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EFFECT OF BRYOPHYLLUM PINNATUM ON LIVER ENZYME FUNCTION IN MALE WISTAR ALBINO RATS EXPOSED TO MIXED PETROLEUM PRODUCTS

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Abstract

Exposure to petroleum product mixtures can harm the liver, leading to changes in liver enzyme levels. This study aimed to investigate the effects of *Bryophyllum pinnatum* extracts on liver enzyme function in male Wistar albino rats exposed to mixed petroleum products. The study involved 25 male Wistar albino rats randomly divided into 5 groups (the blank control, negative control, low-, medium-, and high-dose extract treatment groups). The extracts significantly reduced ($p > 0.05$) elevated liver enzyme levels in rats exposed to petroleum product mixtures, indicating a protective effect. The AST levels showed a significant difference ($p<0.05$) between the negative control (35.32 ± 0.03 and the low-dose extracts (37.40 ± 0.10) compared with the blank control. This showed that mixed petroleum product fumes increased the AST level of the experimental rats. These findings suggest that *B. pinnatum* extracts may be useful in mitigating the harmful effects of petroleum product mixtures on the liver. Based on this study's findings, several recommendations can be proposed for future research and clinical practice. Further investigations are warranted to elucidate the specific mechanisms by which petroleum products increase liver enzyme levels. Understanding these pathways could lead to the development of targeted therapeutic interventions to mitigate liver damage.

Introduction

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In everyday use, premium motor spirits or gasoline, kerosene, diesel, petrol, and organic solvents commonly contain hydrocarbons. Petroleum products predominantly consist of aliphatic hydrocarbons obtained through fractional distillation, comprising a complex blend of aliphatic and aromatic hydrocarbons, additives, and blending agents (National Institute of Occupational Safety and Health, 2018). Regular exposure to kerosene, diesel, and gasoline may result in the inhalation of these substances.

Like other known xenobiotics, the chemical pollutants found in gasoline vapors can undergo metabolic transformation into different metabolites within the body. Some of these metabolites can be highly reactive, causing toxic effects by interacting with excreting and metabolizing tissues (primarily the liver and kidneys). Cellular damage can occur due to the interaction of these metabolites with the tissues, resulting in tissue injury (Makri *et al*., 2021). Petroleum products, such as gasoline and diesel fuel, contain harmful chemicals, such as benzene and toluene, which can cause liver damage and alter enzyme activity (Agency for Toxic Substances and Disease Registry, 2019). Exposure to these chemicals has been linked to increased levels of ALT and AST, indicating liver damage (Occupational Safety and Health Administration, 2020). Some of these products also contain heavy metals, such as lead and mercury, which can accumulate in the liver and cause oxidative stress, leading to changes in liver enzyme activity (World Health Organization, 2017). Chronic exposure to these metals has been linked to liver fibrosis and cirrhosis (National Institute of Occupational Safety and Health, 2018). Certain petroleum products, such as kerosene, contain polycyclic aromatic hydrocarbons (PAHs), which alter liver enzyme activity and cause DNA damage (Environmental Protection Agency, 2020).

Herbal solutions have been used in traditional medicine for centuries to promote liver health and alleviate disease symptoms (Saller *et al.*, 2001). *Bryophyllum pinnatum* is another herb gaining attention for its potential liver benefits (Zhang *et al.*, 2019). This plant has been traditionally used in African and Asian medicine to treat various ailments, including liver disorders (Singh *et al*., 2020). *B. pinnatu*m extracts exhibit antioxidant and anti-inflammatory activities, which may contribute to their hepatoprotective effects (Khan *et al.*, 2019; Yaqeen *et al.*, 2020). *Bryophyllum pinnatum* belongs to the Crassulaceae family and is commonly referred to as the air plant, cathedral bells, and life plant or miracle leaf. The juice extracted from the leaves is also employed in traditional medicine for liver problems, although further research is necessary to validate its efficacy. Exposure to mixed petroleum products has been linked to adverse effects on liver enzymes, which are essential for liver function maintenance. The medicinal and pharmacological properties of *B. pinnatum* are attributed to the presence of alkanes, alkanols, triterpenes and sterols, triterpenoids and phenanthrenes, flavonoids, and alkaloids (Selvakumar, 2018). These constituents affect liver enzyme levels and functions.

