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Orthosiphon stamineus Benth. Impedes Carbon Tetrachloride-Induced Hepatic Damage by Modulating Oxidative Stress

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Abstract

Orthosiphon stamineus Benth. (Lamiaceae) is an herb traditionally used in folk medicine for protecting against various diseases such as oxidative stress. This study aimed to assess the antioxidant and hepatoprotective properties of the ethanol extract of *O. stamineus* (EEOS) in rats with hepatic injury induced by carbon tetrachloride (CCl_4). The antioxidant properties of EEOS were evaluated through total phenolic content (TPC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays. Sprague-Dawley rats were orally administered EEOS at doses of 100, 500, and 1000 mg/kg b.wt. for two weeks, followed by exposure to CCl_4 (1.2 mL/kg). The rats were then euthanised for biochemical analyses. In vitro studies demonstrated the robust antioxidant potential of EEOS, revealing effective scavenging of DPPH free radicals and reducing power. Additionally, EEOS exhibited a high TPC of 127.28 ± 1.57 mg GAE/g db, contributing significantly to its antioxidant activities. Administration of EEOS significantly mitigated CCl_4-induced toxicity, as evidenced by reduced alanine transaminase (1–91-fold recovery) and aspartate transaminase (1–28-fold recovery) levels of hepatic damage in rats. Moreover, EEOS alleviated the heightened malondialdehyde levels (40–80% recovery) and elevated reduced glutathione levels (30–80% recovery) in CCl_4-induced rats, while also restoring levels of various antioxidant enzymes: catalase (12–20% recovery), glutathione peroxidase (23–41% recovery), glutathione reductase (6–30% recovery), glutathione S-transferase (11–25% recovery), and quinone reductase (8–35% recovery). The study conclusively demonstrates the strong antioxidant potential of EEOS, with 1000 mg/kg b. wt. exhibiting efficacy in restoring antioxidant enzymes. These compelling findings highlight the potential of EEOS as a promising candidate for preventing liver damage associated with reactive oxygen species.

INTRODUCTION

Oxidative stress-induced liver injuries pose a substantial medical dilemma due to the incompatibility between the body's ability to neutralise reactive oxygen species (ROS) and their production. This condition arises when the liver is exposed to a burden of harmful chemicals, leading to cellular damage and disruption of essential liver processes. Numerous factors, including prolonged alcohol use, infections, certain drugs, pollutants, and

other harmful substances, are implicated in this condition [1]. Oxidative imbalance influences processes such as apoptosis, ischemia/regeneration, and necrosis. The presence of oxidative stress has a substantial impact on the aetiology of non-alcoholic fatty liver disease [1]. According to a 2019 report by the World Health Organisation, cirrhosis and hepatocellular carcinoma, the most prevalent type of cancer of the liver, accounted for the majority of the predicted 290,000 deaths due to hepatitis C [2]. It is noteworthy that synthetic medications used to treat liver diseases can also exacerbate liver damage. Consequently, there has been a growing trend in the use of herbal drugs, which is now widespread. Herbal medications have a longstanding history in treating liver problems, offering a holistic approach to maintaining a healthy liver [3,4].

Orthosiphon stamineus Benth., also known as *O. aristatus* (Blume) Miq., is a perennial herbaceous plant classified within the family Lamiaceae (Figure 1). Widely distributed in Borneo, it is frequently encountered in the wild, forest fringes, and along roadsides. Additionally, it is found in regions such as Papua New Guinea, Singapore, the Philippines, Thailand, Cambodia, and Vietnam. In English, it goes by the name Java tea, while in Bahasa Melayu, it is referred to as 'Misai Kucing' [5,6]. *O. stamineus* is renowned for its rich array of secondary metabolites, encompassing polyphenols like sinensetin and eupatorin, as well as diterpenes such as orthosiphols R–T, norstaminolactone A, and norstaminols B and C. Triterpenes like α-amyrin, maslinic acid, hydroxybetulinic acid, betulinic acid, and ursolic acid, along with phenolic acids such as chlorogenic acid and rosmarinic acid, and essential oils have also been identified [5,6]. The plant extract has been documented to showcase various beneficial properties, including anticlastogenic, antimutagenic, cytoprotective, antiapoptotic, antioxidant, hepatoprotective, and antidiabetic effects [7–10]. Furthermore, studies suggest that the extract exhibits no toxicity within concentrations ranging from 0.5 to 5 g/kg [11].

