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Original Article

Acute and repeated-dose oral toxicity evaluation of *Caulerpa racemosa* (Forsskål) J.Agardh ethanolic extract

[Evaluación de la toxicidad oral aguda y a dosis repetidas del extracto etanólico de *Caulerpa racemosa* (Forsskål) J.Agard]

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Abstract

Context: *Caulerpa racemosa* is a green alga showing significant potential as a medicinal raw material without any currently available associated safety information.

Aims: To assess the safety of *C. racemosa* ethanolic extract (CRE) through oral administration to mice and rats in acute and subchronic stages.

Methods: In the acute toxicity test, mice were divided into the control group, and a group was administered 5000 mg/kg CRE. Mortality and signs of toxicity were observed over 14 day period. For the repeated dose oral toxicity test, rats were divided into control, satellite, and treatment groups, each consisting of five rats. The treatment groups received CRE at doses of 50, 350, and 1050 mg/kg b.w., respectively. The satellite group was observed for 35 days, while other groups were monitored for 28 days.

Results: No mortality or signs of toxicity were observed in the animal subjects administered with acute and repeated-dose treatments. Additionally, the treatments did not result in statistically significant changes in body weight and relative organ sizes, compared to the control group. The hematological and biochemical analyses showed no substantial changes, with all parameters remaining within normal limits. However, significant discrepancies were noted in the male animal group for red blood cell and platelet count, as well as alanine transaminase and triglyceride levels, although these values remained within normal ranges. Morphological examination of organs did not reveal any significant alterations.

Conclusions: Based on an LD_{50} value exceeding 5000 mg/kg body weight and all other results obtained, CRE was considered safe for consumption and could serve as a potential candidate for medicinal use derived from natural sources.

Keywords: acute toxicity; *Caulerpa racemosa;* ethanolic extract; repeated-dose oral toxicity; mice; rat.

Resumen

Contexto: *Caulerpa racemosa* es un alga verde que muestra un potencial significativo como materia prima medicinal sin ninguna información de seguridad asociada disponible actualmente.

Objetivos: Evaluar la seguridad del extracto etanólico de *C. racemosa* (CRE) mediante administración oral a ratones y ratas en etapas agudas y subcrónicas.

Métodos: En la prueba de toxicidad aguda, los ratones se dividieron en el grupo de control y a un grupo se le administró CRE 5000 mg/kg. Se observó la mortalidad y los signos de toxicidad durante un período de 14 días. Para la prueba de toxicidad oral de dosis repetidas, las ratas se dividieron en grupos de control, satélite y tratamiento, cada uno compuesto por cinco ratas. Los grupos de tratamiento recibieron CRE en dosis de 50, 350 y 1050 mg/kg de peso corporal, respectivamente. El grupo satélite se observó durante 35 días, mientras que los otros grupos se monitorearon durante 28 días.

Resultados: No se observó mortalidad ni signos de toxicidad en los sujetos animales administrados con tratamientos agudos y de dosis repetidas. Además, los tratamientos no resultaron en cambios estadísticamente significativos en el peso corporal y el tamaño relativo de los órganos, en comparación con el grupo control. Los análisis hematológicos y bioquímicos no mostraron cambios sustanciales, y todos los parámetros permanecieron dentro de los límites normales. Sin embargo, se observaron discrepancias significativas en el grupo de animales machos en el recuento de glóbulos rojos y plaquetas, así como en los niveles de alanina transaminasa y triglicéridos, aunque estos valores se mantuvieron dentro de los rangos normales. El examen morfológico de los órganos no reveló ninguna alteración significativa.

Conclusiones: Con base en un valor de LD_{50} superior a 5000 mg/kg de peso corporal y todos los demás resultados obtenidos, se consideró que CRE era seguro para el consumo y podría servir como un candidato potencial para uso medicinal derivado de fuentes naturales.

Palabras Clave: *Caulerpa racemosa*; extracto etanólico; toxicidad aguda; toxicidad oral a dosis repetidas; rata; ratones.

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Abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; CAT: catalase; BW: body weight; CRE: *Caulerpa racemosa* extract; DMSO: dimethylsulfoxide; ERK: extracellular signal-regulated kinase; GSH-PX: seleno-dependent glutathione peroxidase; Hb: hemoglobin; HBa1c: glycated hemoglobin; HCT: hematocrit; JNK: c-Jun N-terminal kinase; LD_{50}: lethal dose 50; MUFA: monosaturated fatty acids; p38 MAPK: mitogen-activated protein kinase; PI3K/Akt: phosphoinositide 3-kinase protein kinase B; PLT: platelet; PUFA: polyunsaturated fatty acids; RBC: red blood cell; SFA: saturated fatty acids; SOD: superoxide dismutase; WBC: white blood cell.

