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Therapeutic potential of combined *Gongronema latifolium* fruit and leaf extracts against oxidative stress and organ damage

CHIAMAKA VIVIAN OKOCHI[1,♥], S. C. UDEDI[1], K. K. ASOGWA[1], E. J. OTOVBO[2], M. S. OBI[1], U. A. EZEAKU[1]

[1]Department of Applied Biochemistry, Nnamdi Azikiwe University. Along Enugu-Onitsha Expressway, Ifite Road, Awka 420110, Anambra, Nigeria. Tel.: +234-8132743397, ♥email: Chiamakaokochi808@gmail.com

[2]Department of Biochemistry, Dennis Osadebay University. Along Asaba-Benin Expressway, Anwai Road, Asaba 320242, Delta State, Nigeria

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Abstract. *Okochi CV, Udedi SC, Asogwa KK, Otovbo EJ, Obi MS, Ezeaku UA. 2025. Therapeutic potential of combined* Gongronema latifolium *fruit and leaf extracts against oxidative stress and organ damage. Asian J Nat Prod Biochem 23: 38-46.* This study aims to evaluate the therapeutic potential of combined extracts of the fruit and leaves of *Gongronema latifolium* in managing oxidative stress and protecting against liver and kidney damage. Forty albino rats were randomly assigned to eight groups (A-H), each consisting of 5 rats. Groups A-C served as the control groups, with Group B as the positive control group, receiving silymarin. Groups D-H were administered different ratios of *G. latifolium* fruit and leaf extracts at 100 mg/kg BW. The biochemical analysis and histological study of the liver were conducted using standard procedures. Results from the acute toxicity study showed that the LD_{50} value of the extract was >5000 mg/kg BW, categorized as relatively harmless; the combined extracts significantly increased antioxidant enzyme activities (CAT, GSH, SOD) and reduced malondialdehyde (MDA) levels. Most combined extracts demonstrated anti-hepatotoxic effects by lowering ALT, AST, and ALP levels. The combined extracts of fruit and leaf of *G. latifolium* demonstrated potential for renal protection, as evidenced by reduced urea and creatinine levels, particularly in groups receiving a higher ratio of fruit extract. Histological analysis showed normal liver tissue, indicating no adverse effects from the extracts. In conclusion, these combinations of fruit and leaf extracts of *G. latifolium* could be natural antioxidants and protective agents against environmental toxins, with possible applications in traditional medicine and therapeutic strategies.

Keywords: Anti-hepatotoxicity, anti-nephrotoxicity, antioxidant, drug discovery, *Gongronema latifolium*, oxidative stress

INTRODUCTION

WHO (2023) stated that approximately 80% of the world's population uses traditional medicine for primary healthcare. It supports research showing that healthcare globally has historically relied heavily on traditional medical systems for their potential therapeutic and medicinal benefits (Aladejana 2023). In recent years, there has been a growing global interest in the toxicity associated with environmental pollutants, the consumption of highly processed food, and counterfeit drinks, which can accumulate in the body for a long time, leading to oxidative stress, inflammation, and organ damage (Sokan-Adeaga et al. 2023). There is a need to investigate alternative therapies to combat this detrimental process by exploring natural plant products. Oxidative stress has been recognized as a deleterious process associated with various health issues, including cell damage, autoimmune disorders, aging, cancer, cardiovascular disease, and degenerative diseases (Asogwa et al. 2020).

Naphthalene, a white, crystalline Polycyclic Aromatic Hydrocarbon (PAH) with the formula $C_{10}H_8$, comprising two fused benzene rings, exhibits high melting and boiling points. Its aromatic character makes it susceptible to various reactions, including electrophilic aromatic substitution, oxidation, and reduction, leading to valuable derivatives (Giri and Moon 2024), such as 1-methylnaphthalene (1-MN) and 2-methylnaphthalene (2-MN), which are the most abundant airborne PAHs (Chen et al. 2016; Fang et al. 2021), primarily emitted from biomass burning, combustion of fossil fuels, and industrial sectors (Fang et al. 2021). A study by Ye et al. (2024) revealed the significance of naphthalene and its derivatives contributing to the formation of Secondary Organic Aerosol (SOA) and other secondary pollutants in the Yangtze River Delta (YRD) region during summer, which may pose environmental risks and result in adverse health effects. On the other hand, silymarin is a natural compound derived from *Silybum marianum* (L.) Gaertn. (milk thistle) that has been well-researched for liver diseases (Abenavoli and Milic 2017). It's an isomeric combination of flavonolignans, exhibiting inherent hepatoprotective and antioxidant activity by controlling free radicals produced during the hepatic metabolism of toxic chemicals like ethanol, acetaminophen, or carbon tetrachloride (Qadir and Sahoo 2021).

