

Influence of Methanol Extract of *Dennettia tripetala* on Hepatic Redox Homeostasis and Detoxification Pathways in Carbon Tetrachloride–Intoxicated Wistar Rats

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ABSTRACT

Background: Therapeutic plants are rich sources of bioactive compounds useful in combating various human diseases. This study investigated the *in vitro* antioxidant activity, gas chromatography–mass spectrometry (GC-MS) profile, and *in vivo* protective effects of the methanolic leaf extract of *Dennettia tripetala* in a carbon tetrachloride (CCl₄)-intoxicated rat model.

Methods: Twenty-five male Wistar rats (6–8 weeks old, 150–190 g) were randomly allocated into six groups (n = 5 per group). Group A (normal control) received liquid paraffin (1 mL/kg); Group B (intoxicated control) received 30% CCl₄ in liquid paraffin (1 mL/kg); Group C received silymarin (100 mg/kg) plus CCl₄; Groups D, E and F received the methanolic leaf extract at 100, 200 and 300 mg/kg, respectively, plus CCl₄. Extract or silymarin was administered orally for six consecutive days; CCl₄ was given from day 2 to day 6. Liver function indices (ALT, AST, ALP, total protein, total bilirubin) and antioxidant markers (SOD, CAT, GSH, MDA) were measured. Data were analysed by one-way ANOVA followed by Bonferroni multiple-comparison test (GraphPad Prism 8.0.1) after confirmation that the assumptions of normality and homogeneity of variance were met; *p* < 0.05 was considered significant. Values are presented as mean ± standard error of the mean (SEM).

Results: The extract exhibited strong free-radical scavenging activity (IC₅₀ = 4.12 µg/mL against DPPH versus 3.33 µg/mL for ascorbic acid; IC₅₀ = 5.00 µg/mL against ABTS versus 3.87 µg/mL for ascorbic acid). GC-MS analysis identified 37 compounds accounting for 99.7% of the total peak area. The major constituents (≥ 2%) were linoleic acid (36.10%), palmitic acid (18.96%), vitamin E (7.15%), stearic acid (6.06%), caryophyllene (4.24%), γ-sitosterol (3.98%), squalene (3.75%), phytol (2.28%) and α-amyrin (2.12%). These compounds are candidate contributors to the observed antioxidant activity. *In vivo*, CCl₄ significantly elevated serum ALT, AST, ALP and total bilirubin and reduced total protein relative to the normal control (*p* < 0.05). Concurrent administration of the extract (100–300 mg/kg) significantly attenuated these elevations and restored total protein toward control values (*p* < 0.05 versus Group B). The extract also significantly increased hepatic SOD, CAT and GSH while decreasing MDA levels compared with the intoxicated control (*p* < 0.05). Acute oral toxicity testing indicated an LD₅₀ > 3000 mg/kg.

Conclusions: The methanolic leaf extract of *D. tripetala* demonstrates preclinical hepatoprotective activity against CCl₄-induced oxidative liver injury in rats, consistent with its *in vitro* antioxidant capacity and the

presence of candidate phytochemicals. These findings provide experimental support for further mechanistic and dose–response studies; they do not constitute justification for therapeutic use in humans.

Keywords: *Dennettia tripetala*; hepatoprotection; carbon tetrachloride; antioxidant; GC-MS; Wistar rats

INTRODUCTION

Plants are complex chemical storehouses of biodynamic compounds with substantial potential for modern medicine (Wang et al., 2002). Plant-derived products provide a veritable source of phytochemicals with antioxidant properties, including flavonoids, alkaloids, saponins, steroids and glycosides (AlSheikh et al., 2020; Doughari, 2012; Leitzmann, 2016). Isolated compounds such as uvariopsine, argentinine, dennettine and a 2,6-dimethoxychromene have been characterised from the root of *Dennettia tripetala* (Lopez et al., 2002). 1-Nitro-2-phenylethane and linalool have also been identified as constituents (Adeoti et al., 2000).

