



## Article Info

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## *In Vitro* Antioxidant Properties of *Ficus exasperata* Leaves and Its Effect on 1,2-dimethylhydrazine-Induced Liver Toxicity

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*Ficus exasperata*, locally referred to as the sandpaper plant, belongs to the *Moraceae* family. This plant has been used traditionally in the treatment of malaria, bacterial infections, anti-diabetic, and lipid-lowering effects. This study investigates the *in vitro* antioxidant capacity of this plant. This study further investigates the influence of *F. exasperata* on the liver antioxidant status of healthy adult Wistar rats and 1,2-dimethylhydrazine-induced liver toxicity. A total of 24 Wistar rats (150-200g) were divided into four groups of 6 animals each. Group 1 was the control, Groups 2, 3, and 4 were given oral doses of methanol extracts of the leaf (each) at 125, 250, and 500mg/kg body weight respectively, for a total of twenty-eight days. For the 1,2-dimethylhydrazine-induced liver toxicity study, a total of 48 male Wistar rats were divided into 8 groups of 6 rats each, with group 1 serving as the control, while groups 2-8 received either methanol extract of *F. exasperata*, 1,2-dimethylhydrazine, or 5-fluorouracil for 84days. At the end of the experimental period, biochemical assays were conducted, including the determination of total protein, catalase, superoxide dismutase, glutathione peroxidase activities and MDA level. The results of rats treated with the plant extract only at different concentrations exhibited no changes. The DMH-induced toxicity shows that the plant was able to mitigate the effect of the toxicant on the liver biomarkers. *In vitro* antioxidants study shows clearly that the extract could scavenge free radicals as seen in the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Ferric Reducing Antioxidant Power (FRAP), nitric oxide, thiobarbituric acid reactive substance (TBARS), and reducing power. The results suggest that the extracts possess appreciable antioxidant activity and ameliorating capacity of toxicants, which may be of high pharmaceutical importance.

**Keywords:** Antioxidants, 1,2-dimethylhydrazine, liver biomarkers, *Ficus exasperata*, toxicity.

## 1. Introduction

A large percentage of the world's population solely uses the extracts obtained from various parts of plants as the main source of their health care (Okeke *et al.*, 2024; Emmanuel *et al.*, 2022; Ekweogu *et al.*, 2024). The strong belief and confidence that makes people resort to the use of plants is due to the high level of phytochemicals present in these plants. The phytochemicals contribute largely to the medicinal properties of plants, without causing little or no side effects in humans. Ekweogu *et al.* (2024) termed these plant-derived treatments "man-friendly medicines". Materials from plants also serve as a source of medicine for various ailments, which is a result of the increase in population, drug resistance, and the high cost of modern drugs and treatments (Adeogun

*et al.*, 2019; Olude *et al.*, 2020). Herbal medicine has been in existence for decades and the valuable pharmaceutical capacities embedded in plants have long been noted Omoregie *et al.*, 2020; Umeh *et al.*, 2014). It is not surprising that a good percentage of the global population relies on naturally derived medicines from plants and their extract for the treatment and management of diseases. In recent research, more attention has been channeled to the use of herbal products as alternative medicine (Olude and Chukwu, 2023). Newer and recent drug therapies used in liver cell healing and regeneration are obtained from plants, which implies their usefulness in the management of some disorders and injuries that affect the liver (Adetuyi *et al.*, 2022).

*Ficus exasperata* is a medicinal plant popularly known as the African sandpaper tree due to its scabrous leaf surface. The extract from this plant has been reported to be rich in numerous medicinal values (Adeyemi *et al.*, 2024; Okon *et al.*, 2024; Olude and Emeninwa, 2025). It is used in the treatment of ailments such as renal dysfunction (Olude and Emeninwa, 2025), colorectal cancer (Olude and Okolie, 2025), rheumatism, blood pressure, bleeding, wounds, diabetes, asthma, ulcers, fungal infections, arthritis and lipid lowering (Sonibare *et al.*, 2006; Abotsi *et al.*, 2010). Different species of this plant contains some secondary metabolites like flavonoids, tannins, alkaloids, terpenoid, sterols, anthraquinones and phenols (Erhirhie *et al.*, 2018; Salehi *et al.*, 2020).