Gongronema latifolium NIF 2345 is a plant that belongs to the family Asclepiadaceae with significant nutritional and medicinal properties, particularly in managing diabetes and oxidative stress, as shown in recent studies on its leaf extract's antidiabetic and antioxidant effects (Ojo et al. 2020). It is also a tropical rainforest plant whose leaves have a characteristic sharp, bitter, and slightly sweet taste, especially when eaten fresh (Omodale et al. 2017). In comparison, its fruit has smooth, greenish skin

when unripe, which then turns brown and black at maturity (Okochi et al. 2024). Their common names in Nigeria are bushbuck in English, *utazi* in Igbo, *madumaro* or *arokeke* in Yoruba, and *utasi* among the Ibibios and Efik (Elijah et al. 2022). Different parts of *G. latifolium* NIF 2345, including leaves, fruit, root, and stem, contain distinct chemical substances that can exert specific physiological effects, making them valuable sources of therapeutic agents (Parsaeimehr et al. 2017). It is used for culinary practice, such as vegetables in soups and salads, or spices in different food preparations (Amrelia et al. 2022), and polyherbal decoction for hepatitis and malaria in traditional medicines because it helps in cleansing the liver (Ihesie 2022), alleviating symptoms of catarrh, congestion, and cough (Juliani et al. 2013), treating hyperglycemia (Ogunyemi et al. 2020), stomach ache, laxative (Osuagwu et al. 2023), and fruit preservative (Ejembi et al. 2022). Previous research on *G. latifolium* NIF 2345 fruit and leaves exhibits good nutritive value, anti-helminthic activity, and anti-malarial activity (Orumwensodia and Uadia 2022; Ojo et al. 2023), anti-bacterial, anti-fungal, and anti-oxidative properties (Onwukeme et al. 2023; Bassey et al. 2020).

Limited research has been conducted on the specific impact of *G. latifolium* NIF 2345 leaf and fruit extracts, or their combination, on naphthalene-induced oxidative stress in the liver and kidneys. This study aims to investigate the natural antioxidant, anti-nephrotoxicity, and hepatoprotective properties of combined *G. latifolium* NIF 2345 leaf and fruit extracts in mitigating naphthalene-induced oxidative stress. The present study investigates the effects of combined *G. latifolium* NIF 2345 fruit and leaf extracts on naphthalene-induced oxidative stress in rats at 100 mg/kg. This study was conducted to develop innovative therapeutic approaches for oxidative stress-related health issues, provide scientific validation for the traditional use of *G. latifolium* NIF 2345, and promote the inclusion of *G. latifolium* fruit in medicinal and dietary applications.

MATERIALS AND METHODS

Materials

Fruit and leaf samples of *G. latifolium* NIF 2345 were collected from Okochi's compound in Umudunu Village, Abagana, Njikoka L.G.A., Anambra State, Nigeria. The plant samples were sent to the Department of Botany Herbarium at Nnamdi Azikiwe University, Awka, for identification and authentication by Mr. Iroka Chisom, a department taxonomist. A voucher specimen was deposited with voucher numbers NAUH-34D and NAUH-34A.

Study area

This study was conducted at the Natural Products Laboratory, Unizik Awka, Animal House Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, and Ifex's Diagnostics laboratory, Regina Caeli Road, Awka.

Duration

The study was completed in 70 days, equivalent to 10 weeks, including 14 days for air-drying, 5 days for pulverization and extraction, 7 days for acclimatization, 30 days for animal studies, and 14 days for in vivo assays, data collection, and data analysis.

Procedures

One kilogram (1 kg) of the pulverized *G. latifolium* NIF 2345 leaves and fruits was soaked separately in 10 L of deionized water and left for 24 hours. After 24 hours, the sample was filtered and concentrated at 60°C using a water bath (Memmert WTB). The crude extracts were transferred into a sample bottle, stored at 4°C in the refrigerator, and used for further laboratory test analysis.

$$\text{Percentage of extract yield} = \frac{\text{Weight of extract}}{\text{Weight of dried homogenized sample}} \times 100\%$$

(Barros et al. 2008)

Animal studies

Ethical approval for all experimental protocols was obtained from the Animal Research Ethics Committee (AREC), Nnamdi Azikiwe University, Awka, with the NAU/AREC/2024/0081 reference number. This research study followed the guidelines for laboratory animal use and care provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

Purchase, acclimatization, and feeding of animals

The experiment followed a completely randomized design. Forty (40) albino rats were purchased from Onyewuchi Farm, Ifite, Awka. These animals were acclimatized for one week and fed only food and water.

