Global Journal of Medical and Health Science
Volume. 12, Number 2; April-June 2025;
ISSN: 2836-5577| Impact Factor: 10.26
https://zapjournals.com/Journals/index.php/Medical-Health
Published By: Zendo Academic Publishing

EFFECTS OF *Bryophyllum pinnatum* EXTRACTS ON LIVER ENZYME FUNCTION IN MALE WISTAR ALBINO RATS EXPOSED TO PREMIUM MOTOR SPIRIT (PMS)

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Article Info

Keywords: PMS, *Bryophyllum Pinnatum*, ALT, AST, ALP, GGT.

DOI
10.5281/zenodo.15520078

Abstract

Exposure to Premium Motor Spirit (PMS) can cause hepatotoxicity, which is typified by changes in liver enzyme levels. In this work, male wistar albino rats subjected to PMS were used to examine the hepatoprotective effects of *Bryophyllum pinnatum* extracts on liver enzyme activity. Five (5) groups of rats were created: low-dose extract, medium-dose extract, high-dose extract, blank control, and negative control. Every group—aside from the blank control group—was subjected to PMS inhalation. Following a 28-day experiment, liver enzyme activity (ALT, AST, ALP, and GGT) were measured. Exposure to PMS markedly elevated liver enzyme activity, suggesting hepatotoxicity. Hepatoprotection was suggested by the considerable reduction in liver enzyme activity following treatment with *Bryophyllum pinnatum* extract. Compared with the blank control (30.48 ± 0.01) in week 2, the results indicated a dosage-dependent decrease in AST enzyme activity, with the highest dose (29.85 ± 0.12) showing the most significant reduction ($p>0.05$), followed by the medium-dose at (31.74 ± 0.32). At week 4, the outcomes showed the same trend. These results suggest that *Bryophyllum pinnatum* extracts can be used as a medicinal agent because they can lessen PMS-induced hepatotoxicity.

Introduction

The organic material known as premium motor spirit (PMS) is composed of hydrogen and carbon molecules. Because the liver is involved in the metabolism of xenobiotics, prolonged exposure to premium motor spirits, especially through ingestion or inhalation, has been associated with systemic toxicity (Farombi *et al.*, 2007). Like other recognized xenobiotics, the chemical contaminants in PMS vapors can change into various metabolites within the body. Oxidative stress and changes in liver enzyme activity, such as increased levels of the indicators of liver injury alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase

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(ALP), are frequently linked to PMS-induced hepatotoxicity. An increase in AST/ALT and alkaline phosphatase levels is referred to as a mixed injury pattern (Vagvala, 2018).

Often referred to as "life plant" or "miracle leaf," *Bryophyllum pinnatum* is a well-known medicinal plant that is a member of the Crassulaceae family. Because of its abundance of bioactive components, *Bryophyllum pinnatum* is used in traditional medicine to treat a variety of conditions, including earaches, burns, abscesses, ulcers, insect bites, whitlow, diarrhea and lithiasis (Okwu, 2019). Triterpenes, steroids, phenanthrene, flavones, chalcones, taraxasterol, aurones, phenolic acid, caffeic acid, syringic acid, malic, oxalic, ferulic acid, and organic acid have all been found in *Bryophyllum pinnata* through phytochemical investigations. These phytochemicals have been correlated with antioxidant, anti-inflammatory, and hepatoprotective effects, and they may be useful in countering chemically induced liver damage (Anaga and Onehi, 2010).

Liver dysfunction and damage are associated with exposure to premium motor spirits (PMS) (Ogbonna *et al*., 2019). One of the most important indicators of liver health is liver enzyme levels. Liver function is frequently evaluated using key enzymes like gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The liver is necessary for metabolism, detoxification, and the production of critical proteins. By producing reactive oxygen species (ROS), which overpower the liver's natural antioxidant defenses, the hazardous chemicals in PMS harm liver cells and cause oxidative damage to cellular lipids, proteins, and DNA (Afolabi *et al*., 2018; Ejikeme *et al*., 2019). Oxidative stress is a major pathway leading to liver dysfunction and the elevation of liver enzymes in the blood (Olaniyi *et al*., 2020).

