Journal of Tropical Biology and Conservation 22: 76–87, 2025 *ISSN 1823-3902*
E-ISSN 2550-1909

Research Article

Modulation of Oxidative Stress by *Centella asiatica* (L.) Urb. Leaves Against Carbon Tetrachloride-Induced Hepatic Damage in Rats

Mohammad IQBAL [1], Mohammad Amil Zulhilmi BENJAMIN [2] and Ping-Chin LEE [1,3]*

[1]*Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.*
[2]*Borneo Research on Algesia, Inflammation and Neurodegeneration (BRAIN) Group, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.*
[3]*Faculty of Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.*

*Corresponding author email address: miqbal@ums.edu.my/driqbalmohammad@gmail.com

Received 16 April 2025| Accepted 23 June 2025| Published 22 August 2025
Associate Editor: Ng Shean Yeaw
DOI: https://doi.org/10.51200/jtbc.v22i.6289

ABSTRACT

Liver injury induced by oxidative stress remains a significant global health concern, necessitating the exploration of safe and effective hepatoprotective agents derived from natural sources. *Centella asiatica* (L.) Urb., frequently referred to as 'pegaga', is a plant renowned for its notable medicinal attributes. The primary aim of this study is to assess the antioxidant capacity and hepatoprotective activity of the ethanolic extract of *C. asiatica* (EECA). The assessments used to evaluate EECA for its antioxidant properties included total phenolic content (TPC), as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays. Additionally, the study evaluated the ability of EECA to mitigate experimentally induced hepatic injury in Sprague-Dawley rats caused by carbon tetrachloride (CCl_4) at 100, 500, and 1,000 mg/kg b.wt. doses. Biochemical assays included measuring hepatic marker enzymes alanine transaminase (ALT) and aspartate transaminase (AST) in serum, as well as evaluating malondialdehyde (MDA) and reduced glutathione (GSH) levels in liver homogenates. The TPC of EECA was 106.55 ± 2.23 mg GAE/g. Its DPPH and reducing power assays exhibited moderately potent antioxidant attributes compared to standard references. Nonetheless, groups subjected to CCl_4 displayed notably elevated levels of ALT and AST, increased MDA, and decreased GSH. Pretreatment with EECA resulted in a marginal decrease in hepatic marker enzyme activity, with reductions in ALT (14% to 2-fold), AST (25% to 3-fold), and MDA (15–57%), along with an increase in GSH (3–23%). These findings imply that EECA contains active constituents capable of mitigating the hepatotoxic effects induced by CCl_4.

Keywords: Oxidative stress; hepatoprotection; ethanolic extract; *Centella asiatica*; antioxidants; carbon tetrachloride.

INTRODUCTION

Oxidative stress-induced liver injuries represent a significant medical challenge, arising from an imbalance between the ability of the body to neutralise reactive oxygen species (ROS) and their production. This condition occurs when the liver is exposed to harmful chemicals, resulting in cellular damage and disruption of essential liver functions. Contributing factors include prolonged alcohol consumption, infections, specific medications, environmental pollutants, high-calorie diets, and exposure to toxins, ultraviolet radiation, or heavy metals. Oxidative imbalance plays a critical role in driving inflammation, necrosis, apoptosis, fibrosis, and malignant transformation, and is a major contributor to the pathogenesis of non-alcoholic fatty liver disease (Allameh et al., 2023). According to the WHO (2024), cirrhosis and hepatocellular carcinoma—the most prevalent form of liver cancer—account for the majority of the estimated 290,000 hepatitis C-related deaths. Synthetic chemicals and medications used to treat or model liver diseases, such as carbon tetrachloride (CCl_4) and acetaminophen, have been reported to aggravate liver damage (Hota et al., 2022). This has led to an increased reliance on herbal drugs, which are now widely utilised. Herbal medications have a long-standing history in the treatment of liver conditions and offer a holistic approach to promoting liver health (Abdel-Hamid et al., 2018).

Centella asiatica (L.) Urb., commonly referred to as 'pegaga', is a medicinal plant with a long-standing role in traditional medicine, indigenous to Southeast Asian countries, including Malaysia and Indonesia (Orhan, 2012). For centuries, this herb has been utilised for its pharmacological properties, particularly due to its triterpene content. Studies have identified triterpene and phenolic compounds as the active constituents in *C. asiatica*, highlighting their relevance to the biological effects of the plant (Gnanapragasam et al., 2004). *C. asiatica* contains various bioactive compounds, such as madecassoside, madecassic acid, asiaticoside, and asiatic acid, which significantly contribute to its antioxidant, anti-inflammatory, and wound-healing activities (Hashim et al., 2011; Su et al., 2015). The rich phytochemical composition of *C. asiatica*, particularly its abundance of phenolic compounds, is associated with a broad spectrum of pharmacological effects. The presence of phenolics, including flavonoids, has been linked to antioxidant activities that play a vital role in protecting against oxidative stress and lipid peroxidation (LPO) (Zainol et al., 2003). Additionally, the phenolic compounds in *C. asiatica* have demonstrated antioxidant and cytotoxic activities, reinforcing the association between these compounds and the pharmacological properties of the plant (Nazmi & Sarbon, 2020). This establishes the significance of *C. asiatica* as a valuable herbal remedy in both traditional and modern medicine (Niamnuy et al., 2013; Ratz-Lyko et al., 2016).

