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EMBLICA OFFICINALIS ENHANCES HEPATOPROTECTIVE PROPERTIES OF SILYMARIN IN DUAL ACTION AGAINST HEPATIC OXIDATIVE STRESS

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ABSTRACT
This research investigated the phytochemical composition and biological activities of a novel plant extract, extract of the leaves of *Emblica officinalis* Gaertn. (EOME-L). Preliminary phytochemical analysis revealed the presence of alkaloids, terpenoids, glycosides, phytosterols, flavonoids, phenols, saponins, and tannins, suggesting potential therapeutic benefits such as anti-inflammatory, antioxidant, antimicrobial, laxative, and cholesterol-lowering effects. The Borntrager test confirmed the presence of anthraquinones, further enhancing the extract's antimicrobial properties. Spectrophotometric analysis quantified the total phenolic content of EOME-L, providing valuable insights into its antioxidant capacity. In vitro assays demonstrated significant antioxidant activity, metal chelating ability, and lipid peroxidation inhibition, highlighting EOME-L's potential in combating oxidative stress-related conditions. Additionally, EOME-L exhibited potent hepatoprotective effects in CCl4-intoxicated HepG2 cells, comparable to the standard hepatoprotective agent Silymarin. Furthermore, combination therapy with EOME-L and Silymarin demonstrated synergistic hepatoprotective effects, offering a promising strategy for improving liver health. In conclusion, this research elucidated the phytochemical profile and therapeutic potential of EOME-L, emphasizing its utility as a natural remedy for oxidative stress and liver-related disorders.
Keywords: *Emblica officinalis,* Antioxidant, Total antioxidant activity, Metal chelating, carbon tetrachloride, hepatoprotective, Liver damage

INTRODUCTION

Hepatotoxicity, a condition characterized by liver damage, is a significant health concern worldwide due to its association with various etiological factors, including drugs, alcohol, viral infections, and environmental toxins. The liver, a vital organ responsible for metabolism, detoxification, and synthesis of essential proteins, is particularly susceptible to damage from xenobiotics and oxidative stress. Oxidative stress, a state of imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, plays a pivotal role in the pathogenesis of hepatotoxicity. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, can inflict cellular damage by oxidizing lipids, proteins, and DNA, leading to mitochondrial dysfunction, inflammation, and cell death (Halliwell and Gutleridge, 1984).

Drug-induced hepatotoxicity represents a major challenge in clinical medicine, accounting for a significant proportion of acute liver failure cases. Many drugs, including acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), and chemotherapeutic agents, exert hepatotoxic effects by generating ROS, depleting cellular antioxidants, and disrupting mitochondrial function. Acetaminophen, a commonly used analgesic and antipyretic agent, is a well-known cause of drug-induced liver injury, particularly in cases of overdose. Its metabolite, N-acetyl-p-benzoquinone imine (NAPQI), can overwhelm the liver's detoxification capacity, leading to oxidative stress, hepatocyte necrosis, and potentially fatal acute liver failure (Li et al., 2007, Gülçin et al., 2002).

Alcohol abuse is another major contributor to hepatotoxicity, as chronic ethanol consumption can induce oxidative stress by promoting ROS production, impairing antioxidant defense mechanisms, and altering mitochondrial function. The metabolism of ethanol generates acetaldehyde, a highly reactive and toxic metabolite that can damage cellular macromolecules and trigger inflammatory responses in the liver. Chronic alcohol consumption also disrupts lipid metabolism, leading to the accumulation of free fatty acids and the development of alcoholic fatty liver disease (AFLD), a precursor to more severe conditions such as alcoholic hepatitis and cirrhosis.

Viral hepatitis, caused by hepatitis viruses (e.g., hepatitis B and C viruses), is a leading cause of chronic liver disease and hepatocellular carcinoma worldwide. Hepatitis viruses can induce hepatotoxicity by directly infecting hepatocytes, triggering immune-mediated responses, and promoting oxidative stress. Chronic hepatitis B and C infections can lead to progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma, highlighting the importance of early diagnosis and treatment to prevent liver damage and complications (Aligita et al., 2023, Dinesh and Kumar, 2023, Guemmogne Temdie et al., 2023).

Environmental toxins, such as heavy metals (e.g., lead, mercury) and industrial chemicals (e.g., carbon tetrachloride, aflatoxins), pose significant health risks due to their hepatotoxic effects. Heavy metals can accumulate in the liver, disrupt cellular functions, and induce oxidative stress by interfering with antioxidant defense mechanisms. Similarly, exposure to industrial chemicals can lead to liver damage by promoting ROS production, lipid peroxidation, and inflammation.

Mitochondrial dysfunction is a hallmark feature of hepatotoxicity and oxidative stress, as mitochondria play a critical role in energy production, redox balance, and apoptosis regulation in hepatocytes. ROS-mediated damage to mitochondrial DNA, proteins, and lipids can impair mitochondrial function, leading to ATP depletion, oxidative phosphorylation dysfunction, and the release of pro-apoptotic factors. Mitochondrial dysfunction exacerbates oxidative stress and hepatocellular injury, contributing to the progression of liver diseases (Ellnain-Wojtaszek et al., 2003, Clement et al., 1998).

