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Hepatoprotective Effect of Ethanolic Leaves Extract of *Musa Paradisiaca* on CCL_4-Induced Hepatotoxicity in Albino Rats

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Abstract

Musa paradisiaca (banana) leaves have been extensively used in traditional medicine for the treatment and management of disorders related to oxidative stress, including diabetes, neurodegenerative diseases, and cancer. However, there is limited scientific evidence supporting these claims. This study aimed to evaluate the hepatoprotective and antioxidant properties of Musa paradisiaca leaf extract in albino rats with carbon tetrachloride (CCl_4)-induced liver damage. Fresh leaves of Musa paradisiaca were collected, shade-dried, and extracted using 60% ethanol. Thirty albino rats were randomly assigned to five groups: control, CCl_4-induced, CCl_4 + silymarin (100 mg/kg), CCl_4 + Musa paradisiaca extract (200 mg/kg), and CCl_4 + Musa paradisiaca extract (400 mg/kg). The extract was administered orally, while CCl_4 was given intraperitoneally (IP) to induce liver damage. Phytochemical screening of the

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extract revealed the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones, and steroids. Liver function biomarkers—alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—were analyzed spectrophotometrically. The results indicated that the Musa paradisiaca extract significantly reduced ALT, AST, and ALP levels ($p \leq 0.05$) compared to the CCl_4-treated group, demonstrating its hepatoprotective effects. The 400 mg/kg dose exhibited a more pronounced effect, comparable to that of silymarin. These findings provide scientific validation for the traditional use of Musa paradisiaca leaves in managing liver disorders and conditions associated with oxidative stress. Further studies are recommended to isolate and characterize the bioactive compounds responsible for these effects.

Keywords: Musa paradisiaca, Hepatoprotection, Oxidative stress, Antioxidant activity, Phytochemicals, Liver function markers.

INTRODUCTION

Liver diseases, especially those caused by toxins such as carbon tetrachloride (CCl_4), are a significant global health issue, leading to high rates of morbidity and mortality (Marc G, 2019). Oxidative stress and the generation of free radicals play a crucial role in hepatotoxicity, contributing to liver damage and dysfunction (Ahmad *et al.*, 2015). While synthetic antioxidants are widely used to combat oxidative stress, concerns regarding their hepatotoxicity, carcinogenicity, and high cost have sparked interest in finding safer and more accessible alternatives (Abbas *et al.*, 2017). Traditional medicine has long utilized plant-based therapies for liver protection, with the leaves of Musa paradisiaca (banana) being a notable example. Despite their common use in ethnomedicine for treating oxidative stress-related disorders, scientific validation of their efficacy remains limited (Simonikova *et al.,* 2020).

Several studies have investigated the hepatoprotective properties of plant-based antioxidants. Ahmad et al. (2015) highlighted the antioxidant potential of Musa species, demonstrating their ability to scavenge free radicals. Similarly, Abbas *et al.*, (2017) explored

the therapeutic role of plant-derived polyphenols in managing liver diseases. However, most research has concentrated on banana fruit and its bioactive compounds, leaving a significant gap concerning the hepatoprotective effects of the leaves. Furthermore, while traditional medical practitioners (TMPs) assert that Musa paradisiaca leaves possess pharmacological activity against oxidative stress-related diseases such as diabetes, neurodegenerative disorders, and cancer, empirical evidence supporting these claims is lacking (Lewis, 2017).

This research aims to fill this gap by scientifically evaluating the hepatoprotective effects of ethanolic extracts of Musa paradisiaca leaves against CCl_4-induced hepatotoxicity in albino rats. Unlike previous studies that primarily focused on the fruit, this study emphasizes the bioactive properties of the leaves, assessing their potential as a natural and cost-effective alternative to synthetic antioxidants. The study is grounded in antioxidant theories, which suggest that plant polyphenols and flavonoids are crucial in reducing oxidative stress-induced liver damage (Carvalho & Machado, 2018).

The objectives of this research were to investigate the hepatoprotective effects of ethanolic extracts of Musa paradisiaca leaves by evaluating key liver function markers, including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP), in CCl_4-induced hepatotoxicity in albino rats (Ringehan, *et al.,* 2017). The study also examined the qualitative phytochemical composition of the plant extract to identify the bioactive compounds responsible for its hepatoprotective effects. Through this research, we aimed to provide scientific validation for the traditional use of Musa paradisiaca leaves as a hepatoprotective agent and contribute to the development of alternative natural antioxidants.

MATERIALS AND METHODS

All chemicals and reagents used in this study were of analytical grade, as was the laboratory equipment.