Despite existing literature highlighting the potential of *C. asiatica* in liver protection, scientific confirmation remains limited, partly due to the narrow focus of studies relying predominantly on CCl_4-induced models, which may not fully reflect the complexity of hepatic injury in clinical settings. This limitation also stems from the absence of parameters involving antioxidative enzymes in the proposed mechanisms. Therefore, the present study aims to assess the antioxidant and hepatoprotective effects of the ethanolic extract of *C. asiatica* (EECA), with a focus on its potential to mitigate hepatic dysfunction and oxidative stress induced by CCl_4 in a rat model.

METHODOLOGY

Preparation of EECA

Whole *C. asiatica* plants were obtained from Papar, Sabah, Malaysia, verified by an ethnobotanist, and stored at the Biotechnology Research Institute, Universiti Malaysia Sabah, with the voucher number MI 001. Following rinsing with distilled water, the leaves were dried at 37 °C for 72 h until a constant weight was achieved, and then finely ground into powder. A total of 100 g of dried powder was extracted with 400 mL of 80% ethanol by shaking in a water bath at 40 °C for 4 h. The mixture was centrifuged at 3,000 rpm for 10 min and filtered through Whatman No. 1 filter paper. Ethanol was removed using a rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) at 40 °C for 30 min under reduced pressure. The sample was frozen overnight at −80 °C, lyophilised, and the resulting EECA powder was stored at −80 °C for further analysis.

Chemical antioxidant assays

The total phenolic content (TPC) was analysed using a modified Folin–Ciocalteu procedure reported by Awang et al. (2023). Absorbance was measured at 720 nm using a spectrophotometer (PerkinElmer, Waltham, MA, USA), and results were expressed as mg GAE/g of extract. The free radical scavenging activity of EECA was evaluated using a slightly modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay based on the method of Jinoni et al. (2024). Absorbance was measured at 517 nm following vortexing and 60 min of incubation at room temperature, with results expressed as percentage inhibition. The reducing power assay followed the method of Bhalodia et al. (2013), with absorbance measured at 700 nm against a blank, where higher absorbance indicated greater reducing capacity. Ascorbic acid (AA) served as the positive control in both DPPH and reducing power assays.

Experimental design

The animal experiment complied with the ethical guidelines of the university and federal laws governing animal experimentation. Approval was obtained from the Animal Ethics Committee under protocol number UMS/IP7.5/M3/4-2012. Male Sprague-Dawley rats (7–8 weeks old, 200–250 g) were acclimated prior to the study and housed under controlled conditions at the Biotechnology Research Institute, Universiti Malaysia Sabah, with free access to tap water and standard rodent chow. Rats were randomly divided into five groups (n = 6 per group) based on treatment and hepatotoxicity protocols, as shown in Fig. 1. All animals received treatments by oral gavage once daily for 14 consecutive days. On days 13 and 14, rats (except those in the normal group) received CCl_4 (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:1 in corn oil, administered orally via gavage at a dose of 1 mL/kg b.wt. to induce hepatotoxicity. The selection of experimental groups and the CCl_4 dosing regimen were based on established hepatotoxicity models in rodents, as described by Vun-Sang et al. (2024).

Each treated animal was euthanised 24 h after the final administration of CCl_4. Following anaesthesia induction with mild ether, the animal was decapitated, and blood was collected via cardiac puncture into tubes coated with lithium heparin to obtain plasma samples. The liver was excised, connective tissue removed, and the organ rinsed with saline to eliminate potential blood contamination. Liver tissue was then stored at –80 °C in a freezer (Thermo Fisher Scientific, Waltham, MA, USA) for biochemical analyses to assess the activity of hepatic antioxidant enzymes.