Figure 1. *Orthosiphon stamineus* plants.

Although existing literature reviews discuss the hepatoprotective potential of *O. stamineus*, there is a lack of scientific confirmation of its liver-protective properties. This is mainly because the mechanism for its protective effects lacks parameters involving antioxidative enzymes. Therefore, the primary aim of this research endeavour is to evaluate the hepatoprotective properties of the ethanol extract of *O. stamineus* (EEOS). The purpose of this evaluation is to examine the antioxidant and chemopreventive properties of the substance in question, with a specific focus on its ability to mitigate hepatic dysfunction and oxidative stress caused by carbon tetrachloride (CCl_4) in rats.

MATERIALS AND METHODS

Chemicals, Reagents and Equipment

The following substances were acquired from Sigma-Aldrich (Burlington, MA, USA): ascorbic acid (AA), dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, sodium carbonate ($NaCO_3$), iron trichloride, hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), potassium ferricyanide, nicotinamide adenine dinucleotide phosphate (NADPH), 2,6-dichlorophenolindophenol (2,6-DCP), 1-chloro-2,4-dinitrobenzene (CDNB), and other biochemical assays. Furthermore, all chemicals and solvents utilised, including CCl_4, were of analytical grade or the highest purity currently available on the market.

The equipment utilised in this study was procured from the Biotechnology Research Institute, Universiti Malaysia Sabah, Sabah, Malaysia. It encompasses a water bath shaker (Jeio Tech, Daejeon, South Korea), centrifuge (Eppendorf, Hamburg, Germany), oven (Binder, Tuttlingen, Germany), Whatman No. 1 filter paper (Maidstone, Kent, UK), freezer (Thermo Fisher Scientific, Waltham, MA, USA), rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland), freeze dryer (Labconco, Kansas City, MO, USA), spectrophotometer (PerkinElmer, Waltham, MA, USA), and chemical analyser (Reflotron, Roche, Basel, Switzerland).

Sample Preparation

The samples were sourced from Papar, Sabah, Malaysia (Coordinates: 6°02'16.4" N 116°07'38.8" E) and were identified by an ethnobotanist from the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (voucher specimen number: OS001). After rinsing with distilled water, the leaves were dried at 37 °C until reaching a powdery consistency. Subsequently, 100 g of the dried sample was agitated with 400 mL of 80% ethanol and incubated at 40 °C in a water bath shaker for 4 h. Following centrifugation process at 3,000 rpm for 10 min, the extracts were filtered through Whatman No. 1 filter paper. The ethanol residue from the sample was then extracted using a rotary evaporator operating at 40 °C for 20–30 min under reduced pressure. After overnight freezing at –80 °C, the resulting sample was lyophilised using a freeze dryer. The freeze-dried powder was stored in a freezer at –80 °C for further analysis.

Total Phenolic Content

To determine the total phenolic content (TPC), the Folin-Ciocalteu method was employed. In this procedure, 0.2 mL of EEOS was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted at a 1:10 ratio) and allowed to stand for 5 min at room temperature. Following that, this mixture was combined with 1.5 mL of $NaCO_3$ (60 g/L) and left to incubate at room temperature for 90 min in the absence of light. The absorbance at 725 nm was measured using a spectrophotometer, with a blank used for comparison. The TPC, expressed as milligrams of gallic acid equivalents per gram of dry basis (mg GAE/g db), was determined using a reference standard curve containing gallic acid [12].

DPPH Assay

The antioxidant activity of EEOS was evaluated using the DPPH assay, following the protocol outlined by Hatano et al. [13]. The EEOS, prepared by dissolving it in DMSO at a concentration of 5 mg/mL, was mixed with the DPPH reagent (6×10^{-5} mol/L) in an ethanol solution. AA was employed as the positive control. The percentage of radical scavenging activity was determined using the following formula:

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_s represents the absorbance of the control, and A_c represents the absorbance of the sample.

Reducing Power Assay

The reducing power of EEOS was determined following the method outlined by Oyaizu [14]. Initially, 1.0 mL of EEOS suspended in distilled water was mixed with 2.5 mL of potassium ferricyanide (1%, w/v) and phosphate buffer (0.2 M, pH 6.6). After incubating the resulting solution at 50 °C for 20 min, 2.5 mL of (10%, w/v) TCA was added. Following a 10-minute centrifugation at 3,000 rpm, the upper portion (2.5 mL) of the solution was combined with 0.5 mL of iron trichloride (0.1%, w/v) and 2.5 mL of distilled water. The absorbance at 700 nm against a blank sample was then measured. An increase in absorbance within the reaction mixture indicated the presence of reducing power.