However, not much has been reported about the ethnomedicinal benefits of *Ficus exasperata* on liver function and 1,2-dimethylhydrazine-induced liver toxicity. This study investigated the *in vitro* antioxidant activity of this plant. The study also investigated the possible influence of this plant on the liver antioxidants of healthy Wistar rats and the liver function markers of rats induced with 1,2-dimethylhydrazine toxicity.

## 2. Materials and Methods

### 2.1 Preparation of *Ficus exasperata*

A large quantity of the leave of *Ficus exasperata* was collected from trees in household gardens in Benin City and around the University of Benin vicinity, Edo state, Nigeria. The plant was identified in the Department of Plant Biology and Biotechnology, University of Benin, authenticated and a voucher specimen number UBH-F319 was deposited at the Herbarium. The leaves were washed, air-dried for three weeks at room temperature and then pulverized using the milling machine. The sample (2kg) was macerated in methanol (10L) for 72 hours after which it was filtered through cheesecloth. The obtained extracts were then concentrated *in vacuo* using a freeze drier. The powdered extract was stored in an air-tight container in a refrigerator at 4°C until use.

### 2.2 *In Vitro* Antioxidants

The free radical scavenging capacity of the methanol extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). The method of Garrat (1964) was followed to determine the nitric oxide radical scavenging activity of the extracts. Estimation of thiobarbituric acid reactive substances (TBARS) was estimated according to the method of Ohkowa *et al.* (1979).

### 2.3 Experimental rats

Male Wistar rats (150g — 200g) were used for this study. The rats were allowed to acclimatize after purchase for two weeks before the commencement of the experiment in the Department of Biochemistry animal house. They were fed with standard rat chow and water *ad libitum*. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the Faculty of Life Sciences, University of Benin, Benin City, and was strictly adhered to.

### 2.4 Acute and Sub-chronic toxicity test

Lorke's method (1983) was followed in determining the LD<sub>50</sub>. A total of twelve Wistar rats were used in the two phases. In phase 1, there were 3 groups of 3 rats each. Group 1 to 3 were treated with 10, 100 and 1000mg/kg bw of *F. exasperata* respectively. In the second phase, there were also three groups with a rat in each group administered 1600, 2900 and 5000mg/kg of the plant extract respectively. The rats were watched closely for 24 hours for likely changes in movements, behaviour and mortality. In the repeated dose oral toxicity study following the Organization for Economic Co-operation and Development (OECD) guideline 407, 24 animals were divided into four groups of six animals each. Group 1 served as a control, while Groups 2, 3, and 4 received extract doses of 125, 250, and 500mg/kg body weight, respectively, for 28 days. Daily evaluations included monitoring mortality, body weights, feed, and water consumption, as well as general toxicity signs. After the 28 days, the animals were sacrificed. The liver was excised and washed in ice-cold saline, and some portions were preserved in 10% formalin for histopathology. The remaining tissues were homogenized in normal saline and centrifuged. The obtained supernatant was used for enzyme activity assays.

#### 2.4.1 Assays

Malondialdehyde (MDA) levels were estimated by the method of Beuge and Aust (1978). Total protein (TP) was determined using lowry's method (1951) and catalase (CAT) was assayed according to the method of Cohen *et al.* (1970). Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972). Glutathione Peroxidase (GPx) was determined according to the method of Paglia and Valentine (1969) and reduced glutathione (GSH) was estimated according to the method of Ellman (1959).

#### 2.4.2 Histopathological Evaluation

Tissue fixation followed Kiernan's method (2008): liver tissue was fixed in 10% buffered formalin, dehydrated, and paraffin-embedded. Sections were obtained, mounted on slides, and haematoxylin-eosin stained for light microscopic examination. Slides were coded and analyzed by a histopathologist.

## 2.5 DMH Induced Liver Toxicity design

Forty-eight (48) Wistar albino rats were used for this study.

