

Ethanollic extract of stems of *Gnetum ula* Brongn ameliorates carbon tetrachloride induced liver toxicity in wistar rats

Sharath Rajshekarappa¹, Preetham Jinadatta², Kiran Sundera³, Sujan Ganapathy Pasura Subbaiah⁴, Sivakami Sundari Ponnusamy⁵, Mruthunjaya Kenganora^{6*}

¹Department of Food Technology, Davangere University, Bangalore, India.

²Department of Biotechnology, Dayananda Sagar College of Engineering, Bangalore, India.

³Academics and Planning, Jain (Deemed to be University), Bengaluru, India.

⁴Department of Product Development, Nutriform Wellness Private Limited, Bengaluru, India.

⁵College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore, India.

⁶Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India.

ARTICLE HISTORY

Received on: 18/01/2025

Accepted on: 01/05/2025

Available Online: 05/07/2025

Key words:

Hepatoprotective, CCl₄,
Gnetum ula, antioxidant.

ABSTRACT

Gnetum ula is used to cure several disorders, whereas stem is used for treating jaundice. The goal of this study is to assess the ethanollic extract of stems of *G. ula* for *in vivo* hepatoprotective and antioxidant efficacy against hepatotoxicity caused by carbon tetrachloride. Wistar rats were employed in the investigation, and silymarin was used as the reference drug to assess liver function tests in CCl₄-treated and untreated groups. Antioxidant enzyme systems involving CAT, GSH, SOD, and malondialdehyde levels were examined. Histopathology studies were carried out. Ethanollic stem extract of *G. ula* (200 mg/kg) blocked the CCl₄-induced elevation of biochemical markers levels of AST (154.5 ± 24.0 IU/l), ALT (125.9 ± 3.5 IU/l), alkaline phosphatase (177.1 ± 3.8 IU/l), and total bilirubin (1.06 ± 0.1 g/dl). Total protein (6.86 ± 0.1 g/dl) and albumin (4.23 ± 0.7 g/dl) was also increased in ethanollic stem extract-treated animals. A significant decline in the CAT, SOD, GSH, and elevation in MDA levels was observed in CCl₄-intoxicated animals. Antioxidant enzyme levels in plant extract-treated groups were comparable to standard and normal. The present report of ethanollic stem extract of *G. ula* in shielding the liver from adverse effects of CCl₄ supports the traditional use to cure liver disease and has been reported foremost.

INTRODUCTION

Liver ailment is one among the foremost causes for death, distressing humans of all ages [1]. New Global Burden of Disease has estimated annual mortality levels, patterns, and temporal trends in liver cirrhosis between 1980 and 2010 in 187 countries covering 99.7% of the global population, which says cirrhosis has led to millions of deaths [2]. Irrespective of advancements in current medicine, the hepatic disorder remains as a worldwide health issue. Hence research for new

drugs to treat this illness is carried out. Herbal medicines are important as hepatoprotective drugs and participate as the most important in curing several ailments with minimum information of their scientific mechanisms. The usage of synthetic drugs causes adverse reactions and are also of high cost. Therefore, treating liver complications with plant-based drugs is of utmost importance now [3].

Gnetum ula belongs to the family Gnetaceae. It is a woody climber and *G. ula* is believed by Kodavas Karnataka, India, as a sacred plant. *Gnetum ula* is an important indigenous plant aided for medication. Stem extracts are used in treating jaundice [4–6] and leaf extracts are used in the treatment of liver enlargement. Stem and root used are used as antiperiodic [7]. Stem is also administered for piles, hemicranias, and piercing wounds brought on by horn thrust [8]. Rheumatism

*Corresponding Author
Mruthunjaya Kenganora, Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India. E-mail: kmruthunjaya@jssuni.edu.in

is treated with seed oil and roasted fruit [9–11]. The fruits of *G. ula* are edible. Additionally, the seeds yield an oil that can be applied to burns and used as medicine [12,13]. Stem yields gnetol characterized as 2, 6, 11, 13-tetrahydroxy-trans stilbene [14], 3, 4, methylenedioxy-4- methoxy -trans-stilbene [15], and gnetulin, a dimer of 3, 4, 5''- trihydroxy -3- methoxystilbene [16,17]. Based on the usage of stem extracts by tribes and medical practitioners to treat jaundice and other ailments we have selected *G. ula* to investigate antihepatotoxic activity by using *in vivo* parameters.

