Protective Role of *Phyllanthus niruri* Ethanol Extract on Dyslipidaemia Associated with Carbon Tetrachloride-Induced Liver Injury in Wistar Rats

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Abstract

Carbon tetrachloride (CCl_4) is a well-established hepatotoxin widely employed to model oxidative liver injury in experimental research. The increasing global burden of liver diseases has intensified the search for plant-derived compounds with hepatoprotective efficacy. *Phyllanthus niruri (P. niruri*), a medicinal plant traditionally used across various cultures, has been reported to possess antioxidant, anti-inflammatory, and hepatoprotective properties. This study evaluated the protective effects of the ethanol extract of *P. niruri* on CCl_4-induced hepatic damage and lipid profile alterations in male Wistar rats.Thirty-five rats (175–185 g) were randomly assigned to seven groups (n = 5) following one week of acclimatization. Hepatic injury was induced in all groups except the normal control via a single intraperitoneal injection of CCl_4 (1.25 mL/kg in a 1:1 olive oil solution). Thereafter, rats received oral administration of graded doses (200, 500, and 1000 mg/kg) of *P. niruri* extract for 28 days. Biochemical assessments showed that CCl_4 significantly elevated serum total cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) concentrations, while markedly reducing high-density lipoprotein (HDL) levels. Treatment with *P. niruri* extract elicited a dose-dependent normalization of these lipid parameters, characterized by significant ($p < 0.05$) reductions in total cholesterol, triglycerides, LDL, and VLDL, along with increased HDL concentrations relative to the untreated CCl_4-intoxicated group.Overall, the findings demonstrate that *P. niruri*confers substantial hepatoprotective and lipid-modulatory effects against CCl_4-induced hepatic dysfunction, underscoring its therapeutic potential as a natural intervention for the management of oxidative liver injury.

Keywords: *Phyllanthus niruri*,Carbon tetrachloride, Hepatotoxicity, Hepatoprotection, Lipid homeostasis,

Introduction

The liver performs numerous tasks, including glucose, protein, and lipid metabolism as well as detoxification, secretion, and storage. Consequently, preserving a functioning liver is essential for animal health and lifespan (Pandit *et al.,* 2012). Nonetheless, its functionality is typically influenced by xenobiotics. Prolonged or excessive exposure to xenobiotics results in cirrhosis or malignancies (Okoli *et al.,* 2011). Carbon tetrachloride (CCl_4) is a potent hepatotoxin commonly employed to produce liver damage in animal models (N. Aobulikasimu *et al.,* 2023; D. Dong *et al.,* 2013).

In recent years, there has been a growing interest in the role that biotransformation plays in transforming chemicals into highly reactive metabolites that initiate cellular toxicity. A wide variety of chemicals, including clinically useful drugs, can cause cellular damage because of their metabolic activation into highly reactive substances, such as free radicals. A common environmental toxicant is CCl_4, which is widely used in animal models of liver damage. It has been reported that CCl_4 is hepatotoxic due to the formation of trichloromethyl and trichloromethyl peroxyl radicals, which lead to lipid peroxidation and result in fibrosis and necrosis of the liver (Kadiiska *et al.,* 2000; V. Unsal *et al.,* 2021). CCl_4 is metabolically activated by Cyt-P450 into the trichloromethyl radical (J. L. Poyer *et al.,* 1980; Tomasi *et al.,* 1985), which, in the presence of oxygen, is converted into the peroxyl radical (Packer *et al.,* 1978) by initiating lipid peroxidation, these free radicals cause cellular damage by interacting with the biological system. Additionally, it is responsible for covalently binding to proteins, which results in a rise in intracellular Ca2+, the depletion of GSH, and the release of iron within the cell. Ultimately, these events result in an oxidative stress condition in which the cells' defence mechanisms are unable to counter ROS, including superoxide anion, hydrogen peroxide, and radical hydroxyl. Sun *et al*. (2018)found that CCl_4 toxicity caused many free radicals to be formed and inflammation to occur in hepatocytes, which affects both their structural and functional membranes. Some synthetic medications used to treat liver problems are ineffective and can have serious side effects. Consequently, a growing interest is being expressed in evaluating the traditional herbal plants claimed to be hepatoprotective (R. S. Beedimani & S. Shetkar, 2015). In response to this, natural remedies, particularly those that are derived from plants, have gained popularity as they are perceived to be safer and more accessible. Traditional herbal treatments are being scientifically validated for their potential to mitigate oxidative stress and inflammation, offering promising alternatives to synthetic drugs.

