ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF *EURYCOMA LONGIFOLIA* JACK ROOT EXTRACT IN PARACETAMOL-INDUCED HEPATOTOXICITY IN SWISS MICE

HOẠT TÍNH CHỐNG OXY HÓA VÀ BẢO VỆ GAN CỦA DỊCH CHIẾT RỄ CÂY MẬT NHÂN (*EURYCOMA LONGIFOLIA* JACK) TRÊN MÔ HÌNH GÂY ĐỘC GAN BẰNG PARACETAMOL Ở CHUỘT SWISS

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Abstract - *Eurycoma longifolia* Jack (commonly known as Tongkat Ali) is a valuable medicinal plant widely used in Europe, the United States, and several Asian countries as a health-supporting supplement. In Vietnam, it has long been applied in traditional medicine, particularly for conditions associated with impaired liver function. This study evaluated the hepatoprotective potential of a root extract of *E. longifolia* in Swiss mice with paracetamol-induced liver injury. Animals were assigned to control and treatment groups receiving different extract doses. Serum AST and ALT levels, together with hepatic malondialdehyde (MDA), were measured to assess liver damage and lipid peroxidation. Paracetamol markedly elevated all biomarkers ($P < 0.05$), whereas co-administration of the extract significantly reduced them, with the 125 mg/kg dose bringing values close to controls. These findings indicate that *E. longifolia* extract confers notable antioxidant and hepatoprotective effects against paracetamol toxicity.

Key words - *Eurycoma longifolia* Jack; hepatoprotective potential; alanine aminotransferase; aspartate aminotransferase; hepatic malondialdehyde

Tóm tắt - Mật nhân (*Eurycoma longifolia Jack)* là dược liệu quý, được sử dụng phổ biến tại châu Âu, Hoa Kỳ và nhiều nước châu Á như một thực phẩm bổ sung hỗ trợ sức khỏe. Ở Việt Nam, cây thuốc này được ứng dụng trong y học cổ truyền để cải thiện nhiều bệnh lý, đặc biệt là những rối loạn liên quan đến chức năng gan. Nghiên cứu này nhằm đánh giá khả năng bảo vệ gan của dịch chiết rễ mật nhân trên chuột Swiss bị gây tổn thương gan bởi paracetamol. Chuột được chia thành nhóm đối chứng và các nhóm điều trị với liều dịch chiết khác nhau. Các chỉ số AST, ALT trong huyết thanh và malondialdehyde (MDA) ở gan được đo để phản ánh mức độ tổn thương và mức peroxy hóa lipid. Kết quả cho thấy, paracetamol làm tăng mạnh AST, ALT và MDA ($P < 0,05$). Việc dùng đồng thời dịch chiết mật nhân giúp giảm rõ rệt các giá trị này, đặc biệt ở liều 125 mg/kg. Điều đó cho thấy mật nhân có tác dụng chống oxy hóa và bảo vệ gan trước độc tính do paracetamol.

Từ khóa - Mật nhân; tác dụng bảo vệ gan; alanine aminotransferase; aspartate aminotransferase; MDA ở gan

1. Introduction

Eurycoma longifolia Jack (*E. longifolia*), commonly known as "mật nhân" in Vietnam, is a traditional medicinal plant with a long history of use across Southeast Asia. In recent decades, it has gained global recognition for its therapeutic properties and is now widely utilized in dietary supplements and health drinks, especially in Europe and the United States. In Vietnamese traditional medicine, *E. longifolia* is frequently used to treat various ailments, including digestive disorders, fatigue, infections, and liver-related conditions [1, 2, 3].

Liver injury, particularly drug-induced hepatotoxicity, remains a significant public health concern. Paracetamol (acetaminophen) is one of the most widely used analgesic and antipyretic agents worldwide; however, its overdose can lead to severe liver damage. Oxidative stress caused by the metabolite NAPQI (N-acetyl-p-benzoquinone imine) is the main mechanism underlying paracetamol-induced hepatotoxicity [4, 5].