MATERIALS AND METHODS

Chemicals

Petroleum ether, chloroform, and ethanol were procured (Ranbaxy Fine Chemicals New Delhi, India). Carbon tetrachloride and Silymarin from Sigma-Aldrich (USA). Biochemical analysis kit was procured from ERBA Diagnostics Inc. Transasia Bio-Medicals Ltd. Other chemicals were analytical grade (Sigma-Aldrich, USA and E-Merck, Mumbai).

Plant material and extraction

Stem of *G. ula* was collected from Biligirirangana Hills (B.R. Hills) of Chamaraanagar district, Karnataka, India, and authenticated at the Department of AYUSH, Govt. of India Bangalore by Dr. Shiddamallayya. N. (Ref No: RRCBI-MUS-0107).

A plant specimen of 500 gm was dried and powered using a mechanical blender, defatted with petroleum ether, and extracted using ethanol using a soxhlet apparatus. The extracts of *G. ula* were filtered and the solvent was evaporated by using a rotary evaporator [18]. For the present study, ethanol extract was used to evaluate its hepatoprotective potential.

In vivo studies of *Gnetum ula*.

A Wistar strain rat of both sex and female Swiss albino mice was procured from Sri Raghavendra Enterprises, Bangalore, Karnataka, India. The animals were granted by the Institutional Animal Ethics Committee (DSCBS/Ph.D./IAEC/01/14-15) and were nursed with a standard pellet diet and water *ad libitum*, accustomed prior to the experimentation.

Determination of acute toxicity

Acute oral toxicity studies were conducted for ethanolic extract of *G. ula* as per the OECD guidelines 423 [19]. Female Swiss albino mice weighing 20–30 g were used for the study. These mice were fasted for 4 hours providing only water. Later GUE was administered orally at a limited dose of 2,000 mg/kg body weight. The animals were monitored continuously for 4 hours for gross behavioral changes and finally for 48 hours and then daily for 14 days for any signs of toxicity including death.

Evaluation of Hepatoprotective activity of *G. ula*

Hepatoprotective activity was conducted using both the sex of Wistar albino rats (150–180 g) and were divided into five groups with six animals each. Group 1 was provided with 0.5% Carboxymethyl cellulose (CMC) (10 ml/kg body

weight) functioned as normal control and Group 2 with 0.5% CMC (10 ml/kg body weight) as toxicant control. Group 3 as positive control served with the standard silymarin (200 mg/kg). Groups 4 and 5 were treated with ethanolic extracts of *G. ula* at doses of 100 and 200 mg/kg body weight, respectively. As mentioned, all the drugs were dispensed orally using gavage for 7 days. On the eighth day, apart from group 1, all other groups were treated with CCl₄ intraperitoneally (1 ml/kg body weight in olive oil). On the ninth day, blood was collected by retro-orbital puncturing under mild anesthetic conditions (diethyl ether) followed by separation of serum by centrifugation at 2 000g for 10 minutes. Serum was preserved in vials at 4°C, and soon used for the estimation of biochemical markers. The animals were sacrificed after 24 hours of CCl₄ treatment by overdosing with diethyl ether. The liver was taken off all animals, perfused with phosphate buffer, homogenized with 10% potassium chloride (KCl) solution, and centrifuged for 10 minutes at 2,000 rpm. The supernatant was used for the estimation of antioxidant assays. A portion of liver tissue was preserved in a 10% formalin solution for histopathological studies to observe gross necrosis, fatty acid changes, and inflammatory cells [20,21].

Lipid Peroxidation assay

Breaking of polyunsaturated fatty acids gives malondialdehyde and forms a pink-colored compound upon reaction with thiobarbituric acid, which is measured at 532 nm [22]. Its concentration in the test can be calculated using an extinction coefficient of $1.6 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Effect of GUE on serum biochemical parameters

Serum was subjected to biochemical investigations like serum glutamic pyruvic transaminase or ALT, serum glutamic-oxaloacetic transaminase or AST, alkaline phosphatase (ALP), total bilirubin, total protein, and albumin by kit methods as per instructions provided by the company (Erba diagnostics inc).

Effect of GUE on liver endogenous antioxidant enzymes

Superoxide dismutase activity assay (SOD)

SOD inhibits auto-oxidation of epinephrine to adrenochrome at alkaline pH [23]. SOD activity was expressed as unit/mg protein. One unit of the enzyme is defined as the amount of enzyme required to inhibit 50% of the rate of epinephrine auto-oxidation.

