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# EFFECTS OF *Bryophyllum Pinnatum* EXTRACTS ON LIVER ENZYME FUNCTION IN MALE WISTAR ALBINO RATS EXPOSED TO KEROSENE

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**Abstract**

This study examined the hepatoprotective effects of *Bryophyllum pinnatum* extracts on the liver enzyme function of male wistar albino rats exposed to kerosene. The rats were divided into five (5) groups: blank control, negative control, low-dose extract, medium-dose extract, and high-dose extract. All groups were exposed to kerosene inhalation (except the blank control group, and the experiment lasted for 28 days. Then, liver enzyme activities (ALT, AST, ALP, and GGT) were measured, and the results showed a significant increase in liver enzyme activities, indicating hepatotoxicity. Treatment with *Bryophyllum pinnatum* extract significantly reduced liver enzyme activity, suggesting hepatoprotection. Results showed a dose-dependent reduction in AST enzyme activities, with the highest dose (28.85 ± 0.12) exhibiting the most significant reduction ($p<0.05$) followed by the medium-dose at (30.74 ± 0.32) when compared with the blank control (30.48 ± 0.01)) at week 2. The results followed the same pattern at week 4. These findings indicate that *Bryophyllum Pinnatum* extracts have the ability to mitigate kerosene-induced hepatotoxicity, supporting its potential use as a therapeutic agent.

## Introduction

The liver is responsible for several functions, including primary detoxification of various metabolites, synthesizing proteins, and digestive enzyme production (Iuz, 2020). The liver also plays a significant role in the metabolism, regulation of red blood cells (RBCs), and glucose synthesis and storage. Typically, liver function, the discussion includes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) (Ribeiro, 2019). Elevations in ALT and AST to ALP, and bilirubin denotes a hepatocellular disease. An elevation in ALP and bilirubin in disproportion to ALT and AST would characterize cholestasis patterns. A mixed injury pattern was defined as an elevation in alkaline phosphatase and AST/ALT levels (Vagvala, 2018).

Kerosene, a common hydrocarbon fuel, can be harmful to both human and animal health if inhaled or absorbed through the skin. Long-term exposure to kerosene can impair liver function by causing oxidative stress,

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inflammation, and hepatotoxicity. Several substances found in liquid kerosene fuel, such as n-hexane and naphthalene, may be harmful to human health.

Research has repeatedly demonstrated that exposure to kerosene increases the levels of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT), which are indicators of liver injury (Singh *et al.,* 2013). Kerosene exposure also decreases glutathione-S-transferase (GST) activity, compromising detoxification pathways (Rao *et al.,* 2015), increases lipid peroxidation, resulting in oxidative stress and liver damage (Patel *et al.,* 2019), and increases alkaline phosphatase (ALP) activity, indicating cholestasis or liver dysfunction (Ahmed *et al.,* 2018).

The perennial medicinal herb *Bryophyllum pinnatum* is found in different places. Numerous names for the plant exist, including the miracle leaf, maternity plant, air plant, life plant, and love plant. It is highly prized in traditional medicine and is known as "Never Die" in Nigeria. The hepatoprotective, therapeutic, and anti-oxidative properties of this agent are attributed to its bioactive constituents, which include flavonoids, alkaloids, triterpenes, and phenolic compounds. These advantages include reducing blood glucose levels, having anti-inflammatory and antioxidative qualities, improving erythropoiesis, protein synthesis, and immunological protection.

According to studies, *B. pinnatum* extracts can lower levels of aspartate transaminase (AST) and alanine transaminase (ALT), suggesting liver protection (Olaleye, 2016). Additionally, it has been observed to boost detoxification pathways by increasing glutathione-S-transferase (GST) activity (Raji, 2017). Using male Wistar albino rats exposed to kerosene, this study aimed to assess the impact of *Bryophllum pinnatum* extract on liver function. The study aim was to ascertain the extract's possible restorative and protective effects by examining biochemical indicators of liver function.

