet EEED would primarily include ethanol-soluble constituents such as phenolics, pigments, and other low molecular weight compounds that contribute to antioxidant activity, as supported by seaweed metabolite databases (Tanna & Mishra, 2018; Gins et al., 2019). While ι-carrageenan itself is not extracted by ethanol, other bioactive constituents present in the seaweed may contribute to reducing oxidative stress and supporting liver health.

CCl_4 is a hepatotoxin that induces liver damage and primarily enters aquatic systems through industrial waste generated during chlorofluorocarbon synthesis. It promotes adipose tissue accumulation in the liver and leads to centrilobular necrosis. Metabolism by the cytochrome P-450 system converts CCl_4 into trichloromethyl free radicals (CCl_3•), which react with lipids and proteins to form trichloromethyl peroxyl radicals. These radicals initiate LPO, disrupt calcium homeostasis, and cause cell death (Unsal et al., 2021). Free radicals are electrically charged molecules that stabilise by gaining or donating electrons, whereas antioxidants neutralise them to prevent oxidative damage (Gulcin, 2020). ALT catalyses the conversion of

alanine to pyruvate, while AST catalyses the conversion of aspartate to oxaloacetate. The antioxidant activity of EEED in rat CCl_4-induced liver injury was evaluated using ALT and AST serum biomarkers to indicate hepatocyte integrity (Li et al., 2015). The reduction in transaminase levels observed in EEED-treated rats reflects hepatoprotective potential because the extract inhibited the leakage of ALT and AST.

GSH is an important antioxidant that protects the liver from chemical injury through the maintenance of thiol groups in membrane proteins and through reduction of oxidative stress inside and outside the cell (Vairetti et al., 2021). In the current study, CCl_4-treated rats had significantly lower GSH activity compared with EEED-treated rats, demonstrating that EEED can restore the reduction in GSH activity induced by CCl_4. Additionally, metabolites of CCl_4 induce LPO, a process that requires strong antioxidant activity or inhibition of free radical formation to counteract. Peroxidation of polyunsaturated fatty acids by excessive free radicals leads to the accumulation of MDA, a key indicator of LPO (Cordiano et al., 2023). In this study, rats exposed to CCl_4 showed elevated MDA activity, indicating increased LPO, tissue injury, and weakened antioxidant defence. In contrast, administration of EEED markedly lowered MDA activity, demonstrating its effectiveness in enhancing antioxidant protection.

Protecting against free radical damage through the reduction of reactive oxygen species such as hydrogen peroxide (H_2O_2) into non-toxic molecules, CAT is a phase II enzyme with the highest activity in the liver and limits hydroxyl radical formation (Nandi et al., 2019). By promoting the accumulation of superoxide radicals and H_2O_2, CCl_4 exposure reduced hepatic CAT activity in the current findings. This effect was reversed by EEED pretreatment, which significantly increased CAT activity and indicated a protective effect. Similarly, GST plays a crucial role in the detoxification of reactive radicals and xenobiotics and is an important phase II detoxification enzyme located primarily in the cytosol (Singhal et al., 2015). Reduced GST activity was associated with enzyme inhibition, membrane damage, and increased peroxyl radical formation in CCl_4-treated rats. However, EEED pretreatment enhanced GST activity, reaffirming its ability to elevate phase II enzyme function, reduce oxidative stress, and minimise free radical-induced damage.

The current study is the first to demonstrate the hepatoprotective effect of EEED through its antioxidant activity in mitigating oxidative stress. Nevertheless, the use of TPC and DPPH alone limits the ability to fully assess antioxidant potential because these assays do not characterise the mechanisms that regulate oxidative stress. Additional assays such as ORAC and superoxide dismutase are required to provide a more comprehensive evaluation of the antioxidant activity of EEED. Histopathological examination is also needed to validate hepatic tissue integrity and quantify CCl_4-induced inflammation, and its absence represents a limitation of this study. Another limitation is the small sample size of $n = 4$ per group, which was determined through an a priori power analysis and ethical considerations to minimise the number of animals used. Future research should incorporate a wider range of antioxidant assays together with detailed histological examinations to further elucidate the antioxidant capacity of EEED and its effect on liver function.

CONCLUSIONS

Overall, EEED effectively protects rats against liver damage caused by CCl_4 through reduction of LPO and elevation of hepatic antioxidant enzyme activity, even though it exhibits poor free radical scavenging activity. The findings indicate that the antioxidant activities of EEED are

closely correlated with its hepatoprotective action. Dietary inclusion of antioxidant-rich marine materials such as EEED may help restore oxidative balance and improve liver function. The current study provides a foundation for future investigations on *E. denticulatum* as a potential candidate for the prevention and treatment of oxidative stress-induced liver injury.

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DECLARATIONS

Research permit(s). Not applicable.
Ethical approval/statement. All experimental procedures were approved by the Animal Ethics Committee of Universiti Malaysia Sabah under approval code UMS/IP7.5/M3/4-2012.
Generative AI use. AI was not used in this study or in the writing of this article.

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