Group	Treatment
1	Feed + water
2	Extract (500mg/kg)
3	DMH only
4	DMH + extract (500mg/kg) for 12 weeks
5	DMH +extract (750mg/kg) for 12 weeks
6	Extract (500mg/kg) for four (4) weeks, then DMH for eight (8) weeks
7	DMH for eight (8) weeks, then extract (500mg/kg) for four (4) weeks
8	DMH + standard drug for 12 weeks

After 12 weeks, the animals were fasted overnight and sacrificed. Blood was collected by heart puncture and dispensed into plain bottles. The samples were centrifuged and the serum was obtained. This was used immediately for the liver function test. Alanine transaminase activity (ALT), Aspartate transaminase activity (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin, direct bilirubin, and total bilirubin were all assayed using a standard colorimetric kit. The manufacturer's protocol was strictly followed.

### 2.5.1 Preparation and Administration of 1,2-dimethyl hydrazine (DMH)

Freshly prepared DMH solution was used for each administration. The toxicant was dissolved in 1 mM EDTA, and 1 M sodium hydroxide was used to achieve pH 6.5. Twice a week, the toxicant was subcutaneously injected at a dose of 40 mg/kg body weight. (Chari *et al.*, 2018).

### 2.6 Statistical Analysis of Data

The obtained data underwent statistical analysis, with values presented as Mean  $\pm$  Standard error of mean. Comparisons between samples were conducted using ANOVA, Tukey's multiple comparison test (SPSS, version 17), and GraphPad Prism. Significance was determined at p-values below 0.05 ( $p < 0.05$ ).

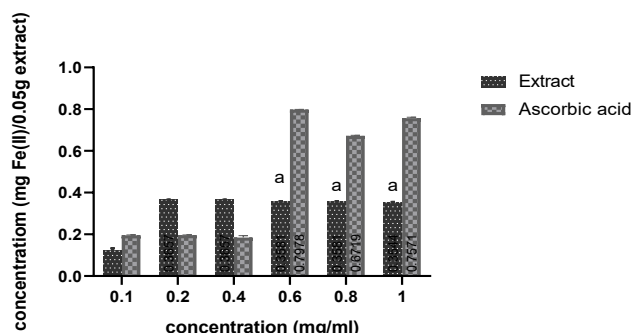
## 3. Results and Discussion

### 3.1 Results

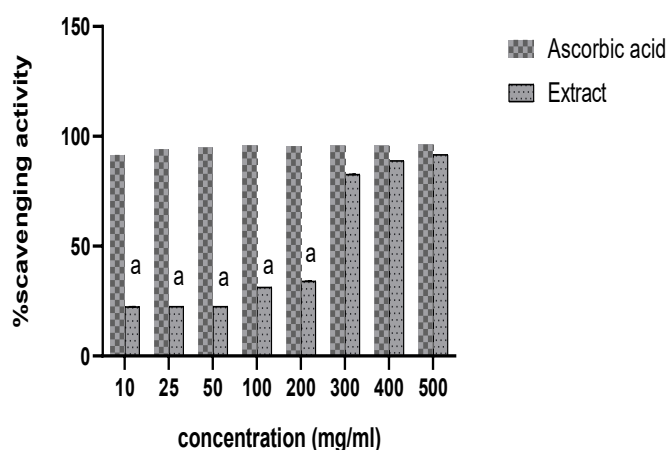
#### 3.1.1 *In vitro* antioxidant activity of methanol extract of *Ficus exasperata* leaves

The antioxidant (Figure 1 – Figure 4) ability of the plant extract was checked by assaying for Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant assay (FRAP), nitric oxide assay, and thiobarbituric acid reactive substances (TBARS) The plant exhibited detoxifying potency against free radicals. The extract showed an appreciable ability in scavenging free radicals as

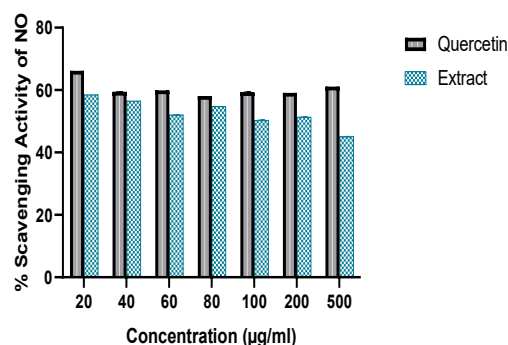
showed in its FRAP activity and TBARS inhibition. The plant also showed a promising antioxidant activity by scavenging DPPH radicals and nitric oxide.