Catalase assay (CAT)

Catalase activity was approached by Aebi methodology [24]. The decrease in optical density at 0 time and 1 minute time is read at 240 nm. Units of catalase were expressed as the amount of enzyme that decomposes 1 mM H₂O₂/minute at 25°C. Catalase activity was expressed as U/mg of protein.

Estimation of Glutathione (GSH)

GSH is a sulfhydryl-containing non-protein substance. DTNB (5,5' di thio bis (2-nitrobenzoic acid) is a disulphide chromagen reduced by sulfhydryl compounds to a yellow color compound and is measured at 412 nm. This is directly proportional to the GSH concentration [25].

Histopathological studies

Liver samples were used to study the effect of GUE on the CCl₄-induced rats. Phosphate phosphate-buffered 10% formaldehyde solution was used to fix the liver sample of different groups. It was then embedded in paraffin wax to cut thin sections of 5 µm thickness and stained with hematoxylin–eosin. Treated sections were observed by using a high-resolution microscope and photomicrographs were captured [26].

Statistical analysis

The results were provided as the mean ± S.E.M. of six animals that were employed in each group. One-way analysis of variance was used to do a statistical analysis of the data. Followed by Tukey's multiple comparison test using Graph pad Prism 6.0 software at the level of $p < 0.05$.

RESULTS

Extraction of *G. ula*

Soxhlet extraction of the stem (500 gm) of *G. ula* with ethanol yielded 4.45%w/w. During this extraction process, plant material was defatted with petroleum ether first and extraction with ethanol led to the accumulation of highly polar

molecules with an increase in the yield, which may result in good hepatoprotective activity. Ethanol extract was studied for hepatoprotective activity GUE.

In vivo studies of *G. ula*.

In recent years, several findings have been supported by traditional medicines in an attempt to find new drugs for liver disorders. In the present study, ethanol extract of the stem of *G. ula* was evaluated for hepatoprotective activity against CCl₄-induced hepatotoxicity to find out the therapeutic efficacy of the extract.

Acute toxicity studies

Ethanol extract of *G. ula* GUE did not show symptoms of mortality or toxicity at the dose of 2,000 mg/kg body weight. Therefore, 110 (200 mg/kg) and 120 (100 mg/kg) of GUE were chosen as high dose and low dose, respectively.

Effect of GUE on hepatic biochemical markers

The hepatoprotective effect of GUE (Groups 4 and 5) is summarised in Table 1. CCl₄-induced elevation of serum AST, ALT, ALP, total bilirubin, and demotion of total protein, albumin was observed in the toxicant group. GUE in

Table 1. Biochemical parameters of ethanolic extract *G.ula* in CCl₄ treated animals.

Groups	AST(IU/l)	ALT(IU/l)	ALP(IU/l)	Albumin (g/dl)	TP (g/dl)	TB (mg/dl)
Normal—1	56.1 ± 4.1	60.1 ± 6.3	89.48 ± 4.5	5.79 ± 0.8	8.44 ± 0.41	0.52 ± 0.05
Toxicant-CCl ₄ - 2	300 ± 56.6	249.8 ± 35.4	279.23 ± 40.6	0.89 ± 0.8	2.55 ± 1.2	2.76 ± 0.6
Silymarin+ CCl ₄ (200 mg/kg)—3	102.4 ± 4.07 ^b	95.48 ± 6.28 ^b	146.8 ± 5.32 ^{a,b}	5.03 ± 0.82 ^b	7.66 ± 0.27 ^b	0.90 ± 0.06 ^b
GUE (100 mg/kg) + CCl ₄ —4	169.5 ± 13.8 ^{ab}	134.7 ± 1.6 ^{ab}	190.76 ± 6.8 ^{ab}	3.02 ± 1.1 ^{ab}	5.89 ± 1.18 ^{a,b}	1.47 ± 0.07 ^{ab}
GUE (200 mg/kg) + CCl ₄ —5	154.5 ± 24.0 ^{ab}	125.9 ± 3.5 ^{ab}	177.1 ± 3.8 ^{ab}	4.23 ± 0.7 ^{ab}	6.86 ± 0.1 ^{a,b}	1.06 ± 0.1 ^{ab}
GUE- Ethanol extract of <i>G.ula</i> ;			ALP-Alkaline phosphatase;			
AST-Aspartate aminotransferase;			TP-Total protein;			
ALT-Alanine amino transaminase;			TB-Total bilirubin			

Values are expressed as mean ±SD; $n = 6$.