Dennettia tripetala (Annonaceae), commonly known as pepper fruit, is widely distributed in the rainforest zone of West Africa and is cultivated in southern, eastern and western Nigeria for nutritional and economic purposes (Ejechi and Akpomedaye, 2005; Burkill, 1995; Gill, 1992). Traditional uses include remedies for fever, cough, toothache and related ailments (Adedayo et al., 2015). Phytochemical screenings have shown that the plant is rich in alkaloids, saponins, flavonoids, steroids, cardiac glycosides and terpenoids. Essential-oil extracts possess analgesic and anti-inflammatory effects in rodents; additional studies have reported anti-hyperglycaemic activity and reduction of intraocular pressure (Anaga et al., 2011; Timothy and Okere, 2008). Root extracts display in vitro antioxidant activity comparable to ascorbic acid and vitamin E. A previous report indicated that methanolic leaf extract normalised abnormal bile secretion in rats under toxic challenge (Ofem et al., 2014).

Despite these reports, adequate scientific evidence linking the in vitro antioxidative activity, chemical profile and in vivo protective effects of the crude leaf extract remains limited. Free-radical-mediated liver injury constitutes a global health concern, and treatment options for many liver ailments remain constrained by the scarcity of fully satisfactory drugs. Carbon tetrachloride (CCl_4) is a classical hepatotoxin that undergoes cytochrome-P450-mediated bioactivation to the trichloromethyl radical ($\text{CCl}_3\bullet$) and subsequently the trichloromethyl peroxy radical ($\text{CCl}_3\text{OO}\bullet$), initiating lipid peroxidation, covalent binding to macromolecules and acute hepatocellular injury (Weber et al., 2003).

The present study therefore aimed to (i) profile the methanolic leaf extract of *D. tripetala* by GC-MS, (ii) determine its acute oral toxicity (LD_{50}), (iii) evaluate its in vitro free-radical scavenging capacity, and (iv) assess its protective effect on serum liver function indices and hepatic enzymatic and non-enzymatic antioxidant markers in a CCl_4 -intoxicated Wistar rat model.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical grade. Silymarin was used as the reference hepatoprotective agent.

Plant collection, identification and extract preparation

Fresh leaves of *Dennettia tripetala* were collected from Ankpa Local Government Area, Kogi State, Nigeria, and authenticated at the Herbarium Unit, Department of Plant Science and Biotechnology, Prince Abubakar Audu University (voucher number BC-002). Leaves were air-dried under shade, reduced to small pieces and extracted with methanol (1:5 w/v) at room temperature for 48 h. The mixture was filtered through Whatman No. 1 filter paper and the filtrate concentrated to dryness using a rotary evaporator. The dried extract was stored in a closed container until reconstitution for subsequent assays.

In vitro free-radical scavenging assays

DPPH radical scavenging activity was assessed according to the protocol of Simelane et al. (2010) with minor modifications. Methanolic extract solutions (50–200 µg/mL) were mixed with an equal volume of DPPH solution and absorbance was read at 517 nm after 30–60 min; ascorbic acid served as the reference standard. ABTS radical scavenging activity was determined by the method of Re et al. (1999); absorbance was measured at 734 nm. Results are expressed as IC₅₀ values (µg/mL).

Gas chromatography–mass spectrometry (GC-MS) analysis

An Agilent 6890N gas chromatograph coupled to a 5973 mass-selective detector and fitted with an HP-5MS column (30 m × 250 µm × 0.25 µm) was used. Helium was the carrier gas (1 mL/min). The oven programme started at 70 °C (2 min hold) and was ramped at 30 °C/min to 300 °C (total run time 49.67 min). Compounds were identified by comparison of retention times and mass fragmentation patterns with the NIST 2014 library.