In our previous studies, we successfully isolated several quassinoid and alkaloid compounds from the roots of *E. longifolia* collected in the Iagrai District, Gialai Province, including *β*-carboline-2*N*-oxide-1-propionic acid,9,10-dimethoxycanthin-6-one, *β*-carboline-1-propionic acid, infractine, eurylene, eurycomanone, 14,15-*β*-hydroxyklaineanol. Their structures were determined by means of spectroscopic methods (UV, IR, HR-ESIMS, 1D and 2D NMR). Among these, the compound 9,10-dimethoxycanthin-6-one (designated as EL4) was found in high concentration and has been reported to possess multiple beneficial biological activities. Quassinoids and alkaloids are the most important bioactive components contributing to the pharmacological effects of *E. longifolia* [6].

A number of quassinoid and alkaloid compounds were successfully isolated from the roots of *E. longifolia* collected in the Iagrai district, Gialai province, based on our earlier research. These compounds represent the key bioactive constituents responsible for the pharmacological activities of *E. longifolia* [6]. Our reported research also demonstrated that the aqueous extract exhibited significant inhibition of nitric oxide

(NO) production in macrophage cells, with an inhibition rate of 50.41% at a concentration of 200 µg/mL and an IC_{50} value of 198.87 ± 9.05 µg/mL. This extract also showed a statistically significant inhibition of the pro-inflammatory cytokine IL-8 by 57.55% at a concentration of 50 µg/mL ($P < 0.05$). The 80% ethanol extract of *E. longifolia* roots exhibited mild to moderate cytotoxic activity against several cancer cell lines, including KB, Hep-G2, LU-1, and MCF7. The strongest cytotoxic activity was observed against the Hep-G2 liver cancer cell line, with an IC_{50} value of 50.0 ± 2.25 µg/mL. The root extract from Gialai also showed mild antioxidant activity, as demonstrated by its DPPH radical scavenging capacity [7]. These findings serve as a preliminary basis for our further investigations. In this study, we report on the hepatoprotective and antioxidant activities of *E. longifolia* root extract collected from ChuProng, Gialai (Vietnam), against paracetamol-induced liver damage in Swiss mice. By assessing biochemical markers such as AST, ALT, and hepatic MDA, the study aims to validate the traditional use of this plant and explore its potential as a natural hepatoprotective agent.

2. Materials and methods

2.1. General

AAS was carried out using a Shimadzu AA 7000**.** HPLC was carried out using a Hitachi – HPLC system, Japan**.** Chromatographic conditions: Mobile phase: Methanol:Water = 70:30; Flow rate: 0.8 mL/min; Injection volume: 5 µL; Detector: DAD, wavelength: 254 nm; Column: C8 - 250 mm. OD was measured by the OD measuring device - V750. Serum biochemical parameters were carried out using an Automatic biochemistry machine AGD 2260 (address: Gia Viet Lab - medical testing center, Danang city).

2.2. Plant material

Roots of *E. longifolia* were collected from the ChuProng District, Gialai Province, Vietnam. The roots were cleaned, chopped, and oven-dried at 50°C for 24 hours. Dried materials were milled into a fine powder (0.5–1 mm) and stored at room temperature. Methanolic extraction was conducted using ultrasonic-assisted extraction, filtered, and removed solvent under reduced pressure to obtain residue.

The extraction was carried out using a solid–liquid maceration method. The extraction procedure was based on the authors' previous studies, with modifications made to accommodate the use of methanol as the extraction solvent. In addition, ultrasonic assistance was applied during the extraction process to enhance the extraction efficiency [3, 6, 7].

2.3. Quality evaluation of extract residue

The quality parameters of *E. longifolia* extract were determined according to the methods specified in the Vietnamese Pharmacopoeia V [8].

Total ash determined according to the method in Appendix 9.8 in [8].

Heavy metals were analyzed using Atomic Absorption Spectrophotometry (AAS) after dry ashing, according to the method in Appendix 9.4.11 in [8].

Qualitative analysis: Chemical identification reactions conducted according to the method in Appendix 8.1 in [8]. Alkaloid identification was performed using two reagents - Wagner's and Dragendorff's reagents. Flavonoid identification was performed using two reagents - concentrated sulfuric acid and sodium hydroxide solution. Steroid identification **was c**onducted using two reactions - Libermann-Burchard and Salkowski reactions.

Quantitative analysis: active compound 9,10-dimethoxycanthin-6-one was quantified by using the HPLC method described in Appendix 5.3 in [8].

2.4. Experimental animals

Sixty healthy adult *Swiss albino* mice (8–10 weeks old, 40–45 g) were used in this study. Animals were housed under standard laboratory conditions with free access to food and water, and acclimatized for one week prior to experimentation. All procedures were performed in accordance with ethical guidelines for animal care and use.