^aSignificance level: $p < 0.05$, compared to Normal group.

^bSignificance Level: $p < 0.05$, compared to Toxicant group.

Table 2. *In vivo* antioxidant assays of ethanolic extract of *G. ula* in CCl₄ treated animals.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)	GSH (nmol/mg protein)
Normal—1	9.40 ± 0.46	23.3 ± 1.09	2.14 ± 0.74	9.26 ± 0.37
Toxicant (CCl ₄)—2	3.45 ± 0.74	9.84 ± 0.29	7.60 ± 0.64	4.17 ± 0.60
Silymarin (200 mg/kg) + CCl ₄ - 3	8.56 ± 0.48 ^b	21.24 ± 0.73 ^b	3.97 ± 0.27 ^{a,b}	8.06 ± 0.83 ^b
GUE (100 mg/kg) + CCl ₄ - 4	4.50 ± 0.76 ^a	17.57 ± 3.01 ^{a,b}	6.12 ± 0.96 ^a	7.03 ± 0.70 ^{a,b}
GUE (200 mg/kg) + CCl ₄ - 5	7.88 ± 0.93 ^b	19.81 ± 0.79 ^b	4.59 ± 0.45 ^{a,b}	7.54 ± 0.85 ^b
GUE –Ethanol extract of <i>G.ula</i> ;		CAT- Catalase;		
SOD- Superoxide dismutase;		MDA-Malondialdehyde;		
		GSH-Glutathione		

Values are expressed as mean ±SD; $n = 6$.

^aSignificance Level: $p < 0.05$, compared to Normal group.

^bSignificance Level: $p < 0.05$, compared to Toxicant group.

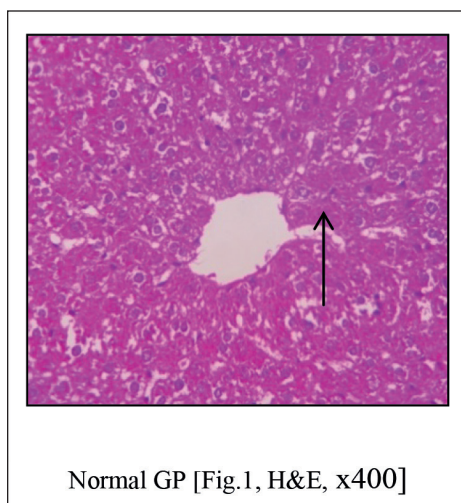


Figure 1. Normal group shows normal architecture of liver.

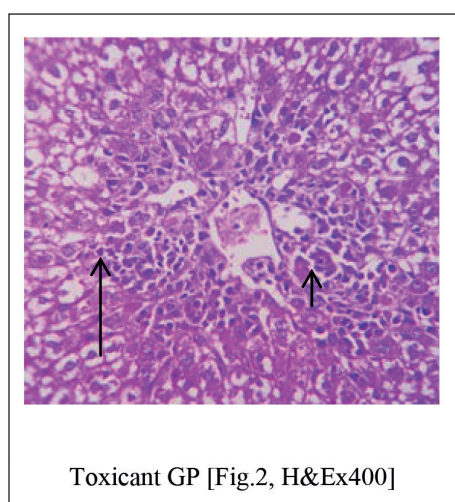


Figure 2. Distorted architecture of liver the perivenular region shows severe necrosis [Short Arrow], the central veins and sinusoids appear distorted. [Long Arrow].

comparison to group 2, reversed the condition significantly ($p < 0.05$) decreasing the serum AST, ALT, ALP, and total bilirubin and elevating total protein and albumin. Silymarin treated group (200 mg/kg) revealed significant protection against CCl_4 induced hepatic damage. Treatment with GUE decreased the serum markers level at the dose of 100 mg/kg and 200 mg/kg only, which is backed by histological changes.