INTRODUCTION

C. racemosa is a species of marine green alga native to coastal regions of the Indo-Pacific region and is commonly used as a food ingredient in Southeast Asia, East Asia, and Oceania. This alga belongs to the phylum *Chlorophyta*, class *Ulvophyceae*, and genus *Caulerpa* (WoRMS, 2022). *C. racemosa* is one of the popular marine products currently being developed from *Caulerpa Spp*, which are abundant in the Indonesian seas (Nursidika and Rachmawati, 2023). Furthermore, it offers various benefits for medicinal and traditional applications, while also serving as a source of fresh vegetables and processed foods. Due to its high nutritional content, it is valued as a nutritious food, particularly in Indonesia, Thailand, Malaysia, the Philippines, China, Korea, and Japan (Moreira et al., 2022; Palaniyappan et al., 2023). Additionally, *C. racemosa* is known as a beauty product and beauty food (Nurjanah et al., 2016; Putri et al., 2023).

The desire for a soft and juicy texture leads to increased consumption of *C. racemosa*, which provides a healthy source of nutrients, including polysaccharides, fiber, protein, minerals, vitamins, and fatty acids. The high nutritional and phytochemical contents have facilitated the advanced cultivation of this alga (Ganesan et al., 2019; Magdugo et al., 2020; Peñalver et al., 2020). Numerous studies have focused on optimizing the alga growth, and while several others have explored the discovery of new drug compounds, cosmetics, and food supplements. Compounds present in *C. racemosa* are recognized for antioxidant, antimicrobial, and antiviral properties (Yap et al., 2019), as well as the ability to function as immunomodulators, anti-inflammatories, antidiabetics, anti-obesity agents (Kurniawan et al., 2023; Pakki et al., 2020), anti-coagulants (Rodrigues et al., 2017), and anti-cancer agents (Permatasari et al., 2022; Sanger et al., 2023). Additionally, the compounds possess hepatoprotective effects (Qudus B Aroyehun et al., 2020) and show promise for cardiovascular treatment (Nurkolis et al., 2023).

C. racemosa has high potential as a medicinal raw material; however, safety information regarding the alga is currently unavailable. Therefore, this study aimed to address the gap by assessing the acute and repeated-dose oral toxicity of *C. racemosa* ethanol extract (CRE) in mice and rats. The results are expected to provide valuable insights into the safety profile of the extract for potential human use.

MATERIAL AND METHODS

Alga material

C. racemosa specimens were collected from Jepara Bay, Central Java, Indonesia, at latitude 6˚ 31′ 59.99″ S and longitude 110˚ 39′ 59.99″ E, during the dry season from February to June 2023. The authentication of this alga was conducted at the Herbarium of Plant Taxonomy Laboratory, Faculty of Biology, Padjadjaran University, Sumedang, Indonesia, where it was assigned number No.26/HB/04/2022. The collected specimens were then thoroughly washed and air-dried away from direct sunlight.

Animals

Subjects used in this study were obtained from the Animal Laboratory at Jenderal Achmad Yani University, Cimahi, Indonesia. Female Swiss Webster mice, weighing between 20 and 30 g, and Wistar rats (both female and male), weighing between 200 and 300 g, were selected for the acute and repeated-dose oral toxicity assessments, respectively. A one-week acclimatization period preceded the initiation of the experiments. The animals were housed under controlled laboratory conditions with a controlled room temperature, provided daily with food, and given unrestricted access to water. Additionally, approval was obtained from the Preclinical Ethics Committee of the Faculty of Pharmacy, Jenderal Achmad Yani, Cimahi, Indonesia, under the assigned number No: 9019/KEP-UNJANI/IV/2023.

Extraction of *C. racemosa* extract

A total of 10 kg of dried *C. racemosa* was finely ground and subjected to extraction with 96% ethanol using a maceration method. The extraction process was repeated three times, with each session lasting 24 hours. The resulting ethanol extract (CRE) was concentrated using a vacuum evaporator.

Acute oral toxicity test

Acute toxicity was assessed following the guidelines provided by the Acute Oral toxicity-Fixed dose procedure (OECD, 2002). The study included the control group, which received a vehicle of 1% DMSO, and the test group, consisting of five female mice, administered CRE at a dose of 5000 mg/kg body weight (b.w.). The animals were continuously monitored for 1 hour, followed by intermittent observa-

tions at 4 and 24 hours. Daily observations continued for 14 days to assess any behavioral changes, signs of toxicity, or mortality. On the 15th day, the treated animals were sacrificed, and their vital organs were extracted, cleaned, and weighed.