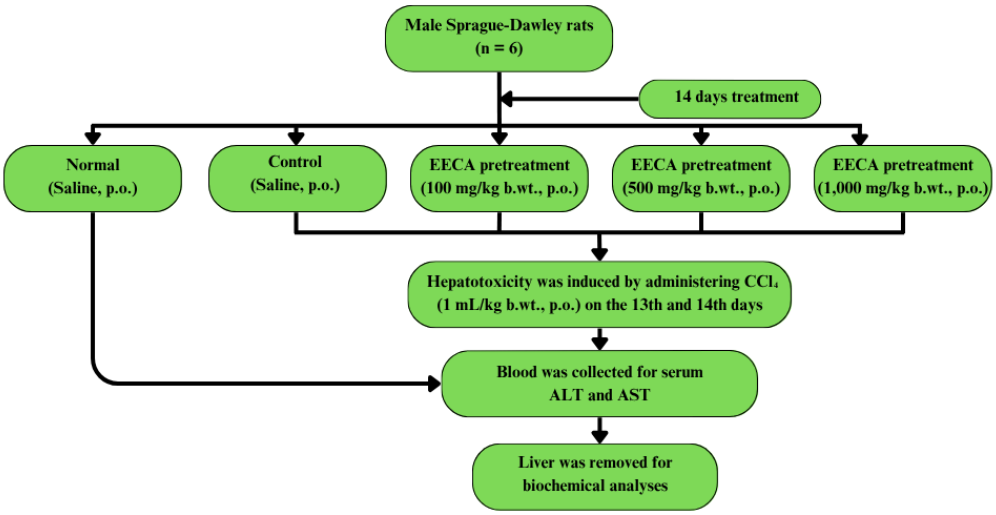

Figure 1: Summary of experimental design.

Preparation of post-mitochondrial supernatant

Post-mitochondrial supernatant was prepared from liver tissue using a standard procedure adapted from Iqbal et al. (1999). The tissue was homogenised using a homogeniser (Kinematica AG, Malters, Switzerland) in cold phosphate buffer (0.1 M, pH 7.4) containing 1.17% (w/v) potassium chloride. The homogenate was centrifuged at 2,000 rpm for 10 min at 4 °C to remove nuclei and cell debris. The resulting supernatant was further centrifuged at 10,000 rpm for 30 min at 4 °C to obtain the post-mitochondrial fraction, which was used for the assessment of endogenous antioxidant enzymes. Protein concentration was determined prior to enzyme assays using the Bradford method, with bovine serum albumin as the standard.

In vitro antioxidant and oxidative stress biomarkers

Serum obtained by centrifuging blood at 1,500 rpm for 15 min and stored at –20 °C was used to analyse alanine transaminase (ALT) and aspartate transaminase (AST) through separate enzymatic assays. According to Reitman and Frankel (1957), colour development in the reaction mixtures was measured at 510 nm after 30 min. LPO was evaluated by determining malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method described by Buege and Aust (1978), with absorbance measured at 535 nm. MDA levels were calculated using a molar absorptivity of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol MDA formed/g tissue. Reduced glutathione (GSH) levels were determined using the method described by Jollow et al. (1974), with absorbance measured at 412 nm and results reported as µmol reduced GSH/g tissue.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical comparisons between groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test. Statistical evaluation was performed using GraphPad Prism (version 10), with $p < 0.05$ considered significant.

RESULTS

Effect of EECA on antioxidant activity

Leaves and other plant components are rich in phenolic compounds, which contribute to stable antioxidant enzyme activity. The TPC of EECA was 106.55 ± 2.23 mg GAE/g, indicating high phenolic content. As shown in Fig. 2, EECA exhibited concentration-dependent DPPH scavenging activity, with the highest inhibition recorded at 80.00 ± 0.01% at 600 µg/mL. Similarly, Fig. 3 shows a concentration-dependent increase in reducing power by EECA (10–200 µg/mL), compared to AA. Although EECA demonstrated significant antioxidant potential, its reducing capacity was markedly lower than that of AA.

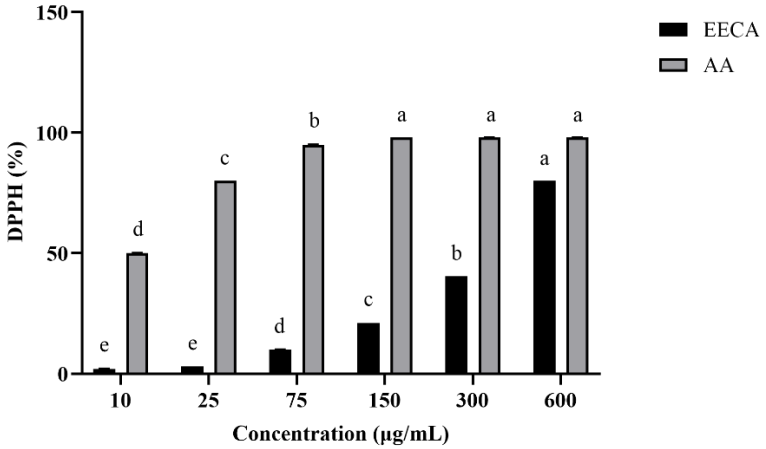

Figure 2: Effects of EECA on DPPH radical scavenging activity across various concentrations (10–600 µg/mL). Data are presented as mean ± standard deviation (n = 3). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$).