Effect of GUE on hepatic antioxidant markers

The antioxidant defense system in the liver (SOD, CAT, and GSH) is affected by lipid peroxidative degradation of bio membrane, the basis for hepatotoxicity [27]. CCl_4 produced a significant reduction in the SOD, CAT, and GSH activities and increases in the MDA level compared to the normal group. GUE (100 mg/kg and 200 mg/kg) made a significant ($p < 0.05$) increase in the enzyme activity of SOD,

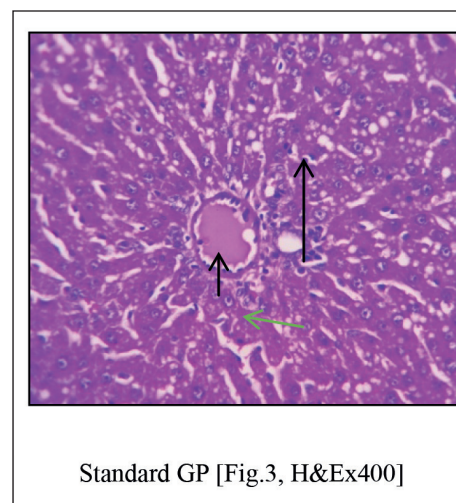


Figure 3. Standard group liver shows undamaged architecture of liver parenchyma [Long Arrow]. The perivenular region shows mild necrosis consisting of degenerative hepatocytes [Green Arrow] and mixed inflammatory cells. Veins appear congested [Short Arrow].

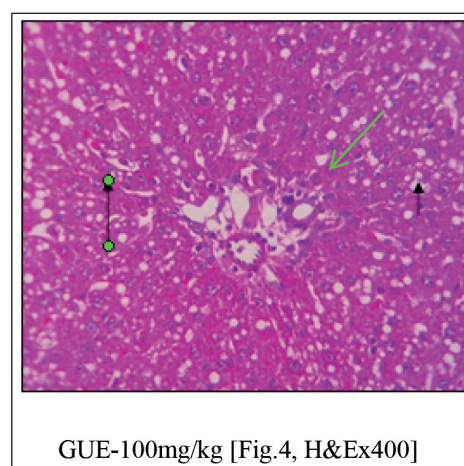


Figure 4. GUE at 100 mg/kg treated group shows micro vesicular steatosis [Green Arrow] with regeneration of hepatocytes. The perivenular region shows moderate necrosis consisting of degenerative hepatocytes [Long Arrow] and mixed inflammatory cells [Short Arrow].

CAT, and GSH and a decrease in MDA. Group III treated with standard silymarin also showed comparable results. Results are tabulated in Table 2.

Histopathological studies (Fig. 3)

Normal Group of rats studied shows intact architecture of liver parenchyma (Fig. 1). Toxicant Group showed distorted architecture of liver the perivenular region shows severe necrosis (Fig. 2). The central veins and sinusoids appear distorted (Fig. 2). Standard group liver Section studied from shows undamaged architecture of liver parenchyma (Fig. 3). The perivenular region shows mild necrosis consisting of degenerative hepatocytes (Fig. 3) and mixed inflammatory cells. Few regenerative hepatocytes are also seen. Some of the veins appear congested (Fig. 3). GUE at 100 mg/kg treated group

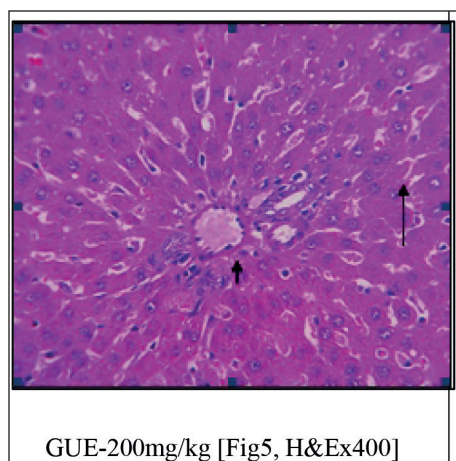


Figure 5. Normal liver parenchyma architecture [Long Arrow]. The periportal hepatocytes [Short Arrow] and midzonal hepatocytes appear unremarkable.

shows micro vesicular steatosis (Fig. 4) with the regeneration of hepatocytes. The perivenular region shows moderate necrosis consisting of degenerative hepatocytes (Fig. 5) and mixed inflammatory cells (Fig. 5). GUE treated group (200 mg/kg) directs the normal liver parenchyma architecture (Fig. 5). The periportal hepatocytes (Fig. 4) and midzonal hepatocytes appear unremarkable.