**Figure 1:** Ferric acid Reducing Antioxidant Potential (FRAP) Assesment of methanol extract of *Ficus exasperata* leaves. Values are expressed as mean  $\pm$  SEM,  $n=3/\text{group}$ . Lowercase letters represent a significant difference at  $P < 0.05$ .

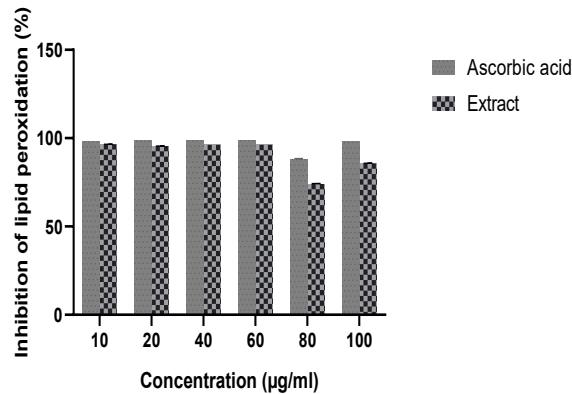


**Figure 2:** DPPH's radical scavenging activity of the methanol extract of *Ficus exasperata* leaves. Values are expressed as mean  $\pm$  SEM,  $n=3/\text{group}$ . Lowercase letters represent a significant difference at  $P < 0.05$ .



**Figure 3:** Nitric Oxide scavenging activity of methanol extract of *Ficus exasperata* leaves. Values are expressed as mean  $\pm$  SEM,  $n=3/\text{group}$ . Data

were considered statistically significantly different at  $P < 0.05$ .



**Figure 4:** Estimation of lipid peroxidation inhibition (TBARS) of methanol extract of *Ficus exasperata* leaves. Values are expressed as mean  $\pm$  SEM,  $n=3$ /group. Data were considered statistically significantly different at  $P < 0.05$ .

**Table 1:** Liver antioxidants, total protein and MDA levels

ASSAYS	GRP 1 (CONTROL)	GRP 2 (125mg/kg bw extract)	GRP 3 (250mg/kg bw extract)	GRP 4 (500mg/kg bw extract)
SOD (units/g wet tissue)	0.112 $\pm$ 0.28	0.114 $\pm$ 0.14	0.117 $\pm$ 0.06	0.152 $\pm$ 0.03
CAT (units/g wet tissue)	1.868 $\pm$ 0.66	1.75 $\pm$ 0.676	1.71 $\pm$ 0.756	2.72 $\pm$ 0.717
GPX (units/g wet tissue)	7.83 $\pm$ 2.92	7.11 $\pm$ 0.84	12.496 $\pm$ 4.32	4.2 $\pm$ 0.98 <sup>c</sup>
GSH Mm/g tissue)	127.08 $\pm$ 31.20	105.55 $\pm$ 20.03	137.50 $\pm$ 10.48	119.79 $\pm$ 13.125
TP (g/dl)	20.99 $\pm$ 4.92	17.61 $\pm$ 2.04	31.33 $\pm$ 10.77	14.75 $\pm$ 2.55
MDA ( $\times 10^{-4}$ ) mmol/g tissue	9.17 $\pm$ 3.08	13.92 $\pm$ 3.3	14.65 $\pm$ 10.5	10.57 $\pm$ 4.6

Values are expressed as mean  $\pm$  SEM,  $n=6$ /group. Lowercase letters represent a significant difference at  $P < 0.05$ .