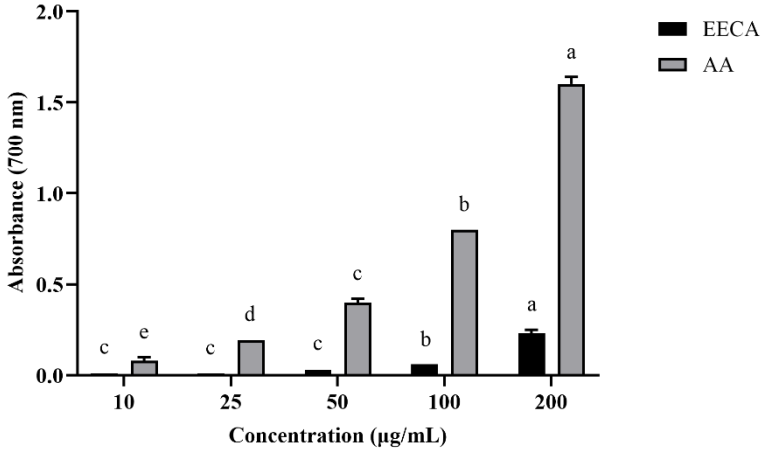

Figure 3: Effects of EECA on reducing power across various concentrations (10–200 µg/mL). Data are presented as mean ± standard deviation (n = 3). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$).

Effects of EECA on serum ALT and AST

Assessment of liver structural integrity relies on evaluating aminotransferase activities, namely ALT and AST. Serum ALT and AST levels in the control group increased significantly ($p < 0.05$) by 99% and 97%, respectively, compared to the normal group, as shown in Fig. 4. EECA pretreatment at 100 and 500 mg/kg b.wt. significantly ($p < 0.05$) reduced the CCl_4-induced increase in serum ALT by 14% and 35%, and AST by 25% and 91%, respectively. Notably, EECA at 1,000 mg/kg b.wt. demonstrated greater efficacy ($p < 0.05$), reducing ALT and AST levels by approximately 2- and 3-fold, respectively, in a dose-dependent manner compared to the lower doses.

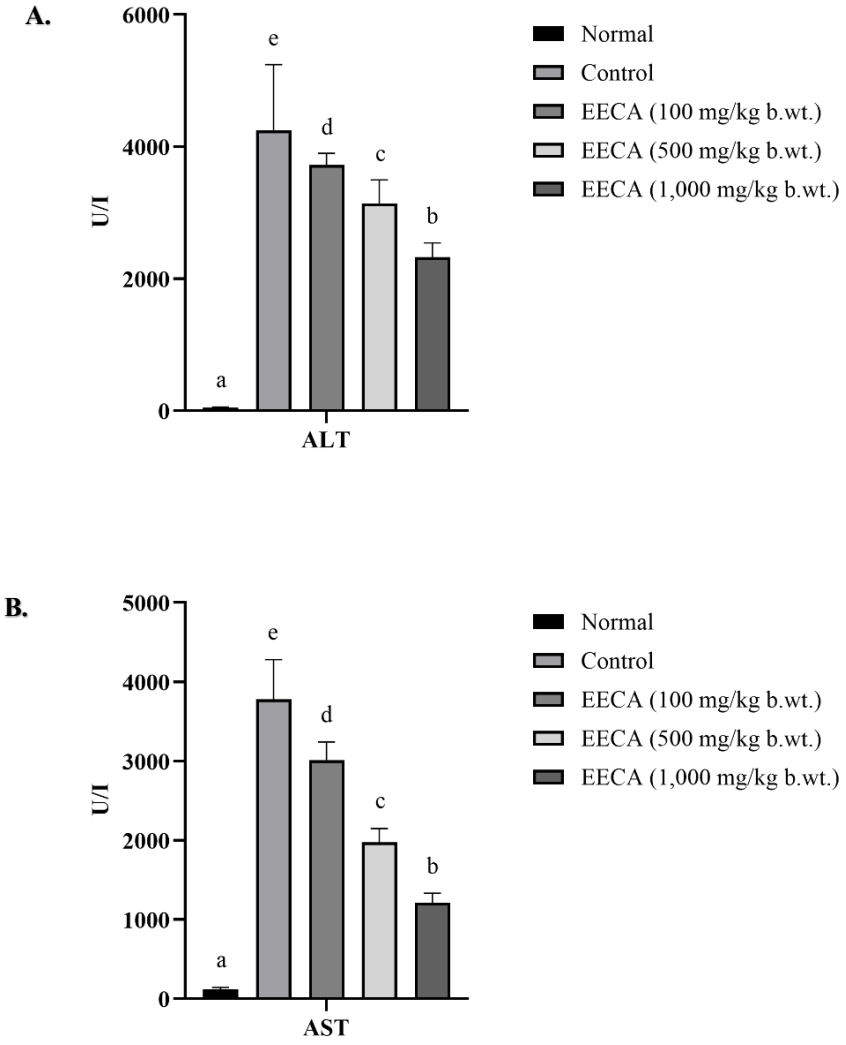

Figure 4: Effects of EECA on serum **A.** ALT and **B.** AST levels across various groups. Data are presented as mean ± standard deviation (n = 6). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$).