Experimental Protocol

The animal experiments strictly adhered to ethical guidelines, university standards, and federal legislation regarding animal research (Animal Ethics Committee: UMS/IP7.5/M3/4-2012). Male Sprague-Dawley rats, obtained from Universiti Sains Malaysia, Penang, Malaysia, weighing between 120 to 150 g, were housed in the Animal Research and Service Centre Health Campus. After acquisition, the rats underwent a one-week acclimated period in an animal room under a 12-hour light-dark cycle with ad libitum access to food and water. To induce hepatic injury, a solution containing CCl_4 and maize oil in a 1:1 ratio with a dose of 1.2 mL/kg b.wt. was formulated. Subsequently, the rats were orally administered EEOS at doses of 100, 500, and 1000 mg/kg b.wt. using oral gavage needles.

Approximately 30 adult male rats were randomly divided into 5 groups, each comprising 6 rats. These groups were designated as follows: the first group (normal) and the second group (control) received saline, the third group received EEOS (100 mg/kg b.wt.), the fourth group received EEOS (500 mg/kg b.wt.), and the fifth group received EEOS (1000 mg/kg b.wt.). All treatments were administered orally during the experimental period for 14 days, followed by the CCl_4 (1.2 mL/kg b.wt.) inducer on the 13th and 14th day, except for normal group.

After 24 h from the final CCl_4 administration, each rat was euthanised, and blood samples were collected via cardiac puncture using sterile disposable needles. Serum components were then separated by centrifugation at 5,000 rpm for 15 min. To remove any impurities, the hepatic tissues of the animals were promptly extracted, rinsed with cold saline solution (0.85%, w/v), and stored at –80 °C until biochemical analyses could be conducted.

Serum Transaminases Analysis

The activities of serum transaminases, alanine transaminase (ALT) and aspartate transaminase, (AST) obtained from centrifuged blood samples, were analysed using a chemical analyser.

Liver Post-Mitochondrial Supernatant Preparation

After homogenising 10% (w/v) hepatic tissues extracted from the samples in a phosphate buffer (0.1 M, pH 7.4), the nuclei were removed by centrifugation at 3,000 rpm at 4 °C for 20 min. Subsequently, an additional 30-minute centrifugation at 10,000 rpm and 4 °C was performed with the obtained supernatant, and the resulting post-mitochondrial supernatant was utilised to measure a variety of biochemical parameters.

Biochemicals Assays

Lipid peroxidation (LPO) was evaluated using the method proposed by Buege & Aust [15] to quantify the production rate of reactive substances containing thiobarbituric acid, expressed as malondialdehyde (MDA) equivalents. The procedure for quantifying reduced glutathione (GSH) followed the methodology described by Jollow et al. [16]. Catalase (CAT) activity was evaluated following the procedure described by Claiborne [17], and glutathione peroxidase (GPx) activity was assessed using the approach outlined by Mohandas et al. [18]. Glutathione reductase (GR) activity was assessed