Sample Collection

Fresh leaves of Musa paradisiaca (banana leaves) were collected from Dadin Kowa, Yamaltu Deba LGA, Gombe State, in September 2023. The samples were transported to

the Department of Biochemistry at Gombe State University, where the research was conducted.

Sample Identification

The plant samples were submitted to the herbarium unit of the Botany Department at Gombe State University for identification and authentication. The samples were assigned a voucher number (GSU-H 102).

Preparation of Musa paradisiaca (Banana) Leaf Extract

The fresh banana leaves were washed and shade-dried. The dried samples were then pulverized into a fine powder using a mortar and pestle. A total of 120g of the powdered sample was macerated in a 60% ethanol solution, allowed to stand for an extended period, and shaken daily. The mixture was then filtered using Whatman no. 1 filter paper to obtain the pure ethanolic extract of Musa paradisiaca. The filtrate was evaporated using a rotary evaporator at 40°C for 5 hours, after which the extract was collected for subsequent treatment.

Experimental Animals

Thirty (30) albino rats of both sexes were purchased from the animal farm of the National Veterinary Research Institute in Vom LGA, Plateau State, Nigeria. During the experiment, the rats were housed in steel cages under controlled laboratory conditions. They were fed a standard chick finisher diet and provided with water ad libitum. The rats were allowed to acclimatize for two weeks before the administration of the leaf extract, with monitoring for 10 days, followed by the induction of liver damage using carbon tetrachloride (CCl_4) for one month. The cages were cleaned daily to prevent infection among the animals. Animal care and treatment were conducted in accordance with institutional guidelines that comply with the United States National Research Council's (2015) standards for the care of laboratory animals.

During the experiment, the animals were weighed frequently to determine the appropriate dosage of the extract to be administered. The thirty albino rats (n=30) were randomly divided into five groups (n=6), and treatments were conducted over four consecutive weeks (Table 1).

Table 1: Experimental Animals and design

GROUPINGS	TREATMENTS
Control No of rats =5	Were administered with physiological saline orally three times a week and olive oil intraperitoneally (IP) twice weekly.
CCl4 No of rats =5	Received an IP injection of CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5ml/kg body weight twice a week.
CCL4 + silymarin No of rats =5	Were administered with 100mg/kg body weight Silymarin for 5 days/week orally for 3 successive weeks and an IP injection of CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5ml/kg body weight, twice weekly.
CCL_4 + 200mg/kg No of rats =5	Plant extract was given orally at a dose of 200mg/kg body weight daily then after 1h received an IP injection of CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5ml/kg body weight, twice weekly.
CCL_4 + 400mg/kg No of rats =5	Plant extract was given orally at a dose of 400mg/kg body weight daily then after 1h received an IP injection of CCL_4 and olive oil (1:1 v/v) mixture at a dose of 0.5ml/kg body weight twice weekly

Preliminary (Qualitative) Test for Phytochemicals in *Musa paradisiaca* crude Extract (Reitman S., & Frankel, S. 1957)

a) Test for Alkaloids

Dragendoff's Test: A 0.5 g portion of the stem bark powder was stirred in 5 mL of 1% Ag.HCl on a steam bath for about 5 minutes. The mixture was filtered through Whatman No. 1 filter paper. To 1 mL of the filtrate, 2–4 drops of Dragendoff's reagent were added. Change of the solution's color to orange indicates the presence of alkaloids. The test is valid only if the plant material is not colored.

b) Test for Saponins

To a test tube containing 5 mL of distilled water, 0.5 g of bark powder was added. The solution was mixed vigorously and observed for frothing (bubbles). The mixture was then warmed by placing it in a water bath at 50°C for 10 minutes. The persistence of frothing indicates the presence of saponins.

c) Test for Tannins

In a test tube with 5 mL of distilled water, 2 g of bark powder was added and stirred. The mixture was then filtered through Whatman No. 1 filter paper. Gradually add 2–3 mL of ferric chloride solution to the filtrate. A deep olive-green colour indicates the presence of tannins.

d) Test for Flavonoids

A 0.5 g portion of the bark powder was added to 2 mL of dilute NaOH solution in a test tube and shaken to dissolve. The formation of an intense yellow colour, which turns colourless upon the addition of concentrated H_2SO_4, indicates the presence of flavonoids.

e) Test for Anthraquinones

In a test tube, 3 g of bark powder was added to 5 mL of benzene and allowed to soak for 10 minutes. The mixture was then filtered, and 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds. A pink-red or violet colour in the ammonia layer indicates the presence of anthraquinones.

f) Test for Steroids

To 1 mL of the extract in a test tube, 2 mL of chloroform was added, followed by an equal volume of concentrated H_2SO_4 poured down the side of the tube. The appearance of a red colour in the upper layer and a yellow colour with green fluorescence in the sulfuric acid layer indicates the presence of steroids.