Effects of EECA on MDA activity

Hepatic TBARS levels were assessed by measuring MDA formation through its reactivity with TBA, producing a pink chromophore. Rats subjected to CCl_4 treatment exhibited a substantial ($p < 0.05$) increase in hepatic TBARS levels (expressed as MDA) by 44% compared to the normal group, as shown in Fig. 5. However, pretreatment with EECA at 100, 500, and 1,000

mg/kg b.wt. significantly ($p < 0.05$) reduced TBARS levels in a dose-dependent manner by 15%, 33%, and 57%, respectively, with the highest dose showing the greatest inhibition.

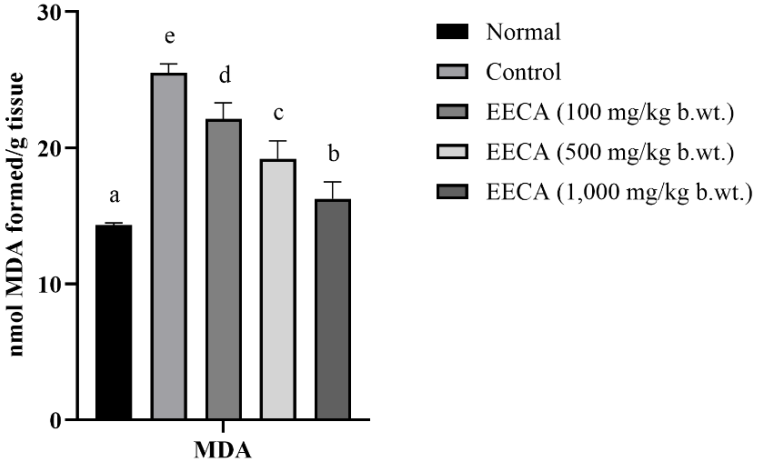

Figure 5: Effects of EECA on MDA levels across various groups. Data are presented as mean ± standard deviation (n = 6). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$).

Effects of EECA on GSH activity

Hepatic GSH levels showed a significant ($p < 0.05$) reduction of 40% in the control group compared to the normal group, indicating oxidative stress (Fig. 6). However, pretreatment with EECA at 100, 500, and 1,000 mg/kg b.wt. significantly ($p < 0.05$) mitigated GSH depletion, increasing GSH levels by 3%, 17%, and 23%, respectively, in a dose-dependent manner. Notably, the 1,000 mg/kg b.wt. dose provided the highest level of protection.

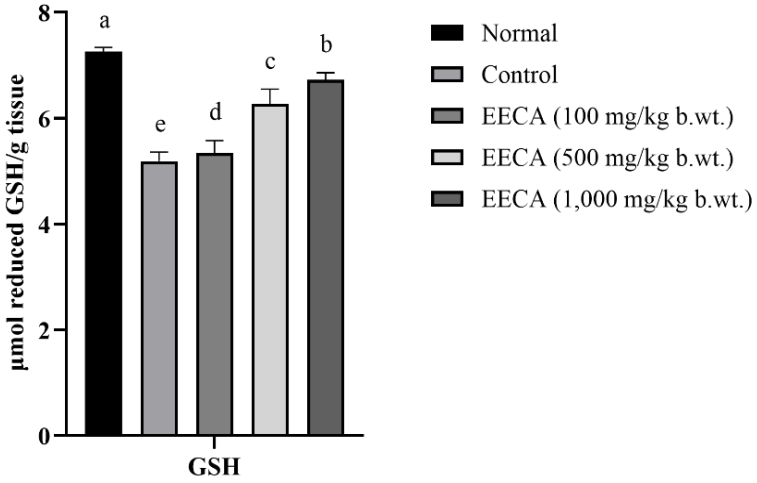

Figure 6: Effects of EECA on GSH levels across various groups. Data are presented as mean ± standard deviation (n = 6). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$).