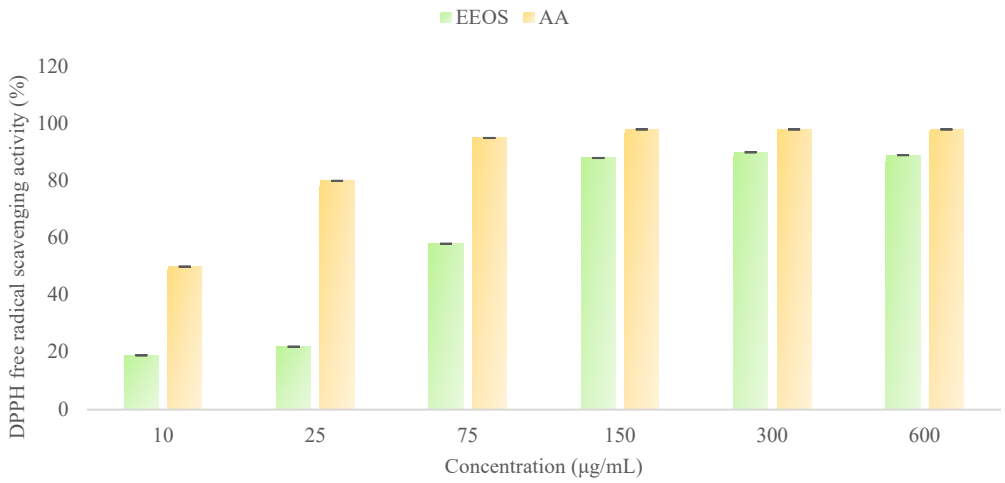

Figure 2. DPPH free radical scavenging activity of EEOS at various concentrations (10 to 600 µg/mL) in comparison to AA. Each value is presented as the mean ± SD (n = 3).

using the approach outlined by Carlberg & Mannervik [19]. Glutathione S-transferase (GST) activity was estimated in accordance with the methodology described by Habig et al. [20], utilising CDNB as the substrate, while quinone reductase (QR) activity was evaluated as per the procedure outlined by Benson et al. [21], with modifications by Iqbal et al. [22]. All biochemical assays were conducted using a spectrophotometer.

Statistical Analysis

The results were reported as means ± standard deviations (SD). Statistical comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test. Levene's test was employed to assess the homogeneity of variance. Data analysis was performed using IBM SPSS Statistics (Version 17) software. A significance level of p-value < 0.05 was considered statistically significant.

RESULTS

Effect of EEOS on Antioxidant Activity

Phenolic compounds are commonly found in leaves and various plant parts. Leaves were selected for this study due to their consistent levels of antioxidative enzyme activities associated with phenolic compounds. The study revealed the TPC of EEOS to be 279.18 ± 1.23 mg GAE/g db, indicating a substantial concentration. In addition, the DPPH assay is frequently employed for assessing the scavenging activity of plant samples. The scavenging capacity of EEOS against DPPH increased in a concentration-dependent manner, as depicted in Figure 2, ranging from concentrations of 10 to 600 µg/mL. The scavenging activity of EEOS peaked at 300 µg/mL, reaching 90.00 ± 0.00%. In comparison, AA demonstrated higher activity at concentrations between 150 and 300 µg/mL, with values ranging from 98.00 ± 0.00% to 98.00 ± 0.04%. Similarly, the reducing power is an important indicator for

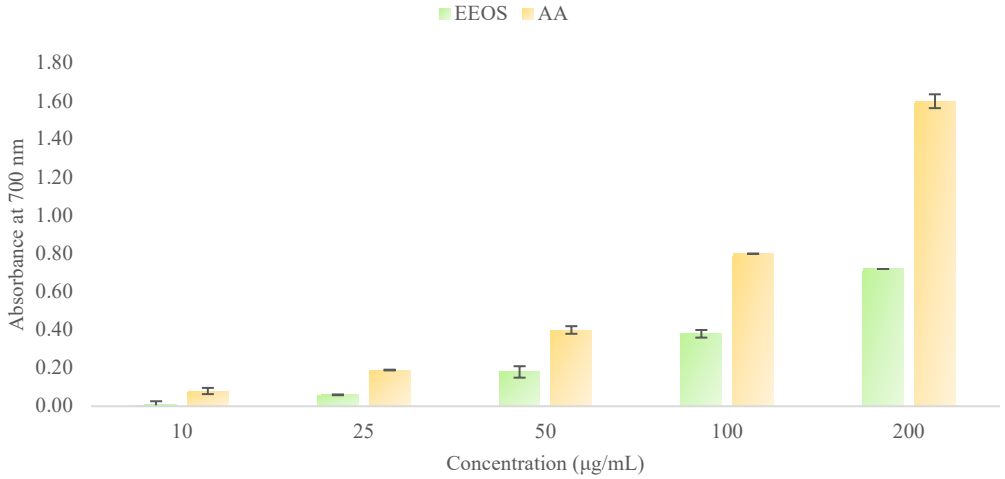

Figure 3. Reducing power of EEOS at various concentrations (10 to 200 µg/mL) in comparison to AA. Each value is presented as the mean ± SD (n = 3).