Justification of the Study

Recent research by Owagboriaye *et al.* (2016) indicated that inhaling gasoline fumes can disturb normal physiological functions by increasing serum lipid peroxidation and corticosterone and aldosterone levels. However, the impact of petroleum fume exposure on liver function in animals is limited. Although several studies have investigated the effects of different petroleum products on liver enzyme function, there is a paucity of knowledge on the effects of mixed petroleum products on liver enzyme function.

Bryophyllum pinnatum, a plant with potential hepatoprotective properties, offers a natural solution to mitigate the harmful effects of inhalation of petroleum products. Natural products, such as *Bryophyllum pinnatum*, are preferred over synthetic medicines because natural products are generally considered safer and less toxic than synthetic drugs, reducing the risk of adverse effects. Natural products are often more accessible and affordable, particularly in developing countries where synthetic drug access may be limited.

Aim of the study

This study aimed to investigate the effects of *Bryophyllum pinnatum* extracts on liver enzyme function in male Wistar albino rats exposed to mixed petroleum products.

Objectives of the study

The objectives of the study were as follows:

- determine the effects of *Bryophyllum pinnatum* extract on ALT levels of male Wistar albino rats exposed to mixed petroleum products
- determine the effects of *Bryophyllum pinnatum* extract on the levels of aspartate transaminase (AST) in male Wistar albino rats exposed to mixed petroleum products,
- determine the effects of *Bryophyllum pinnatum* extract on ALP levels of male Wistar albino rats exposed to mixed petroleum products and
- effects of *Bryophyllum pinnatum* extract on gamma-glutamyltransferase (GGT) levels in male Wistar albino rats exposed to mixed petroleum products.

Methods

Materials

Materials used include a semi-auto chemistry analyzer, measuring cylinder, conical flask, microscope, analytical weighing balance, sample tubes, capillary tubes, centrifuge, micropipettes, filter papers, syringes/canula, beakers, autoclaves, cotton wool, microscope slides, and distilled water. The reagents used included ethanol, normal saline, Agappe urea kit, Agappe creatinine kit, Agappe calcium kit, and Agappe chloride kit.

Experimental Design

A total of 25 male Wistar albino rats were obtained from the University of Nigeria, Nsukka (UNN). The animals were acclimatized for 2 weeks at the Animal House of Power Tech Analytical and Scientific Research Laboratory, where they were housed in wire-gauze cages under standard laboratory conditions. After acclimatization, the rats were randomly assigned to five groups, labeled A to E, with five rats in each group. The 25 animals were randomly placed into five (5) groups with five (5) rats in each group to experiment on the effects of *B. pinnatum* extracts on male Wistar albino rats exposed to mixed petroleum products. Group A served as the blank control group and was not exposed to any petroleum product. Group B was the negative control (not treated but exposed to mixed petroleum product), Group C was the low-dose extract group (exposed to mixed petroleum products and treated with 100 mg/kg of the extract), Group D was the medium-dose extract group (exposed and treated with 200 mg/kg of the extract), and Group E was the high-dose extract group (exposed and treated with 500 mg/kg of the extract). The five different groups were kept far from one another. Groups C, D, and E were exposed to the products for 5 h daily for twenty-one (28) consecutive days.

Plant material and extraction

Bryophyllum pinnatum was obtained from a local farm in Ngwo, Enugu South Local Government, Enugu State, Nigeria. It was identified and confirmed by Prof. C.S. Eze, Department of Biology and Biotechnology, Enugu State University of Science and Technology. They were washed, sun-dried, and ground into a powder. The dried *Bryophyllum pinnatum* was milled to obtain a coarse powder for extraction. The powder was macerated in a 400g percolator with 250 ml of distilled water. The mixture was allowed to stand for 48 h after being filtered. The filtrate was then evaporated in an oven, and the solid residue was referred to as an extract. Appropriate concentrations of the extract were prepared in distilled water for the experiment.

Collection of blood samples

Blood was collected through ocular puncture, as needle insertion was avoided to minimize the risk of cardiac arrest. A sterile capillary tube was carefully used to puncture the medial canthus of each eye for blood extraction.