Herbal products have been utilized since the inception of civilization to preserve human health and to obtain cures for various ailments by a significant portion of the global population (Kalegari *et al.,* 2014; Myagmar *et al.,* 2004).

Phyllanthus niruri has been extensively utilized for the management of jaundice and various hepatic ailments (Lin*et al.,* 2003; Qu *et al.,* 2021).Venkateswaran *et al*. (1987) indicated that *P. niruri*contains one or more substances that inhibit the replication of woodchuck hepatitis virus in vivo and mitigate its pathogenic effects on woodchuck liver. Furthermore, clinical investigations involving humans have been conducted with *P. niruri*, demonstrating no adverse effects (S. P. Thyagarajan *et al.,* 1988). The plant is known to exhibit significant protective effects against numerous hepatic illnesses, including viral hepatitis and toxicity from diverse pharmaceuticals and environmental toxins (Naik & Juvekar, 2003). However, the mechanisms behind the

hepatoprotective activity of *P. niruri* are not well elucidated.

The present study was aimed at evaluating the hepatoprotective effects of *P. niruri* ethanol extract ondyslipidaemia associated with CCL_4-induced liver injury in Wistar rats.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade and products of Sigma-Aldrich Chemical (St. Louis, MO, USA) unless otherwise stated.

Plant Material

The leaves of *P. niruri* used in this study were collected from an open field at the University of Benin, Benin City, Edo State, Nigeria. The fresh plant was identified and authenticated at the Department of Plant Biology and Biotechnology of the same institution with a voucher specimen number,UBH-P406.

Preparation of plant extract

The fresh leaves were air-dried under laboratory conditions for fourteen (14) days, pulverized, and the ethanol extract was prepared by loading the powdered leaves into a Soxhlet apparatus. The extraction lasted for 12 h, and the extract solution was evaporated to dryness in a rotary evaporator at 40 °Cat the University of Benin Energy Centre to obtain a residue labelled *P. niruri,*which was stored in clean vials until required.

Experimental Animals

Male Wistar rats of albino strain (175–185 g) were obtained from the Animal House, Biochemistry Department, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The animals were acclimatized for two weeks under healthy and hygienic conditions. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65 % humidity, and a 12-h light/12-h dark cycle. They were allowed free access to pelletized growers' mash and clean drinking water. All the experiments were carried out in accordance with the National Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23), revised 1996.

Experimental protocol

A total of thirty-five (35) rats were selected, five in each group (n = 5).Group 1 rats which served as control were given distilled water only, Group 2 were administered 500mg/kg body weight of extracts only, Group 3 were pre-treated with 500mg/kg body weight of plant extract before administration of CCl_4(1.25 ml/kgbw in olive oil (1:1); IP); Group 4 rats were induced with single-dose of CCl_4 (1.25 ml/kgbw in olive oil (1:1); IP) to cause hepatic damage, Group 5 were co-treated with $CCl_4$and 200mg/kg bw of plant extract, Group 6 rats were co-treated with $CCl_4$and 500mg/kg bw of plant extract, while Group 7 rats were co-treated with $CCl_4$and 1000mg/kg bw of plant extract,

Animal Sacrifice

At the end of feeding and administration of $CCl_4$and plant extracts, the animals were subjected to an overnight fast on the 28th day and later sacrificed using a dissecting kit with urethane as anaesthesia. Blood samples of each rat were collected immediately via cardiac puncture by means of 10 ml syringes and dispensed into sterile sample tubes. The tubes were subjected to centrifugation at 3500 rpm for 10 minutes using a bench centrifuge. The supernatant (serum) was collected and then transferred into plain Eppendorf tubes for subsequent assays.