**3.1.4 Histology of the Effect of MEFE on Healthy Liver Tissues**

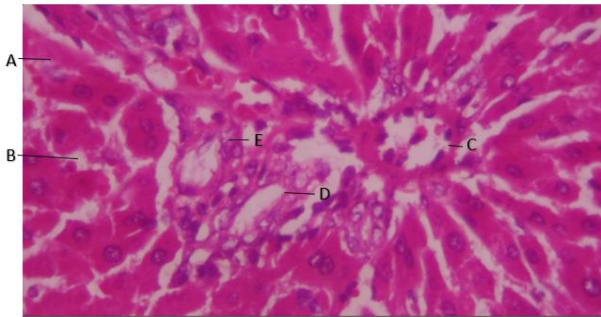
Plates 1 to 4 show the effect of the methanol extract of the plant on different tissues. The plant appeared non-toxic to the tissues even at 500mg/kg body weight. The normal architecture of the tissues was not tampered with. However, Kupffer cells activation and lymphocytes mobilization were observed in the liver tissues of rats given different doses of the extract

**3.1.2 Acute toxicity result**

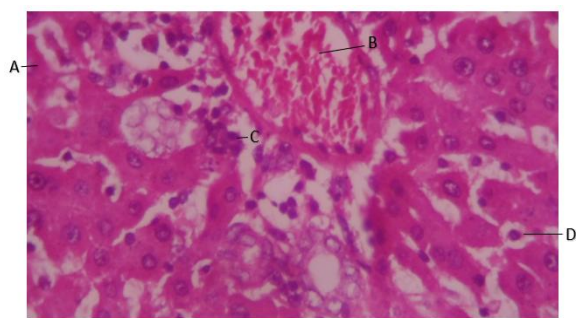
There was no record of mortality in both phases I and 2. The animals in phase 1 remain active even after the administration of 1000mg/kg bw of the extract, but the highest dose administration (5000mg/kg bw) in phase 2 rats resulted in weakness and sluggish movements.

**3.1.3 Tissue enzymatic antioxidants and lipid peroxidation marker**

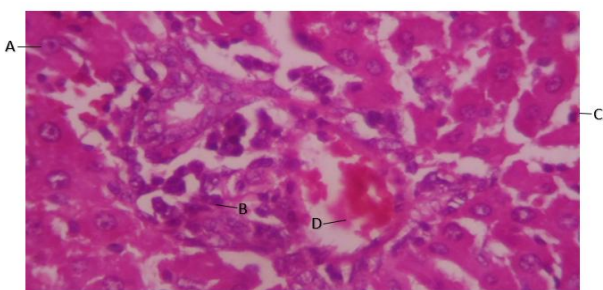
The results for the antioxidant parameters, total protein and lipid peroxidation marker (MDA) of the subacute toxicity test on the liver are presented in Table 1. Catalase (CAT), superoxide dismutase (SOD) activities and glutathione peroxidase (GSH) level in the liver showed no significant difference across the groups. Lipid peroxidation marker (MDA) and total protein also showed no significant difference across various groups when compared with the control.



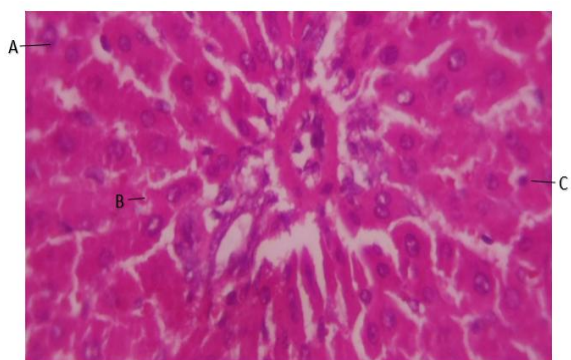
**Plate 1:** Liver section from rat (control) composed of normal tissue: A. hepatocytes, B. sinusoids, C. portal vein, D. bile ducts, E. hepatic artery (H&E x 400)



**Plate 2:** Liver section of rat given 125mg/kg bw extract showing normal architecture: A..hepatocytes, B. active portal vascular congestion, C. periportal lymphocyte mobilization, D. sinusoidal Kupffer cell activation (H&E x 400)



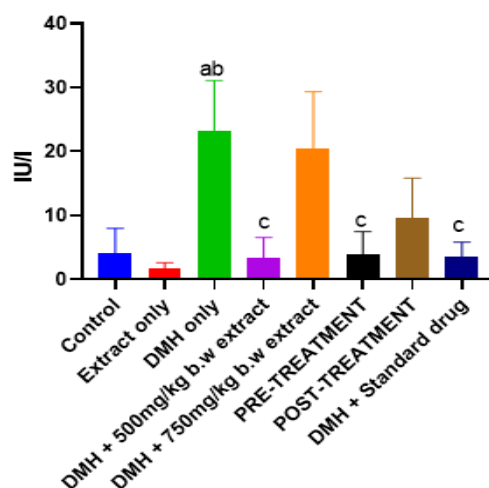
**Plate 3:** Liver section of rat given 250mg/kg bw extract showing normal architecture: A. hepatocytes, B. periportal lymphocyte mobilization, C. Kupffer cell activation, D. active vascular congestion (H&E x 400)