Repeated-dose oral toxicity

The repeated-dose oral toxicity test included a control group administered a vehicle of 1% DMSO and a treatment group receiving doses of 50, 350, and 1050 mg/kg body weight (b.w.) for 28 days. Additionally, a satellite group received a daily dose of 1050 mg/kg for 28 days and was observed for an additional 7 days post-treatment. On days 28 and 35, all rats were sacrificed by cervical dislocation, and the blood samples were collected for the evaluation of hematological and biochemical parameters (OECD, 2008). Approximately 2 mL of blood samples were collected with EDTA tubes, homogenized, and analyzed for hematological parameters. Another 2-3 mL blood was collected in non-anticoagulant tubes, and centrifuged at 3000 rpm for 10 minutes, and the resulting serum was used for biochemical analysis.

Hematological analysis

The collected samples were analyzed to determine white blood cell (WBC) count, red blood cell (RBC) count, platelet count (Plt), hematocrit (Hct), and hemoglobin (Hb) following the guidelines of the Repeated-Dose 28 days Oral Toxicity (OECD, 2008). These hematological parameters were measured using the Mindray BC 1800 analyzer, providing a comprehensive panel.

Biochemical analysis

Biochemical analysis was conducted to measure levels of creatinine, urea, AST, ALT, total cholesterol, triglycerides, and glucose using the Mindray BA-88 analyzer.

Histopathological evaluation

The animal organs were extracted, weighed to determine their relative weight (g/100 body weight), and preserved in 10% neutral buffered formalin for histopathological observations. The tissues examined included the kidney, liver, and heart, and microscopic analysis was performed using hematoxylin and eosin (HE) staining. Histopathological evaluation was conducted at the Cytohistotechnology Laboratory of the Medical Laboratory Technology Department, Faculty of Health Sciences and Technology, Jenderal Achmad Yani University.

Statistical analysis

The collected data were analyzed using an independent t-test and one-way analysis of variance (ANOVA), followed by a post hoc Tukey's test, performed using GraphPad Prism 9.5.1 (GraphPad Software Inc., California, USA) for Mac. The results are presented as mean ± SD, and statistical significance was set at p-values of less than $^{*}0.05$ and $^{**}0.001$.

RESULTS

Acute oral toxicity of CRE

The animals did not experience any mortality from oral doses of CRE during the experimental period, and no indications of signs of adverse effects or toxicity were observed. There were no significant differences in body weight between the treated mice and the control group (Fig. 1). However, mice administered with CRE showed improved physical conditions, including cleaner and whiter fur, with a healthier body appearance.

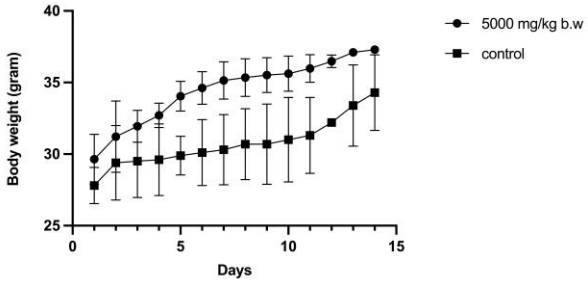


Figure 1. Body weight of female mice during the acute toxicity assessment of *Caulerpa racemosa* extract (CRE).

Data are shown as mean ± SD (n = 5). Statistical analysis was performed using the t-test. A significant difference was observed between the test and control groups (p<0.0001).

Repeated-dose oral toxicity of CRE

No mortalities or signs of toxicity were observed during the 28 and 35 days of treatment, based on body and organ weights, as well as hematological profiles, biochemistry, and histology results. Fig. 2 shows the body weight data of female and male rats. Statistic analysis results in the male group showed significant differences observed between the dose of 50 mg/kg b.w, 1050 mg/kg b.w, and satellite group compared to control (p<0.0001). Significant differences were observed between a dose of 1050 mg/kg b.w. and satellite compared to the control group (p<0.0001). Additionally, compared to the control group, no substantial differences in organ weight were observed between both genders of rats following CRE administration.

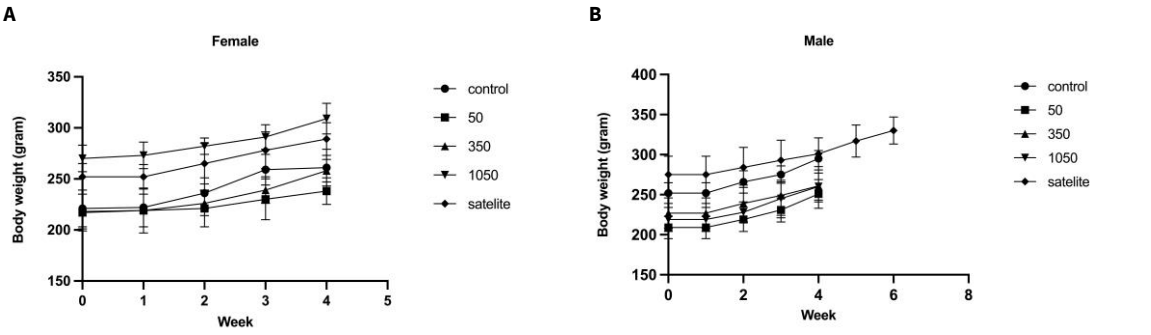


Figure 2. Body weight of rats after exposure to *Caulerpa racemosa* extract (CRE).