**Justification for the study**

According to a recent study by Owagboriaye *et al*. (2016), breathing in gasoline fumes may be detrimental to normal bodily physiology by increasing the levels of corticosterone, aldosterone, and serum lipid peroxidation. However, nothing is known about how exposure to petroleum fumes affects an animal's liver function. Because the liver is the primary organ that chemically changes every molecule that enters the body, it stands to reason that environmental pollutants may target the liver in particular, impairing its ability to perform its metabolic function. The purpose of this study was to elucidate the potential hepatotoxic and genotoxic consequences of albino rat inhalation exposure to petroleum fumes.

**Aim of the study**

This study aimed to evaluate the effects of *Bryophyllum pinnatum* extracts on liver enzyme function in male Wistar albino rats exposed to kerosene.

**Objectives of the study**

The specific objectives of the study were as follows:

- the effects of *Bryophyllum pinnatum* extracts on alanine transaminase levels in male Wistar albino rats exposed to kerosene
- the effects of *Bryophyllum pinnatum* extracts on aspartate transaminase levels in male Wistar albino rats exposed to kerosene
- the effects of *Bryophyllum pinnatum* extracts on alkaline phosphatase levels in male Wistar albino rats exposed to kerosene
- the effects of *Bryophyllum pinnatum* extracts on gamma-glutamyl transferase levels of male wistar albino rat exposed to kerosene.

## Method

### Experimental Design

The animals in the experiment were exposed to fumes from kerosene. For this study, we used 25 adult male albino rats (*Rattus norvegicus*) weighing 200–250 g who appeared to be in good health, as previously described (Uboh *et al.*, 2019). After two weeks of acclimatization in the experimental animal home at 25 ± 5°C and 65 ± 5% relative humidity, the rats were randomly assigned to five experimental treatment groups (A, B, C, D, and E), each consisting of five rats. Each animal had unrestricted access to food and water. The purpose of exposing the animals to petroleum products for five hours every day is to account for the fact that most employees at conventional or well-known gas stations work between four and five hours every day. The essence of exposing the animals to petroleum products for five hours daily is to accommodate the fact that most workers in standard or well-established petrol stations work for about four (4) to five (5) hours daily, although some also work up to eight hours daily.

### Experimental Animals

Twenty-five male albino rats aged 7 weeks (between 130 g-160 g body weight) were used in this study. Rats were procured and kept in the animal house of the Department of Physiology, University of Nigeria, Nsukka. The animals were allowed to acclimatize for 14 days under standard laboratory conditions with free access to commercial rat feed and water.

### Petroleum Products

Kerosene was purchased from the Nigerian National Petroleum Corporation (NNPC) Mega Filling Station in Enugu State, Nigeria.

### Petroleum inhalation protocol

The rats were individually kept in wooden cages (65 cm × 35 cm × 50cm) in a well-ventilated animal house and were allowed free access to clean drinking water and food. Groups B, C, D and E were exposed to kerosene fume for 5 hours at a room temperature for four weeks. Group A was housed separately in a section of the experimental animal house that was free from kerosene fume. The exposure chambers were equipped with an inhalation method of exposure. For each experimental treatment, 500 milliliters of kerosene were added to the exposure chambers containing the cages containing the treatments. During their respective daily hours, the rats in each treatment were permitted to breathe in the vapors evaporated from the cans. The rats were moved to the fume-free area of the experimental animal house in the presence of control treatment (Group A) after the exposure hours. The ethical criteria for animal experiments (regulation CEE 86/609) were followed when conducting the experiment.

### Collection of Plant Materials

Fresh leaves of *Bryophyllum pinnatum* were harvested from a local farm in Ngwo, Enugu South Local Government in Enugu State, Nigeria. The leaves were identified and authenticated by a Professor of Botany, Prof. C.S Eze, in the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology.

### Serum collection

The animals were anesthetized with chloroform and fasted for the whole night after being exposed to petroleum compounds by inhalation. After an ocular puncture near the eye, blood was drawn and drained into dry test tubes. After allowing the blood sample to coagulate for approximately 15 minutes, it was centrifuged. To assess specific liver function markers, serum was extracted from the clot using a Pasteur pipette and transferred into sterile sample tubes.

**Biochemical Analysis**

The colorimetric technique outlined by Schmidt and Schmidt (1971) was used to measure the serum amounts of AST and ALT levels. Gamma-glutamyl transferase was measured using Sherlock's method, whereas alkaline phosphatase (ALP) was measured using the Tietz (1991) method.