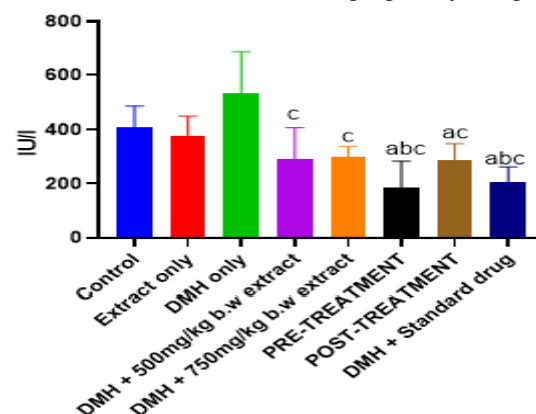
**Plate 4:** Liver section of rat given 500mg/kg bw extract showing normal architecture: A. hepatocytes, B. active vascular congestion, C. Kupffer cell activation (H&E x 400)

### 3.1.5 Effect of MEFE on DMH-Induced Liver Toxicity

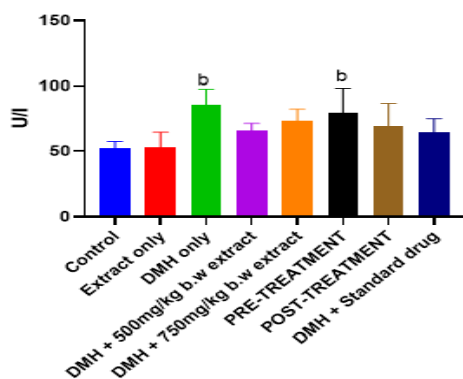
Liver function markers; aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and albumin gamma glutamyl transferase (GGT) and bilirubin (direct and total) were assayed for in the serum. These results are represented in Figures 5 to 11.



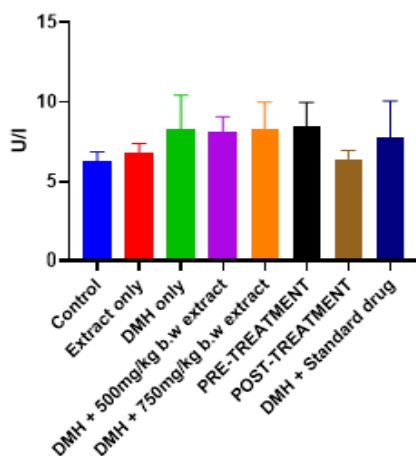
**Figure 5:** GGT activity of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Groups 4 and 6 were not statistically different from the group treated with standard drugs. The MEFE-treated groups show that the plant extract has hepatoprotective properties but is deleterious at a dose of 750mg/kg body weight.



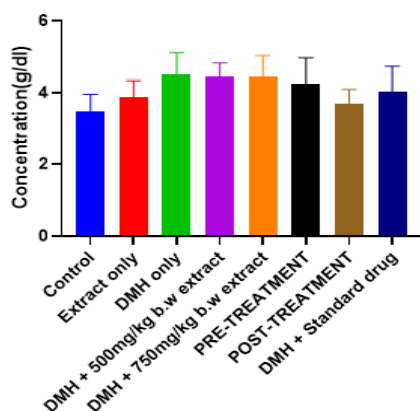
**Figure 6:** Alkaline phosphatase activity of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The ALP enzyme activity was high in DMH only group. The exposed rats that were treated with MEFE showed a significant reduction in the activity of this enzyme, the levels were not different from the group treated with standard drug.



**Figure 7:** Alanine transaminase activity of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . The other groups except the pretreatment group have a lowered level of ALT compared to group 3.