2.5. In vivo experimental design and biochemical analyses

Mice were randomly divided into nine groups (n = 5 per group).

Group 1: Physiological control, received distilled water.

Group 2: Liver injury control, received distilled water.

Group 3: Positive control, received silymarin (50 mg/kg, p.o.).

Groups 4–6: Treated with *E. longifolia* methanolic extract at 125, 250, and 500 mg/kg, respectively, via oral gavage.

All treatments were administered once daily for 8 consecutive days.

On day 8, three hours after the final treatment, mice in Groups 2–6 were given a single oral dose of paracetamol (400 mg/kg) to induce acute liver injury. Food was reinstated one hour post-paracetamol administration. After 24 hours, blood samples were collected via retro-orbital puncture under light anesthesia. Serum was obtained by centrifugation at 3000 rpm for 15 minutes and stored at 0–3°C [9, 10].

2.6. Lipid peroxidation assay

Lipid peroxidation was assessed using a modified thiobarbituric acid reactive substances (TBARS) assay, based on the methods described by Stroev and Makarova [11] and Badmus et al. [12]. This assay quantifies malondialdehyde (MDA), a primary end-product of lipid peroxidation, which reacts with thiobarbituric acid (TBA) to form a pink chromogen measurable at 532 nm.

Procedure: Liver tissues were homogenized in phosphate-buffered saline (PBS, pH 7.4; 1:10 w/v) at 0–4°C. Each reaction mixture contained 1 mL of homogenate, 0.1 mL of the test extract at various concentrations, 0.8 mL PBS, and 0.1 mL of Fenton's

reagent ($FeSO_4$ 0.1 mM and H_2O_2 15 mM, 1:1 v/v). Samples were incubated at 37 °C for 15 minutes. The reaction was stopped with 1 mL of 10% trichloroacetic acid (TCA), followed by centrifugation at 12,000 rpm for 5 minutes. The supernatant was then mixed with 1 mL of 0.8% TBA (2:1 v/v), heated at 100 °C for 15 minutes, cooled, and the absorbance was measured at 532 nm.

Trolox was used as a reference antioxidant. The percentage inhibition of lipid peroxidation was calculated as:

$$\textbf{Inhibition (\%)} = [(OD_{control} - OD_{test}) / OD_{control}] \times 100$$

Half-maximal inhibitory concentration (IC_{50}) values were calculated using Microsoft Excel.

3. Results and discussions

3.1. Physicochemical parameters of the extract

Total ash content: The total ash of the *E. longifolia* root extract was 0.16% based on dry material. This result meets the standard maximum ash content for *E. longifolia* root (not more than 2.5% of dry material) as specified in the Vietnamese Pharmacopoeia V.

Heavy metals: The analytical results of heavy metal content in the *E. longifolia* extract are presented in Table 1. The levels of heavy metals were all below the permissible limits for heavy metals regulated according to Vietnamese standards QCVN 8-2:2011/BYT [13].

Table 1. *Content of heavy metal ions in E.longifolia root*

Heavy metal ion	Content in *E.longifolia* root (mg/kg)	Permissible limit (mg/kg)
Pb^{2+}	0.015	≤ 3.0
As^{2+}	$3.35.10^{-4}$	≤ 1.0
Cd^{2+}	$1.88.10^{-4}$	≤ 1.0
Hg^{2+}	0	≤ 0.1

Qualitative analysis: Alkaloid, flavonoid, polyphenol and steroid compounds were clearly identified in "mật nhân" extract with specific reagents.

Quantitative analysis: The compound 9,10-dimethoxycanthin-6-one (coded EL4) showed a retention time of T_R = 6.8 minutes in HPLC chromatogram (Figure 1). The content was determined using a calibration curve method (Figure 2).