Determination of LD_{50} of extract

The acute toxicity study used Lorke's method (Lorke 1983). In the first phase, six rats were randomly divided into three groups (n = 1 rat/group). The groups received oral doses of 10, 100, and 1,000 mg/kg BW of the extract via a cannula. The rats were monitored for 24 hours for adverse effects and mortality. In the second phase, the procedure was repeated using six rats randomly divided into three groups (n = 1 rat/group). The groups received oral doses of 1,600, 2,900, and 5,000 mg/kg BW of the extract. The rats were observed for signs of mortality and toxicity effects.

Induction of oxidative stress

Oxidative stress was induced using the method of Vijayavel et al. (2007) by administering 1,100 mg/kg of naphthalene to the experimental animals and monitoring the results within 24 hours after naphthalene administration. The level of oxidative stress was determined by analyzing the concentration of malondialdehyde in the serum.

Body weight of experimental animal

The weight of the experimental subjects was measured using an electronic weighing scale. The body weight was monitored before, during, and after the experiment.

Grouping of the animal

Forty (40) albino rats were divided into eight (8) groups of five (5) animals in each group. They were grouped as

follows: Group A-normal control; Group B-positive control (induced and treated with silymarin); Group C-negative control (induced but not treated); while Groups D, E, F, G, and H were induced with naphthalene and treated with fruit and leaf extract at different ratios of 100 mg/kg; where Group D-(20 leaf + 80 fruit); Group E-(40 leaf + 60 fruit); Group F-(50 leaf + 50 fruit); Group H-(80 leaf + 20 fruit), The study was carried out for 28 days.

Blood collection

At the end of the experiment, the animals were anesthetized with chloroform. The blood was collected in a plain bottle via cardiac puncture and then centrifuged for 15 minutes at 4,000 rpm. The serum was collected and used for further analyses.

In vivo antioxidant activity

The effect of the combined fruit and leaf extract ratio was analyzed for antioxidant enzymes. Malondialdehyde was determined using the spectrophotometric method at 532 nm following the procedure of Buege and Aust (1978); reduced glutathione level was determined at 412 nm according to the method of Exner et al. (2000). Superoxide dismutase activity was determined by the increase in absorbance at 480 nm described by the method of Sun and Zigma (1978). Catalase activity was measured by the increase in absorbance at 620 nm, as described by Sinha's method (Sinha 1972).

Kidney function test

Urea and creatinine were analyzed using Randox test kits. The procedures followed the manufacturer's instructions.

Liver function test

Serum biochemical indices routinely estimated for liver functions, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total protein, were determined using Randox diagnostic kits. The procedures followed the manufacturer's instructions.

Histological analysis

Histopathological evaluations of the liver were carried out after the completion of the 4-week experimental procedure. The experimental animal was sacrificed by diethyl ether inhalation, and the abdominal cavity was exposed. Thereafter, liver tissues were processed for light histological microscopy, and conventional histopathological evaluations of the liver were conducted using the hematoxylin-eosin technique, as previously described by Akinlolu et al. (2017).

Data analysis

The data were expressed as mean ± Standard Error of the Mean (SEM) and subjected to One-Way Analysis of Variance analysis (ANOVA). Values were considered statistically significant at ($p < 0.05$) using Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Percentage yield of extract

The percentage yields of the crude extract of *G. latifolium* NIF 2345 fruit and leaf were 15.3 and 15.0%, respectively.

Acute toxicity study via oral administration

The results of the acute toxicity study revealed no observable signs of toxicity at doses ranging from low to high (10-5,000 mg/kg) within the first 24 hours of exposure, as shown in Table 1.

Result on mean-weight parameters

Body weights of the rats were recorded on day 0 (before the acclimatization period), day 7 (before the induction of naphthalene), day 9 (24 hours after naphthalene administration), day 18 (after 9 days of treatment), and day 28 (after 18 days of treatment) (Table 2). The induction of naphthalene showed a significant difference in the body weight of the rats between the negative control, positive, and normal control groups. The body weight was increased significantly ($p < 0.05$) in the normal group (A) and positive control (B) on days 18 and 28 compared to days 0 and 7. The body weight in group D (20 leaf: 80 fruit mg/kg) and F (50 leaf: 50 fruit mg/kg) was significantly higher than that of the normal and negative control groups at day 28. The body weight of other treatment groups exhibited no significant difference compared to normal and positive control groups.