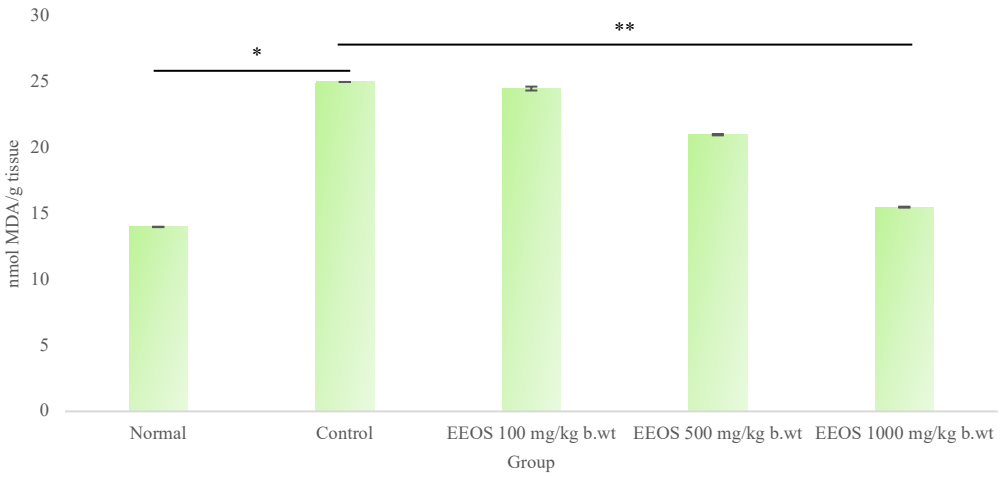

Figure 4. Effect of EEOS on the LPO levels. Each value is presented as the mean ± SD (n = 6). * indicates a statistically significant difference between the normal and control groups, while ** indicates statistically significant differences between the control group and the various EEOS doses (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

evaluating the potential antioxidant activity of EEOS. Figure 3 illustrates the effect of EEOS on reducing power in a concentrations-dependent manner, ranging from 10 to 200 µg/mL, reaching its peak at 200 µg/mL (0.72 ± 0.00 Abs). Compared to AA at the same highest concentration of 200 µg/mL (1.60 ± 0.04 Abs), EEOS exhibited considerable reducing capacity, indicating its potential as an effective antioxidant agent.

Effect of EEOS on Serum Transaminases

Table 1. Protective effects of EEOS on CCl_4-induced alterations in serum transaminases.

Group	ALT (IU/L)	AST (IU/L)
Normal	48.28 ± 9.57 *	115.90 ± 26.45 *
Control	4248.00 ± 994.0 *,**	3780.00 ± 502.3 *,**
EEOS 100 mg/kg b.wt.	3138.00 ± 86.17 **	3144.00 ± 175.6 **
EEOS 500 mg/kg b.wt.	2640.00 ± 371.9 **	1484.00 ± 200.2 **
EEOS 1000 mg/kg b.wt.	46.43 ± 9.92 **	113.90 ± 11.7 **

Each value is presented as the mean ± SD (n = 6). * Indicates a statistically significant difference between the normal and control groups, while ** indicates statistically significant differences between the control group and the various EEOS doses (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

The principal category of clinical examinations employed to assess hepatocellular dysfunction consists of initial indicators represented by serum ALT and AST levels. Compared to the normal group, the control group exhibited significantly increased ($p < 0.05$) levels of serum transaminases (ALT and AST), with increases of 99% and 97%, respectively, indicating hepatic damage (Table 1). In contrast, the administration of EEOS at 100 mg/kg b.wt. resulted in a significant 1-fold reduction ($p < 0.05$) in both ALT and AST levels compared to the control group. Furthermore, EEOS at 500 mg/kg b.wt. led to a significant 2-fold reduction ($p < 0.05$) in both ALT and AST levels. Notably, at 1000 mg/kg b.wt., EEOS demonstrated the most substantial hepatoprotective effect, with a significant decrease of 91-fold in ALT and 28-fold in AST levels, highlighting its potent efficacy in mitigating CCl_4-induced hepatic damage.

Effect of EEOS on LPO Levels

MDA, a by-product and typical indicator of LPO, was measured. The data presented in Figure 4 illustrates a notable increase ($p < 0.05$) of 85% in MDA levels within the livers of rats exposed to CCl_4 (control group) compared to the normal group. However, MDA levels were significantly decreased ($p < 0.05$) after pretreatment with EEOS: at 100 mg/kg b.wt. by 40%, 500 mg/kg b.wt. by 60%, and 1000 mg/kg b.wt. by 80% compared to the control group. These findings suggest that the administration of EEOS effectively reduced the oxidative stress caused by CCl_4.