Determination of Liver Marker Enzymes

AST & ALT

Test: A 0.5 mL portion of the buffer substrate solution was incubated at 37°C for 30 minutes. After that, 0.1 mL of serum sample was added, mixed, and incubated for an additional 60 minutes. Following this, 0.5 mL of 2,4-Dinitrophenyl Hydrazine was added, and mixed, and the solution was removed from the incubator to stand at room temperature for 20 minutes. Finally, 5.0 mL of 0.4N NaOH was added, and after standing for 5 minutes at room temperature, the absorbance was measured using a UV spectrophotometer at 546 nm.

Test Blank: A 0.5 mL portion of the buffer substrate solution was incubated at 37°C for 30 minutes. Then, 0.1 mL of distilled water was added after the 60-minute incubation. The

method was completed as described above for the test, and this solution was used to blank the UV spectrophotometer at 546 nm.

Alkaline Phosphatase (ALP) Test (King Armstrong, 1964)

Test: A 0.5 mL portion of alkaline buffer was incubated at 37°C for 3 minutes. Next, 0.05 mL (50 µL) of the serum sample was added to the test tube, mixed, and incubated at 37°C for 10 minutes. After incubation, 2.5 mL of alkaline phosphatase colour developer was added, and the mixture was mixed well. The absorbance was then measured at 590 nm.

Test Blank: As with the test, a 0.5 mL portion of alkaline buffer was incubated at 37°C for 3 minutes. Following that, 0.05 mL of distilled water was added, mixed, and incubated at 37°C for 10 minutes. After that, 2.5 mL of alkaline phosphatase colour developer was added, and mixed, and this solution was used to blank the UV spectrophotometer at 590 nm.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for significant differences between groups at $P \leq 0.05$ using the Statistical Package for Social Sciences (SPSS Version 16.0). Post-hoc analysis was performed using Tukey's test, with values of $P \leq 0.05$ considered statistically significant. Results were expressed as Mean ± Standard Error of Mean (SEM).

RESULTS

Percentage Yield of Musa paradisiaca Leaf Extract

The percentage yield of the Musa paradisiaca leaf extract following extraction was found to be 11.65%.

Phytochemical Analysis of Musa paradisiaca Leaf Extract

Phytochemical screening of the Musa paradisiaca leaf extract revealed the presence of alkaloids, flavonoids, phenols, steroids, tannins, and terpenes. However, saponins were not detected, as summarized in Table 2.

TABLE 2: Results of phytochemical analysis of *Musa paradisiaca* leaves extract

PHYTOCHEMICALS	RESULTS
Saponin	–
Tannins	+
Steroids	+
Flavonoid	+
Phenols	+
Alkaloid	+
Terpenes	+

Effect of *Musa Paradisiaca Leaves extract on serum AST, ALT and ALP in CCl_4* induced Albino rats.

Administration of carbon tetrachloride (CCl_4) resulted in a significant increase ($P < 0.05$) in the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) compared to the control group (Mahmood *et al.,* 2018). In contrast, the group treated with CCl_4 and 100 mg/kg body weight of silymarin demonstrated a significant decrease ($P < 0.05$) in liver enzyme levels, indicating potential hepatoprotective effects. Moreover, the groups treated with CCl_4 along with Musa paradisiaca extract at doses of 200 mg/kg and 400 mg/kg, administered five days per week for 30 days, exhibited a dose-dependent reduction in levels of ALT, AST, and ALP (Mahmood *et al.,* 2018). This finding suggests that Musa paradisiaca has protective effects against CCl_4-induced liver damage.

Table 3: Effect of Oral Administration *Musa Paradisiaca* leaves extract on liver parameters (AST, ALT, and ALP) in CCl_4 Albino rats.

GROUPS	ALT U/L (a)	AST U/L (b)	ALP U/L (c)
Control	37.8 ± 1.714^{a}	24.6 ± 0.979^{a}	57.5 ± 0.376^{a}
CCl_4	86.0 ± 2.846^{b}	82.0 ± 4.516^{b}	250.8 ± 13.2^{b}
CCL_4 + silymarin	41.4 ± 0.979^{c}	24.0 ± 3.130^{a}	60.7 ± 2.180^{c}
CCL_4 + 200mg/kg	54.0 ± 1.225^{d}	37.0 ± 2.449^{c}	151.3 ± 1.920^{d}
CCL_4 + 400mg/kg	48.6 ± 3.715^{e}	36.2 ± 5.718^{c}	72.7 ± 6.935^{e}

***** All values are expressed as mean ± standard error of the mean (SEM). Values with different superscripts (^{a}, ^{b}, ^{c}, etc.) are significantly different ($P < 0.05$) within the same column.