Experimental animals and housing

Twenty-five male Wistar rats (6–8 weeks old) weighing 150–190 g were obtained from the animal house of Prince Abubakar Audu University, Kogi State, Nigeria. Animals were housed in a well-ventilated room under standard laboratory conditions with free access to standard rodent feed and clean water ad libitum. They were allowed to acclimatise for two weeks before the commencement of experiments. All procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Acute oral toxicity (LD₅₀)

Acute oral toxicity was evaluated using the Up-and-Down Procedure (OECD Guideline 425, 2001). A limit dose of 3000 mg/kg body weight of the extract was administered to four randomly selected rats. Animals were observed for 8 h, 24 h and up to 48 h for signs of toxicity (tremors, depression, weakness, salivation, refusal of food/water) or mortality. No deaths occurred; therefore the median lethal dose (LD₅₀) was concluded to be greater than 3000 mg/kg.

Experimental design for the intervention study

Hepatotoxicity was induced according to the method of Guntupalli et al. (2006). Rats were randomly allocated into six groups of five animals each (n = 5):

Group A (normal control): liquid paraffin (1 mL/kg body weight) daily for six days.

Group B (intoxicated control): 30% CCl₄ in liquid paraffin (1 mL/kg) from day 2 to day 6.

Group C (positive control): silymarin (100 mg/kg) daily for six days plus CCl₄ from day 2 to day 6.

Group D: methanolic leaf extract (100 mg/kg) daily for six days plus CCl₄ from day 2 to day 6.

Group E: methanolic leaf extract (200 mg/kg) daily for six days plus CCl₄ from day 2 to day 6.

Group F: methanolic leaf extract (300 mg/kg) daily for six days plus CCl₄ from day 2 to day 6.

The three extract doses (100, 200 and 300 mg/kg) were selected to span a threefold range for dose-response assessment, with the highest dose corresponding to one-tenth of the acute oral toxicity threshold established in this study (LD₅₀ > 3000 mg/kg), remaining well within the established margin of safety. Extracts and silymarin were administered orally. On day 7 animals were sacrificed under diethyl ether anaesthesia. Blood was collected, allowed to clot and centrifuged (3000 rpm, 5 min) to obtain serum for biochemical assays. A portion of each liver was homogenised in ice-cold phosphate buffer for antioxidant assays.

Determination of biochemical parameters

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by the method of Reitman and Frankel (1957). Total protein was determined by the biuret method (Gornall et al., 1949). Total bilirubin was measured by the colorimetric method of Jendrassik and Grof (1938). Alkaline phosphatase (ALP) activity was determined by the colorimetric method of Sood (1999). Catalase activity was assayed according to Beers and Sizer (1952). Reduced glutathione (GSH) was estimated by the method of Patterson and Lazarow (1955). Malondialdehyde (MDA) was quantified as described by Hartman (1983). Superoxide dismutase (SOD) activity was measured by a modified Kakkar et al. (1984) method.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM) of five replicates ($n = 5$). Statistical comparisons among groups were performed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple-comparison test using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, USA). Prior to ANOVA, data were assessed for normality using the Shapiro–Wilk test (Shapiro & Wilk, 1965) and for homogeneity of variance using the Brown–Forsythe test (Brown & Forsythe, 1974); the assumptions of ANOVA were satisfied for all parameters. A p -value < 0.05 was considered statistically significant. In the result tables, superscript “a” denotes significant difference versus Group A (normal control) and superscript “b” denotes significant difference versus Group B (intoxicated control). To formally test for a dose-dependent response, a linear trend across the CCl₄-exposed groups (0, 100, 200 and 300 mg/kg extract; Groups B, D, E and F) was assessed for each parameter using an orthogonal polynomial linear contrast (coefficients -3, -1, +1, +3 for equally spaced doses), evaluated against the pooled within-group error term from the one-way ANOVA ($df = 24$).

RESULTS

Body-weight changes

Body weights of animals in all groups showed progressive changes over the six-day experimental period (Table 1). No mortality was recorded in any group. The short duration of the study limited pronounced weight differences among treatment groups.