DISCUSSION

Carbon tetrachloride, a well-studied hepatotoxin is used to induce liver damage in rats reported in 1936 [28], widely used and accepted by many researchers. The cytochrome P450 activates CCl_4 to create the trichloromethyl radical (CCl_3^\bullet), which combines with a molecule of oxygen to produce trichloromethylperoxyl radical ($\text{CCl}_3\text{O}_2^\bullet$) (Cameron and Karunaratne, 1936). $\text{CCl}_3\text{O}_2^\bullet$ binds to macromolecules and causes per oxidative degradation of lipid membranes, leading to the formation of lipid peroxides like MDA causing loss of integrity of cell membrane and hepatic damage [29,30]. Stress and injury caused by CCl_4 induce the activation of other cells like natural killer cells, kupffer cells, and NKT cells, progressing to liver injury by producing various inflammatory cytokines like tumor necrosis factor- α , Interferon- γ , and Interleukins-1 β [31]. However, interleukins (IL-10 and IL-6) and prostaglandins exhibit a liver-protective role [32]. AST, ALT, ALP, and total bilirubin in plasma are sensitive to liver injury [33] and cause leakage from cells due to the altered permeability of the membrane. AST is normally found in heart, liver, and kidney and in skeletal muscles. AST helps in the conversion of L-aspartate and Alpha-ketoglutarate to L-glutamate and oxaloacetate. ALT is found in liver disorders, cirrhosis, myocardial infarction, and high levels of liver issues. It catalyzes the transamination of L-alanine and Alpha-ketoglutarate to form pyruvate and L-glutamate. Serum ALP is found in bone, liver, intestine placenta and excreted in bile. An increased level is noted in the case of hepatobiliary and bone diseases, [34]. The present investigation revealed that ethanolic extract of *G. ula* has therapeutic values in treating

liver damage induced by CCl_4 in rats. GUE attempted to recover the damage caused by increased significantly serum marker levels.

Bilirubin, metabolite from heme breakdown also affects the hepatic cell [35]. A raised level of bilirubin is found in hepatic diseases, hemolysis, and biliary excretion. GUE at 200 mg/kg b.wt dose was able to control from dropping down of bilirubin levels. Total protein concentration will be deprived in the hepatotoxic condition by defective protein biosynthesis [36,37]. Hepatotoxic CCl_4 causes disruption of polyribosomes on the endoplasmic reticulum, thereby bringing down the process of protein synthesis. In this study, ethanolic extract of *G. ula* at 200 mg/kg significantly stabilizes the membrane, repairs the hepatic tissue, protects the polyribosomes, and restores protein synthesis.

Whenever the cellular capacity of removal of reactive oxygen species diminishes then the cell gets injured [38,39]. Our results showed significant depletion of SOD, CAT, and GSH levels in rat liver cells with CCl_4 induction. However, antioxidants can inhibit CCl_4 -induced toxicity at various levels by directly scavenging the CCl_3^\bullet , donating hydrogen to CCl_3^\bullet , and donating hydrogen $\text{CCl}_3\text{O}_2^\bullet$. Antioxidant enzymes include SOD, CAT, and GSH are the first line of the defense system, converting oxygen molecules its non-toxic compounds [27].

SOD eliminates superoxide radicals by turning them into H_2O_2 , which CAT and GSH can quickly convert into water. Thus, CAT and GSH inhibit the formation of hydroxyl radicals.

MDA is one of the endproducts in the lipid peroxidation process. An increase in MDA levels indicates the extent of lipid peroxidation leading to tissue damage and preventing the antioxidant system from working through [40]. GUE-treated groups enhanced enzymatic activity thereby protecting the hepatocytes and diminishing the lipid peroxidation.

Since their chemical structures are interrelated, a direct connection between antioxidant activity and total phenol concentration has been identified. Therefore, *in vivo* antioxidant activity of the ethanolic extract of *G. ula* is associated with the total phenol content present in the extract. Hepatoprotective activity has been linked to the antioxidant activity of the plant extracts. Most of the naturally occurring phenolic compounds possess antioxidant activity [41,42]. Stilbenes are a group of polyphenols and many of them have been isolated from the genus *Gnetum* [43,44] and also from the *G. ula* [14,15,16]. Stilbenes are a rich source of lead phytoconstituent for newer drugs and treatments. Many of the naturally occurring stilbenes exhibit uniqueness by possessing strong antioxidant activity/radical scavenging properties. Most recently isolated stilbene is known to possess antioxidant, antimicrobial, and anti-inflammatory properties and some of them have the potential to be developed as a new drug [45]. In our present study, the stem of *G. ula* exhibited antihepatotoxicity and the stilbenes content of the plant might be contributing to the protection of hepatic cells, thereby bringing back to normal functioning of liver cells.