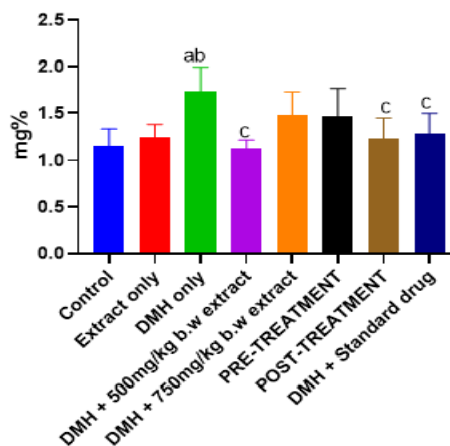


**Figure 8:** Aspartate transaminase activity of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . There were no significant differences in AST activities but group 7 had the lowest AST activities.

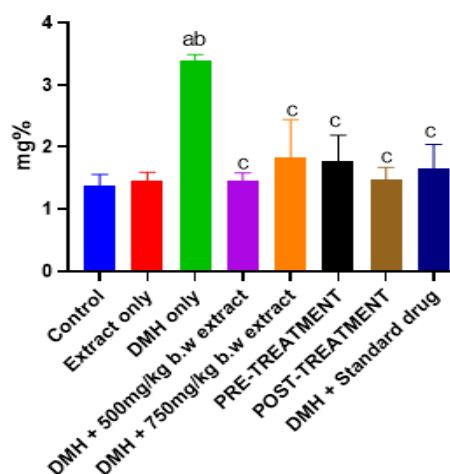


**Figure 9:** Albumin level of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference

at  $P < 0.05$ . No significant difference in the data obtained for serum albumin level. The post treated group showed the lowest albumin level compared to other groups ( $P > 0.05$ ).



**Figure 10:** Direct bilirubin levels of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Post-treated and co-treated (500mg/kg b.w MEFE) direct bilirubin levels were not significantly different from the group treated with standard drugs. The DMH-only treated group showed the highest level of direct bilirubin which was statistically different from groups 1, 2, 4, 7 and 8 ( $P < 0.05$ ).



**Figure 11:** Total bilirubin level of rats in different groups. Values are expressed as mean  $\pm$  SEM, n=6/ group. Lowercase letters represent a significant difference at  $P < 0.05$ . Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the

lowercase letter "c" denote significant differences from the DMH Only group. MEFE was as effective as the standard drug in reducing the effect of the toxicant on the liver tissue. This was evident in the result obtained from total bilirubin levels of MEFE-treated rats when compared to the control.

### 3.2 Discussion

1,2-dimethylhydrazine (DMH) is the toxicant used in this study. DMH is a colon-specific carcinogen used to induce colorectal cancer in rodents, but the liver plays an essential role in metabolizing and activating this procarcinogen into its active form (Olude *et al.*, 2023). During the activation, methyl diazonium ion and reactive oxygen species are released (Olude and Emeninwa, 2025). There is a continuous generation of free radicals in the biological system due to spontaneous metabolism that takes place in the mitochondria (Adeogun *et al.*, 2019). Exposure to different xenobiotics from the environment also yields free radicals generation in the body, with the liver being the major organ responsible for xenobiotics detoxification, hence receiving the largest share of the toxic effect of any toxicant it metabolizes (Bojic *et al.*, 2023). However, antioxidants are known to combat free radicals generated by cells, hence inhibiting lipid peroxidation and the deleterious effects accompanying reactive oxygen and nitrogen species production (Olude and Omoregie, 2024).

In this study, the values obtained from the DPPH scavenging capacity of *Ficus exasperata* were relatively comparable to the control. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. These plant extracts may thus have potent radical scavenging properties. The standard antioxidant (Ascorbic acid) was observed to have a significantly higher ( $p < 0.05$ ) FRAP value than the leaf extract. However, FRAP values were still recorded for the extract. *F. exasperata* significantly inhibited nitric oxide (NO) production, which has health implications and benefits. Cardiovascular diseases, cancer, inflammation, and the pathogenesis of various human diseases have been linked to nitric oxide. The NO scavenging capacity of the methanol extract of *Ficus exasperata* may help to arrest the chain of reactions initiated by the excess generation of NO that are detrimental to human health (Adeogun *et al.*, 2019). TBARS production was greatly inhibited by the extract. There was no statistically significant difference ( $p > 0.05$ ) between the extract and quercetin (Standard antioxidant). A recent study showed that the methanol leaf of *F. exasperata* contains some biological bioactive molecules such as quercetin, kaempferol, rutin, isoquinoline, alpha-caryophyllene, garcin, caffeic acid, luteolin, catechin, linalool, epigallocatechin, stigmasterol, sitosterol, orientin, naringerin, hesperidin, isoitexin and isorhamnetin (Okolie and Olude, 2025). Quercetin, a potent antioxidant, is capable of scavenging free radicals