According to Misra *et al*. (2018), *Bryophyllum pinnatum*, a plant with traditional medicinal usage, may be able to treat liver damage because of its hepatoprotective qualities. Serious health consequences may result from liver injury, which is frequently indicated by increased liver enzyme levels (Guyton and Hall, 2019). Research has been conducted on how *Bryophyllum pinnatum* extract affects liver enzymes and how it can be used to cure liver damage.

Aim of the Study

This study aimed to evaluate the effects of *Bryophyllum pinnatum* extracts on the liver enzyme function of male wistar albino rats exposed to premium motor spirit (PMS).

Objectives of the Study

The objectives of this study were to;

- the effects of *Bryophyllum pinnatum* extract on aspartate transaminase (AT) levels in male Wistar albino rats exposed to motor spirit
- the effects of *Bryophyllum pinnatum* extract on Alkaline phosphatase levels in male Wistar albino rats exposed to motor spirit
- the effects of *Bryophyllum pinnatum* extract on the levels of alanine transaminase in male Wistar albino rats exposed to motor spirit and
- Effects of *Bryophyllum pinnatum* extract on Gamma-glutamytransferase levels in male Wistar albino rats exposed to motor spirit

Methods

Experimental Animals

Twenty-five male albino rats weighing between 130 and 160 grams at 7 weeks of age were used in this study. The rats were bought and housed in the animal house at the Faculty of Applied Natural Science, Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology.

The rats were allowed unlimited access to commercial rat food and water for 7 days as they were used for the typical laboratory conditions.

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 a week 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. Animal investigations were conducted according to standard laboratory procedures. 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.

Collection of Plant Materials

Fresh *Bryophyllum pinnatum* leaves were collected from a nearby farm in Ngwo, Enugu South Local Government and Enugu State, Nigeria. Prof. C.S. Eze, a botany professor in the Department of Applied Biology and Biotechnology at Enugu State University of Science and Technology, identified and verified the leaves.

Petroleum Products

Premium motor spirits were purchased from the Nigerian National Petroleum Corporation (NNPC) Mega Filling Station, Enugu State, Nigeria.

Serum sample 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. Two portions of the blood sample were separated. 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 Rietman and Frankel (1957) was used to measure the serum concentrations of aspartate transaminase (AST) and alanine transaminase (ALT). The Bessey (1946) method was used to determine alkaline phosphatase (ALP), and the Szasz (1969) method to measure gamma-glutamyl transferase.

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 various petroleum products on the parameters under study were ascertained 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 AST (mg/dl)

Compared with the control, which was at 32.51 ± 0.01 mg/dl, the treatment groups B, C, D, and E (31.57 ± 0.01, 31.62 ± 0.01, 28.51 ± 0.04, and 29.54 ± 0.00) did not significantly differ ($p>0.05$) from the baseline result, which was at week 0. In comparison to the baseline and control groups, the negative control, medium-dose, and high-dose treatments exhibited significant differences ($P<0.05$) at week 2, but the low-dose treatment did not show any significant differences from the blank control (30.48 ± 0.01).

Compared with the control group, all treatment groups exhibited a significant difference in aspartate transaminase levels at the end of the experiment, which was four weeks long (table 1).

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

Groups	**Week 0**	**Week 2**	**Week 4**
A (Blank Control)	32.51 ± 0.01^{a1}	30.48 ± 0.01^{a1}	30.50 ± 0.00^{a1}
B (Negative Control)	31.57 ± 0.00^{a1}	36.32 ± 0.03^{b2}	56.70 ± 0.03^{b3}
C (Low-dose Extracts)	31.62 ± 0.01^{a1}	37.41 ± 0.10^{b1}	50.79 ± 0.021^{c2}
D (Medium-dose Extracts)	28.51 ± 0.04^{a1}	31.74 ± 0.32^{a2}	48.75 ± 0.01^{c2}
C (High-dose Extracts)	29.54 ± 0.00^{a1}	29.85 ± 0.12^{a2}	31.45 ± 0.02^{a3}