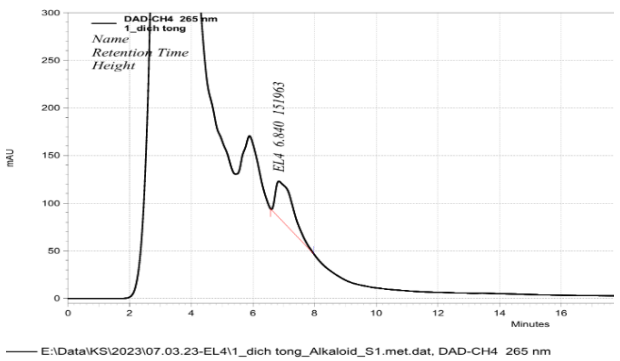

Figure 1. *HPLC chromatogram of EL4 (9,10-dimethoxycanthin-6-one)*

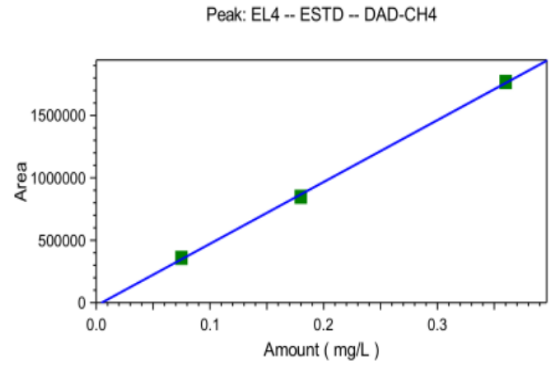

Figure 2. *Standard curve for the determination of EL4 (9,10-dimethoxycanthin-6-one)*

Results showed that 9,10-dimethoxycanthin-6-one was present at 240 mg/kg of dried extract residue, consistent with previously published data on *E. longifolia* roots collected from Ia Grai District [6].

3.2. Acute toxicity assessment of E. longifolia J. root extract in mice

The acute toxicity of the methanolic extract derived from *E. longifolia* roots was assessed in laboratory mice via oral administration at graded doses ranging from 100 to 500 mg/kg body weight. The highest dose (500 mg/kg) was selected based on the maximum feasible volume for oral gavage in mice.

Table 2. *Results of the acute toxicity test of the total extract methanol of the E. Longifolia J. root*

Lot	Dosage (mg/kg mice)	Number of mice dead/ number of live mice (after 72 hours	Expression function within 24 hours
1	Control	0/5	Mice is healthy, moves and eats normally responds to light and sounds well
2	100	0/5	Mice is healthy, moves and eats normally responds to light and sounds well
3	200	0/5	Mice is healthy, moves and eats normally responds to light and sounds well
4	300	0/5	Mice is healthy, moves and eats normally responds to light and sounds well
5	400	0/5	Mice is healthy, moves and eats normally responds to light and sounds slowly
6	500	0/5	Mice eat decrease and move slowly, after 24 hours, the mice's activity returned to normal, movement and eating returned to normal, and light and sound reflexes were normal

No mortality was observed in any treatment group within 72 hours following administration (Table 2). Mice receiving doses up to 300 mg/kg exhibited normal physiological and behavioral parameters, including locomotion, food intake, and responsiveness to auditory and visual stimuli. At 400 mg/kg, animals displayed mild

hypoactivity and delayed reactions, though feeding behavior remained unaffected. At 500 mg/kg, mice showed transient reductions in activity and appetite during the first 24 hours post-administration; however, these effects were completely resolved thereafter, with full recovery of normal behavior.

These results indicate that the methanolic root extract of *E. longifolia* does not induce acute toxicity in mice at doses up to 500 mg/kg. The temporary and reversible behavioral alterations at higher doses suggest a low level of systemic toxicity. Therefore, 500 mg/kg was chosen as the upper safety limit for subsequent *in vivo* experiments.

3.3. Antioxidant activity of E. longifolia root extract via lipid peroxidation inhibition

The antioxidant capacity of *E. longifolia* root extract was evaluated through its ability to inhibit lipid peroxidation, as reflected by malondialdehyde (MDA) levels in liver tissue. The results are summarized in Table 3.

MDA concentrations were measured in murine liver homogenates following paracetamol-induced oxidative stress and subsequent treatment with either silymarin or *E. longifolia* extract at varying doses. As a stable end-product of lipid peroxidation, MDA is widely recognized as a reliable biomarker of oxidative hepatic injury.

Paracetamol administration significantly increased hepatic MDA levels in the pathological control group (85.60 ± 1.75 nmol/mL), confirming the induction of oxidative damage when compared with the physiological baseline (72.48 ± 1.85 nmol/mL). Treatment with silymarin (50 mg/kg) markedly reduced MDA accumulation to 72.51 ± 1.32 nmol/mL ($P < 0.05$), demonstrating a strong hepatoprotective and antioxidant effect.