Table 1. Body weights (g) of rats before and during the experimental period

Group	Day 1 (initial)	Day 3	Day 6
A (n=5)	158.6–177.2	151.6–174.8	155.2–176.2
B (n=5)	187.4–196.2	178.2–186.3	179.8–186.1
C (n=5)	174.2–186.3	170.4–179.9	172.8–184.3
D (n=5)	171.3–182.8	167.4–179.5	169.9–180.8
E (n=5)	166.4–177.2	166.9–178.0	170.7–180.7
F (n=5)	173.4–188.4	171.2–187.3	172.8–187.0

Values represent the range of individual animal weights (g) recorded in each group. Group A: liquid paraffin; Group B: 30% CCl₄; Group C: silymarin 100 mg/kg + CCl₄; Group D: extract 100 mg/kg + CCl₄; Groups E & F: extract 200 & 300 mg/kg + CCl₄, respectively. All groups $n = 5$.

GC-MS profile of the methanolic leaf extract

GC-MS analysis revealed 37 identifiable compounds accounting for 99.7% of the total peak area (Table 2). The major constituents ($\geq 2\%$ relative abundance) were linoleic acid (36.10%), palmitic acid (18.96%), vitamin E (7.15%), stearic acid (6.06%), caryophyllene (4.24%), γ -sitosterol (3.98%), squalene (3.75%), phytol (2.28%) and α -amyrin (2.12%) (Table 3). These compounds belong principally to the classes of fatty acids, isoprenoid chromanols, sterols, diterpenoids and triterpenes. The presence of vitamin E, phytol, squalene and unsaturated fatty acids is consistent with potential antioxidant capacity; however, GC-MS profiling alone does not establish that any individual compound mediates the observed biological effects.

Table 2. Complete bioactive profile of the methanolic leaf extract of *D. tripetala* identified by GC-MS

RT (min)	Bioactive constituent	% Area	MF	Class of compound
13.40	α -Copaene	0.99	C15H24	Sesquiterpenes
14.05	Caryophyllene	4.24	C15H24	Sesquiterpenes
14.52	Humulene	1.66	C15H24	Sesquiterpenes
15.26	Cadina-1(10),4-diene	1.13	C15H24	Sesquiterpenes
15.69	Lauric acid	0.18	C12H24O2	Fatty acid
16.15	Caryophyllene oxide	0.74	C15H24O	Oxygenated sesquiterpenoid
16.48	Humulene epoxide	0.17	C15H24O	Cyclic ethers
18.01	Myristic acid	0.37	C14H28O2	Fatty acid
18.32	Loliolide	0.17	C11H16O3	Monoterpenoid lactone
18.37	5-Methyltridecane	0.29	C14H30	Hydrocarbon
18.69	2,4,4-Trimethyl-2-but-2-enolide	0.27	C7H10O2	Ester
18.82	Neophytadiene	1.10	C20H38	Diterpene
18.88	2-Phytene, isomer 1	0.29	C20H38	Hydrocarbon
19.27	11-Hexadecen-1-ol, acetate, (Z)	0.24	C18H34O2	Ester
19.37	n-Cetyl alcohol	0.23	C16H34O	Fatty alcohol
19.94	Palmitoleic acid	0.82	C16H30O2	Fatty acid
20.29	Palmitic acid	18.96	C16H32O2	Fatty acid
20.44	Ethyl palmitate	0.41	C18H36O2	Ester
20.77	Nerolidol	0.46	C15H26O	Oxygenated sesquiterpene

21.11	Margaric acid	0.30	C17H34O2	Fatty acid
21.48	Methyl elaidate	0.21	C19H36O2	Ester
21.57	Phytol	2.28	C20H40	Diterpenoid
22.04	Linoleic acid	36.10	C18H32O2	Fatty acid
22.22	Stearic acid	6.06	C18H36O2	Fatty acid
23.26	Tricosane	0.16	C23H48	Hydrocarbon
23.83	Arachidic acid	0.27	C20H40O2	Fatty acid
24.92	Heneicosane	0.27	C21H44	Hydrocarbon
25.07	Glycerol β -palmitate	1.28	C19H38O4	Fatty acid ester
26.45	Heptacosane, 1-chloro	0.68	C27H55Cl	Hydrocarbon chloride
27.51	Squalene	3.75	C30H50	Triterpene
27.90	Nonacosane	0.58	C28H60	Hydrocarbon
29.22	γ -Tocopherol	0.49	C28H48O2	Phenol lipid
29.67	Nonacosene	0.58	C29H58	Hydrocarbon
30.02	Vitamin E	7.15	C29H50O2	Isoprenoid chromanol
31.50	Stigmasterol	0.72	C29H48O	Sterol
32.35	γ -Sitosterol	3.98	C29H50O	Sterol
33.70	α -Amyrin	2.12	C30H50O	Triterpenol
	Total	99.7		

RT = retention time; MF = molecular formula. Compounds identified by comparison with NIST 2014 library.