In vivo antioxidant enzyme activity

MDA, CAT, SOD, and reduced GSH activity of the experimental animals were assayed to determine the effect of the administration of a combination extract of *G. latifolium* NIF 2345 on these antioxidant enzymes. The results reveal no significant difference ($P > 0.05$) among the treated groups (D to H) when compared to the control groups (A-C), as seen in Table 3 and Figure 1.

Table 1. Signs of toxicity and mortality rate of rats treated with aqueous extract of *Gongronema latifolium* NIF 2345 fruit and leaf

Extract dose	*G. latifolium* leaf		*G. latifolium* fruit	
(mg/kg)	Observation	Mortality	Observation	Mortality
10	Normal	0/1	Normal	0/1
100	Normal	0/1	Normal	0/1
1,000	Normal	0/1	Normal	0/1
1,600	Normal	0/1	Normal	0/1
2,900	Normal	0/1	Normal	0/1
5,000	Slightly weak	0/1	Normal	0/1

Table 2. The effect of treatment on the body weight (g) of experimental rats

Groups (leaf:fruit)	Body weight (mg)				
	0 day	7 days	9 days	18 days	28 days
A (normal control)	113.50±2.90	116.50±2.90	117.75±3.09	119.75±5.38	122.25±3.09
B (positive control)	108.00±3.93	110.00±3.93	106.75±4.11	112.50±3.30	117.00±3.10
C (negative control)	108.00±5.35	110.25±5.23	105.25±5.12	102.5±4.94	98.25±4.87
D (20:80)	114.5±2.96^{bdf}	117.25±2.76^{bdf}	114.75±2.85^{bde}	119.75±2.55^{bde}	125.25±2.40^{ace}
E (40:60)	113.00±3.58^{bdf}	116.25±3.56^{bdf}	114.25±3.32^{bde}	116.25±3.32^{bde}	122.25±1.93^{bde}
F (50:50)	115.25±1.79^{bdf}	118.25±1.79^{bdf}	115.25±1.79^{bde}	120.25±1.79^{bde}	124.00±2.16^{ace}
G (60:40)	113.75±5.32^{bdf}	116.50±5.37^{bdf}	111.28±6.65^{bde}	114.00±6.72^{bde}	113.75±5.32^{bde}
H (80:20)	116.50±4.51^{bdf}	118.75±4.41^{bdf}	110.50±4.41^{bde}	114.00±4.60^{bde}	116.50±4.51^{bde}

Note: Each value represents the Mean ±SEM of five rats per group. [a]Significant difference compared to normal control, [b]No significant difference compared to normal control, [c]Significant difference compared to positive control, [d]No Significant difference compared to positive control, [e] Significant difference when compared to negative control, [f]No Significant difference when compared to negative control

Table 3. In vivo antioxidant enzyme activity of rats treated with various combinations of leaf and fruit extract of *Gongronema latifolium* NIF 2345 extracts

Groups (leaf:fruit)	MDA±SEM (µmol/L)	GSH±SEM (IU/L)	SOD±SEM (IU/L)	CAT±SEM (IU/L)
A (normal control)	6.25±0.59	9.29±1.38	7.35±0.32	1.90±0.34
B (positive control (silymarin)	6.38±0.28	9.56±0.63	7.55±0.33	1.92±0.46
C (negative control)	7.40±0.03	5.81±0.79	5.66±0.23	1.36±0.29
D (20:80 mg/kg)	7.26±0.14^{bdf}	8.97±0.69^{bdf}	6.18±1.37^{bdf}	1.91±0.68^{bdf}
E (40:60 mg/kg)	6.36±0.19^{bdf}	7.85±1.32^{bdf}	8.29±0.00^{bdf}	2.36±1.11^{bdf}
F (50:50 mg/kg)	6.62±0.56^{bdf}	6.25±0.1^{bdf}	7.92±0.37^{bdf}	2.22±0.69^{bdf}
G (60:40 mg/kg)	6.87±0.36^{bdf}	8.56±2.14^{bdf}	6.86±0.13^{bdf}	2.21±0.51^{bdf}
H (80:20 mg/kg)	6.68±0.29^{bdf}	6.78±0.76^{bdf}	6.89±0.50^{bdf}	2.08±0.65^{bdf}

Note: Each value represents the Mean ±SEM of five rats per group. [b]No significant difference when compared to normal control, [d]No significant difference when compared to positive control, and [f]No significant difference when compared to negative control