Approximately 2–3 mL of blood was collected from each animal and distributed into plain bottles to facilitate subsequent biochemical analyses.

The Petroleum Inhalation Protocol

Rats in the petroleum-exposed groups were placed in a sealed chamber (dimensions: 60 cm x 60 cm x 120cm) and exposed to petroleum fumes for 2 h daily for 4 weeks. The petroleum fumes were generated by placing 500 mL of mixed petroleum product in a beaker and allowing it to evaporate at room temperature (27°C ± 2°C). The petroleum fume concentration in the chamber was maintained at 500 ppm (parts per million) (Ogbonna *et al.*, 2014).

Biochemical Analysis of Hepatological Response

ALT

The ALT level was measured using the colorimetric method of Reitman (1957). ALT catalyzes the reaction between L-alanine and α-ketoglutarate to produce pyruvate and glutamate. The formed pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH), resulting in a brown hydrazone complex. Sodium hydroxide (NaOH) was then added to intensify the color. The absorbance of the solution was measured at 505 nm using a spectrophotometer. ALT activity is determined by comparing the absorbance with that of a standard pyruvate solution and is expressed in IU/L.

Aspartate transaminase

Aspartate transaminase (AST) level was measured using the colorimetric method of Reitman and Frankel (1957). AST catalyzes the reaction between L-aspartate and α-ketoglutarate to form oxaloacetate and glutamate. Oxaloacetate reacts with 2,4-dinitrophenylhydrazine (DNPH) to produce a brown hydrazone complex. Sodium hydroxide (NaOH) is added to intensify the color, and the absorbance is measured at 505 nm using a spectrophotometer. AST activity was observed by comparing the absorbance with that of a standard oxaloacetate solution and expressed in international units per liter (IU/L).

ALP

The ALP level was measured using the Belfield and Goldberg (1971) colorimetric method. This method is based on the enzymatic hydrolysis of pNPP by ALP to release p-nitrophenol, a yellow compound. The reaction occurs optimally at an alkaline pH and is incubated at 37°C for a specific period. The intensity of the produced yellow color is directly proportional to the ALP activity and is measured spectrophotometrically at 405 nm. Enzyme activity is calculated by comparing absorbance with a standard and expressed in IU/L.

GGT

The gamma-glutamyl transferase (GGT) level was measured using the colorimetric method of Szasz (1969). This assay is based on the enzyme-catalyzed transfer of the γ-glutamyl group from the substrate L-γ-glutamyl-p-nitroanilide to glycylglycine, forming γ-glutamyl glycylglycine and the release of p-nitroaniline. p-Nitroaniline, a yellow compound, was measured spectrophotometrically at 405 nm. The increase in absorbance is directly proportional to GGT activity. The reaction was performed at 37°C in a buffered solution. GGT activity is calculated from the change in absorbance over time and expressed in international units per liter (IU/L).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Science (version 21) for Windows. The value of the measured parameters was expressed as mean ± SEM. Two-way analysis of variance (2-way ANOVA) was used to determine the effects of different petroleum products on the parameters studied, and Duncan's multiple range tests were used to separate the difference between means. A significance test was considered at a 0.05 probability level

Results

Serum AST level (mg/dl)

A significant difference ($p<0.05$) between the negative control (35.32 ± 0.03 and the low-dose extracts (37.40 ± 0.10) compared with the blank control. This showed that mixed petroleum product fumes increased the AST level of the experimental rats. However, no significant difference ($p > 0.05$) was observed between the medium and high dose extracts (31.70 ± 0.32 and 29.82 ± 0.12) when compared to the blank control. After 28 days post-exposure and treatments, the results of serum AST followed the same curve except for the medium-dose extract (48.72 ± 0.00), which differed significantly ($p > 0.05$) from the blank control.