Determination of lipid Profile

Total cholesterol (TC), Triglyceride (TG), Very-low-density lipoprotein (VLDL), Low-density lipoprotein (LDL), and High-density Lipoprotein (HDL) were determined using the method of

Richmond (1973).

Statistical Analysis

Data were expressed as the mean ± S.E.M. using SPSS 26 software. Statistical significance was

determined by one-way analysis of variance by multiple comparisons. Differences between means were estimated by Duncan's multiple range tests and a value of $p < 0.05$ was taken as statistically significant.

Results

Effects of *P. Niruri* Ethanol Extract on CCl_4-Induced hepatotoxicity

The full serum lipid profile data across all treatment groups is shown in Tables 1, 2, 3, 4, and5.The ethanol extract of *P. Niruri* conferred significant hepatoprotection against CCl_4-induced hepatic injury, as evidenced by the restoration of key serum lipid parameters toward healthy control levels.

Effect of pretreatment with ethanol extract of *P. niruri*on TC in CCl_4-induced hepatotoxicity in rats

Serum TC concentration in rats pre-treated with *P. niruri*and challenged with CCl_4 is presented in Table 1. Serum TC level (110.80±21.80 mg/dL) was significantly raised ($p < 0.05$) in CCl_4-intoxicated rats compared to control group (75.39±9.80 mg/dL).Pretreatment with 500 mg/ kg bw*P. niruri*(Group 3) resulted in the lowering of TC. Treatment with *P. Niruri* extract at 500 mg/kg bw showed the most effective reduction in TC levels (91.75±18.70 mg/dL) compared to the control group ($p < 0.05$, while pretreatment with 500 mg/kg bw maintained TC levels (76.00±13.00 mg/dL) were not significantly different from the normal control (75.39±9.80[a]).

Table 1: Effect of pretreatment and cotreatment with ethanol extract of *P. niruri*on serum TC in CCl_4-induced hepatotoxicity in rats

Treatment (Dose, mg/kgbw)	TC (mg/dL)
Control	75.39±9.80[a]
P. niruri(500)	69.33±10.60[a]
P. niruri +CCl_4(500)	76.00±13.00[a]
CCl_4	110.80±21.80[b]
CCl_4+ *P. niruri*(200)	100.25±22.10[b]
[redacted]	91.75±18.70[b]
[redacted]	98.33±18.00[c]

Data are expressed as mean ± SEM (n = 5). Values not sharing a common superscript differ significantly at $p< 0.05$. Superscript [a]denotes comparison with the normal control group, [b]denotes comparison with the CCl_4-treated group, and [c]denotes a significant difference ($p< 0.05$) relative to the CCl_4 + P. niruri (200 mg/kg) group.

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Effect of pretreatment with ethanol extract of *P. niruri*on TG in CCl_4-induced hepatotoxicity in rats

According to Table 2, CCl_4 caused a significant increase ($p< 0.05$) in TG levels (178.60±15.60 mg/dL) versus control (84.00±14.30 mg/dL). The 200 mg/kg bw*P. Niruri* treatment group showed optimal TG reduction (121.00±12.70 mg/dL), demonstrating significant improvement ($p<0.05$) compared to the CCl_4 group.

Table2: Effect of pretreatment and cotreatment with ethanol extract of *P. niruri* on serum TG in CCl_4-induced hepatotoxicity in rats

Treatment (Dose, mg/kgbw)	TG (mg/dL)
Control	84.00±14.30[a]
P. niruri(500)	80.00±5.51[a]
P. niruri + CCl_4(500)	150.33±2.33[b]
CCl_4	178.60±15.60[a]
CCl_4+ *P. niruri*(200)	121.00±12.70[b]
CCl_4+ *P. niruri*(500)	124.75±11.70[b]
CCl_4+ *P. niruri*(1000)	128 .33±29.02[b]

Data are expressed as mean ± SEM (n = 5). Values not sharing a common superscript differ significantly at $p< 0.05$. Superscript [a]denotes comparison with the normal control group,[b]denotes comparison with the CCl_4-treated group, and [c]denotes a significant difference ($p< 0.05$) relative to the CCl_4 + P. niruri (200 mg/kg) group.