(A) Body weight change of female rats; **(B)** Body weight change of male rats.

Data represent mean ± SD (n = 5). The data were analyzed using ANOVA, followed by a Tukey test. Results showed a significant difference between each group of female and male rats. Negative control: DMSO 1%; The CRE doses 50, 350, 1050 mg/kg b.w.

Table 1. Hematological analysis of female and male rats after exposure to *Caulerpa racemosa* extract (CRE).

Gender	Control	CRE (mg/kg b.w)			Satellite CRE
		50	350	1050	1050
Female					
WBC (x10^3/µL)	9.23 ± 1.36	12.67 ± 0.69	14.93 ± 1.05	11.63 ± 1.96	12.67 ± 1.48
RBC (x10^6/µL)	7.31 ± 0.30	7.44 ± 0.23	7.75 ± 0.41	6.99 ± 0.14	7.57 ± 0.47
PLT (x10^3/µL)	389.00 ± 29.00	598.00 ± 41.00	546.00 ± 222.00	544.00 ± 72.00	551.00 ± 58.00
HCT (%)	42.60 ± 0.73	38.87 ± 1.20	40.53 ± 2.76	39.40 ± 1.13	42.17 ± 1.73
Hb (g/dL)	14.40 ± 0.32	13.30 ± 0.43	14.83 ± 0.28	13.77 ± 0.69	14.17 ± 0.58
Male					
WBC (x10^3/µL)	8.70 ± 1.67	12.56 ± 0.48	10.23 ± 1.08	8.03 ± 0.39	9.27 ± 0.83
RBC (x10^6/µL)	7.52 ± 0.55	8.73 ± 0.12*	8.36 ± 0.23	6.74 ± 0.53	7.64 ± 0.31**
PLT (x10^3/µL)	844.00 ± 68.00	774.00 ± 54.00*	657.00 ± 52.00	556.00 ± 48.00	663.00 ± 65.00
HCT (%)	38.17 ± 3.24	46.60 ± 0.52	42.60 ± 1.10	38.30 ± 3.72	42.53 ± 3.48
Hb (g/dL)	12.93 ± 1.14	15.83 ± 0.08	14.87 ± 0.28	13.17 ± 1.34	15.00 ± 0.79

The data are defined as mean ± SD (n = 5). Statistically significant differences of test and satellite groups with respect to the control group according to ANOVA followed by Tukey's test ($^*p<0.05$, $^{**}p<0.01$).

Hematological analysis

Hematological parameters for both female and male rats are presented in Table 1. Although the female rats exhibited higher levels of RBC, WBC, and PLT, no significant differences were observed in any of these parameters when compared to the control group. In male rats, disparities were noted in RBC and PLT levels. Specifically, the RBC count was lower in the CRE-treated groups at doses of 50 and 350 mg/kg compared to other groups; however, the difference was not statistically significant. The PLT count varied between the control and test groups, with the control group displaying a higher count than both the test and satellite groups.

Biochemical analysis

The biochemical analysis revealed no significant changes in creatinine, urea, AST, ALT, total cholesterol, triglyceride, and glucose levels among the female rats (Table 2). However, differences were observed in triglyceride and ALT levels within the male group. Specifically, a significant variation in triglyceride levels was observed between the male group receiving 50 mg/kg CRE and the satellite group. Significant differences in ALT activity were also identified between the group receiving 50 mg/kg b.w. and 350 mg/kg b.w. CRE compared to the control group. Furthermore, triglyceride concentrations were elevated in the satellite group administered 1050 mg/kg b.w. compared to the control and treatment groups.

Table 2. Biochemical analysis of female and male rats after exposure to *Caulerpa racemosa* extract (CRE).