DISCUSSION

Phenolic compounds are widely recognised for their antioxidant activity, chain-breaking properties, physiological functions, and hepatoprotective effects. These effects arise from redox potential, enabling neutralisation of free radicals, decomposition of peroxides, and suppression of singlet and triplet oxygen (Shahidi & Ambigaipalan, 2015). The Folin–Ciocalteu method, employed to determine TPC, produces a blue solution with maximum absorption at 765 nm through the reduction of metal oxides by phenolic acids (Pérez et al., 2023). According to Moon and Shibamoto (2009), numerous studies have utilised these assays to evaluate antioxidant capacity in various food products. The DPPH assay is widely used due to its simplicity, high sensitivity, minimal technical expertise required, and reliance on a basic spectrophotometer. The reaction involves a colour change from purple to pale yellow as DPPH radicals are reduced and hydrogen atoms are abstracted from antioxidants, indicating effective scavenging activity (Moon & Shibamoto, 2009). Similarly, the ferric reducing antioxidant power assay assesses the ability of antioxidants to convert ferric iron (Fe^{3+}-TPTZ) into the more stable divalent Fe^{2+} ion at low pH, producing a violet-blue colour that ensures consistent and timely results (Nwachukwu & Aluko, 2019). Overall, the study demonstrated the antioxidant properties of EECA, as shown by its high TPC and effective elimination of the stable free radical DPPH, although its reducing power, despite being concentration-dependent, was comparatively weak. Previous research has established a correlation between TPC, DPPH radical scavenging activity, and reducing power (Benjamin et al., 2022), suggesting that the observed antioxidant effects of EECA are attributed to its high concentration of phenolic compounds.

CCl_4 has long been recognised as a hepatotoxin capable of causing liver damage (Thanebal et al., 2021), primarily entering water sources through industrial waste due to its use in chlorofluorocarbon synthesis (Borzelleca et al., 1990). Its toxicity is associated with accumulation in liver adipose tissue and centrilobular necrosis, with hepatotoxicity initiated in the endoplasmic reticulum of hepatocytes via cytochrome P-450 enzymes. Frequently employed as a model for studying hepatotoxicity (Vun-Sang et al., 2024), CCl_4 induces tissue damage through oxidative stress mediated by LPO. Cytochrome P-450 converts CCl_4 into highly reactive trichloromethyl (CCl_3•) and trichloromethylperoxyl (CCl_3O_2•) radicals. These unpaired electron species trigger chain reactions targeting lipid-rich membranes, including mitochondria and the endoplasmic reticulum, disrupting cellular structures and leading to oxidative, mitochondrial, and endoplasmic reticulum stress. These events contribute to apoptosis, necrosis, ferroptosis, and autophagy (Unsal et al., 2021). Free radicals, produced under specific environmental conditions and normal cellular metabolism, lack an electron and achieve stability by acquiring or donating one to nearby molecules. Antioxidants neutralise free radicals before chain reactions responsible for oxidative damage can begin (Chaudhary et al., 2023). To evaluate the potential of EECA in mitigating oxidative stress and free radical-induced damage, its antioxidant properties were assessed in a rat model of CCl_4-induced hepatotoxicity.

Liver health can be assessed by measuring serum ALT and AST levels, as these cytoplasmic enzymes are released into circulation following hepatocyte membrane damage (Wang et al., 2017). ALT catalyses the conversion of alanine to pyruvate, while AST catalyses the conversion of aspartate to oxaloacetate. EECA may have protected hepatocyte membranes from damage caused by reactive metabolites during CCl_4 exposure, thereby reducing enzyme leakage (McGill, 2016). The lower expression of these transaminases in EECA-treated rats supports this hypothesis. One proposed mechanism suggests that free radical derivatives of

CCl_4 contribute to LPO, leading to hepatopathy, and that protection against CCl_4 toxicity requires significant antioxidant activity or inhibition of free radical generation (Yin et al., 2011). A substantial increase in MDA levels was observed in the CCl_4-only group, indicating that elevated LPO levels contributed to tissue damage and overwhelmed antioxidant defence systems. Treatment with EECA significantly reduced MDA levels, demonstrating its antioxidant potential. GSH levels are critical in determining tissue susceptibility to oxidative injury, as this tripeptide acts as a non-enzymatic antioxidant, providing protection on both intracellular and extracellular surfaces (Kurutas, 2016). GSH, a non-protein sulphydryl compound, helps maintain the reduced state of membrane protein-SH groups, while persistent oxidative stress can lead to cellular deformities (Bansal, 2015). The lower GSH levels observed in rats exposed to CCl_4, compared to those treated with both EECA and CCl_4, suggest that EECA mitigated susceptibility to CCl_4-induced hepatic injury. These findings are consistent with those reported by Sivakumar et al. (2018) and Park et al. (2021), who observed reduced serum hepatic markers and LPO, along with restoration of endogenous antioxidant enzymes, following treatment with *C. asiatica* extract in acetaminophen-induced hepatotoxicity. Hence, the hepatoprotective effects observed in this study further support the therapeutic potential of *C. asiatica*.

Prior research has established that antioxidative enzymes act as a primary defence against ROS and other free radicals. This study evaluated the antioxidant potential of EECA using a rat hepatotoxicity model induced by CCl_4 intoxication, with a focus on serum ALT and AST levels, as well as MDA and GSH in hepatic tissues. The findings contribute to understanding the role of *C. asiatica* administration in mitigating oxidative liver damage, suggesting a potential nutritional alternative to pharmacological interventions for reducing hepatotoxicity. However, a key limitation of the study is the absence of histopathological analysis, which is crucial for providing comprehensive insights into hepatic tissue integrity and for complementing biochemical assessments. Such analyses are essential for evaluating structural damage and tissue inflammation caused by toxic substances such as CCl_4. Future research should incorporate more extensive histopathological examinations to gain deeper insights into liver tissue architecture, the mechanisms underlying CCl_4-induced damage, and the hepatoprotective effects of EECA.