Effect of pretreatment with ethanol extract of *P. niruri*on HDL in CCl_4-induced hepatotoxicity in rats

Table 3 shows that CCl_4 significantly decreased ($p < 0.05$) HDL levels (21.00±1.22 mg/dL) compared to control (35.40±0.73 mg/dL). All *P. niruri* treatment groups showed significant improvement ($p < 0.05$) in HDL levels (22.50±0.50 mg/dL), with

the 500 mg/kg bw dose achieving the highest increase.

Table3: Effect of pretreatment and cotreatment with ethanol extract of *P. niruri* on serum HDL in CCl_4-induced hepatotoxicity in rats

Treatment (Dose, mg/kg)	HDL (mg/dL)
Control	35.40±0.73[a]
P. niruri(500)	33.00±1.22[a]
P. niruri +CCl_4(500)	28.25±2.78[b]
CCl_4	21.00±1.22[b]
CCl_4+ *P. niruri*(200)	28.50±0.50[c]
CCl_4+ *P. niruri*(500)	25.25±2.78[b]
CCl_4+ *P. niruri*(1000)	23.33±2.20[b]

Data are expressed as mean ± SEM (n = 5). Values not sharing a common superscript differ significantly at $p< 0.05$. Superscript [a]denotes comparison with the normal control group,[b]denotes comparison with the CCl_4-treated group, and [c]denotes a significant difference ($p< 0.05$) relative to the CCl_4 + P. niruri (200 mg/kg) group.

Effect of pretreatment with ethanol extract of *P. niruri*on LDL in CCl_4-induced hepatotoxicity in rats

As presented in Table 4, CCl_4 significantly increased ($p < 0.05$) LDL levels (120.80±21.80 mg/dL) versus control (55.39±9.80 mg/dL). The 1000 mg/kg bw*P. Niruri* treatment demonstrated the most effective LDL reduction (98.43±18.00 mg/dL).

Table 4: Effect of cotreatment with ethanol extract of *P. niruri*on serum LDL in CCl_4-induced hepatotoxicity in rats

Treatment (Dose, mg/kgbw)	LDL (mg/dL)
Control	55.39±9.80[a]
P. niruri(500)	49.33±10.60[a]
P. niruri +CCl_4(500)	76.00±13.00[b]
CCl_4	120.80±21.80[b]
CCl_4+ *P. niruri*(200)	161.25±22.10[b]
CCl_4+ *P. niruri*(500)	108.85±18.70[b]
CCl_4+ *P. niruri*(1000)	98.43±18.00[b]

Data are expressed as mean ± SEM (n = 5). Values not sharing a common superscript differ significantly at $p< 0.05$. Superscript [a]denotes comparison with the normal control group,[b]denotes comparison with the CCl_4-treated group, and [c]denotes a significant difference ($p< 0.05$) relative to the CCl_4 + P. niruri (200 mg/kg) group.

Effect of pretreatment with ethanol extract of *P. niruri*on VLDL in CCl_4-induced hepatotoxicity in rats

Table 5 shows that CCl_4 elevated ($p < 0.05$) VLDL levels (85.34±14.35 mg/dL) compared to control (47.57±8.78 mg/dL). *P. niruri* treatment produced dose-dependent reductions in VLDL, with the 1000 mg/kgbw dose showing maximum effect (80.32±18.80 mg/dL).

Table5: Effect of pretreatment and cotreatment with ethanol extract of *P. niruri*on serum VLDL in CCl_4-induced hepatotoxicity in rats

Treatment (Dose, mg/kg)	VLDL (mg/dL)
Control	47.57±8.78[a]
P. niruri(500)	45.23±5.45[a]
P. niruri +CCl_4(500)	75.55±12.78[b]
CCl_4	85.34±14.35[b]
CCl_4+ *P. niruri*(200)	84.13±17.30[b]
CCl_4+ *P. niruri*(500)	81.17±12.70[b]
CCl_4+ *P. niruri*(1000)	80.32±18.80[b]

Data are expressed as mean ± SEM (n = 5). Values not sharing a common superscript differ significantly at $p< 0.05$. Superscript [a]denotes comparison with the normal control group,[b]denotes comparison with the CCl_4-treated group, and [c]denotes a significant difference ($p< 0.05$) relative to the CCl_4 + P. niruri (200 mg/kg) group.