Parameter	Control	CRE (mg/kg bw)			Satellite CRE
		50	350	1050	
Female					
Creatinine (mg/dL)	0.36 ± 0.15	0.55 ± 0.34	0.61 ± 0.16	0.63 ± 0.29	0.51 ± 0.13
Urea (mg/dL)	18.67 ± 2.40	17.63 ± 2.15	18.10 ± 1.31	15.40 ± 5.42	18.96 ± 2.40
AST (U/L)	76.00 ± 12.53	88.07 ± 8.25	81.97 ± 11.58	95.30 ± 9.08	83.23 ± 17.09
ALT (U/L)	69.47 ± 3.01	43.67 ± 8.50	61.30 ± 26.97	56.13 ± 3.01	82.80 ± 9.26
Cholesterol (mg/dL)	77.00 ± 5.57	88.00 ± 24.43	76.00 ± 8.19	72.67 ± 1.52	105.00 ± 16.46
Triglyceride (mg/dL)	83.00 ± 10.82	90.00 ± 8.54	80.00 ± 13.89	59.00 ± 30.00	77.00 ± 5.57
Glucose (mg/dL)	136.67 ± 16.80	169.33 ± 16.77	134.33 ± 15.17	144.50 ± 8.76	160.00 ± 33.95
Male					
Creatinine (mg/dL)	0.38 ± 0.38	1.40 ± 0.60	1.13 ± 0.72	0.60 ± 0.52	0.68 ± 0.15
Urea (mg/dL)	18.30 ± 1.43	16.92 ± 4.24	15.23 ± 3.00	15.17 ± 1.40	16.10 ± 3.61
AST (U/L)	58.33 ± 24.90	92.33 ± 6.25	85.63 ± 6.25	79.67 ± 11.55	85.33 ± 4.16
ALT (U/L)	77.33 ± 7.58*	37.90 ± 8.69*	61.77 ± 12.57**	77.33 ± 7.58**	85.23 ± 13.95
Cholesterol (mg/dL)	81.00 ± 11.79	92.33 ± 3.21**	81.67 ± 10.41	110.67 ± 30.66**	104.00 ± 10.53
Triglyceride (mg/dL)	77.00 ± 5.67	78.42 ± 4.02	89.67 ± 5.69	74.33 ± 16.56	116.67 ± 30.10
Glucose (mg/dL)	158.63 ± 9.84	147.67 ± 110.60	159.67 ± 33.55	135.17 ± 13.36	135.33 ± 35.71

The data are defined as mean ± SD (n = 5). Statistically significant differences of test and satellite groups with respect to the control group according to ANOVA followed by Tukey's test (*p<0.05, **p<0.01).

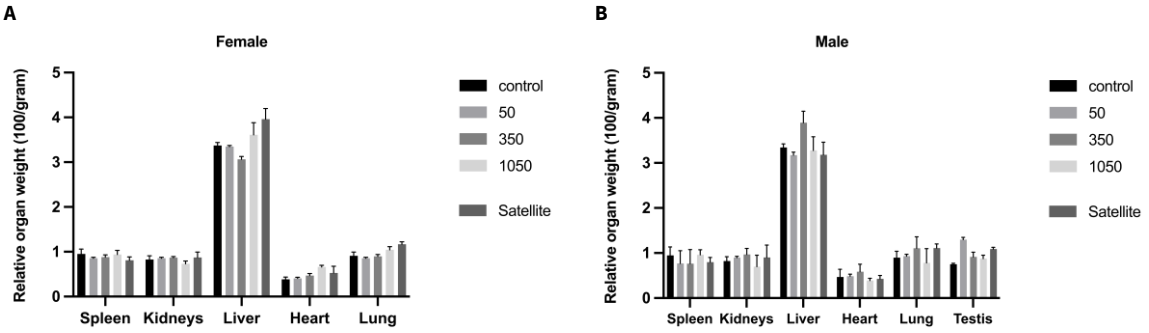


Figure 3. Relative organ weights (100/g BW) of **(A)** female and (**B)** male rats after exposure to *Caulerpa racemosa* extract (CRE).

The data are defined as mean ± SD (n=5). Statistically significant differences of test and satellite groups with respect to the control group according to ANOVA.

Macropathological observation

Macroscopic examination of organs from animals treated with CRE revealed no abnormalities in color or texture compared to the control group. Additionally, the relative organ weights in all test groups did not differ significantly from those of the control group (Fig. 3).

Histopathological observation

The liver, kidney, and heart exhibited normal morphology and relative organ weights, with no lesions observed. Microscopic examination revealed no abnormalities (Figs. 4, 5, and 6), consistent with the results of the biochemical analysis.

DISCUSSION

In this study, the acute and repeated-dose oral toxicity of CRE was assessed in mice and rats. The results demonstrated that CRE did not cause mortality or toxic effects in test animals during acute and repeated-dose testing.

Toxicity tests are primarily conducted to evaluate the safety of extracts, a critical step in the drug development process. If the results confirm safety, the substance can proceed to subsequent stages of develop-

ment. Safety data are derived from a series of toxicity assessments conducted on animal models, including acute, subchronic, and chronic oral toxicity, as well as teratogenicity studies. The main objective is to provide robust evidence regarding the safety of CRE (Prasesti and Fisheri, 2021).

C. racemosa is a marine alga known for its diverse applications, including use as a functional food, cosmetics ingredient, and medicinal resources (Qudus B Aroyehun et al., 2020). However, limited data are available on the safety of *C. racemosa*, which is crucial for the future development of drugs derived from *C. racemosa*.