In conclusion, hepatotoxicity and oxidative stress represent major pathophysiological mechanisms underlying liver damage and disease. Understanding the molecular mechanisms involved in hepatotoxicity and oxidative stress is crucial for developing effective preventive and therapeutic strategies for liver diseases. Targeting oxidative stress pathways, enhancing antioxidant defences, and mitigating mitochondrial dysfunction hold promise for protecting the liver from the harmful effects of hepatotoxic agents and improving clinical outcomes for patients with liver diseases (Bose Mazumdar Ghosh et al., 2022, Sparzak-Stefanowska and Krauze-Baranowska, 2022). The need for an alternative hepatoprotective modality derived from natural sources arises from several factors. Firstly, conventional treatments for liver diseases often involve pharmaceutical agents with potential side effects and limited efficacy, necessitating the exploration of safer and more effective alternatives. Natural compounds derived from plants,

herbs, and other natural sources offer promising hepatoprotective properties with fewer adverse effects, making them attractive candidates for therapeutic intervention.

Furthermore, natural hepatoprotective agents exhibit a multifaceted mode of action, targeting multiple pathways involved in liver damage and disease progression. These compounds possess antioxidant, anti-inflammatory, and detoxifying properties, which help combat oxidative stress, reduce inflammation, and enhance liver function. Additionally, natural hepatoprotective agents often contain a complex mixture of bioactive compounds that act synergistically to exert their beneficial effects, offering a comprehensive approach to liver protection (Ahsan et al., 2023, Kumar et al., 2023, Hussain et al., 2023, Kamel et al., 2023, Sowunmi and Gonzo, 2023). Moreover, the growing interest in traditional and complementary medicine has fueled research into the pharmacological potential of natural products for liver health. Traditional herbal remedies have been used for centuries in various cultures to treat liver ailments, providing a rich source of knowledge for the discovery of novel hepatoprotective agents. By harnessing the therapeutic potential of natural compounds, we can develop alternative hepatoprotective modalities that are not only effective but also sustainable, affordable, and accessible to a wider population (Khoshakhlagh et al., 2023, Kim et al., 2023, Shahrajabian and Sun, 2023, Ahsan et al., 2023, Kumar et al., 2023).

The objective of this study was to assess the antioxidant, lipid peroxidation inhibition, and hepatoprotective properties of the methanolic fraction obtained from *Emblica officinalis* Gaertn. leaves. Preliminary phytochemical screening was conducted to determine the presence of bioactive compounds in the extract, and the total phenolic content was quantified. Investigating the combined hepatoprotective effects of the extract and silymarin in HepG2 cells added an interesting dimension to the research. Furthermore, this study aimed to provide robust evidence supporting the traditional folklore and ayurvedic use of *Emblica officinalis* Gaertn. in treating various ailments, including liver diseases.

MATERIALS AND METHODS

Plant

The leaves of *Emblica officinalis* Gaertn. were collected in Dehradun area of Uttarakhand, India. The scientific validity of the plant had been confirmed by botanists and herbalists. A voucher specimen (BKS/DEHEO/033/2023) was stored in the pharmacy department for later use.

Drugs and chemicals

The reagents and solvents used in the study were sourced from various suppliers, primarily located in India. Carbon tetrachloride (CCl4), methanol, ammonium thiocyanate, trichloroacetic acid (TCA), gallic acid, α-tocopherol, butylated hydroxyanisole (BHA), dimethylsulphoxide (DMSO), linoleic acids, ascorbic acid, and malondialdehyde (MDA) were obtained from reputable suppliers such as E. Merck (India) Limited and Loba Chem Company, ensuring analytical-grade quality. Petroleum ether and Tween 80 were procured from suppliers including SRL Mumbai, Himedia, and E. Merck India. Additionally, Silymarin, utilized as a standard hepatoprotective agent, was provided as a gift sample by Newlife Pharmaceuticals located in

Himachal Pradesh, India. This meticulous sourcing of reagents from trusted suppliers helps ensure the reliability and consistency of the experimental procedures and results.

Preparation of extracts

The leaves of *Emblica officinalis* Gaertn. were crushed into a powder after being shade dried. Using the cold maceration process, methanol was utilized as the solvent to extract the mechanically ground and powdered plant leaves, which were subsequently concentrated in a vacuum. From 50 g of dried leaf material, approximately 7.1 g of dried methanolic extract of *Emblica officinalis* Gaertn. leaves (EOME-L) were generated (Yield, 14.2 percent).

Preliminary and initial phytochemical evaluation

Preliminary and initial phytochemical evaluation were done by using chemical techniques in accordance with the methodology suggested elsewhere (Harborne, 1973). The preliminary and initial phytochemical evaluation of plant material involves a series of meticulous steps to identify and analyze the presence of various chemical compounds. Once the extracts are prepared, preliminary phytochemical screening is conducted using specific tests to detect various classes of compounds. Alkaloids are identified using reagents such as Mayer's, Wagner's, and Dragendorff's, which form precipitates if alkaloids are present. Flavonoids are detected through tests like the Shinoda test, Alkaline reagent test, and Lead acetate test, which induce characteristic color changes. Tannins are identified using the Ferric chloride test and Gelatin test, which result in blue-black or green precipitates. Saponins are detected by the Froth test, where persistent froth indicates their presence. Steroids and terpenoids are identified through Salkowski's test and Liebermann-Burchard test, which produce specific color changes. Glycosides are detected using the Keller-Killiani test and Legal's test, with color changes or precipitate formation indicating their presence. Phenols are identified by the Ferric chloride test, which produces a blue or green color. Proteins and amino acids are detected using the Biuret test and Ninhydrin test, forming violet or purple colors. Carbohydrates are identified using Molisch's test, Benedict's test, and Fehling's test, where color changes or precipitate formation indicate their presence.