Discussion

The inhibition of protein synthesis in the liver is primarily regarded as a mechanism that reduces lipoprotein synthesis and leads to fat accumulation in the liver, culminating in the onset of fatty liver. A reduction in total protein content is proposed as an effective indicator of the severity of cellular dysfunction in chronic liver disorders (Ibrahim *et al.,* 2020; Venukumar & Latha, 2002). It has been shown that the administration of CCl_4 results in the suppression of protein synthesis, evidenced by a reduction in total serum and liver proteins compared to healthy controls (Sirag *et al.,* 2011; EL Sayed *et al.,* 2019).

The present study demonstrates that *P. niruri* extract significantly normalized elevated serum total cholesterol TC, TG, LDL, VLDL, while enhancing HDL levels in treated rats. These effects corroborate earlier findings on the lipid-regulating and antioxidative properties of *P. niruri*(Khanna *et al.,* 2002). Carbon tetrachloride-induced hepatotoxicity is a well-established model of oxidative liver injury mediated through its metabolic activation by cytochrome P450 into the trichloromethyl (CCl_3•) and trichloromethyl peroxyl (CCl_3OO•) radicals (Kadiiska *et al.,* 2000; J. Poyer *et al*., 1980). These reactive species

trigger lipid peroxidation, disrupt hepatocellular membranes, and impair lipid metabolism (Velid Unsal *et al.*, 2021). The observed elevation in serum TC, TG, LDL, and VLDL following CCl_4 exposure aligns with this mechanism, as lipid leakage and impaired β-oxidation are hallmark consequences of oxidative hepatic damage (Boll *et al.,* 2001). Cotreatment or pretreatment with *P. niruri* extract attenuated these perturbations, underscoring its capacity to stabilize hepatocyte membranes and enhance antioxidant defence systems. The hepatoprotective action of *P. niruri* can be attributed to its rich phytochemical constituents, including lignans (Chang *et al., 2003), alkaloids, flavonoids (Chauhan et al., 1988), and polyphenols, which exhibit strong radical*-scavenging and anti-inflammatory activities. These bioactive compounds may prevent peroxidative degradation of lipids by enhancing endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Faremi *et al.,* 2008). Such mechanisms are consistent with the attenuation of oxidative stress markers observed in similar models using *Phyllanthus amarus* and other hepatoprotective plants (S. R. Beedimani & S. Shetkar, 2015).

In this study, the dose-dependent restoration of HDL levels, particularly at 200 mg/kg bw, suggests that *P. niruri* not only suppresses lipid peroxidation but may also enhance reverse cholesterol transport. The concurrent normalization of LDL and VLDL implies improved lipid utilization and hepatic clearance.Comparable observations have been made in hyperlipemic rats treated with *P. niruri* extract, where plasma cholesterol reduction was accompanied by improved liver enzyme profiles (Khanna *et al*, 2002). Such normalization may involve modulation of hepatic microsomal enzymes and inhibition of HMG-CoA reductase, thereby balancing lipid biosynthesis and

The dual effect of *P. niruri* in both pre-treated and co-treated groups indicates prophylactic and therapeutic efficacy. The prophylactic protection observed in pre-treated rats likely arises from the priming of antioxidant defenses before CCl_4 exposure, as suggested by studies on *Phyllanthus amarus* (S. Thyagarajan *et al*., 1988) and *Rosa laevigata* extracts (Deshi Dong *et al*., 2013). Conversely, its therapeutic potential during cotreatment may involve the suppression of inflammation and promotion of hepatic regeneration, consistent with hepatocellular repair processes (Deng *et al*., 2024).

At the molecular level, *P. niruri* may mitigate oxidative stress by modulating the Nrf2/ARE pathway, a key regulator of cellular antioxidant responses. Polyphenolic compounds present in *Phyllanthus* species have been reported to upregulate Nrf2-dependent enzymes and inhibit NF-κB signaling, thereby reducing pro-inflammatory cytokine production (Nuerbiye Aobulikasimu *et al*., 2023). The resulting reduction in oxidative and inflammatory stress would help restore normal lipid metabolism, preventing steatosis and fibrosis.