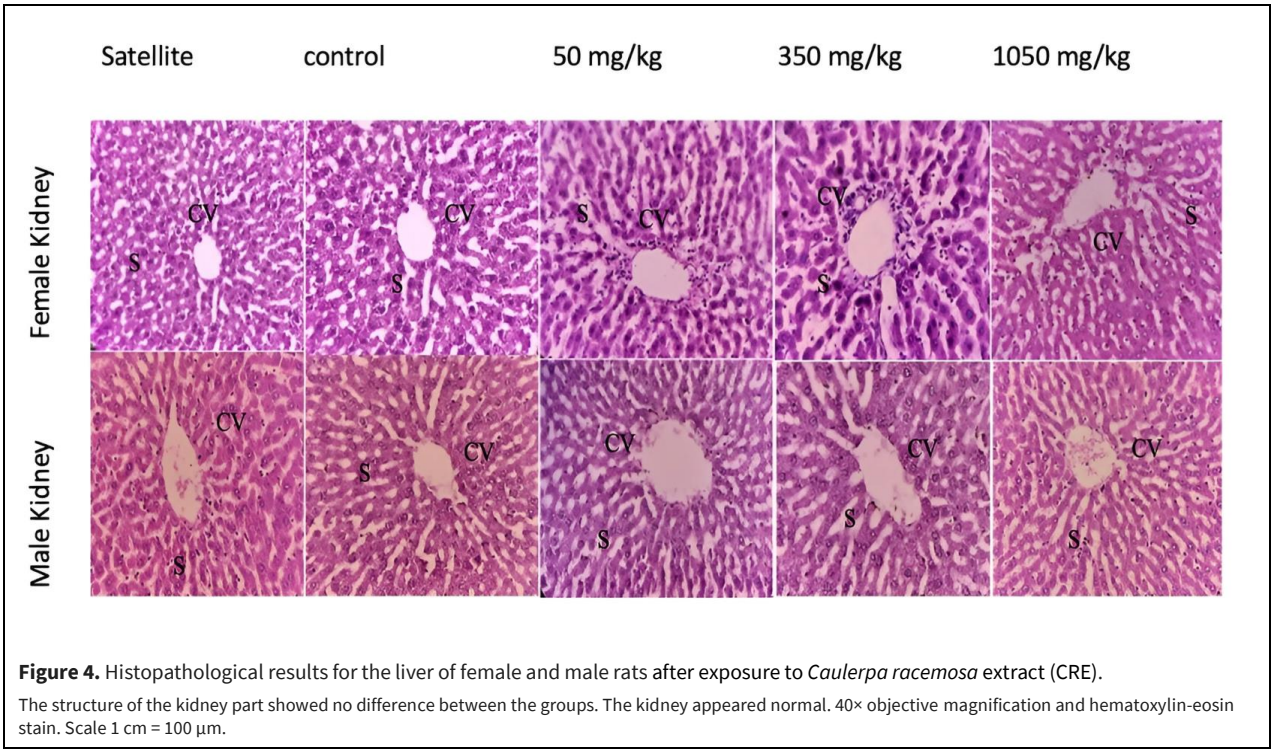


Figure 4. Histopathological results for the liver of female and male rats after exposure to *Caulerpa racemosa* extract (CRE).

The structure of the kidney part showed no difference between the groups. The kidney appeared normal. 40× objective magnification and hematoxylin-eosin stain. Scale 1 cm = 100 µm.