DISCUSSION

The liver is a vital organ responsible for maintaining metabolic balance and detoxifying the body (Ozougwu, 2017). However, it is susceptible to various toxic insults, including hepatotoxicity induced by carbon tetrachloride (CCl_4) (Carvalho & Machado, 2018). This study evaluated the hepatoprotective effects of Musa paradisiaca leaf extract against CCl_4-induced liver damage and found that the extract significantly reduced liver enzyme levels, indicating its protective properties.

Hepatic injury caused by CCl_4 occurs primarily due to its metabolism by cytochrome P450 enzymes (CYP2E1, CYP2B1, CYP2B2), which leads to the formation of trichloromethyl free radicals ($CCl_3\cdot$) (Targher *et al.,* 2020). These radicals initiate lipid peroxidation, resulting in membrane damage, oxidative stress, and elevated serum levels of liver enzymes such as ALT, AST, and ALP (Hamza et al., 2014). The significant increase in these liver biomarkers in the CCl_4-treated group confirms extensive hepatocellular damage. However, treatment with Musa paradisiaca extract at doses of 200 mg/kg and 400 mg/kg significantly mitigated these effects, suggesting that its bioactive compounds play a role in reducing oxidative stress and preserving liver function.

The hepatoprotective effects of Musa paradisiaca leaves are likely attributed to its rich phytochemical composition, which includes flavonoids, phenols, tannins, and alkaloids, all known for their antioxidant and anti-inflammatory properties (Panigrahi, 2017). Previous studies have demonstrated that flavonoids and phenolic compounds can neutralize free radicals, decrease oxidative damage, and stabilize hepatocyte membranes (Maisarah et al., 2013). Similar hepatoprotective effects were reported by Mahmood *et al.,* (2018), which found that extracts from banana leaves exhibited strong antioxidant activity, reducing oxidative stress in liver injury models. Our study aligns with these findings. The 400 mg/kg dose of Musa paradisiaca provided protection comparable to silymarin (100 mg/kg), a standard hepatoprotective agent recognized for its free radical scavenging and membrane-stabilizing effects (Daniel *et al.,* 2018). This reinforces the potential of Musa paradisiaca as a

natural hepatoprotective agent, particularly in resource-limited settings where synthetic drugs may be less accessible.

While previous research has established the antioxidant and anti-inflammatory properties of banana leaf extracts, this study further supports their hepatoprotective effects in an in vivo model of CCl_4-induced hepatotoxicity. The dose-dependent reduction in ALT, AST, and ALP levels highlights the efficacy of Musa paradisiaca leaf extract as a potential therapeutic agent for liver diseases. Additionally, unlike prior studies that focused mainly on in vitro antioxidant assays, this research provides in vivo evidence, enhancing its clinical relevance. Future studies should investigate the specific molecular mechanisms by which Musa paradisiaca mitigates hepatotoxicity, particularly its effects on oxidative stress markers such as MDA, GSH, SOD, and CAT. Additionally, the long-term safety and efficacy of the extract in chronic liver diseases, including non-alcoholic fatty liver disease (NAFLD) and drug-induced liver injury, should be explored. Bioavailability and pharmacokinetic studies are necessary to determine optimal dosages for therapeutic applications.

CONCLUSION

This study demonstrated the hepatoprotective potential of Musa paradisiaca leaf extract against liver damage induced by carbon tetrachloride (CCl_4) in albino rats. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenols, steroids, tannins, and terpenes, which may contribute to its protective effects (Ketiku, 2017). Administering 200 mg/kg and 400 mg/kg of the extract significantly reduced serum levels of AST, ALT, and ALP, indicating a protective role against hepatotoxicity. The observed hepatoprotective effect is likely due to the antioxidant properties of the phytochemicals, which may neutralize reactive oxygen species (ROS) and inhibit lipid peroxidation (Hamza et al., 2014). The extract's efficacy was dose-dependent, with higher doses providing greater protection, comparable to the standard drug silymarin (Maisarah et al., 2013).These findings suggest that Musa paradisiaca leaf extract could serve as a natural therapeutic agent for liver protection, particularly against oxidative stress-induced liver damage. However, further studies, including mechanistic investigations and clinical trials, are needed to fully establish its potential for therapeutic applications.

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