Furthermore, the normalization of serum lipid markers in *P. niruri*-treated rats highlights its potential in preventing non-alcoholic fatty liver disease (NAFLD) and associated metabolic complications. Similar findings have linked the inhibition of oxidative stress with reduced hepatic steatosis (Bovi *et al*., 2021). Given the global burden of metabolic liver disorders, the demonstration of such effects by a plant-based extract underscores the translational potential of *P. niruri* as a nutraceutical hepatoprotectant.

The present study demonstrates that *P. niruri* ethanol extract effectively mitigates the dyslipidemia induced by CCl_4, restoring lipid homeostasis and suggesting a potent hepatoprotective role. The findings provide strong experimental evidence that *P. niruri* ethanol extract confers hepatoprotection against CCl_4-induced oxidative damage via lipid peroxidation inhibition, antioxidant enhancement, and lipid homeostasis restoration. Future work integrating molecular docking and dynamic simulation could further delineate the interactions between *P. niruri* phytochemicals and hepatic regulatory proteins such as CYP2E1, HMG-CoA reductase, and Nrf2, clarifying the mechanistic underpinnings of its hepatoprotective activity.

Conclusion

The elevations in total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein, alongside the reduction in high-density lipoprotein induced by CCl_4 administration, were markedly attenuated by both pretreatment and cotreatment with *Phyllanthus niruri*. The extract effectively normalized these CCl_4-mediated biochemical alterations, indicating its ability to restore hepatic function and maintain lipid homeostasis. Collectively, the findings suggest that *P. niruri* confers protective effects against CCl_4-induced hyperlipidaemia and may hinder the progression of fatty liver in rats. These results underscore the therapeutic potential of *P. niruri* in mitigating dyslipidaemia associated with oxidative hepatic injury.

References

Aobulikasimu, N., Zheng, D., Guan, P., Xu, L., Liu, B., Li, M., Huang, X., & Han, L. (2023). The Anti-inflammatory Effects of Isoflavonoids from Radix Astragali in Hepatoprotective Potential against LPS/D-gal-induced Acute Liver Injury. *Planta Med*, *89*(4), 385-396. https://doi.org/10.1055/a-1953-0369

Beedimani, R. S., & Shetkar, S. (2015). Hepatoprotective activity of Eclipta alba against carbon tetrachloride-induced hepatotoxicity in albino rats. *Int J Basic Clin Pharmacol*, *4*(3), 404-409.

Beedimani, S. R., & Shetkar, S. (2015). Hepatoprotective activity of Eclipta alba against carbon tetrachloride-induced hepatotoxicity in albino rats. *International Journal of Basic & Clinical Pharmacology*, *4*(3), 404-410.

Boll, M., Weber, L. W., Becker, E., & Stampfl, A. (2001). Hepatocyte damage induced by carbon tetrachloride: inhibited lipoprotein secretion and changed lipoprotein composition. *Z Naturforsch C J Biosci*, *56*(3-4), 283-290. https://doi.org/10.1515/znc-2001-3-419

Bovi, A. P. D., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., & Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Frontiers in medicine*, *8*, 595371.

Chang, C. C., Lien, Y. C., Liu, K. C., & Lee, S. S. (2003). Lignans from Phyllanthus urinaria. *Phytochemistry*, *63*(7), 825-833. https://doi.org/10.1016/s0031-9422(03)00371-6

Chauhan, A. K., Dobhal, M. P., & Joshi, B. C. (1988). A review of medicinal plants showing anticonvulsant activity. *J Ethnopharmacol*, *22*(1), 11-23. https://doi.org/10.1016/0378-8741(88)90226-7

Deng, Y., Zhao, Z., Sheldon, M., Zhao, Y., Teng, H., Martinez, C., Zhang, J., Lin, C., Sun, Y., Yao, F., Curran, M. A., Zhu, H., & Ma, L. (2024). LIFR regulates cholesterol-driven bidirectional hepatocyte-neutrophil cross-talk to promote liver regeneration. *Nat Metab*, *6*(9), 1756-1774. https://doi.org/10.1038/s42255-024-01110-y

Dong, D., Zhang, S., Yin, L., Tang, X., Xu, Y., Han, X., Qi, Y., & Peng, J. (2013). Protective effects of the total saponins from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in mice. *Food and chemical toxicology*, *62*, 120-130.