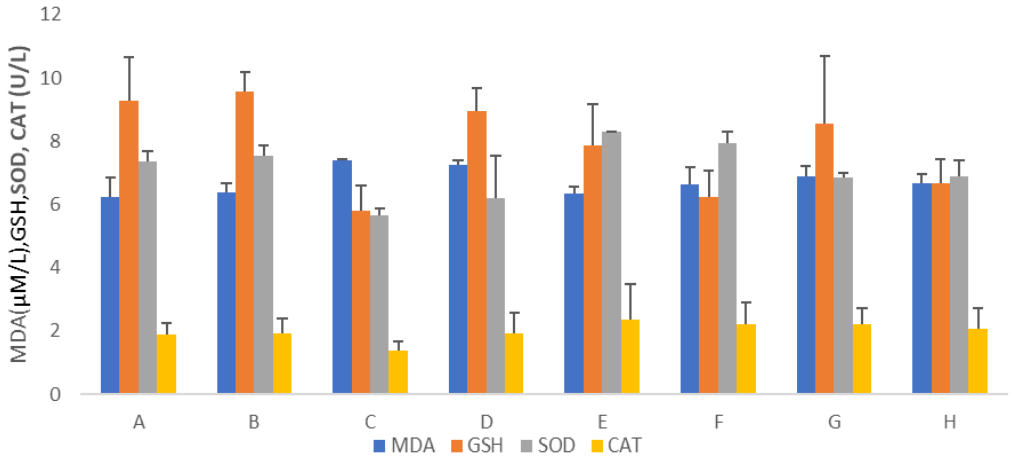

Figure 1. Effect of combined *Gongronema latifolium* fruit and leaf NIF 2345 extract on in vivo antioxidant enzyme activity

Liver analysis results

The induction of naphthalene results in elevated liver function parameters (ALP, AST, ALB, TP, and ALT). The results showed that there was no significant increase ($p < 0.05$) in the levels of AST and ALT among all groups (D to H) treated with *G. latifolium* extracts compared to the normal and positive controls. However, they indicated a significant reduction compared to the negative control. There were no notable changes in ALP, TP, and ALB levels among the groups receiving combined *G. latifolium* fruit and leaf extract compared to the normal, positive, and negative controls ($P > 0.05$). It suggests that the combination of *G. latifolium* fruit NIF 2345 and leaf extract does not significantly impact ALP, TP, and ALB levels compared to the controls. Therefore, these specific treatments may not significantly alter the liver function parameters, as presented in Table 4 and Figure 2.

Table 4. Effects of the treatment of combined *Gongronema latifolium* NIF 2345 fruit and leaf extract on the liver function parameters

Groups (leaf:fruit)	ALT±SEM (U)	AST±SEM (U/L)	ALP±SEM (U/L)	TP±SEM (g/dl)	ALB±SEM (g/dl)
A (normal control)	57.60±4.14	30.49±5.89	143.05±3.61	7.50±1.01	5.08±0.54
B (positive control (silymarin)	57.82±0.59	19.90±2.29	107.16±16.17	7.82±0.75	5.04±0.49
C (negative control)	77.52±7.19	51.12±3.34	190.39±25.36	7.39±0.72	5.25±0.42
D (20:80 mg/kg)	50.61±6.37^{bde}	18.86±2.89^{bde}	98.74±19.53^{bdf}	8.08±1.00^{bdf}	4.80±0.60^{bdf}
E (40:60 mg/kg)	40.19±4.95^{bde}	27.72±4.54^{bde}	160.73±24.05^{bdf}	8.23±0.93^{bdf}	4.75±0.55^{bdf}
F (50:50 mg/kg)	35.93±1.26^{ace}	27.82±1.40^{bde}	121.32±6.80^{bdf}	9.84±1.50^{bdf}	5.41±0.85^{bdf}
G (60:40 mg/kg)	56.68±1.31^{bde}	20.42±6.04^{bde}	130.69±9.32^{bdf}	6.91±1.37^{bdf}	4.03±0.50^{bdf}
H (80:20 mg/kg)	53.65±1.14^{bde}	17.65±2.34^{bde}	136.16±23.00^{bdf}	7.49±0.43^{bdf}	5.01±0.50^{bdf}

Note: Each value represents the Mean ± SEM of four rats per group. [b]No significant difference compared to normal control, [d]No significant difference compared to positive control, [e]Significant difference compared to negative control, [f]No significant difference compared to negative control