Table 3. *Results of MDA content in the liver of white mice*

Group	MDA content (nmol/ml)	%vs. Pathological control	Significance
Physiological control	72.48±1.85	-	-
Pathological control	85.60±1.75	100%	-
Silymarin control (50mg/kgP)	72.52±1.32	84.71%	P<0.05 vs. pathological condition
Total extract (125mg/kgP)	72.64±2.0	84.71%	P<0.05 vs. pathological condition
Total extract (250mg/kgP)	80.75±1.98	99.33%	P<0.05 vs. pathological condition
Total extract (500mg/kgP)	84.54±1.03	98.76%	P<0.05 vs. pathological condition

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Similarly, administration of *E. longifolia* extract at a dose of 125 mg/kg yielded a comparable antioxidant response, with MDA levels reduced to 72.64 ± 2.00 nmol/mL ($P < 0.05$), indicating a similar efficacy to that of silymarin in mitigating lipid peroxidation. However, higher doses of the extract (250 mg/kg and 500 mg/kg) showed diminished antioxidant effects, with MDA concentrations measured at 80.75 ± 1.98 and 84.54 ± 1.03 nmol/mL, respectively. Although these values remained significantly lower than those of the pathological control group ($P < 0.05$), the absolute reductions were minimal, with percentage decreases of only 1–2%.

These findings suggest a non-linear, biphasic dose-response pattern, in which antioxidant efficacy is maximal at lower doses and diminishes at higher concentrations. This phenomenon may be attributed to factors such as metabolic saturation, reduced bioavailability, or the emergence of pro-oxidant effects at elevated concentrations - responses frequently reported in studies involving complex phytochemical mixtures.

3.4. Hepatoprotective effect of E. longifolia root extract in a Paracetamol-induced liver injury model

The hepatoprotective potential of *E. longifolia* root extract was evaluated by measuring serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - two well-established biomarkers of hepatic injury - across experimental groups (Table 4).

Table 4. *Changes in liver enzyme activities in mice subjected to paracetamol-induced hepatotoxicity*

Group	AST (UI/L)	ALT (UI/L)
Physiological control	17±1.13	17.25±1.30
Pathological control	25.5±2.21	26±1.40
Silymarin control (50mg/kgP)	18.75±1.22 (79.41)	17.75±1.72 (94.29)
Total extract (125mg/kgP)	22±1.63 (41.18)	21.75±0.5 (48.57)
Total extract (250mg/kgP)	19±1.29 (76.47)	18.25±0.91 (88.57)
Total extract (500mg/kgP)	17.75±1.61 (91.18)	18.15±1.34 (89.71)

The pathological control group (paracetamol only) exhibited significant elevations in AST (25.5 ± 2.21 U/L) and ALT (26 ± 1.40 U/L) compared to the physiological control group (AST: 17 ± 1.13 U/L; ALT: 17.25 ± 1.30 U/L), confirming the successful induction of liver injury in accordance with previously validated models [14].

Treatment with silymarin at 50 mg/kg significantly reduced both AST and ALT levels to 18.75 ± 1.22 U/L and 17.75 ± 1.72 U/L, respectively, corresponding to inhibition rates of 79.41% and 94.29%. These findings reaffirm the established hepatoprotective role of silymarin [15].

Administration of the *E. longifolia* root extract resulted in a dose-dependent protective effect. At 125 mg/kg, the extract moderately reduced AST and ALT levels (41.18% and 48.57% inhibition, respectively). A more pronounced effect was observed at 250 mg/kg, with inhibition rates of 76.47% for AST and 88.57% for ALT, approaching the efficacy of silymarin. Notably, at 500 mg/kg, enzyme levels were nearly restored to baseline physiological values (AST: 17.75 ± 1.61 U/L; ALT: 18.15 ± 1.34 U/L), with both enzymes inhibited by more than 89%.

These findings demonstrate that *E. longifolia* root extract exerts substantial hepatoprotective effects, particularly at higher doses. The extract may thus serve as a promising candidate for the prevention or management of chemically induced liver injury [16].