Measuring and calculating the total phenolic content

The measurement and calculation of total phenolic content in plant extracts typically involve using the Folin-Ciocalteu reagent, a widely accepted method for its simplicity and effectiveness. The procedure begins with the preparation of standard solutions of a known phenolic compound, such as gallic acid, dissolved in distilled water at various concentrations. Each standard solution is then mixed with Folin-Ciocalteu reagent and allowed to react for 5-8 minutes before adding sodium carbonate solution. After incubating the mixture for 30 minutes in the dark, the absorbance is measured at 765 nm using a UV-Vis spectrophotometer, and a standard curve of absorbance versus gallic acid concentration is plotted. For the plant extract, a similar process is followed: the extract is diluted with distilled water to ensure the phenolic content falls within the standard curve range, then mixed with the Folin-Ciocalteu reagent and sodium carbonate solution. After a 30-minute incubation in the dark, the absorbance is measured at 765 nm. The

concentration of phenolics in the plant extract is determined from the standard curve, and the total phenolic content is calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of the plant material. The formula used is

Total Phenolic Content (mg GAE/g dry weight) = C*V/m

where C is the concentration of phenolics from the standard curve, V is the volume of extract used, and m is the mass of the plant material. This method provides an accurate measure of the phenolic content, offering valuable insights into the antioxidant properties and potential health benefits of the plant extracts.

Evaluating the Total antioxidant activity

The thiocyanate approach, which was previously reported with some modifications, was used to measure the extract's total antioxidant activity (Mitsuda et al., 1996). Evaluating the total antioxidant activity of plant extracts using the thiocyanate method involves a series of precise steps aimed at measuring the inhibition of lipid peroxidation. This method assesses the ability of antioxidants to prevent the oxidation of lipids, a common process in biological systems and food products. The procedure begins with the preparation of necessary reagents, including a linoleic acid emulsion, ferrous chloride solution, and ammonium thiocyanate solution. Standard antioxidant solutions, such as Trolox or ascorbic acid, are prepared in ethanol at various concentrations to create a standard curve. Each standard solution is mixed with the linoleic acid emulsion and incubated at 37°C in the dark to initiate lipid peroxidation. After a set incubation period, ammonium thiocyanate and ferrous chloride solutions are added to each test tube, and the absorbance is measured at 500 nm using a UV-Vis spectrophotometer. A standard curve of absorbance versus antioxidant concentration is then plotted. For the plant extract, a similar process is followed: the extract is diluted, mixed with the linoleic acid emulsion, incubated, and then reacted with ammonium thiocyanate and ferrous chloride solutions. The absorbance of this mixture is measured at 500 nm. The antioxidant activity is determined by comparing the decrease in absorbance caused by the plant extract to the standard curve, and the results are expressed as micromoles of Trolox equivalents (TE) per gram of dry weight of the plant material. The calculation uses the formula:

Total Antioxidant Activity (μmol TE/g dry weight) = C*V/m

where C is the concentration of antioxidant equivalents, V is the volume of extract used, and m is the mass of the plant material. This method provides an accurate measure of the plant extract's ability to inhibit lipid peroxidation, highlighting its potential health benefits and antioxidant capacity.

Metal chelating activity

The metal chelating activity of plant extracts or test compounds is a crucial measure of their antioxidant capability, particularly in binding metal ions and preventing the catalysis of reactive oxygen species formation. This activity is commonly assessed using the ferrous ion (Fe^{2+}) chelating assay, which evaluates the ability of an extract to compete with ferrozine for binding ferrous ions. The procedure involves preparing a 2 mM $FeCl_2$ solution and a 5 mM ferrozine

solution, followed by dissolving the plant extract or test compound in methanol at various concentrations. A standard chelating agent, such as EDTA, is also prepared for comparison. In the assay, 1 mL of $FeCl_2$ solution is mixed with 1 mL of the plant extract or test compound solution, followed by the addition of 1 mL of ferrozine solution. After vortexing and a 10-minute incubation at room temperature, the absorbance of the mixture is measured at 562 nm using a UV-Vis spectrophotometer. A control solution without the plant extract and a blank solution with methanol are also prepared to account for maximum absorbance and background absorbance, respectively.

The metal chelating activity is calculated as the percentage inhibition of ferrozine-Fe^{2+} complex formation using the formula:

Chelating Activity (%)=(Control-Sample/Control)*100

A higher percentage indicates a greater ability of the extract to bind ferrous ions and prevent oxidative damage. By comparing the chelating activity of the plant extract with that of a standard chelating agent like EDTA, the relative effectiveness of the extract can be evaluated. This method provides valuable insights into the antioxidant properties of plant extracts and their potential to mitigate metal-catalyzed oxidative damage.