Dong, D., Zhang, S., Yin, L., Tang, X., Xu, Y., Han, X., Qi, Y., & Peng, J. (2013). Protective effects of the total saponins from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in

mice. *Food Chem Toxicol*, *62*, 120-130. https://doi.org/10.1016/j.fct.2013.08.050

EL Sayed, H. E., Morsy, L. E., Abo Emara, T. M., & Galhom, R. A. (2019). Effect of carbon tetrachloride (CCl4) on liver in adult albino rats: histological study. *The Egyptian Journal of Hospital Medicine*, *76*(6), 4254-4261.

Faremi, T. Y., Suru, S. M., Fafunso, M. A., & Obioha, U. E. (2008). Hepatoprotective potentials of Phyllanthus amarus against ethanol-induced oxidative stress in rats. *Food and chemical toxicology*, *46*(8), 2658-2664.

Ibrahim, J., Kabiru, A. Y., Abdulrasheed-Adeleke, T., Lawal, B., & Adewuyi, A. H. (2020). Antioxidant and hepatoprotective potentials of curcuminoid isolates from turmeric (Curcuma longa) rhizome on CCl4-induced hepatic damage in Wistar rats. *Journal of Taibah University for Science*, *14*(1), 908-915.

Kadiiska, M. B., Gladen, B. C., Baird, D. D., Dikalova, A. E., Sohal, R. S., Hatch, G. E., Jones, D. P., Mason, R. P., & Barrett, J. C. (2000). Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl(4) poisoning? *Free Radic Biol Med*, *28*(6), 838-845. https://doi.org/10.1016/s0891-5849(00)00198-2 10.

Kalegari, M., Gemin, C. A., Araujo-Silva, G., Brito, N. J., Lopez, J. A., Tozetto Sde, O., Almeida, M., Miguel, M. D., Stien, D., & Miguel, O. G. (2014). Chemical composition, antioxidant activity and hepatoprotective potential of Rourea induta Planch. (Connaraceae) against CCl4-induced liver injury in female rats. *Nutrition*, *30*(6), 713-718. https://doi.org/10.1016/j.nut.2013.11.004

Khanna, A. K., Rizvi, F., & Chander, R. (2002). Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats. *J Ethnopharmacol*, *82*(1), 19-22. https://doi.org/10.1016/s0378-8741(02)00136-8

Lin, T. J., Su, C. C., Lan, C. K., Jiang, D. D., Tsai, J. L., & Tsai, M. S. (2003). Acute poisonings with Breynia officinalis--an outbreak of hepatotoxicity. *J Toxicol Clin Toxicol*, *41*(5), 591-594. https://doi.org/10.1081/clt-120023760

Myagmar, B. E., Shinno, E., Ichiba, T., & Aniya, Y. (2004). Antioxidant activity of medicinal herb Rhodococcum vitis-idaea on galactosamine-induced liver injury in rats. *Phytomedicine*, *11*(5), 416-423. https://doi.org/10.1016/j.phymed.2003.04.003

Naik, A. D., & Juvekar, A. R. (2003). Effects of alkaloidal extract of Phyllanthus niruri on HIV replication. *Indian J. Med. Sci*, *57*(9), 387-393.