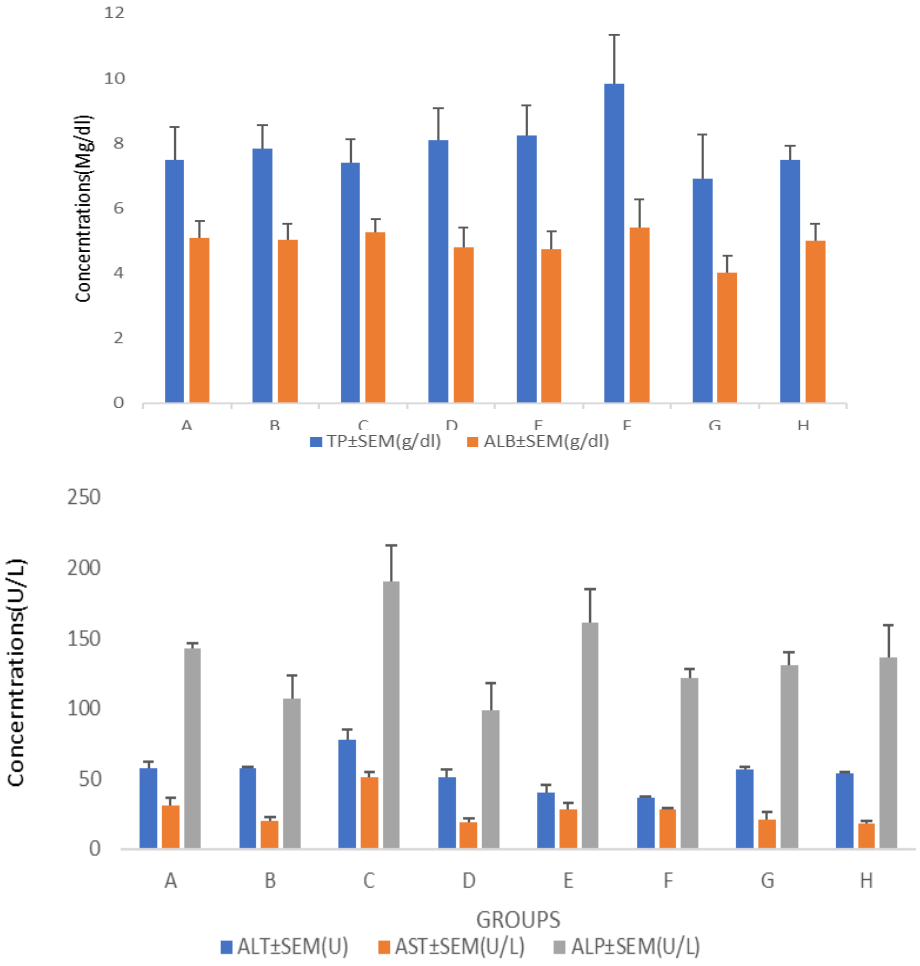

Figure 2. Liver parameters of the group treated with combined *Gongronema latifolium* NIF 2345 extract at 100 mg/kg

The effect of administration of *Gongronema latifolium* leaf and fruit extract on the parameter of kidney function

The effects of *G. latifolium* leaf and fruit extracts on kidney function showed that all groups treated with *G. latifolia* showed significant reductions in urea and creatinine levels ($p < 0.05$) compared to the negative control group. The treated groups exhibited kidney function parameters comparable to the positive control group (silymarin). These findings suggest that the leaf and fruit extracts may possess kidney protective properties, as shown in Figure 3 and Table 5.

Results of histology studies of the liver

The following photomicrographs were taken from the various treatment groups to present the effect(s) of *G. latifolium* NIF 2345 on the liver.

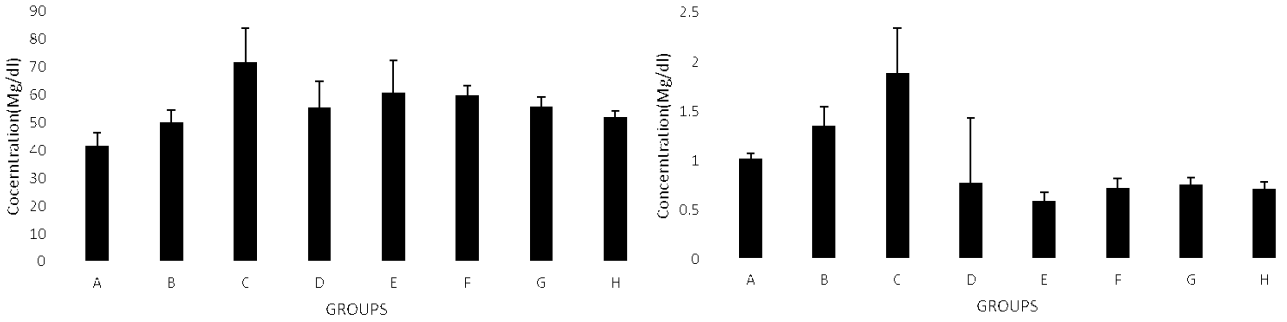

Figure 3. Effect of combined *Gongronema latifolium* fruit and leaf aqueous extract on kidney function parameters