**Statistical Analysis**

The Statistical Package for Social Sciences (SPSS) for Windows (version 21) was used to perform all statistical analyses. The mean ± SEM was used to express the values of the measured parameters. The effects of *Bryophylum pinnatum* and kerosene exposure on the parameters under investigation were assessed using two-way analysis of variance (2-way ANOVA), and Duncan's multiple range tests were employed to separate the differences in means. At the 0.05 probability level, the significance test was considered.

**Results**

**Serum aspartate transaminase AST (mg/dl) count**

The baseline findings demonstrated no significant difference ($p>0.05$) between the experimental and control groups. Compared with the blank control (30.48 ± 0.01) at the end of week 2, the low-dose, medium-dose, and high-dose extracts (31.51 ± 0.11, 30.74 ± 0.32, and 28.85 ± 0.12) did not differ significantly ($p>0.05$), but the negative control varied significantly ($p<0.05$). With the exception of the high-dose (31.45 ± 0.12) extract, which exhibited a range comparable to the blank control, the treatment groups' AST value ranges after 28 days after exposure and treatment differed considerably from the blank control (30.48 ± 0.01). By week four, there was no discernible difference between the high-dose extract therapy and the blank control ($P>0.05$) (Table 1).

**Table 1:** Effect of *Bryophyllum pinnatum* extract on serum aspartate transaminase (AST) (mg/dl) of male wistar albino rats exposed to kerosene.

| Groups | Week 0 | Week 2 | Week 4 |
|---|---|---|---|
| A (Blank Control) | $32.51 \pm 0.01^{a1}$ | $30.48 \pm 0.00^{a1}$ | $30.50 \pm 0.00^{a1}$ |
| B (Negative Control) | $31.57 \pm 0.00^{a1}$ | $36.32 \pm 0.02^{b2}$ | $56.71 \pm 0.03^{b3}$ |
| C (Low-dose Extracts) | $37.62 \pm 0.01^{a1}$ | $31.51 \pm 0.11^{a2}$ | $51.79 \pm 0.021^{b3}$ |
| D (Medium-dose Extracts) | $27.51 \pm 0.00^{a1}$ | $30.74 \pm 0.32^{a2}$ | $45.75 \pm 0.01^{c3}$ |
| C (High-dose Extracts) | $28.44 \pm 0.00^{a1}$ | $28.85 \pm 0.12^{a1}$ | $31.45 \pm 0.02^{a2}$ |

*In a column, mean values with the same letter as the superscript are not significantly different ($p>0.05$). In a row, mean values with the same number as the superscript are not significantly different ($p>0.05$).*

**Serum alanine transaminase (ALT) (mg/dl)**

When comparing the low-, medium-, and high-dose treatment groups to the blank control (21.62 ± 0.01) at week 0, no significant change ($p>0.05$) was found. Although all treatment groups demonstrated a significant difference ($p<0.05$) at the end of week 2 when compared to the blank control (20.84 ± 0.00), the low-dose (39.30 ± 0.01), medium-dose (30.22 ± 0.1), and high-dose (32.92 ± 0.26) treatment groups did not differ significantly ($p>0.05$), suggesting that their ranges in the parameter (alanine transaminase) were similar. Comparing the treatment groups to the blank control (37.54 ± 0.03) at week 4, similar differences were seen, with a significant difference ($p<0.05$) (Table 2).

**Table 2:** Effect of *Bryophyllum pinnatum* extract on serum alanine transaminase (ALT) (mg/dl) of male wistar albino rats exposed to kerosene.

| Groups | Week 0 | Week 2 | Week 4 |
|---|---|---|---|
| A (Blank Control) | $21.62 \pm 0.01^{a1}$ | $20.84 \pm 0.00^{a1}$ | $37.54 \pm 0.03^{a2}$ |
| B (Negative Control) | $29.22 \pm 0.00^{a1}$ | $47.22 \pm 0.01^{b2}$ | $65.22 \pm 0.01^{b3}$ |
| C (Low-dose Extracts) | $17.30 \pm 0.02^{a1}$ | $39.30 \pm 0.01^{c2}$ | $44.30 \pm 0.00^{c3}$ |
| D (Medium-dose Extracts) | $18.22 \pm 0.02^{a1}$ | $30.22 \pm 0.1^{c2}$ | $36.22 \pm 0.21^{a3}$ |
| E (High-dose Extracts) | $17.92 \pm 0.03^{a1}$ | $32.92 \pm 0.26^{c2}$ | $38.92 \pm 0.01^{a2}$ |