Table 3. Major constituents ($\geq 2\%$ relative abundance) of the methanolic leaf extract of *D. tripetala*

Compound	% Area	Class
Linoleic acid	36.10	Fatty acid
Palmitic acid	18.96	Fatty acid
Vitamin E	7.15	Isoprenoid chromanol

Stearic acid	6.06	Fatty acid
Caryophyllene	4.24	Sesquiterpene
γ -Sitosterol	3.98	Sterol
Squalene	3.75	Triterpene
Phytol	2.28	Diterpenoid
α -Amyrin	2.12	Triterpenol

These nine compounds together account for approximately 84.6% of the total identified peak area.

In vitro antioxidant activity

The methanolic extract displayed potent free-radical scavenging activity. IC₅₀ values were 4.12 μ g/mL (DPPH) and 5.00 μ g/mL (ABTS), compared with 3.33 μ g/mL and 3.87 μ g/mL, respectively, for ascorbic acid (Table 4).

Table 4. IC₅₀ (μ g/mL) values of the methanolic leaf extract of *D. tripetala* against DPPH and ABTS radicals

Sample	DPPH	ABTS
Extract	4.12	5.00
Ascorbic acid	3.33	3.87

Liver function indices

CCl₄ administration produced a marked elevation of serum ALT, AST, ALP and total bilirubin and a significant reduction in total protein relative to the normal control (Group A), consistent with acute hepatocellular injury and impaired synthetic function (Table 5). Concurrent treatment with the extract at 100, 200 and 300 mg/kg significantly attenuated the rises in ALT, AST, ALP and total bilirubin and improved total protein levels compared with the intoxicated control (Group B) ($p < 0.05$). The magnitude of protection was broadly comparable to that of silymarin (100 mg/kg). These changes meet conventional biochemical criteria for hepatoprotection (reduction of elevated transaminases and bilirubin together with restoration of protein synthesis). A formal linear trend test across the 0-300 mg/kg dose range (Table 7) confirmed a significant dose-dependent linear response for all five liver function indices ($p < 0.01$).

Table 5. Effect of methanolic leaf extract of *D. tripetala* on serum liver function indices in CCl₄-intoxicated rats

Group	ALT (U/L)	AST (U/L)	TP (g/dL)	ALP (U/L)	TB (mg/dL)
A	11.25 $\pm$ 2.65	11.75 $\pm$ 2.06	7.75 $\pm$ 0.95	63.50 $\pm$ 3.41	6.00 $\pm$ 0.81
B	38.25 $\pm$ 7.43 ^a	32.00 $\pm$ 4.08 ^a	1.75 $\pm$ 0.50 ^a	212.50 $\pm$ 41.95 ^a	11.25 $\pm$ 1.25 ^a
C	17.50 $\pm$ 3.22 ^b	14.75 $\pm$ 3.56 ^b	5.10 $\pm$ 0.57 ^b	124.50 $\pm$ 14.00 ^a	6.22 $\pm$ 0.95 ^b

D	12.50±3.44 ^b	14.75±3.03 ^b	5.75±1.74 ^b	97.25±16.15 ^{ab}	7.75±0.95 ^b
E	15.25±2.98 ^b	14.75±3.30 ^b	6.25±0.50 ^b	115.50±16.70 ^{ab}	7.00±0.81 ^b
F	17.00±1.41 ^b	16.75±3.20 ^b	6.50±0.57 ^b	105.50±18.69 ^{ab}	6.25±1.25 ^b

Values are mean ± SEM (n = 5). ^a significantly different from Group A; ^b significantly different from Group B (p < 0.05, one-way ANOVA + Bonferroni). ALT = alanine aminotransferase; AST = aspartate aminotransferase; TP = total protein; ALP = alkaline phosphatase; TB = total bilirubin.