Lipid peroxidation assay in the egg yolk model

The lipid peroxidation assay in the egg yolk model is a reliable method for evaluating the antioxidant activity of compounds by measuring their ability to inhibit lipid peroxidation. This assay utilizes egg yolk homogenate as a lipid source, and the extent of lipid peroxidation is assessed using the thiobarbituric acid reactive substances (TBARS) assay. Initially, the egg yolk is diluted with phosphate buffer and homogenized to create a uniform mixture. The homogenate is then mixed with the plant extract or test compound, along with ferrous sulfate and ascorbic acid to induce lipid peroxidation. After incubation at 37°C for one hour, trichloroacetic acid and thiobarbituric acid are added to the mixture, which is then heated in a boiling water bath to develop a colored complex. The mixture is cooled, centrifuged to remove precipitated proteins, and the absorbance of the supernatant is measured at 532 nm using a UV-Vis spectrophotometer. Controls without the plant extract or with standard antioxidants are used for comparison. The inhibition of lipid peroxidation is calculated by comparing the absorbance of the sample to the control.

Inhibition of Lipid Peroxidation (%) = (Control-Sample/Control)*100

A higher percentage of inhibition indicates greater antioxidant activity. This assay provides valuable insights into the potential of plant extracts and other compounds to prevent oxidative damage in biological systems, highlighting their effectiveness in inhibiting lipid peroxidation.

Evaluation of hepatoprotective activity *In vitro* using hepatic ($HepG_2$) Cell Line

Evaluating the hepatoprotective activity of plant extracts using the human liver cell line HepG2 involves a detailed in vitro process to assess the protective effects against induced liver cell damage (Arzumanian et al., 2021, Hu et al., 1999). Initially, HepG2 cells are cultured in a

DMEM medium supplemented with fetal bovine serum, penicillin, and streptomycin, and maintained at 37°C in a humidified 5% CO2 atmosphere. Once cells reach approximately 80% confluence, they are subcultured and seeded into 96-well plates. After a 24-hour incubation to allow cell adherence, cells are treated with a hepatotoxic agent, carbon tetrachloride, to induce liver damage. Subsequently, cells are exposed to various concentrations of the plant extract or test compounds, including appropriate controls: untreated cells, cells treated with the hepatotoxic agent alone, and cells treated with the hepatotoxic agent plus a known hepatoprotective agent. Cell viability is assessed using the MTT assay, which involves adding MTT solution to each well, incubating the plates, and then dissolving the formed formazan crystals in DMSO (Thabrew et al., 1997) (Hu et al., 1999). The absorbance is measured at 570 nm using a microplate reader, and cell viability is calculated as a percentage relative to the control group. An increase in cell viability in the presence of the test compound, compared to cells treated with the hepatotoxic agent alone, indicates hepatoprotective activity. This method allows for the evaluation of the protective effects of plant extracts against liver cell damage and helps identify potential candidates for further in vivo studies or therapeutic applications (Arzumanian et al., 2021, Zhu et al., 2022).

Statistical analysis

The total flavonoid content was graphically estimated using a linear regression technique. The hepatoprotective and antioxidant activity data were presented as mean ± SD, derived from three replicates. The average of three different analyses was used to represent all other data. The data were statistically analysed using a Student's t-test as well as one way ANOVA test followed by post hoc Dunnett’s test for comparison analysis, with a p-value of 0.05 or less being considered significant.

RESULTS AND DISCUSSIONS

Preliminary and initial phytochemical evaluation

The phytochemical analysis of EOME-L reveals a rich composition of bioactive compounds. The presence of alkaloids, terpenoids, glycosides, and phytosterols indicates potential therapeutic properties, including anti-inflammatory and antioxidant effects. The Borntrager test confirms the presence of anthraquinones, which are known for their antimicrobial and laxative properties. The abundance of flavonoids and phenols (indicated by ++) suggests strong antioxidant activities, which can protect liver cells from oxidative stress and damage. The presence of saponins (also ++) implies potential benefits in reducing cholesterol levels and enhancing immune function. Tannins, known for their astringent properties, further contribute to the hepatoprotective effects by preventing cellular damage. The absence of coumarins means reduced risk of bleeding complications, making EOME-L a safer option for therapeutic use. Collectively, these phytochemicals synergize to enhance the hepatoprotective efficacy of EOME-L, as observed in the improved cell viability in CCl4-toxicated HepG2 cells.

Table 1. Results of Preliminary phytochemical screening

Phytochemical Constituent	**EOME-L**
Alkaloids	+
Terpenoids	+
Glycosides	+
Borntrager Test	+
Flavonoids	++
Phenols	++
Coumarins	-
Saponins	++
Phytosterols	+
Tannins	+

+: Presence of moderate active constituents**, ++:** Presence of maximum active constituents

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the sample was assessed using the Gallic Acid Equivalent (GAE) method, a common technique for quantifying phenolic compounds (Figure 1). Through spectrophotometric analysis, a regression equation was derived: $y = 0.0037x + 0.074$, where 'y' denotes the absorbance and 'x' represents the concentration of phenolic compounds in mg/mL. The high coefficient of determination ($R^2 = 0.9785$) suggests a robust correlation between absorbance and phenolic concentration, indicating the reliability of the model. For a given absorbance value of 0.462, the corresponding TPC in Gallic Acid Equivalent (GAE) is computed to be approximately 0.092 mg/mL. This quantification provides valuable insights into the phenolic composition of the sample, facilitating its characterization and potential applications in various fields such as food science, pharmaceuticals, and natural product research. The precision of the method underscores its utility in assessing phenolic content, essential for evaluating the antioxidant capacity and health benefits of substances like plant extracts and dietary supplements. Such analyses contribute significantly to understanding the biological properties and functional qualities of phenolic-rich materials, aiding in the development of novel products with enhanced nutritional and therapeutic value (Slinkard and Singleton, 1977).