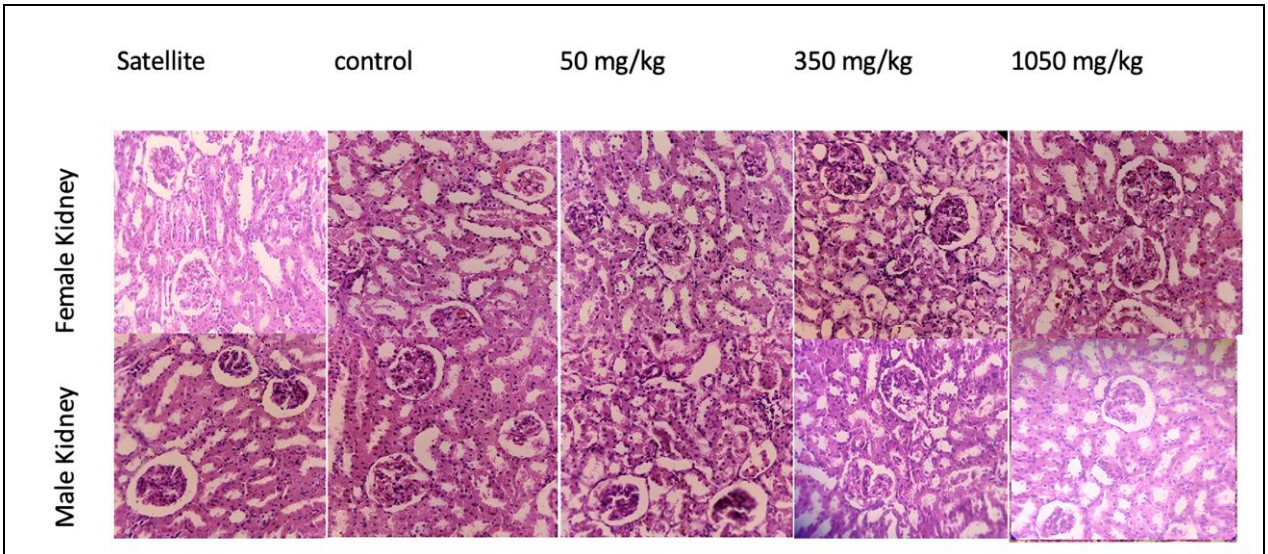


Figure 5. Histopathological results for the kidney of female and male rats after exposure to *Caulerpa racemosa* extract (CRE).

The structure of the kidney part showed no difference between the groups. The kidney appeared normal. 40× objective magnification and hematoxylin-eosin stain. Scale 1 cm = 100 µm.

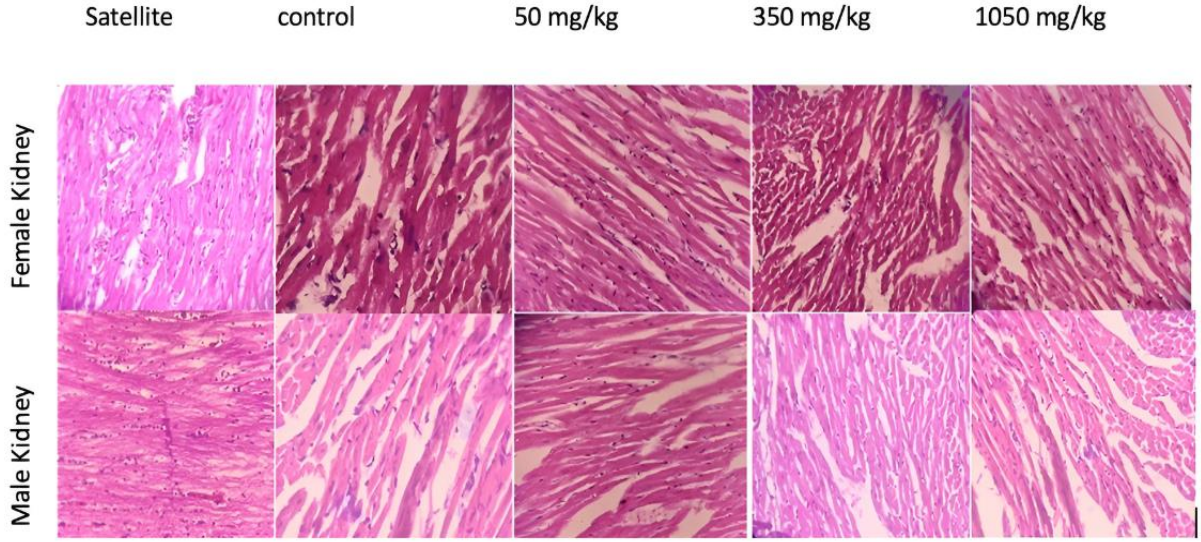


Figure 6. Histopathological results for the heart of female and male rats after exposure to *Caulerpa racemosa* extract (CRE).
The structure of the heart part showed no difference between the groups. The kidney appeared normal. 40× objective magnification and hematoxylin-eosin stain. Scale 1 cm = 100 µm.

This study began with an acute toxicity test using an extract concentration of 5000 mg/kg b.w. No deaths were recorded during the testing process, leading to the conclusion that the LD_{50} value of CRE exceeds 5000 mg/kg b.w., suggesting low toxicity (Gadaleta et al., 2019). Injuries caused by inter-species fights were observed during the study; however, the rate of wound healing was significantly faster in the group receiving 5000 mg/kg b.w. compared to the control group. Post-experiment observations revealed that mice in the test group exhibited healthier bodies and cleaner fur. This effect is attributed to polysaccharides in *Caulerpa*, which have wound-healing properties (Lee et al., 2021). The body weight of the satellite group and the 1050 mg/kg b.w. group was higher than the control group, likely due to an increase in appetite. *Caulerpa,* particularly *C. racemosa,* has been shown to enhance appetite and is recognized as a functional food, often utilized as a dietary supplement (Fithriani, 2013; Nasmia et al., 2022; Rumayomi, et al. 2024).