Hepatic antioxidant status and lipid peroxidation

CCl₄ produced a significant increase in hepatic MDA and significant decreases in GSH, CAT and SOD relative to the normal control (Table 6), indicating oxidative stress and impaired antioxidant defence. Co-administration of the extract (100–300 mg/kg) significantly reduced MDA and restored GSH, CAT and SOD activities toward control levels (p < 0.05 versus Group B). The highest extract dose (300 mg/kg) tended to produce the most favourable antioxidant profile. A formal linear trend test (Table 7) confirmed a significant dose-dependent linear response for MDA, GSH and SOD (p < 0.05), but not for CAT (p = 0.094), consistent with the non-monotonic pattern observed for this marker across the three extract doses. Effects of the extract were comparable to those of silymarin.

Table 6. Effect of methanolic leaf extract of *D. tripetala* on hepatic MDA, GSH, CAT and SOD in CCl₄-intoxicated rats

Group	MDA (liver)	GSH (liver)	CAT (liver)	SOD (liver)
A	2.33±0.57	11.00±1.00	8.00±2.00	8.00±2.30
B	9.33±0.57 ^a	1.33±0.57 ^a	2.00±0.57 ^a	1.33±2.30 ^a
C	4.66±0.51 ^b	6.00±0.00 ^b	8.60±2.51 ^b	9.33±1.33 ^b
D	4.00±0.00 ^b	7.66±0.57 ^b	7.33±1.45 ^b	10.66±4.80 ^b
E	4.33±1.15 ^b	8.00±1.00 ^b	5.33±0.33 ^b	12.00±4.61 ^b
F	3.33±0.57 ^b	9.00±0.00 ^b	6.66±1.15 ^b	13.33±3.52 ^b

Values are mean ± SEM (n = 5). MDA expressed as nmoles/g tissue × 10⁻⁵; GSH as mg/100 mL; CAT as U/mg tissue; SOD as U/g Hb. ^a vs Group A; ^b vs Group B (p < 0.05, one-way ANOVA + Bonferroni).

Table 7. Linear dose-response trend analysis across CCl₄-exposed groups (0, 100, 200 and 300 mg/kg extract)

Parameter	Linear contrast t(24)	p-value	Trend
ALT (U/L)	-3.42	0.0022	Significant
AST (U/L)	-3.14	0.0045	Significant
TP (g/dL)	3.58	0.0015	Significant

ALP (U/L)	-3.10	0.0049	Significant
TB (mg/dL)	-3.45	0.0021	Significant
MDA	-6.05	0.0001	Significant
GSH	7.86	0.0001	Significant
CAT	1.74	0.0940	Not significant
SOD	2.46	0.0214	Significant

Linear trend assessed by orthogonal polynomial contrast (coefficients -3, -1, +1, +3) across Groups B, D, E and F, using the pooled within-group error term from the one-way ANOVA (df = 24). Values are derived from the group means and SEM reported in Tables 5 and 6.

DISCUSSION

The methanolic leaf extract of *D. tripetala* exhibited strong in vitro free-radical scavenging activity against both DPPH and ABTS radicals, with IC₅₀ values only modestly higher than those of ascorbic acid. Such activity is consistent with the presence of antioxidant-capable constituents identified by GC-MS, particularly the major compounds linoleic acid, palmitic acid, vitamin E, stearic acid, caryophyllene, γ -sitosterol, squalene, phytol and α -amyirin (Tables 2 and 3). It must be emphasised, however, that GC-MS profiling demonstrates the presence of candidate phytochemicals; it does not by itself prove that any single compound or class mediates the biological effects observed in vivo.

Acute oral toxicity testing established an LD₅₀ greater than 3000 mg/kg, indicating a wide margin of safety under the conditions of the OECD limit test. Body-weight changes over the short experimental period were modest and did not differ markedly among groups.