Table 5. Effect of the treatment of combined *Gongronema latifolium* NIF 2345 fruit and leaf on kidney function

Groups (leaf:fruit)	Urea ± SEM (mg/dL)	Creatinine ± SEM (mg/dL)
A (normal control)	41.74±4.63	1.02±0.05
B (positive control (silymarin)	50.26±4.34	1.35±0.19
C (negative control)	71.63±12.46	1.89±0.45
D (20:80 mg/kg)	55.35±9.40^{bdf}	0.77±0.66^{bde}
E (40:60 mg/kg)	60.83±11.65^{bdf}	0.59±0.09^{bde}
F (50:50 mg/kg)	59.74±3.72^{bdf}	0.72±0.10^{bde}
G (60:40 mg/kg)	55.9±3.32^{bdf}	0.76±0.07^{bde}
H (80:20 mg/kg)	51.90±2.28^{bdf}	0.71±0.07^{bde}

Note: Each value represents the Mean ± SEM of four rats per group. [a]Significant difference compared to normal control, [b]No significant difference compared to normal control, [c]Significant difference compared to positive control, [d]No significant difference compared to positive control, [e]Significant difference when compared to negative control, [f]No significant difference when compared to negative control

Discussion

Medicinal plants have been used for centuries to prevent and treat diseases, with much of the world's population still relying on traditional or herbal medicine across cultures and continents (Fitzgerald et al. 2020). Phytochemicals are bioactive components in traditional herbal medicines and are formulated in herbal formulations (Tanaka and Kashiwada 2022). An environmental toxicant, naphthalene, has been reported to induce oxidative stress through inhalation, ingestion, and dermal contact, leading to hemolytic anemia, liver damage, retinal damage, and neurological issues (Fang et al. 2021). On the other hand, silymarin is a synthetic drug known for its antioxidant and anti-hepatotoxicity properties (Abenavoli and Milic 2017).

Previous research on *G. latifolium* NIF 2345 shows that *G. latifolium* exhibits analgesic, antitumor, broad-spectrum antimicrobial, and anti-sickling (Damunupola et al. 2014) and may offer protection against hematological complications associated with diabetes and toxic environmental exposures (Effiong et al. 2024). The Lethal Dose (LD50) assessment in measuring acute toxicity and using fewer animals in acute toxicity studies are very important for ethical reasons (Chinedu et al. 2013). The findings showed that the median lethal dose of *G. latifolium* NIF 2345 fruit and leaf extract was ≥ 5,000 mg/kg, categorized as relatively harmless. Administration of aqueous extracts of combined *G. latifolium* NIF 2345 reduced the weight-altering effects of naphthalene (1,100 mg/kg) in rats without significant changes in body and organ weights compared to positive and normal controls. This finding supports previous research on the nutritional value and potential health benefits of *G. latifolium* NIF 2345 (Nneoyi-Egbe et al. 2024).

Antioxidants are the compounds in charge of scavenging free radicals and protecting our bodies from a wide range of diseases associated with free radicals (Alam et al. 2020). The results of our study (Table 3; Figure 1) showed no significant reduction ($p < 0.05$) in the in vivo antioxidant enzyme of the rat groups treated with naphthalene and administered with the combined extract. It revealed that the combined extract exhibits good antioxidant potential. However, group E (40% leaf:60% fruit mg/kg) had lower MDA levels and higher GSH, SOD, and CAT levels compared to other experimental groups. It closely aligns with Analike et al. (2022) findings on the antioxidant potential of an aqueous extract of *G. latifolium* NIF 2345 leaves, which reduced heart malondialdehyde levels and increased antioxidant enzyme levels. The presence of phytochemicals in *G. latifolium* NIF 2345 fruit and leaf, such as phenols, saponins, and tannins, contributed to the antioxidant effects to prevent oxidative damage by scavenging reactive oxygen species (Okochi et al. 2024).

Numerous studies have demonstrated the hepatoprotective benefits of silymarin (Eze et al. 2021). The findings of this study showed that silymarin treatment reduced AST and ALP levels compared to the negative control group (group C) but not to the normal control group (group A). Group D (20% leaf:80% fruit mg/kg) showed a significant decrease in ALT levels compared to the normal and negative control groups. It might relate to the findings of Adeyemi-Doro et al. (2021) that *G. latifolium* decreased liver enzyme activities, indicating the hepatoprotective effect. Previous in vivo studies by (Al-Hindi et al. 2019; Omodamiro et al. 2021) showed that high doses and prolonged use of the alcoholic extract of *G. latifolium* leaf extract may lead to hepatotoxicity.