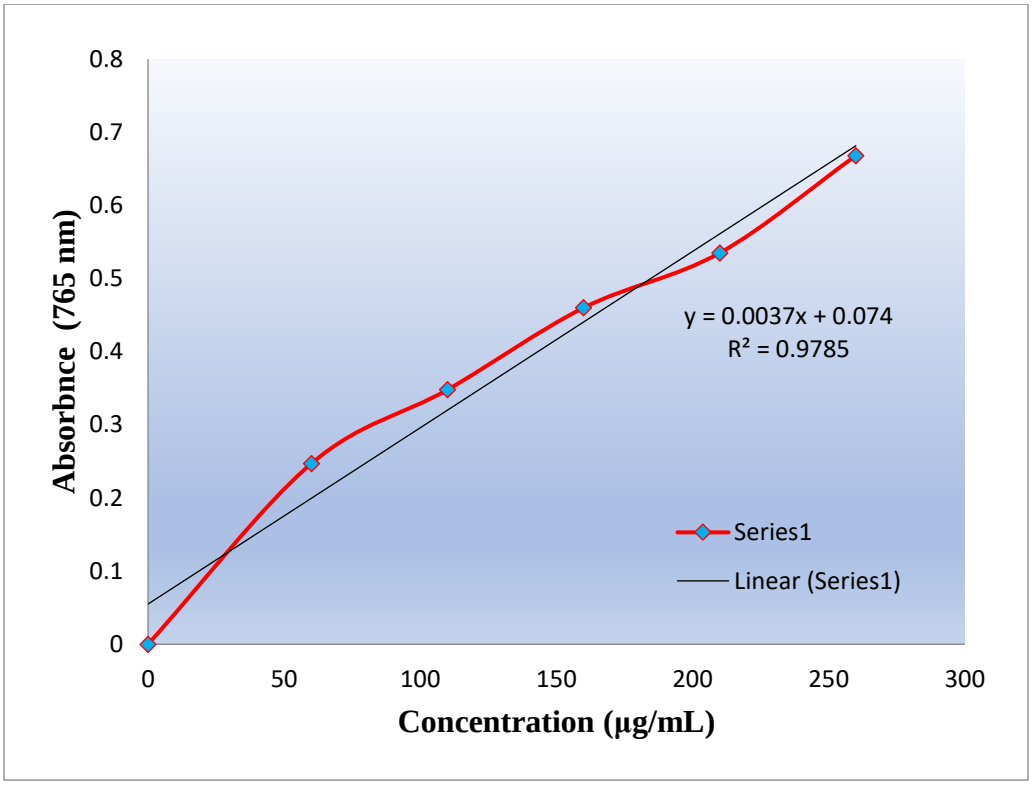

Figure 1. Estimated amount of TPC in the EOME-L.

***In vitro* antioxidant action**

Total antioxidant action estimation in a system of linoleic acid

The provided data represents the estimation of total antioxidant action in a linoleic acid system over various incubation times, comparing the effects of EOME-L, Butylated Hydroxyanisole (BHA), and α-Tocopherol. Initially, at 0 hours, all antioxidants showed negligible activity with absorbance values of 0. However, as the incubation progressed, significant changes were observed.

EOME-L exhibited a notable increase in antioxidant activity, with its mean absorbance peaking at 36 hours before gradually declining. This suggests an initial surge in antioxidant potential, possibly due to the release of active compounds, followed by a gradual decrease, indicating possible depletion or degradation of these compounds over time. BHA consistently demonstrated high and stable antioxidant activity throughout the entire incubation period. Its absorbance remained relatively unchanged over time, indicating its robust and enduring antioxidant properties.

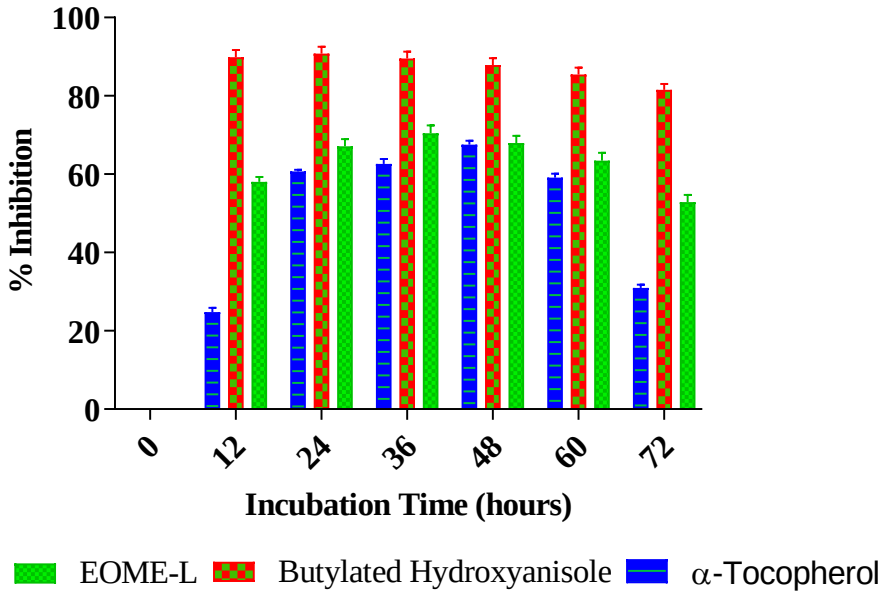

Figure 2. The results of total antioxidant activity of EOME-L.