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) of male wistar albino rats exposed to Premium motor spirit (PMS) was presented

When ALT levels were compared with the blank control (20.62 ± 0.01) at week 0, no discernible difference ($p>0.05$) between any of the treatment groups. At week 2, the results showed no significant difference ($p>0.05$) between treatment groups C, D, and E (39.32 ± 0.02, 30.22 ± 0.10, and 32.92 ± 0.20), with only minor variations in ALT levels noted in each treatment group. In contrast to the control, all treatment groups displayed a significant difference ($p>0.05$) at week four (table 2).

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

Groups	Week 0	Week 2	Week 4
A (Blank Control)	20.62 ± 0.01^{a1}	20.84 ± 0.02^{a1}	21.50 ± 0.02^{a1}
B (Negative Control)	20.22 ± 0.01^{a1}	46.32 ± 0.04^{b2}	61.20 ± 0.04^{b3}
C (Low-dose Extracts)	19.30 ± 0.02^{a1}	39.32 ± 0.02^{c2}	44.30 ± 0.10^{c2}
D (Medium-dose Extracts)	19.22 ± 0.02^{a1}	30.22 ± 0.10^{d2}	36.22 ± 0.20^{d3}
C (High-dose Extracts)	19.92 ± 0.03^{a1}	32.92 ± 0.20^{d2}	25.90 ± 0.02^{e3}

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)

Alkaline phosphatase (ALP) values in treatment groups B, C, D, and E (105.93 ± 0.61, 94.66 ± 0.41, 105.27 ± 5.12, and 97.63 ± 5.10) at week 0 did not change significantly (p>0.05) from the control value.

After two weeks, the medium-dose extract group (113.0.7 ± 0.52) demonstrated a significant difference (P<0.05) from the baseline results, while the low-dose and high-dose treatment groups (132.46 ± 0.45 and 114.73 ± 0.70) showed no significant difference (P>0.05) from the baseline result at week 0. There was no discernible change (P>0.05) in the ALP values between the treatment groups at the conclusion of the experiment (week 4) (table 3).

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

Groups	Week 0	Week 2	Week 4
A (Blank Control)	95.11 ± 0.52^{a1}	100.34 ± 0.62^{a1}	95.27 ± 2.02^{a1}
B (Negative Control)	105.93 ± 0.61^{a1}	138.83 ± 0.51^{b2}	222.33 ± 3.50^{b2}
C (Low-dose Extracts)	94.66 ± 0.41^{a1}	132.46 ± 0.45^{b1}	133.43 ± 2.18^{c2}
D (Medium-dose Extracts)	105.27 ± 5.12^{a1}	113.07 ± 0.52^{c2}	123.65 ± 1.21^{c2}
C (High-dose Extracts)	97.63 ± 5.10^{a1}	114.73 ± 0.70^{c1}	134.40 ± 3.20^{c2}

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)

The baseline results showed no significant change (P>0.05) between treatment groups C, D, and E (39.50 ± 0.04, 44.28 ± 0.06, and 42.74 ± 0.10) and the blank control (39.08 ± 0.02). After two weeks, the serum GGT levels of the test groups did not differ significantly (p>0.05) from those of the blank control (38.23 ± 0.02), with the exception of the negative control and medium-dose extract group. Compared with the blank control (36.45 ± 0.03) at the end of week 4, all treatment groups, with the exception of the negative control group (table 4), showed a significant difference (p<0.05) in blood GGT levels.