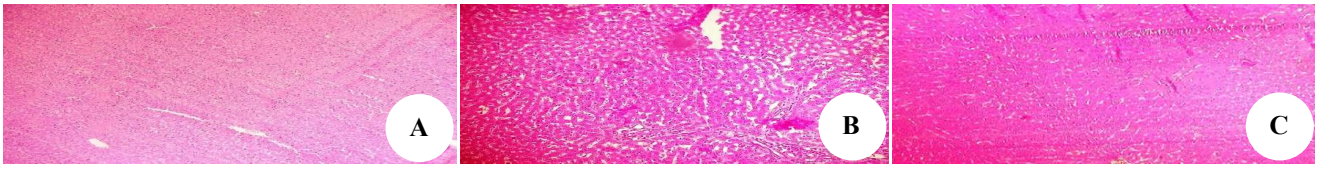

Figure 4. A. Plate 1: Photomicrograph of liver from group B (positive control), 10X (using Hematoxylin & Eosin staining e). It has normal hepatic tissue; B. Plate 2: Photomicrograph of liver from group C (negative control), 10X. It has normal hepatic tissue; C. Plate 3: Photomicrograph of liver from rat in group D (treated with 20% leaf extract and 80 % fruit extract, 10X) indicated normal liver tissue

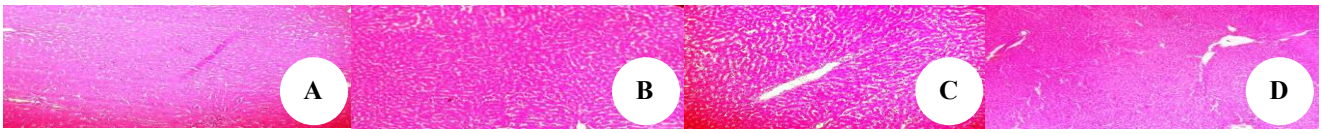

Figure 5. A. Plate 4: Photomicrograph of liver from the rat in group E (treated with 40% leaf extract and 60% fruit extract), 10X. It indicated normal liver tissue; B. Plate 5: Photomicrograph of liver from the rat in group F, 10X, treated with 50% leaf extract and 50% fruit extract, indicating normal liver tissue at 50 leaf:50 fruit extract of *Gongronema latifolium* NIF 2345; C. Plate 6: Photomicrograph of liver from rat in group G, 4X. It revealed normal liver tissue of rats treated with a 60:40 leaf:fruit ratio of 1 mg/kg extract; D. Plate 7: Photomicrograph of liver from the rat in group H (treated with 80% leaves: 20% fruit extract), 10X. It indicated normal hepatic tissue at 100mg/kg of leaf extract

The findings showed that groups D (20% leaf:80% fruit mg/kg), G (60% leaf:40% fruit mg/kg), and H (80% leaf:20% fruit mg/kg) show a decrease in serum creatinine and urea levels compared with the negative control, indicating that this plant may also possess hepato-renal protective function against naphthalene-induced damage (Ighodaro and Akinoye 2017; Ujong et al. 2022). Our findings align with recent studies (Okochi et al. 2024; Oyama and Chukwura 2024), suggesting that the protective effects of *G. latifolium* extract' on the liver and kidney are attributed to their phytochemical content, including alkaloids, tannins, flavonoids, and phenols, which exhibit antioxidant and anti-inflammatory properties.

The histology of the liver of all treatments showed normal hepatic tissue (Figures 4 and 5). The study showed that the extracts did not harm liver tissue in rats with naphthalene-induced oxidative stress, consistent with previous histopathological findings (Agwaramgbo et al. 2014). However, high fruit and leaf extract doses caused vacuolar degeneration, suggesting that moderate consumption is advisable.

In conclusion, the findings of this study highlight the significant hepato-renal protection and antioxidant properties of combined *G. latifolium* NIF 2345 extracts and reduced oxidative stress in naphthalene-induced rats. The study revealed that the LD50 of the *G. latifolium* NIF 2345 extract was ≥ 5000 mg/mL BW, indicating that the extract is relatively safe, and the observed improvements in liver enzyme levels and body weight support its potential as a beneficial therapeutic agent in traditional medicine. The extract's administration reduced malondialdehyde levels and increased antioxidant enzyme activities. These findings suggest moderate consumption of the extracts, as they may offer a natural intervention against oxidative damage and liver toxicity. Further research is needed to explore the specific mechanisms behind the hepatoprotective and antioxidant activities of *G. latifolium* NIF 2345 extracts.

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