In contrast, α-Tocopherol showed fluctuating antioxidant activity, peaking at 36 hours before declining. These fluctuations suggest transient variations in antioxidant efficacy, possibly influenced by factors such as degradation or consumption of α-Tocopherol. Overall, the data demonstrated the dynamic nature of antioxidant activity in the linoleic acid system and highlight differences in the performance of various antioxidants over time. Further analysis could delve into the underlying mechanisms driving these observed trends, aiding in the development of antioxidant formulations with sustained efficacy and stability.

Metal chelating activity

The provided data evaluates the metal chelating activity of EOME-L in comparison to EDTA, a well-known chelating agent used as a positive control. The concentrations of EOME-L range from 0 µg/ml to 250 µg/ml, while EDTA concentrations remain constant throughout the experiment. At 0 µg/ml concentration, both EOME-L and EDTA exhibit negligible metal chelating activity, with absorbance values of 0. As the concentration of EOME-L increases, there is a gradual increase in metal chelating activity, indicated by higher absorbance values. This suggests that EOME-L possesses the ability to chelate metal ions in a dose-dependent manner. Compared to EDTA, EOME-L demonstrates lower metal chelating activity at lower concentrations. However, as the concentration of EOME-L increases, its chelating activity becomes more pronounced and approaches the levels observed with EDTA. This indicates that EOME-L exhibits comparable metal chelating activity to EDTA at higher concentrations. Overall, the data suggest that EOME-L possesses significant metal chelating activity, which may

contribute to its potential therapeutic applications, particularly in the treatment of conditions involving metal toxicity or oxidative stress.

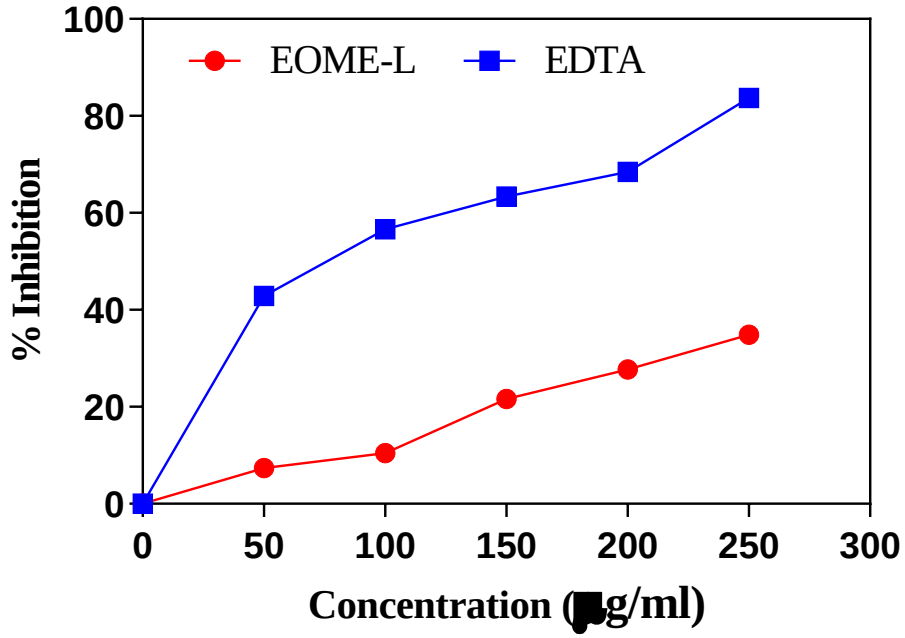

Figure 3. Metal chelating efficacy of EOME-L

Lipid peroxidation assay in the egg yolk model

The data provided demonstrates the lipid peroxidation inhibition percentages of EOME-L, where Quercetin and Catechin are utilized as standards in the egg yolk model. EOME-L exhibits a robust lipid peroxidation inhibition of 84.64% ± 1.48, indicating its potent antioxidant activity in preventing lipid oxidation. Quercetin, a well-known flavonoid standard, shows a significant inhibition percentage of 81.78% ± 1.72, consistent with its established antioxidant properties. Similarly, Catechin, also used as a standard, demonstrates notable lipid peroxidation inhibition at 87.18% ± 1.53, highlighting its efficacy in preventing lipid oxidation. These findings underscore the antioxidant potential of EOME-L in comparison to established standards, emphasizing its promising role in combating oxidative stress-related conditions.