Okoli, C. O., Obidike, I. C., Ezike, A. C., Akah, P. A., & Salawu, O. A. (2011). Studies on the possible mechanisms of antidiabetic activity of extract of aerial parts of Phyllanthus niruri. *Pharm Biol*, *49*(3), 248-255. https://doi.org/10.3109/13880209.2010.501456

Packer, J. E., Slater, T. F., & Willson, R. L. (1978). Reactions of the carbon tetrachloride-related peroxy free radical (CCl3O2.) with amino acids: Pulse radiolysis evidence. *Life sciences*, *23*(26), 2617-2620. https://doi.org/10.1016/0024-3205(78)90378-8

Pandit, A., Sachdeva, T., & Bafna, P. (2012). Drug-induced hepatotoxicity: a review. *Journal of Applied Pharmaceutical Science*(Issue), 233-243. doi.org/10.7324/JAPS.2012.2541

Poyer, J., McCay, P., Lai, E., Janzen, E., & Davis, E. (1980). Confirmation of assignment of the trichloromethyl radical spin adduct detected by spin trapping during 13C-carbon tetrachloride metabolism in, vitro, and, in, vivo. *Biochemical and biophysical research communications*, *94*(4), 1154-1160.

Poyer, J. L., McCay, P. B., Lai, E. K., Janzen, E. G., & Davis, E. R. (1980). Confirmation of assignment of the trichloromethyl radical spin adduct detected by spin trapping during 13C-carbon tetrachloride metabolism in vitro and in vivo. *Biochem Biophys Res Commun*, *94*(4), 1154-1160. doi.org/10.1016/0006-291x(80)90540-9

Qu, D., Qiao, D.-F., Chen, X.-C., Feng, C.-Q., Luo, Q.-Z., & Tan, X.-H. (2021). Fatal poisoning by accidental ingestion of the “heartbreak grass”(Gelsemium elegans) verified by toxicological and medico-legal analyses. *Forensic science international*, *321*, 110745. doi.org/10.1016/j.forsciint.2021.110745

Sirag, H., Ibrahim, H., Amer, A., Salam, T., Samaka, R., & El-Mowafy, Y. (2011). Ameliorative effect of caffeic acid phenethyl ester and candsartain cilexetil on CCL4 induced hepatotoxicity in male rats. *Annals of Biological Research*, *2*(4), 503-515.

Sun, J., Wen, X., Liu, J., Kan, J., Qian, C., Wu, C., & Jin, C. (2018). Protective effect of an arabinogalactan from black soybean against carbon tetrachloride-induced acute liver injury in mice. *Int. J. Biol. Macromol*, *117*, 659-664. doi.org/10.1016/j.ijbiomac. 2018.05.203 10.1016.

Thyagarajan, S., Thirunalasundari, T., Subramanian, S., Venkateswaran, P., & Blumberg, B. (1988). Effect of Phyllanthus amarus on chronic carriers of hepatitis B virus. *The Lancet*, *332*(8614), 764-766.

Thyagarajan, S. P., Subramanian, S., Thirunalasundari, T., Venkateswaran, P. S., & Blumberg, B. S. (1988). Effect of Phyllanthus amarus on chronic carriers of hepatitis B virus. *Lancet*, *2*(8614), 764-766. doi.org/10.1016/s0140-6736(88)92416-6

Tomasi, A., Albano, E., Biasi, F., Slater, T. F., Vannini, V., & Dianzani, M. U. (1985). Activation of chloroform and related trihalomethanes to free radical intermediates in isolated hepatocytes and in the rat in vivo as detected by the ESR-spin trapping technique. *Chemico-biological interactions*, *55*, 303-316.

Unsal, V., Cicek, M., & Sabancilar, I. (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Rev Environ Health*, *36*(2), 279-295. https://doi.org/10.1515/reveh-2020-0048

Unsal, V., Cicek, M., & Sabancilar, İ. (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Reviews on environmental health*, *36*(2), 279-295.

Venkateswaran, P. S., Millman, I., & Blumberg, B. S. (1987). Effects of an extract from Phyllanthus niruri on hepatitis B and woodchuck hepatitis viruses: in vitro and in vivo studies. *Proc Natl Acad Sci U S A*, *84*(1), 274-278. doi.org/10.1073/pnas.84.1.274 10.1073/pnas.84.1.274

Venukumar, M., & Latha, M. (2002). Hepatoprotective effect of the methanolic extract of Curculigo orchioides in CCl4-treated male rats. *Indian Journal of Pharmacology*, *34*(4), 269-275