CCl₄-induced hepatotoxicity is a well-characterised model of oxidative liver injury. Elevated serum ALT, AST and ALP, increased total bilirubin and decreased total protein constitute classical biochemical markers of hepatocellular necrosis, cholestasis and impaired hepatic synthetic function. In the present study these markers were significantly altered by CCl₄ and were significantly improved by concurrent administration of the extract at all three doses tested. The pattern of change meets established criteria for biochemical evidence of hepatoprotection.

Restoration of hepatic GSH, CAT and SOD together with reduction of MDA further supports an antioxidant-based mechanism of protection. The extract appears to counteract CCl₄-driven lipid peroxidation and to support endogenous antioxidant defence systems. While the 300 mg/kg dose frequently produced the most favourable numerical values, a clear monotonic dose–response relationship was not uniformly evident across every parameter; larger sample sizes and additional intermediate doses would strengthen dose–response characterisation.

Taken together, the data provide preclinical evidence that the methanolic leaf extract of *D. tripetala* possesses hepatoprotective activity against CCl₄-induced oxidative injury in rats. These findings justify further mechanistic studies (including isolation of active constituents and evaluation of Nrf2/ARE or cytochrome-P450 pathways) but do not, by themselves, justify clinical use in humans.

CONCLUSION

The methanolic leaf extract of *Dennettia tripetala* demonstrates significant in vitro antioxidant activity and attenuates CCl₄-induced elevations in serum liver enzymes and bilirubin while restoring total protein and

hepatic antioxidant markers in Wistar rats. GC-MS analysis identified 37 compounds (Table 2), of which nine major constituents ($\geq 2\%$) accounted for approximately 84.6% of the total peak area (Table 3). The extract exhibited low acute oral toxicity ($LD_{50} > 3000$ mg/kg). These results constitute preclinical evidence of potential hepatoprotective activity and support continued investigation; they do not constitute a recommendation for therapeutic use of *D. tripetala* products in the management of human liver disease.

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Conflict of interest

The authors declare no conflict of interest.

Ethical Statement

All animal procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals at Prince Abubakar Audu University. Acute toxicity testing followed OECD Guideline 425.

REFERENCES

1. Abo-elhamd, A.M., Abul-Enein, A.M., Mohammed, S.M., Shalaby, A.S. & Konsowa, A. (2016). Chemical characterization, antioxidant and antihepatotoxicity of *Calliandra haematocephala* growing in Egypt. *Journal of Chemical and Pharmaceutical Research*, 8(4), 828–845.
2. Adeoti, S.B., Ayedoun, M.A. & Leclercq, P.A. (2000). Essential oil of *Dennettia tripetala* leaves from Benin. *Journal of Essential Oil Research*, 12(4), 412–414.
3. AlSheikh, H.M.A., Sultan, I., Kumar, V., Rather, I.A., Al-Sheikh, H., Tasleem Jan, A. & Haq, Q.M.R. (2020). Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics*, 9(8), 480.
4. Anaga, A.O. & Asuzu, I.U. (2011). Glucose uptake-enhancing activity of the ethyl acetate extract of *Dennettia tripetala* in 3T3-L1 adipocytes. *Journal of Complementary and Integrative Medicine*, 8(1).
5. Ayeni, G., Ejoba, R., Olajide, J.E. & O.S. (2016). Hepatoprotective activity of silymarin in carbon tetrachloride (CCl_4)-intoxicated rats. *International Journal of Innovation Research*, 4(5), 83–86.
6. Bag, A. & Mumtaz, S.M. (2013). Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylinia* leaves. *Bangladesh Journal of Pharmacology*, 8(3), 263–268.
7. Beers, R.F. & Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195, 133–140.
8. Brown, M.B. & Forsythe, A.B. (1974). Robust tests for the equality of variances. *Journal of the American Statistical Association*, 69(346), 364–367.
9. Burkill, H.M. (1995). *The useful plants of West Tropical Africa*, Vol. 3. Royal Botanic Gardens, Kew.
10. Doughari, J.H. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents*. INTECH Open Access Publisher.
11. Ejechi, B.O. & Akpomede, D.E. (2005). Activity of essential oil and phenolic acid extracts of pepperfruit (*Dennettia tripetala*) against some food-borne microorganisms. *African Journal of Biotechnology*, 4(2), 258–261.
12. Gill, L.S. (1992). *Ethnomedical uses of plants in Nigeria*. Uniben Press, Benin City.
13. Gornall, A.G., Bardawill, C.S. & David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 177(2), 751–766.
14. Guntupalli, M.M.R., Chandana, V.R., Palpu, P. & Annie, S. (2006). Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. *Journal of Ethnopharmacology*, 103, 484–490.