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

Groups	Week 0	Week 2	Week 4
A (Blank Control)	39.08 ± 0.02^{a1}	38.23 ± 0.02^{a1}	36.45 ± 0.03^{a1}
B (Negative Control)	42.42 ± 0.04^{a1}	51.62 ± 0.02^{b2}	55.42 ± 0.00^{b2}
C (Low-dose Extracts)	39.50 ± 0.04^{a1}	36.50 ± 0.02^{a1}	35.50 ± 0.10^{a1}
D (Medium-dose Extracts)	44.28 ± 0.06^{a1}	35.28 ± 0.00^{a2}	34.28 ± 0.02^{a2}
C (High-dose Extracts)	42.74 ± 0.10^{a1}	37.74 ± 0.02^{a1}	34.74 ± 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

The blood AST, ALT, and ALP levels of the experimental rats increased as the number of hours they spent exposed to premium motor spirit fumes each day increased. This rise can be a sign of cellular leakage and breakdown in the ability of liver cell membranes to function properly. According to Abdel-Baset *et al.* (2017), these enzyme groups are sensitive indicators of liver cell damage. According to previous reports, the increased rate of entry of injured liver cells into the serum is reflected by the elevated activity of these enzymes (AST, ALT, and ALP) in serum (Ezejindu *et al.,* 2016).

Similar to Adedeji (2020) research on the hepatoprotective effects of *Bryophyllum pinnatum* against premium motor spirit-induced liver damage in rats, the results of this study showed that the baseline results of all treatment groups B, C, D, and E (Negative control, low-dose, medium-dose, and high-dose extracts) had no significant difference when compared with the control.

The aberrant dynamic characteristics of cellular membranes after exposure to hydrocarbon components of premium motor spirit fumes may be the cause of the observed increase in liver enzyme activity in this investigation. Since benzene and toluene are found in gasoline, the increase in these enzyme activities observed in rats exposed to gasoline fumes may be related to disruptions in metabolite transport or changes in the synthesis of specific enzymes, as observed under other hepatotoxic conditions (Sharma *et al.,* 2016). This could compromise the integrity of the cell membrane. As a result, there has been an impact on the liver's ability to

function normally has been affected, which is crucial for the elimination and degradation of harmful or potentially harmful substances from the blood (Miller and Harley, 2018).

Conclusion

The disruption in liver tissue may also have been caused by the study's finding of a significant increase in the liver enzymes AST, ALT, ALP, and GGT. Therefore, there is a chance that regular exposure to gasoline fumes could result in liver tissue malformation and impaired liver physiology, which could lead to illnesses. Therefore, it is best to protect Nigerians from exposure to gasoline directly.

Recommendations

The study's conclusions suggest that those who frequently come into contact with petroleum products—particularly gasoline fumes—should take appropriate safety measures to reduce their exposure. This involves reducing the amount of hazardous vapors inhaled by wearing protective gear like respirators or masks. By ensuring sufficient ventilation systems and routine health monitoring, employers in petroleum-related sectors should also prioritize their employees' safety and health.

Additionally, more thorough research is required to examine the long-term health impacts of gasoline fume exposure. Research on various demographics and exposure levels can shed important light on the possible dangers of extended interaction with petroleum-based goods. Campaigns to raise public awareness of the risks posed by gasoline fumes and the significance of taking preventative action should also be undertaken.

The findings of this study demonstrate the harmful impact of exposure to gasoline fumes on liver function and general health. The negative effects of petroleum products on people and the environment can be reduced by implementing preventive measures into place and encouraging safety precautions. To protect the health of employees and the public at large, health and safety must be given top priority in industries that handle hazardous materials.

Declaration

We declare that the manuscript titled "Effect of *Bryophyllum Pinnatum* Extracts on Liver Enzyme Function of Male Wistar Albino Rats Exposed to premium motor spirit (PMS)" 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.

Acknowledgment

The authors sincerely appreciate the support of the department of Applied Biology and Biotechnology, Enugu State University of Science and Technology (ESUT) for providing the necessary facilities for this study. We also appreciate the effort of Mr. Aneke Rowland Jachike for providing technical support. Special thanks to the supervisor, Dr. Cyril Onyekachi Edoga for his valuable insights and contributions. We thank our families and friends for their encouragement throughout the research period.

Funding

The research was fully funded and was carried out by the authors.

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 premium motor spirit (PMS)."

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