Effect of EEOS on GSH Levels

GSH plays a vital role in mitigating the harmful effects of free radicals and oxygen radicals. The impact of EEOS on hepatic GSH levels in the control group was investigated (Figure 5). Hepatic GSH levels in this group were significantly reduced ($p < 0.05$) by 70% due to the oxidative stress induced by CCl_4 compared to the normal group. Nonetheless, administering EEOS at doses of 100, 500, and 1000 mg/kg b.wt. demonstrated notably increased ($p < 0.05$) GSH levels by 30%, 50%, and 80%, respectively, compared to the control group.

Effect of EEOS on Antioxidant Enzyme Activities

Antioxidant enzymes play a crucial role in mitigating oxidative stress through the scavenging of ROS and the facilitation of the detoxification process for

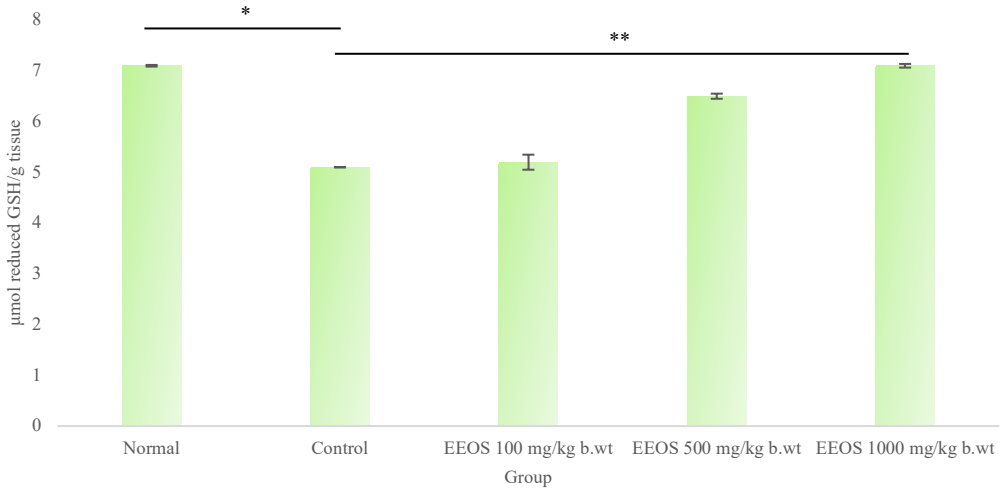

Figure 5. Effect of EEOS on the GSH levels. Each value is presented as the mean ± SD (n = 6). * indicates a statistically significant difference between the normal and control groups, while ** indicates statistically significant differences between the control group and the various EEOS doses (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

detrimental substances. Overall, the activities of antioxidant enzymes (CAT, GPx, GR, GST, and QR) were substantially reduced in the control group relative to the normal group (Table 2). On the contrary, levels of antioxidant enzymes were restored in a dose-dependent manner after pretreatment with EEOS.

The control group exhibited a significant reduction ($p < 0.05$) of hepatic antioxidant enzymes, with CAT, GPx, and GR levels decreasing by 37%, 88%, and 80%, respectively, compared to the normal group. Nevertheless, administration of EEOS at doses of 100, 500, and 1000 mg/kg b.wt. resulted in enhancements ($p < 0.05$) of 12%, 21%, and 20%, respectively, relative to the control group for CAT levels. Similarly, GPx levels improved ($p < 0.05$) by 23%, 36%, and 41% at 100, 500, and 1000 mg/kg b.wt. administered by EEOS. In addition, GR levels showed significantly reductions ($p < 0.05$) at doses of 100 mg/kg b.wt. by 6%, 500 mg/kg b.wt. by 23%, and 1000 mg/kg b.wt. by 30% compared to the control group. Therefore, all hepatic antioxidant enzymes demonstrated the potent antioxidant effect of EEOS in counteracting CCl_4-induced oxidative stress.