4. Discussion

The findings of this study demonstrate that *Eurycoma longifolia* root extract exerts a dose-dependent hepatoprotective effect in a validated murine model of paracetamol-induced liver injury. Consistent with the oxidative nature of paracetamol hepatotoxicity, the pathological control group exhibited significantly elevated hepatic MDA levels (85.60 ± 1.75 nmol/mL), confirming lipid peroxidation as a key pathogenic mechanism. Silymarin, a well-established reference antioxidant, effectively reduced MDA accumulation to 72.51 ± 1.32 nmol/mL, supporting its known capacity to scavenge free radicals and stabilize hepatocyte membranes [15].

Notably, administration of *E. longifolia* extract at 125 mg/kg produced a comparable reduction in MDA levels (72.64 ± 2.00 nmol/mL), indicating similar antioxidant potential. This effect may be attributed to bioactive compounds in the extract, including β-carboline alkaloids, eurycomanone, and benzoquinone derivatives, which have been reported to exert antioxidant effects through mechanisms such as Nrf2 pathway activation, reactive oxygen species (ROS) neutralization, and stabilization of lipid membranes [17, 18, 19]. However, increasing the dose to 250 mg/kg and 500 mg/kg resulted in diminished antioxidant efficacy, as evidenced by higher MDA concentrations. This biphasic dose-response pattern is common among phytotherapeutic agents and may be due to saturation of hepatic uptake, altered metabolic conversion, or the emergence of pro-oxidant effects at higher concentrations [6, 20].

Interestingly, the response of serum liver enzyme biomarkers followed an inverse trend. The most pronounced hepatoprotective effect - reflected in near-normalization of AST and ALT levels - was observed at the highest dose of 500 mg/kg (AST: 17.75 ± 1.61 U/L; ALT: 18.15 ± 1.34 U/L). This divergence between oxidative stress markers and transaminase activity suggests the involvement of distinct protective mechanisms. While lower doses of the extract may primarily modulate redox balance, higher doses may activate additional cytoprotective pathways, including anti-inflammatory responses, membrane stabilization, and inhibition of necrotic or apoptotic signaling.

Furthermore, the dose-dependent improvement in AST and ALT levels - from modest effects at 125 mg/kg (41.18% and 48.57% inhibition, respectively) to near-complete normalization at 500 mg/kg - underscores the therapeutic potential of *E. longifolia* in mitigating hepatocellular damage. Although the extract did not produce a linear effect on lipid peroxidation, its ability to restore functional liver enzyme levels at higher doses strengthens its candidacy as a hepatoprotective agent, with possible application alongside or as an alternative to established therapies such as silymarin.

In summary, *E. longifolia* root extract demonstrates multifaceted hepatoprotective activity, effectively modulating both oxidative stress and enzymatic indicators of liver injury. These properties support its potential development as a botanical therapeutic for the management of drug-induced hepatotoxicity.

In an earlier investigation, we standardized herbal medicine from *E. longifolia* extract and established a technological process for the production of health supplements including *E. longifolia* - pennywort juice [3] and herb tea [21]. The results of the study on the hepatoprotective activity of *E. longifolia* extract will provide further strong scientific evidence for the potential application of *E. longifolia* in Gialai province, Vietnam, in the production of health supplements.

5. Conclusion

This study demonstrated that the methanolic extract of *Eurycoma longifolia* roots exerted notable hepatoprotective effects in a murine model of paracetamol-induced liver injury. At a low dose (125 mg/kg), the extract exhibited antioxidant activity by reducing malondialdehyde (MDA) levels, indicating inhibition of lipid peroxidation. Remarkably, at higher doses (250–500 mg/kg), although MDA reduction was less pronounced, serum AST and ALT levels were nearly restored to physiological values, suggesting the involvement of additional protective mechanisms such as membrane stabilization, anti-inflammatory effects, or anti-necrotic actions.

These findings imply that the hepatoprotective activity of *E. longifolia* is not solely attributed to its antioxidant capacity, but may also result from multiple synergistic biological pathways - an intrinsic feature of complex phytomedicines. This multifaceted mode of action supports its potential as a therapeutic or preventive agent for chemically-induced liver damage.

To the best of our knowledge, this is the first study to report the hepatoprotective properties of *E. longifolia* roots collected from Gialai province, Vietnam. These results contribute new scientific evidence to the medicinal value of this indigenous plant and support its potential application in the development of functional health products, in continuation of previous studies on herbal tea and *E. longifolia* beverages.

Further studies are needed to elucidate the underlying molecular pathways and validate these findings in clinical models.

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