CONCLUSION

In the present study, GUE possesses a strong antioxidant activity. The antioxidant and free radical-scavenging properties

of *G. ula* stem were found to be responsible for the plant's hepatoprotective effects. These reports, therefore, support the tribal use of *G. ula* stem for liver problem treatment. For the first time, *G. ula* has been studied for ant hepatoprotective activity. A further study on the isolation and identification of phytochemical compounds to correlate the antioxidant and hepatoprotective activity has been obtained to support the present study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

There is no funding to report.

CONFLICT OF INTEREST

The author reports no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Ethical approvals details are given in the 'Material and Method' section

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Negi AS, Kumar JK, Luqman S, Shanker K, Gupta MM, Khanuja SP. Recent advances in plant hepatoprotectives: a chemical and biological profile of some important leads. *Med Res Rev*. 2008;28(5):746–72.
2. Ali AM, Alan DL, Saied S, Rafael L, Ali HM, Jeff S, *et al.* Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med*. 2014;12(1):145.
3. Maity T, Maity S, Pahari N, Ranjan DK, Ganguli S. A review on hepatic diseases and development of herbal drugs for the treatment of liver complications. *World J Pharm Res*. 2015;5(5):677–91.
4. Vikneshwaran D, Viji M, Raja LK. Ethanomedicinal plants survey and documentation related to paliyar community. *J Ethnobot Leaflets*. 2008;12(1):1108–11015.
5. Thirumalian T, Elumalai EK, Vijiyan TS, Senthil BK, Ernest. D. Ethnobotanical survey of folklore plants for the treatment of jaundice and snakebites in vellore. *J Ethnobot Leaflets*. 2010;14:529–36.
6. Mohan VR, Rajesh A, Athiperumalsami T, Sutha S. Ethanomedicinal plants of the Tirunelveli District. Tamilnadu, India. *J Ethnobot Leaflets*. 2008;12:79–95.
7. Pushpangadan P, Atal CK. Ethnomedical and ethnobotanical investigations among some scheduled caste communities of Travancore, Kerala. India. *J Ethnopharmacol*. 1986;16(1-2):175–90.
8. Raj Kumar MH, Rajanna MD. Ex-situ conservation of climbing plants at university of agricultural sciences, Bangalore, Karnataka. *Rec Res Sci Tech*. 2011;3(4):18–20.
9. Basu P, Mitra B. Preliminary notes on the climbing taxa of Andaman and Nicobar Islands with special reference to their importance. *J Econ Taxon Bot*. 1992;16:393–9.
10. Pullaiah. T. Biodiversity of India. Paris, France: Regency publications; 1992. Pp 552.
11. Prusti AB, Behera KK. Ethnobotanical exploration of Malkangiri District of Orissa, India. *J Ethnobot Leaflets*. 2007;11:122–40.
12. Warriar PK, Nambiar VPK, Ramankutty C. Indian medicinal plants: a compendium of 500 species. Madras, India: Orient Longman; 1993.
13. Mondal P, Mukerjee PK. Notes on ethnobotany of Keonjhar district, Orissa. *J Econ Tax Bot Addl Ser*. 1992;10:7–18.
14. Zaman A, Prakash S, Wizarat. K. Isolation and structure of gnetol, a novel stilbene from *Gnetum ula*. *Indian J Chem*. 1983;22b:101–4.
15. Prakash S, Khan MA, Khan KZ. Stilbenes of *Gnetum Ula*. *Phytochemistry*. 1985;24:622–4.
16. Siddiqui Z, Rahman S, Khan MA. Gnetulin, a dimer of 3',4,5' – Trihydroxy- 3- methoxy Stilbene from *Gnetum ula*. *Tetrahedron*. 1993;49:10393–6.
17. Tandon N. Reviews on Indian medicinal Plants. Medicinal Plant Unit, Indian Council of Medical Research, New Delhi. 2013;11:1120.
18. Khandewal KR. Practical pharmacognosy. 22nd ed. Pune, India: Nirali publications; 2013.
19. OECD/OCDE. OECD Guideline for testing of chemicals. Acute Oral Toxicity-Acute Toxicity Class methods. Paris, France: OECD Publishing; 2002.
20. Chen Y, Huang B, He J, Han L, Zhan Y, Wang Y. *In vitro* and *in vivo* antioxidant effects of the ethanolic extract of *Swertia chiraytia*. *J Ethnopharmacol*. 2011;136(2):309–15.
21. Pareek A, Godvarthi A, Issarani R, Nagori BP. Antioxidant and hepatoprotective activity of *Fagonia schweinfurthii* (Hadidi) extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. *J Ethnopharmacol*. 2013;150(3):973–81.
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction *Anal. Biochem*. 1979;95(2):351–58.
23. Mishra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972;247(10):3170–5.
24. Aebi H. Catalase. In: Bergmeyer HV. *Methods in enzymatic analysis* Newyork, NY: Academic press; 1974. Vol. 2, pp 674–84.
25. Moran MA, Depierre JW, Mannervick B. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat liver. *Biochimica Biophysica Acta*. 1979;582(1):67–8.
26. Shenoy, KA, Somayaji SN, Bairy KL. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. *Indian J Pharmacol*. 2001;33(4):260–6.
27. Ames BN, Shigenenga MT, Hagen M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci United States Am*. 1993;90(3):7915–22.