15. Hartman, P.E. (1983). Putative mutagens and carcinogens in foods. IV. Malondialdehyde. *Environmental Mutagenesis*, 5(4), 603–607.
16. Hassan, S.W., Ayeni, G., Wasagu, R.S.U., Umar, R.A., Agaie, B.M. & Farouk, U.Z. (2015). Hepatoprotective effect of leaf extracts of *Newbouldia laevis* on carbon tetrachloride-intoxicated rats. *Journal of Global Biosciences*, 4(5), 2258–2281.
17. Jendrassik, L. & Gróf, P. (1938). Simplified photometric methods for the determination of bilirubin. *Biochemische Zeitschrift*, 297, 81–89.
18. Kakkar, P., Das, B. & Viswanathan, P.N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*, 21, 130–132.
19. Lee, I.C., Kim, S.H., Baek, H.S., et al. (2014). The involvement of Nrf2 in the protective effects of diallyl disulfide on carbon tetrachloride-induced hepatic oxidative damage and inflammatory response in rats. *Food and Chemical Toxicology*, 63, 174–185.
20. Leitzmann, C. (2016). Characteristics and health benefits of phytochemicals. *Complementary Medicine Research*, 23(2), 69–74.
21. OECD (2001). Guideline for the testing of chemicals. Acute oral toxicity – Up-and-Down Procedure. No. 425.
22. Ofem, O.E., et al. (2014). Methanolic extract of *Dennettia tripetala* leaf normalises abnormal bile secretion in rats during toxic challenge.
23. Patterson, J.W. & Lazarow, A. (1955). Determination of glutathione. In: Glick, D. (ed.) *Methods of Biochemical Analysis*, Vol. 2. Interscience, New York, pp. 259–278.
24. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237.
25. Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28, 56–63.
26. Shapiro, S.S. & Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3–4), 591–611.
27. Simelane, M.B.C., Lawal, O.A., Djarova, T.G. & Opoku, A.R. (2010). In vitro antioxidant and cytotoxic activity of *Gunnera perpensa* L. from South Africa. *Journal of Medicinal Plants Research*, 4(21), 2181–2188.
28. Sood, R. (1999). *Medical Laboratory Technology: Methods and Interpretations*, 5th ed. Jaypee Brothers Medical Publishers.
29. Timothy, C.O. & Okere, C.O. (2008). Effect of *Dennettia tripetala* (Mmimi) seed intake on the IOP of normotensive emmetropic Nigerian Igbos. *Journal of the Nigerian Optometric Association*, 14(1), 14–17.
30. Wang, M.Y., West, B.J., Jensen, C.J., et al. (2002). *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, 23(12), 1127–1141.
31. Weber, L.W., Boll, M. & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology*, 33(2), 105–136.
32. Yang, J., Li, Y., Wang, F. & Wu, C. (2010). Hepatoprotective effects of apple polyphenols on CCl₄-induced acute liver damage in mice. *Journal of Agricultural and Food Chemistry*, 58(10), 6525–6531.
33. Yi, J., Xia, W., Wu, J., et al. (2014). Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. *Journal of Veterinary Science*, 15(1), 141–148.
34. Zamzami, M.A., Baothman, O.A.S., Samy, F. & Abo-Golayel, M.K. (2019). Amelioration of CCl₄-induced hepatotoxicity in rabbits by *Lepidium sativum* seeds. *Evidence-Based Complementary and Alternative Medicine*, 2019, 5947234.