CONCLUSIONS

The results indicate that EECA exhibits significant antioxidant activity, providing effective protection against CCl_4-induced hepatic toxicity in a dose-dependent manner at 100, 500, and 1,000 mg/kg b.wt. This protective effect is demonstrated by improvements in ALT and AST levels, reductions in MDA levels, and prevention of GSH depletion in hepatic cells. The findings suggest that EECA mitigates CCl_4-induced hepatic injury by enhancing antioxidant defence mechanisms and reducing oxidative stress. These outcomes highlight the potential of *C. asiatica* in counteracting ROS-related liver damage.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the Ministry of Higher Education, Malaysia, for supporting this work through grant no. FRG166-SP-2008. Special appreciation is extended to Lee Ping Chin, Director of the Biotechnology Research Institute, Universiti Malaysia Sabah, for her invaluable guidance and support.

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.

REFERENCES

Abdel-Hamid NM, Abass SA, Mohamed AA, Muneam Hamid D (2018) Herbal management of hepatocellular carcinoma through cutting the pathways of the common risk factors. Biomedicine and Pharmacotherapy 107: 1246–1258. https://doi.org/10.1016/j.biopha.2018.08.104.

Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K (2023) Oxidative stress in liver pathophysiology and disease. Antioxidants 12: 1653. https://doi.org/10.3390/antiox12091653.

Awang MA, Benjamin MAZ, Anuar A, Ismail MF, Ramaiya SD, Mohd Hashim SNA (2023) Dataset of gallic acid quantification and their antioxidant and anti-inflammatory activities of different solvent extractions from kacip fatimah (*Labisia pumila* Benth. & Hook. f.) leaves. Data in Brief 51: 109644. https://doi.org/10.1016/j.dib.2023.109644.

Bansal AK (2015) Antioxidants and other potent strategies to reduce oxidative stress in semen. In Free Radicals in Human Health and Disease, Rani V, Yadav UCS (eds). Springer: New Delhi, India; 381–395. https://doi.org/10.1007/978-81-322-2035-0_24.

Benjamin MAZ, Ng SY, Saikim FH, Rusdi NA (2022) The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules 27: 6458. https://doi.org/10.3390/molecules27196458.

Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ (2013) In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. AYU 34: 209–214. https://doi.org/10.4103/0974-8520.119684.

Borzelleca JF, O'Hara TM, Gennings C, Granger RH, Sheppard MA, Condie LW (1990) Interactions of water contaminants. I. Plasma enzyme activity and response surface methodology following gavage administration of CCl_4 and $CHCl_3$ or TCE singly and in combination in the rat. Fundamental and Applied Toxicology 14: 477–490. https://doi.org/10.1016/0272-0590(90)90252-f.

Buege JA, Aust SD (1978) [30] Microsomal lipid peroxidation. In Methods in Enzymology, Fleischer S, Packer L (eds). Academic Press: Cambridge, MA, USA; 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J (2023) Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry 11: 1158198. https://doi.org/10.3389/fchem.2023.1158198.

Gnanapragasam A, Kumar Ebenezar K, Sathish V, Govindaraju P, Devaki T (2004) Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. Life Sciences 76: 585–597. https://doi.org/10.1016/j.lfs.2004.09.009.

Hashim P, Sidek H, Helan MHM, Sabery A, Palanisamy UD, Ilham M (2011) Triterpene composition and bioactivities of *Centella asiatica*. Molecules 16: 1310–1322. https://doi.org/10.3390/molecules16021310.

Hota RN, Nanda BK, Behera BR, Bose A (2022) Ameliorative effect of ethanolic extract of *Limnophila rugosa* (Scrophulariaceae) in paracetamol- and carbon tetrachloride-induced hepatotoxicity in rats. Future Journal of Pharmaceutical Sciences 8: 6. https://doi.org/10.1186/s43094-021-00397-y.

Iqbal M, Giri U, Giri DK, Alam MS, Athar M (1999) Age-dependent renal accumulation of 4-hydroxy-2-nonenal (HNE)-modified proteins following parenteral administration of ferric nitrilotriacetate commensurate with its differential toxicity: Implications for the involvement of HNE-protein adducts in oxidative stress and carcinogenesis. Archives of Biochemistry and Biophysics 365: 101–112. https://doi.org/10.1006/abbi.1999.1135.