Repeated-dose toxicity testing was conducted to evaluate potential adverse effects of CRE on animals over a repeated administration period (Suwandi et al., 2021), spanning 28 days for the test group and 35 days for the satellite group. The results revealed no fatalities, toxic signs, or significant changes in body weight across all groups and genders. Additionally, vital organs, including the liver, kidneys, and heart, showed no morphological or weight alterations, suggesting that repeated administration of CRE did not impair normal growth or organ function.

Hematological parameter analysis showed no significant differences among the control, test, and satellite groups. All parameters remained within the normal range, although a reduction in RBC count was observed at CRE doses of 350 and 1050 mg/kg b.w. *C. racemosa* is known to contain various macro and micronutrients, including polysaccharides, proteins, fatty acids, vitamins, and minerals (Magdugo et al., 2020). These nutrients play an essential role in hematopoiesis and can influence the hematopoietic process (Tadokoro and Hirao, 2022; Ussishkin and Nachmani, 2023).

Biochemical analysis was performed to evaluate kidney function (urea and creatinine), liver function (AST and ALT), lipid profile (cholesterol and triglycerides), and glucose levels. The kidney function tests showed no statistically significant differences in urea and creatinine levels between genders. Polysaccharides present in *C. racemosa* may confer nephroprotective effects by reducing inflammation and oxidative stress. Additionally, these compounds can enhance antioxidant capacity by increasing the activity of SOD, CAT, and GSH-PX in damaged kidneys, including those affected by diabetic nephropathy (Cao et al., 2021).

ALT activity and triglyceride levels showed significant differences ($p<0.05$) in the male group. However, all values remained within the reference range (63-175 U/L) (Giknis and Clifford, 2008). Variations in ALT were observed in the groups administered with 50 mg/kg b.w. and 350 mg/kg b.w. of CRE compared to the control. The test group treated with 1050

mg/kg b.w. showed lower activity (51.63 U/L) than the other groups.

Statistical analysis of triglyceride levels revealed a significant difference ($p = 0.01$). Post hoc Tukey analysis indicated substantial disparities, with the group treated with 1050 mg/kg b.w. showing a significant increase in triglyceride levels compared to all other groups. Despite these differences, the triglyceride levels remained within the reference range of 27-160 mg/dL (Giknis and Clifford, 2008). Triglyceride levels can be influenced by dietary fatty acids found in food, including those from *C. racemosa*. Saturated fatty acids (SFA) tend to elevate plasma triglyceride levels, compared to polyunsaturated fatty acids (PUFA). *C. racemosa* contains 41.3% SFA, 34.6% monounsaturated fatty acids (MUFA), 21.6% PUFA, and 2.5% unidentified fatty acids (Lopez-Alvarenga et al., 2010; Magdugo et al., 2020), with the PUFA/SFA ratio in this alga being 0.52. To prevent cardiovascular disease (CVD) and chronic diseases such as cancer, it is recommended that foods should comprise ingredients with PUFA/SFA ratio greater than 0.45 (Wołoszyn et al., 2019).

Blood glucose parameter analysis revealed no significant differences between genders, and all values fell in the reference range. Moreover, CRE has been shown to reduce blood glucose levels in diabetic rats as well as normalize HbA1c levels, liver glycogen levels, glucose uptake, and glucose transport by hepatic cells (Mandlik et al., 2022). The glucose lowering effects of CRE are attributed to polysaccharides, which enhance pancreatic β-cell mass and function. These polysaccharides improve insulin signaling by interacting with insulin receptors and activating the PI3K/Akt pathway, which regulates the ERK/JNK/MAPK pathway (Ganesan and Xu, 2019).

During macroscopic histopathological observation, no changes or lesions were detected in the organs, and organ weights showed no significant changes among the control, test, and satellite groups ($p>0.05$). Similarly, microscopic histopathological analysis revealed no significant changes in any of the test groups. Histopathology results of the liver, kidneys, and heart showed no in cell and tissue structure in the test group compared to the control.

Based on the testing results, *C. racemosa* was deemed safe for use during the 28 and 35-day assessment periods. This finding aligns with its traditional use as a functional food in several Asian countries (Qudus B Aroyehun et al., 2020). However, further toxicity studies are necessary before the extract can be considered for use as a drug ingredient.

CONCLUSION

The results of the acute toxicity testing of CRE indicated an LD_{50} value exceeding 5000 mg/kg body weight, while the repeated-dose oral toxicity testing identified no adverse effects on vital organs during the 28-day trial. Therefore, the ethanol extract of *C. racemosa* examined in this study was deemed safe for consumption. However, further safety evaluations should be conducted using higher doses over an extended period to support comprehensive safety profiling.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Perdina N	Elin J	Neng FK
Concepts or ideas			x
Design	x	x	x
Definition of intellectual content			x
Literature search	x	x	x
Experimental studies	x	x	x
Data acquisition	x		x
Data analysis	x	x	x
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Manuscript preparation	x	x	x
Manuscript editing	x	x	x
Manuscript review	x	x	x

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