Moreover, hepatic phase II metabolising antioxidative enzymes, namely GST and QR, exhibited a notable increase ($p < 0.05$) of 78% and 53%, respectively, compared to the normal group. Administration of EEOS at 100 mg/kg b.wt. resulted in a significant increase ($p < 0.05$) in GST and QR levels by approximately 11% and 8%, respectively, relative to the control group. At a dose of 500 mg/kg b.wt., EEOS further enhanced GST and QR levels, demonstrating improvements ($p < 0.05$) of around 17% and 34%, respectively. The highest dose of EEOS, 1000 mg/kg b.wt., led to a substantial increase ($p < 0.05$) in enzyme activities, with GST and QR levels restored to approximately 25% and 35% of the control levels, respectively. These results indicate the dose-dependent efficacy of EEOS in significantly ameliorating the reduced GST and QR levels induced by CCl_4 for hepatic phase II metabolising antioxidative enzymes.

DISCUSSION

The findings suggest that EEOS leaves have the potential to mitigate oxidative stress and prevent CCl_4-induced hepatic damage in rats. Notably, EEOS exhibited significant antioxidant properties, as demonstrated by its ability to effectively scavenge the stable free radical DPPH (90.00 ± 0.00%) and exhibit

Table 2. Protective effects of EEOS on CCl4-induced alterations in hepatic antioxidant enzymes.

Group	CAT [1]	GPx [2]	GR [3]	GST [4]	QR [5]
Normal	21.13 ± 0.74 *	328.66 ± 27.32 *	88.31 ± 13.61 *	134.27 ± 2.10 *	75.26 ± 2.83 *
Control	15.46 ± 0.44 *,**	174.77 ± 26.78 *,**	49.10 ± 6.01 *,**	87.83 ± 2.92 *,**	42.35 ± 4.98 *,**
EEOS 100 mg/kg b.wt.	17.62 ± 1.72 **	226.83 ± 29.02 **	52.48 ± 8.02 **	98.34 ± 2.43 **	46.05 ± 3.29 **
EEOS 500 mg/kg b.wt.	19.46 ± 0.44 **	272.03 ± 16.45 **	63.42 ± 17.59 **	105.29 ± 2.65 **	64.29 ± 2.72 **
EEOS 1000 mg/kg b.wt.	19.37 ± 0.30 **	295.62 ± 13.54 **	70.55 ± 18.27 **	117.23 ± 3.72 **	65.28 ± 4.65 **

Each value is presented as the mean ± SD (n = 6). * indicates a statistically significant difference between the normal and control groups, while ** indicates statistically significant differences between the control group and the various EEOS doses (one-way ANOVA, Tukey's HSD test, p < 0.05). 1 µmol H2O2/min/mg protein. 2 nmol NADPH oxidised/min/mg protein. 3 nmol 2,6-DCP reduced/min/mg protein. 4 nmol CDNB conjugate/min/mg protein. 5 nmol NADP reduced/min/mg protein.

a dose-dependent reduction in power (0.72 ± 0.00 Abs). The measured TPC of EEOS at 279.18 ± 1.23 mg GAE/g db emphasises the importance of phenolics as essential components. Published research attributes the antioxidant activity of plant extracts to their TPC, which serves as a quencher of singlet oxygen, a donor of hydrogen ions, and a scavenger of free radicals [23,24].

The DPPH assay is frequently utilised to assess the antioxidant capacities of samples due to its straightforward and cost-effective nature, minimal operational expertise, and reliance on a basic spectrophotometer [25]. The colour of the solution diminishes after the reduction of the DPPH radical and the abstraction of hydrogen atoms from the antioxidant. The transition from purple to pale yellow signifies that an antioxidant compound in the sample has effectively eliminated the scavenging agent [26]. Furthermore, at low pH, the FRAP assay quantifies the capacity of antioxidants to convert ferric iron (Fe^{3+}-TPTZ) to the divalent Fe^{2+} ion, which is more stable [27]. The process of Fe^{3+} to Fe^{2+} conversion, which generates a violet-blue hue, yields a consistent and timely result. According to Moon & Shibamoto [26], they have been employed in a multitude of investigations to assess the antioxidant capacity of diverse food items.