Table 2. The percentage lipid peroxidation assay results of EOME-L in the egg yolk model

	% Lipid peroxidation Inhibition
EOME-L	84.64±1.48
Quercetin	81.78±1.72
Catechin	87.18±1.53

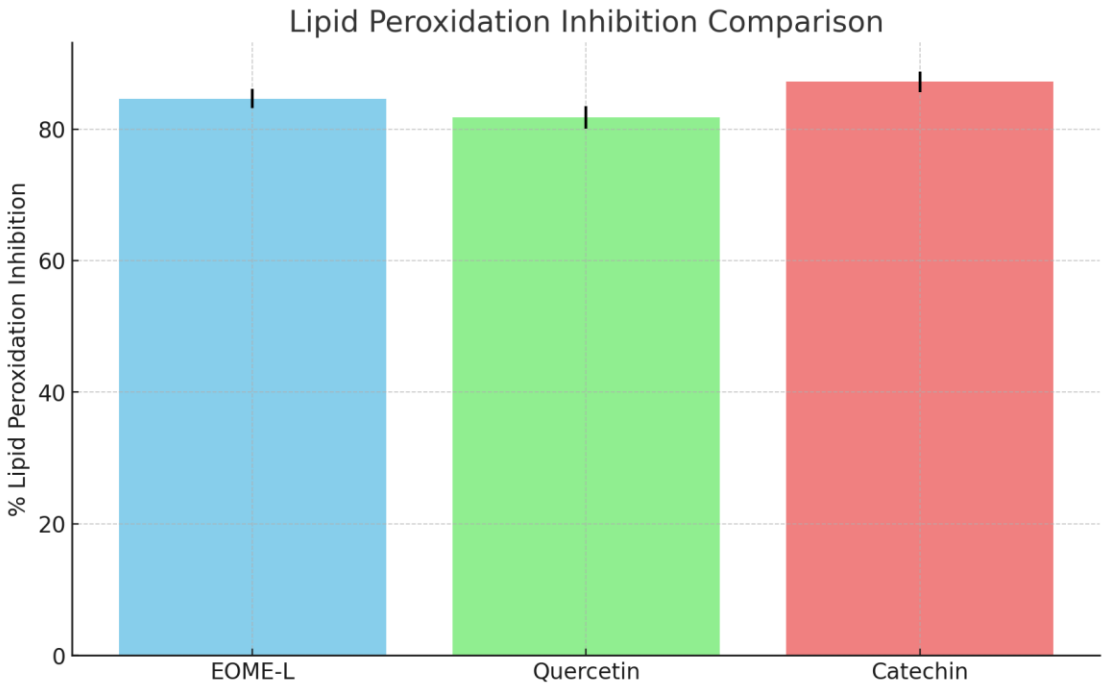

Figure 4. The percentage lipid peroxidation assay results of EOME-L

***In vitro* hepatoprotective activity of the EOME-L**

Appraising the hepatoprotective action in HepG$_2$ Cells

The provided data evaluates the hepatoprotective action of EOME-L in HepG2 cells exposed to carbon tetrachloride (CCl4), a known hepatotoxin. The cell viability percentages indicate the extent to which the treatments preserve cell viability compared to the normal control (untreated cells) and the positive control (CCl4-intoxicated cells). The positive control group, exposed to CCl4, shows a significantly reduced cell viability of 13.47 ± 1.21%, indicating substantial toxicity induced by CCl4. However, when HepG2 cells are treated with EOME-L after CCl4 exposure, a dose-dependent increase in cell viability is observed. At concentrations of 120 µg/ml, 100 µg/ml, and 80 µg/ml of EOME-L, the cell viability percentages are 94.99 ± 3.07%, 89.65 ± 3.62%, and 86.48 ± 3.21%, respectively. Even at lower concentrations of 60 µg/ml and 40 µg/ml, EOME-L still exhibits significant hepatoprotective effects, with cell viabilities of 75.87 ± 3.01% and 72.98 ± 2.98%, respectively. Comparatively, treatment with the standard hepatoprotective agent, Silymarin, at a concentration of 250 µg/ml, also shows a high cell viability of 95.88 ± 2.98%, indicating its efficacy in protecting HepG2 cells from CCl4-induced toxicity. The data suggest that EOME-L possesses hepatoprotective properties against CCl4-induced toxicity in HepG2 cells, with its efficacy comparable to that of the standard hepatoprotective agent Silymarin. The dose-dependent response further supports the potential therapeutic utility of EOME-L in liver protection and warrants further investigation into its mechanism of action and clinical application.

Table 3. When $HepG_2$ cells are exposed to CCl_4, the EOME-L displays hepatoprotective properties.

Treatments	**Concentration (µg/ml)**	**% Cell Viability**
Normal Control	-	99.99
Positive Control (CCl_4 intoxicated)	-	$13.47 \pm 1.21^{*}$
CCl_4 (1%) intoxicated + Treated with Standard Silymarin	250	$95.88 \pm 2.98^{\#}$
CCl_4 (1%) + EOME-L treated	120	$94.99 \pm 3.07^{\#}$
	100	$89.65 \pm 3.62^{\#}$
	80	$86.48 \pm 3.21^{\#}$
	60	$75.87 \pm 3.01^{\#}$
	40	$72.98 \pm 2.98^{\#}$
An average of three determinations and 3 replicates (n = 3); * denotes significant compared to normal cells at $p < 0.001$; # = denotes significant compared to the CCl4-toxicated cells at $p < 0.01$.		