*In a column, mean values with the same letter as the superscript are not significantly different ($p>0.05$).In a row, mean values with the same number as the superscript are not significantly different ($p>0.05$).*

**Serum alkaline phosphatase (ALP) (mg/dl)**

When comparing the treatment groups (low-dose, medium-dose, and high-dose extracts) to the control, no discernible change ($p>0.05$) at the baseline results (94.66 ± 0.01, 105.27 ± 0.12, and 97.63 ± 0.13). At the end of the second week, however, the alkaline phosphatase levels in the low-dose treatment group (128.26 ± 0.005) and the negative control group (138.93 ± 0.00) showed a substantial increase, whereas the medium-dose and high-dose treatment groups showed no significant change in value ranges. Comparing the treatment groups to the blank control at week four revealed a significant difference ($P<0.05$) (Table 3).

**Table 3:** Effect of *Bryophyllum pinnatum* extract on serum alkaline phosphatase (ALP) (mg/dl) of male wistar albino rats exposed to kerosene.

| Groups | Week 0 | Week 2 | Week 4 |
|---|---|---|---|
| A (Blank Control) | $95.11 \pm 0.12^{a1}$ | $102.34 \pm 0.02^{a1}$ | $95.27 \pm 0.02^{a1}$ |
| B (Negative Control) | $101.93 \pm 0.11^{a1}$ | $138.93 \pm 0.00^{b2}$ | $222.33 \pm 0.00^{b3}$ |
| C (Low-dose Extracts) | $94.66 \pm 0.01^{a1}$ | $128.26 \pm 0.005^{b2}$ | $123.43 \pm 0.18^{c2}$ |
| D (Medium-dose Extracts) | $105.27 \pm 0.12^{a1}$ | $103.27 \pm 0.002^{a1}$ | $121.65 \pm 0.01^{c2}$ |
| E (High-dose Extracts) | $97.63 \pm 0.13^{a1}$ | $104.63 \pm 0.001^{a1}$ | $102.40 \pm 0.21^{a1}$ |

*In a column, mean values with the same letter as the superscript are not significantly different ($p>0.05$). In a row, mean values with the same number as the superscript are not significantly different ($p>0.05$).*

**Serum gamma-glutamyl tranferase (GGT) (mg/dl)**

As the duration increased to 2 weeks, the medium-dose and high-dose treatments showed significant variations ($p<0.05$) in comparison to the blank control, but the baseline results show no significant difference ($p>0.05$) between the treatment groups C, D, and E (39.50 ± 0.04, 38.28 ± 0.03, and 38.74 ± 0.03) and the blank control (39.08 ± 0.03). In comparison with the control (35.45 ± 0.03), the negative control, low-dose, and medium-dose groups showed a significant difference ($p<0.05$) in the value at week 4. The low- and medium-dosage treatments often exhibit larger value fluctuations than the high-dose treatment, suggesting that the GGT value is negatively dose dependent (Table 4).

**Table 4:** Effect of *Bryophyllum pinnatum* extract on serum gamma-glutamyl tranferase (GGT) (mg/dl) of male albino rats exposed to kerosene.

| **Groups** | **Week 0** | **Week 2** | **Week 4** |
|---|---|---|---|
| A (Blank Control) | $39.08 \pm 0.03^{a1}$ | $36.23 \pm 0.02^{a1}$ | $35.45 \pm 0.03^{a1}$ |
| B (Negative Control) | $37.42 \pm 0.01^{a1}$ | $38.42 \pm 0.00^{b1}$ | $44.42 \pm 0.00^{b2}$ |
| C (Low-dose Extracts) | $39.50 \pm 0.04^{a1}$ | $36.50 \pm 0.01^{b1}$ | $40.50 \pm 0.10^{c1}$ |
| D (Medium-dose Extracts) | $38.28 \pm 0.03^{a1}$ | $31.28 \pm 0.00^{a2}$ | $41.28 \pm 0.02^{c1}$ |
| E (High-dose Extracts) | $38.74 \pm 0.03^{a1}$ | $31.74 \pm 0.02^{a2}$ | $34.74 \pm 0.11^{a2}$ |