LPO is initiated by the addition of oxygen or removal of hydrogen radicals, causing oxidative damage to polyunsaturated fatty acids [17]. In the liver, cytochrome P450 (CYP)2E1, and to a lesser extent, other CYPs such as CYP2B and CYP3A, participate in the biotransformation of CCl_4 to produce free radicals, primarily trichloromethyl (CCl_3), in the endoplasmic reticulum. The interaction between oxygen and free radicals gives rise to trichloromethyl peroxyl (CCl_3O_2) radicals. These hazardous metabolites may attach to different proteins or lipids, causing LPO [28]. As the peroxidation process advances, lipids break down into small molecules, including MDA or 4-hydroxynonenal, extremely reactive aldehydes capable of forming adducts with proteins and DNA [28]. In the current investigation, the administration of EEOS significantly and dose-dependently reduced the production of the LPO end product (MDA). This demonstrates that EEOS administration substantially mitigated CCl_4-induced LPO.

In the process of protecting the body from damage caused by free radicals, GSH is an antioxidant that plays a significant role. It accomplishes this by acting as a radical scavenger and supplying GSH to enzymes that are responsible for antioxidant activity. Additionally, it contributes to the regeneration of other antioxidants such as vitamins E and C [29,30]. Because of the administration of EEOS, there was an increase in the levels of GSH in the liver. This suggests that EEOS administration effectively restored the reduced GSH levels induced by CCl_4.

CCl_4 intoxication also affects hepatic antioxidant enzyme activity. All organisms utilising oxygen have well-organised antioxidant systems to guard against the damage caused by free radicals. CAT, GPx, GR, dehydrogenase of glutathione-6-phosphate, GST, and QR are among these enzymes, serving as the initial line of defence against oxidative stress induced by free radicals [31,32]. CAT transforms H_2O_2 into molecular water and oxygen, while seleno-dependent GPx expedites the decomposition of H_2O_2 and hydroperoxides generated from unsaturated fatty acids, utilising GSH [33]. GR accelerates the NADPH-dependent reduction of GSSG to GSH, a pivotal process for preserving stable glutathione levels [34]. GST and QR also enhance cellular GSH levels, protecting cells against the toxicities of free radicals [35]. The antioxidant enzyme levels in rats significantly increased in response to EEOS administration at varying levels, as compared to the group that received CCl_4 alone.

Prior research has conclusively established that antioxidative enzymes serve as the principal defence mechanism against ROS and other free radicals. The primary objective of this research is to assess the antioxidant potential of EEOS against ROS in a rat model of CCl_4-induced hepatotoxicity. The objective of this investigation is to assess the levels of ALT and AST in serum, as well as LPO, GSH, CAT, GPx, GR, GST, and QR in hepatic tissues. This research contributes to a better understanding of the potential for *O. stamineus* administration to reduce the amount of oxidative damage that occurs in the liver. Additionally, this nutritional strategy offers a viable alternative to the pharmacological methods that are currently in use with the intention of reducing hepatotoxicity through its use.

Relying solely on TPC, and DPPH and reducing power assays may limit the comprehensive assessment of antioxidant potential in EEOS, as these assays provide valuable but incomplete insights into antioxidant activity, crucial for understanding the oxidative stress mechanism. Thus, additional assays such as hydrogen peroxide free radical scavenging activity and oxygen radical absorbance capacity should be emphasised for EEOS to further delineate its ability to mitigate oxidative stress. In addition, the lack of compulsory histopathological examination presents a notable drawback. This analysis provides valuable insights into the integrity of hepatic tissue and supplements biochemical analyses, which are crucial in animal protocols to assess mechanisms or evidence of tissue inflammation induced by CCl_4. Future research should emphasise a broader range of antioxidant assays and histopathological examinations to achieve a more comprehensive understanding of the hepatoprotective potential of EEOS and its mechanisms on hepatic health.

CONCLUSIONS

Overall, the data illustrate that EEOS exhibits significant antioxidant activity. Moreover, EEOS demonstrates effective protection against hepatic injury induced by CCl_4 in rats. This hepatoprotective effect is apparent in the restoration and reduction of LPO and GSH levels in hepatic cells, the enhancement in the levels of hepatic enzyme markers (ALT and

AST), and the augmentation in antioxidant enzyme activities (CAT, GPx, GR, GST, and QR), with 1000 mg/kg b.wt. demonstrating superior efficacy. These findings suggest that EEOS plays a safeguarding role in hepatic injury induced by CCl_4, potentially attributed to enhanced antioxidant defence mechanisms, restrained inflammatory responses, and mitigated oxidative stress in hepatic tissues. These results underscore the potential efficacy of *O. stamineus* as a functional ingredient in mitigating ROS-induced liver damage.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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