and inhibiting lipid peroxidation (Cizmarova *et al.*, 2023) while kaempferol exhibits antioxidant potency also by reducing free radicals and reactive oxygen species production (Imran *et al.*, 2019). Rutin is known for its chemopreventive and chemosensitizing properties against various cancers (Nouri *et al.*, 2020) and isoquinoline possesses antiproliferative and anticancer properties (Alam *et al.*, 2022). Caffeic acid exhibits diverse pharmacological properties such as anti-inflammatory, antioxidant, reducing oxidative stress, and inhibiting DNA damage by free radicals (Zdunska *et al.*, 2018). These bioactive compounds contributed to the *in vitro* antioxidant properties displayed by the methanol leaf extract of *F. exasperata*.

In the subacute oral toxicity test, the liver enzymatic antioxidant; SOD and CAT activities remained consistent across the groups given different concentrations of MEFE, with no significant differences. MDA, TP, and GSH levels also showed no significant changes, even at the highest dosage of 500mg/kg MEFE. Superoxide and hydrogen peroxide are catalyzed by superoxide dismutase and catalase, respectively. A reduction in the activity of these enzymes might be a pointer to the excess production of free radicals in the cells. ROS damage proteins, lipids, and DNA, and may finally result in different cell death processes. Non-elimination of these free radicals in time results in the formation of lipid peroxides, and the final product of lipid peroxide is MDA (Ku *et al.*, 2021). Changes in the activities of these enzymes can indicate tissue damage by toxicants (An *et al.*, 2025). Thus, non-alteration of enzyme levels in the tissues of rats administered low and moderate doses (125, 250, and 500 mg/kg bw) of the extract suggests that the extract did not pose any threat to the structural and functional integrity of the cells at these doses, and this was consistent with the histology results.

The increase in serum enzymes (ALT, AST, and ALP) activities might be due to the loss of cellular functional integrity of hepatocyte membrane resulting from highly reactive electrophiles i.e., carbonium ions and alkyl free radicals which severely damage the liver causing necrosis and fatty infiltration, methylate nucleobases and disrupt the polysomal assembly and hence enzymes that are located in liver cells leak out and make their way into the general circulation (Abd-Elmoneim *et al.*, 2013; Ansil *et al.*, 2013; Wang *et al.*, 2017; Shebbo *et al.*, 2020). Lonardo and Ndrepepa (2022), reported an increase in GGT levels as a result of exposure to xenobiotics and carcinogens. A similar observation was recorded in group 3 and the levels of all these enzymes were moved to normal after *F. exasperata* treatments, representing the significant hepatoprotective effect of this extract (Sahdev *et al.*, 2017). The GGT, direct, and total bilirubin levels of the group treated with the toxicant only were significantly increased compared to other groups.

The low values seen in other treated groups further applaud the hepatoprotective medicinal qualities embedded in this plant (Moss *et al.*, 2023). Such amelioration in the activities of these enzymes suggests a significant hepatoprotective effect of the plant extract.

#### 4. Conclusion

The liver is an important target of toxic substances and is the organ that is most sensitive to the effects of toxicants. The study shows clearly that the methanol extract of *Ficus exasperata* leaves possesses great *in vitro* and *in vivo* antioxidant properties. The plant was found to be non-toxic to the liver but rather hepatoprotective to the hepatocytes. The liver biomarkers that were increased after DMH administration were reduced after treatment with the plant extract. The plant also attenuated the aberrations inflicted by the toxicant and restored the liver architecture.

#### Conflict of Interest

The authors declare no conflict of interest.

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