*In a column, mean values with the same letter as the superscript are not significantly different ($p>0.05$). In a row, mean values with the same figure or number as the superscript are not significantly different. ($p>0.05$).*

**Discussion**

In line with the findings of Akinrinmade (2021), who conducted a study on the biochemical assessment of kerosene-contaminated diets on liver enzymes and found that a long-term exposure to kerosene showed a significant increase in aspartate transaminase (AST) levels, the experimental rats' serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) increased with an increase in the number of hours of kerosene fume exposure. This rise can be a sign of cellular leakage and breakdown in the ability of liver cell membranes to function properly. Kerosene exposure has been linked to negative effects on gamma-glutamyl transferase (GGT), a crucial liver enzyme.

In a study conducted by Joel (2019) on the influence of a kerosene-contaminated diet on liver function in Wistar albino rats, a notable elevation in the activity of the gamma-glutamyl transferase (GGT) enzyme was identified; this was in agreement with the findings of the present study. According to Sharma (2014), ALP is involved in the transport of metabolites across the membrane, the synthesis of certain enzymes and proteins, secretory activities, and glycogen metabolism. Elevated activity of such enzymes (AST, ALT and ALP) in serum was reported to reflect its increase rate of entrance into the serum from damaged liver cells (Ezejindu *et al.*, 2019). Furthermore, Wang (2024) found that a high ALT level was associated with liver damage or injury, making it a crucial component in the diagnosis and tracking of liver disorders and conditions. The aberrant dynamic characteristics of cellular membranes after exposure to the hydrocarbon components of gasoline fumes may be the cause of the

observed increase in liver enzyme activity in this study. According to reports, the metabolism of aliphatic and aromatic hydrocarbons, including toluene and benzene, which are the main components of petroleum fumes, as well as other xenobiotics, may cause reactive free radical species to alter the cell membrane (Sharma *et al*., 2018). Since benzene and toluene are found in kerosene, the rise in these enzyme activities observed in rats exposed to kerosene fume may be related to disruptions in metabolite transport or changes in the synthesis of specific enzymes, as in other hepatotoxic conditions (Sharma *et al*., 2018), which could compromise the integrity of the cell membrane.

**Conclusion**

The results of this study demonstrated the harmful effects of extended exposure to kerosene fumes on the liver health of albino rats. Exposure to petroleum products causes a disruption in the functional integrity of liver cells, as evidenced by the marked rise in liver enzyme activity, including AST, ALT, and ALP.

**Recommendations**

The study findings indicate that strict safety regulations must be put in place in places of employment where workers are exposed to petroleum vapors. Employers should guarantee the usage of personal protective equipment and provide sufficient ventilation systems to reduce the amount of hazardous vapors that employees inhale. Early diagnosis of liver damage requires routine monitoring of liver enzyme levels among individuals who handle petroleum products. Potential preventive measures and interventions to protect employees from the harmful consequences of hydrocarbon exposure require more investigation. By prioritizing occupational health and safety procedures first priority, we can reduce the dangers of extended exposure to petroleum products and protect the health of employees across a range of industries.

**Declaration**

We declare that the manuscript titled "Effect of *Bryophyllum Pinnatum* Extracts on Liver Enzyme Function of Male Wistar Albino Rats Exposed to Kerosene" is original and has not been published or submitted elsewhere for publication. All data were collected and analyzed following ethical guidelines for animal research. There are no conflicts of interest to declare, and all authors have approved the final version of the manuscript for submission.

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**Conflict of Interest:** This study focused on the hepatoprotective effects of *Bryophyllum pinnatum***.** The authors declare no conflicts of interest regarding the manuscript titled "Effect of *Bryophyllum Pinnatum* Extracts on Liver Enzyme Function of Male Wistar Albino Rats Exposed to Kerosene."

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