Jinoni DA, Benjamin MAZ, Mus AA, Goh LPW, Rusdi NA, Awang MA (2024) *Phaleria macrocarpa* (Scheff.) Boerl. (mahkota dewa) seed essential oils: Extraction yield, volatile components, antibacterial, and antioxidant activities based on different solvents using Soxhlet extraction. Kuwait Journal of Science 51: 100173. https://doi.org/10.1016/j.kjs.2023.100173.

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11: 151–169. https://doi.org/10.1159/000136485.

Kurutas EB (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutrition Journal 15: 71. https://doi.org/10.1186/s12937-016-0186-5.

McGill MR (2016) The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI Journal 15: 817–828. https://doi.org/10.17179/excli2016-800.

Moon J-K, Shibamoto T (2009) Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry 57: 1655–1666. https://doi.org/10.1021/jf803537k.

Nazmi NNM, Sarbon NM (2020) Characterization on antioxidant and physical properties of gelatin based composite films with incorporation of *Centella asiatica* (pegaga) extract. Food Research 4: 224–233.

Niamnuy C, Charoenchaitrakool M, Mayachiew P, Devahastin S (2013) Bioactive compounds and bioactivities of *Centella asiatica* (L.) Urban prepared by different drying methods and conditions. Drying Technology 31: 2007–2015. https://doi.org/10.1080/07373937.2013.839563.

Nwachukwu ID, Aluko RE (2019) Structural and functional properties of food protein-derived antioxidant peptides. Journal of Food Biochemistry 43: e12761. https://doi.org/10.1111/jfbc.12761.

Orhan IE (2012) *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evidence-based Complementary and Alternative Medicine 2012: 946259. https://doi.org/10.1155/2012/946259.

Park DW, Jeon H, et al. (2021) Hepatoprotective effect of *Centella asiatica* 50% ethanol extract against acetaminophen-induced acute liver injury in BALB/c mice. Toxicological Research 37: 261–275. https://doi.org/10.1007/s43188-020-00063-0.

Pérez M, Dominguez-López I, Lamuela-Raventós RM (2023) The chemistry behind the Folin–Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic intake in a mediterranean dietary pattern. Journal of Agricultural and Food Chemistry 71: 17543–17553. https://doi.org/10.1021/acs.jafc.3c04022.

Ratz-Lyko A, Arct J, Pytkowska K (2016) Moisturizing and anti-inflammatory properties of cosmetic formulations containing *Centella asiatica* extract. Indian Journal of Pharmaceutical Sciences 78: 27–33. https://doi.org/10.4103/0250-474X.180247.

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28: 56–63. https://doi.org/10.1093/ajcp/28.1.56.

Shahidi F, Ambigaipalan P (2015) Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Journal of Functional Foods 18: 820–897. https://doi.org/10.1016/j.jff.2015.06.018.

Sivakumar V, Sadiq AM, Bharathi SD (2018) Hepatoprotective activity of *Centella asiatica* Linn. against paracetamol induced liver damage in experimental animals. Emergent Life Sciences Research 4: 19–26. https://doi.org/10.31783/elsr.2018.411926.

Su Z, Ye J, Qin Z, Ding X (2015) Protective effects of madecassoside against doxorubicin induced nephrotoxicity in vivo and in vitro. Scientific Reports 5: 18314. https://doi.org/10.1038/srep18314.

Thanebal SAP, Vun-Sang S, Iqbal M (2021) Hepatoprotective effects of *Pandanus amaryllifolius* against carbon tetrachloride (CCl_4) induced toxicity: A biochemical and histopathological study. Arabian Journal of Chemistry 14: 103390. https://doi.org/10.1016/j.arabjc.2021.103390.

Unsal V, Cicek M, Sabancilar İ (2021) Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Reviews on Environmental Health 36: 279–295. https://doi.org/10.1515/reveh-2020-0048.

Vun-Sang S, Sivapragasam G, Yong WTL, Benjamin MAZ, Iqbal M (2024) Assessment of antioxidant and hepatoprotective effects of *Kappaphycus alvarezii* (Doty) Doty ex Silva against carbon tetrachloride-induced liver injury in rats. Journal of Tropical Biology and Conservation (JTBC) 21: 73–87. https://doi.org/10.51200/jtbc.v21i.5420.

Wang L, Li N, Lin D, Zang Y (2017) Curcumin protects against hepatic ischemia/reperfusion induced injury through inhibiting TLR4/NF-κB pathway. Oncotarget 8: 65414–65420. https://doi.org/10.18632/oncotarget.18676.

World Health Organization (2024) Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c. (Accessed 28 November 2024).

Yin H, Xu L, Porter NA (2011) Free radical lipid peroxidation: Mechanisms and analysis. Chemical Reviews 111: 5944–5972. https://doi.org/10.1021/cr200084z.

Zainol MK, Abd-Hamid A, Yusof S, Muse R (2003) Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. Food Chemistry 81: 575–581. https://doi.org/10.1016/S0308-8146(02)00498-3.