28. Cameron GR, Karunaratne WAE. Carbon tetrachloride cirrhosis in relation to liver regeneration. *J Pathol Bacteriol.* 1936;42(1):1–21.
29. Thabrew MI, Joice PD, Rajatissa WA. Comparative study of the efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Med.* 1987;53(3):239–41.
30. Bishayee A, Sarkar A, Chatterjee M. The hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. *J Ethnopharmacol.* 1995;49(2):69–74.
31. Blazka ME, Wilmer JL, Holladay SD, Wilson RE, Luster MI. Role of pro-inflammatory cytokines in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol.* 1995;133(1):43–52.
32. Bourdi M, Masubuchi Y, Reilly TP. Protection against acetaminophen-induced liver injury and lethality by interleukin 10, role of inducible nitric oxide synthase. *Hepatology.* 2002;35(2):289–98.
33. Molander DWF, Wroblewski, JS. LA-Due. Transaminase compared with cholesterae and alkaline phosphatase an index of hepatocellular integrity. *Clin Res Proc.* 1987;3:20–4.
34. Harsha Mohan. The liver, biliary tract and exocrine pancreas. In: *Text book of pathology*, 4th ed. New Delhi, India: Jaypee Brothers Medical Publishers LTD; 2002. Pp 447–9.
35. Victor J, Navarro MD, John R, Senior MD. Drug-related hepatotoxicity. *N Engl J Med.* 2006;354(7):731–39.
36. Goel A, Dani V, Dhawan DK. Protective effects of zinc on lipid peroxidation, antioxidation, antioxidant enzymes and hepatic histoarchitecture in chlorphrifos induced toxicity. *Chem Biol Interact.* 2005;156(2-3):131–40.
37. Wu YH, Zhang XM, Hu MH, Wu XM, Zhao Y. Effect of *Lagera alata* on hepatocytes damage induced by carbon tetrachloride *in vitro* and *in vivo*. *J Ethnopharmacol.* 2009;126(1):50–6.
38. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem.* 1998;46:4113–7.
39. Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. *Fresen Environ Bull.* 2017;26(7):4757–63.
40. Antonio A, Mario F. Muñoz, Sandro Argüelles. “Lipid Peroxidation: production, metabolism, and signaling mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal”. *Oxid Med Cell Longev.* 2014;2014:31.
41. Preetham J, Kiran S, Sharath R, Sundari S, Ganapathy S, Sushma S Murthy. Pharmacognostic and preliminary phytochemical studies in *Gnetum ula* brongn. *Int Res J Pharm.* 2015;6:377–38.
42. Pereira DM, Valentão P, José AP, Andrade PB. Phenolics: from chemistry to biology. *Molecules.* 2009;14(6):2202–11.
43. Lin M, Li JB, Li SZ, Yu DQ, Liang XT. A dimeric stilbene from *Gnetum parvifolium*. *Phytochemistry.* 1992;31(2):633–8.
44. Tanaka T, Iliya I, Ito T, Furusawa M, Nakaya KI, Iinuma M, *et al.* Stilbenoids in Lianas of *Gnetum parvifolium*. *Chem Pharm Bull.* 2001;49(7):858–62.
45. Tao S, Wang XN, Lou HX. Natural stilbenes: an overview. *Nat Prod Rep.* 2009;26:916–35.

How to cite this article:

Rajshekarappa S, Jinadatta P, Sundera K, Subbaiah SGP, Ponnusamy SS, Kenganora M. Ethanolic extract of stems of *Gnetum ula* Brongn ameliorates carbon tetrachloride induced liver toxicity in wistar rats. *J Appl Pharm Sci.* 2025;15(08):199–205. DOI: 10.7324/JAPS.2025.157972