Synergistic Effects of EOME-L and Silymarin

The study presented in Table 2 demonstrates the significant hepatoprotective effects of EOME-L and Silymarin on HepG2 cells intoxicated with carbon tetrachloride (CCl4). The Normal Control group, representing untreated healthy cells, shows near-complete viability at 99.99%. In stark contrast, the Positive Control group, which was exposed to CCl4, exhibits a drastic reduction in cell viability to 14.02 ± 1.11%, highlighting the severe cytotoxic impact of CCl4. This reduction is statistically significant ($P < 0.001$), underscoring the extent of damage inflicted by CCl4 on liver cells. Treatment with standard Silymarin at a concentration of 250 µg/mL restores cell viability significantly, achieving a viability rate of 96.29 ± 2.74%. This indicates the well-documented hepatoprotective properties of Silymarin. However, the combination treatment of EOME-L and Silymarin demonstrates an even more remarkable recovery of cell viability across various concentrations. At 120 µg/mL, the combination treatment achieves a cell viability of 97.27 ± 3.11%, which is slightly higher than that achieved with the standard Silymarin treatment. This suggests that the combined therapy can offer equivalent, if not superior, protection at a lower overall concentration of active ingredients. As the concentration of the EOME-L and Silymarin combination decreases, there is a corresponding decline in cell viability. However, even at the lowest tested concentration of 40 µg/mL, the combination treatment significantly improves cell viability to 75.75 ± 2.89%. This demonstrates a robust dose-dependent protective effect, with all tested concentrations showing statistically significant improvements compared to the CCl4-intoxicated control group.

The data suggest a synergistic interaction between EOME-L and Silymarin, enhancing hepatoprotection more effectively than either compound alone. This synergy likely arises from complementary mechanisms of action, where EOME-L and Silymarin together provide a more comprehensive protective effect against CCl4-induced cytotoxicity. Such synergistic effects can be advantageous in clinical settings, as they may allow for lower doses of each compound to be used, potentially reducing the risk of side effects and lowering treatment costs. The findings of this study highlight the potential therapeutic benefits of combining EOME-L with Silymarin for treating liver toxicity and damage. The combination therapy's ability to significantly restore cell viability at various concentrations offers a promising strategy for improving liver health. Further research is warranted to explore the molecular mechanisms underlying these synergistic effects and to optimize the therapeutic protocols for clinical application (Cioffi et al., 2019, Hendrix et al., 2020, Li et al., 2016)

Table 4. EOME-L and Silymarin exerts synergistic hepatoprotective effects on CCl_4 - toxicated $HepG_2$ cells.

Treatments	**Concentration (µg/ml)**	**% Cell Viability**
Normal Control	-	99.99
Positive Control (CCl_4 intoxicated)	-	$14.02 \pm 1.11^{*}$
CCl_4 (1%) intoxicated + Treated with Standard Silymarin	250	$96.29 \pm 2.74^{\#}$
CCl4 (1%) intoxicated + Treated with [EOME-L + Silymarin]	120	$97.27 \pm 3.11^{\#}$
	100	$92.49 \pm 3.09^{\#}$
	80	$88.72 \pm 3.13^{\#}$
	60	$78.09 \pm 3.04^{\#}$
	40	$75.75 \pm 2.89^{\#}$

An average of three determinations and 3 replicates (n = 3); * denotes significant compared to normal cells at $p < 0.001$; # = denotes significant compared to the CCl_4-toxicated cells at $p < 0.01$.

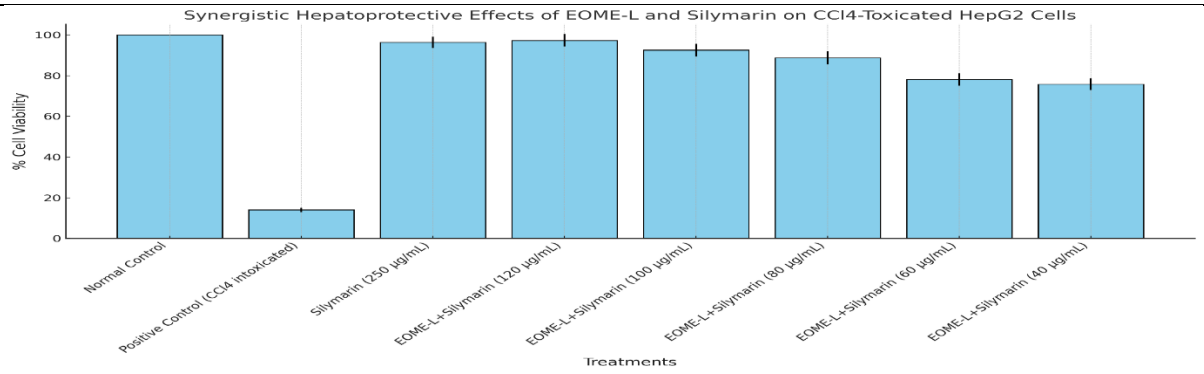

Figure 5. Comparative illustration of EOME-L and Silymarin's combined hepatoprotective benefits.

CONCLUSIONS

In conclusion, extract of the leaves of *Emblica officinalis* Gaertn. (EOME-L) exhibits a rich phytochemical composition and diverse biological activities, including antioxidant, metal chelating, and hepatoprotective effects. The presence of bioactive compounds such as flavonoids, phenols, and saponins contributes to its therapeutic properties, making it a promising candidate for pharmaceutical and nutraceutical applications. The significant antioxidant and hepatoprotective activities of EOME-L highlighted its potential in preventing and treating oxidative stress-related diseases and liver damage. Furthermore, the synergistic effects observed with Silymarin suggested a promising combination therapy for enhancing liver protection. Future research should focus on elucidating the molecular mechanisms underlying the observed effects of EOME-L and optimizing its therapeutic protocols for clinical use. Overall, EOME-L emerges as a valuable natural remedy with multifaceted health benefits, warranting further exploration and development in the field of herbal medicine and pharmacology.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

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