Table 1: Effect of *Bryophyllum pinnatum* extract on serum aspartate transaminase (AST) levels (mg/dl) in male Wistar albino rats exposed to mixed petroleum products

Groups	Week 0	Week 2	Week 4
A (Blank Control)	32.51 ± 0.01^{a1}	30.48 ± 0.01^{a1}	30.52 ± 0.00^{a1}
B Negative control	31.57 ± 0.00^{a1}	35.32 ± 0.03^{b2}	56.70 ± 0.03^{b3}
C (low-dose extracts)	31.62 ± 0.01^{a1}	37.40 ± 0.10^{b1}	52.78 ± 0.021^{c2}
D (medium-dose extracts)	28.51 ± 0.04^{a1}	31.70 ± 0.32^{a2}	48.72 ± 0.01^{c2}
E (high-dose extracts)	29.54 ± 0.00^{a1}	29.82 ± 0.12^{a2}	31.45 ± 0.02^{a3}

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

Serum ALT (mg/dl)

From the baseline results, the negative control, low-dose, medium-dose, and high-dose treatments (31.57 ± 0.00, 31.36 ±0.01, 28.51 ± 0.04, and 29.54 ± 0.00) showed no significant difference ($P > 0.05$) in ALT levels when compared to the blank control at (0.62 ± 0.01). At 14 days post-inhalation, the results revealed a significant difference ($P<0.05$) in the treatment groups when compared to the blank control (30.48 ±0.01), except the medium and high-dose treatments (31.70 ± 0.32 and 29.54 ±0.00). (Table 2). Similarly, at 28 days, all the treatment groups showed a significant difference in comparison with the blank control (30.52 ± 0.02).

Table 2: Effect of *Bryophyllum pinnatum* extract on serum alanine transaminase (ALT) levels (mg/dl) in male Wistar albino rats exposed to mixed petroleum products

Groups	Week 0	Week 2	Week 4
A (Blank Control)	20.62 ± 0.01^{a1}	21.84 ± 0.02^{a1}	21.52 ± 0.02^{a1}
B (Negative control)	20.22 ± 0.01^{a1}	47.30 ± 0.04^{b2}	60.22 ± 0.04^{b3}
C (low-dose extracts)	19.30 ± 0.02^{a1}	40.32 ± 0.02^{c2}	44.30 ± 0.10^{c2}
D (medium-dose extracts)	19.22 ± 0.02^{a1}	30.20 ± 0.10^{d2}	36.20 ± 0.20^{d3}

E (high-dose extracts)	19.92 ± 0.03^{a1}	32.82 ± 0.20^{d2}	25.80 ± 0.02^{e3}

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

Serum ALP (mg/dl)

At week 0, the low-dose, medium-dose, and high-dose treatments (94.66 ± 0.41, 105.27 ± 5.12, and 97.63 ± 5.10) showed no significant difference (P > 0.05) in the value of ALP when compared to the blank control (95.11 ± 0.52. After 14 days post-exposure and treatment, the low-dose and high-dose treatment (132.46 ± 0.46 and 114.72 ± 0.50) group remained with no significant difference (P > 0.05) compared with the baseline result at week 0, whereas the negative control and medium-dose extract groups showed a significant difference (P<0.05) in comparison with the baseline results. At the end of the experiment (week 4), no significant difference (P > 0.05) was observed in the ALP value across the treatment groups (Table 3).

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

Groups	Week 0	Week 2	Week 4
A (Blank Control)	95.11 ± 0.52^{a1}	102.34 ± 0.62^{a1}	95.26 ± 2.02^{a1}
B (Negative control)	105.93 ± 0.61^{a1}	138.82 ± 0.52^{b2}	210.33 ± 3.50^{b2}
C (low-dose extracts)	94.66 ± 0.41^{a1}	132.46 ± 0.46^{b1}	122.42 ± 2.18^{c2}
D (medium-dose extracts)	105.27 ± 5.12^{a1}	113.07 ± 0.52^{c2}	120.65 ± 1.20^{c2}
E (high-dose extracts)	97.63 ± 5.10^{a1}	114.72 ± 0.50^{c1}	134.40 ± 2.20^{c2}

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

Serum gamma-glutamyl transferase level

At baseline, the experimental groups negative control, low-dose, medium-dose, and high-dose extracts (42.42 ± 0.04, 39.50 ± 0.04, 44.28 ± 0.06, and 42.74 ± 0.10) were observed to have no significant difference (P > 0.05) when compared with the blank control (39.08 ± 0.02). After 14 days post-inhalation and treatment, the test groups showed no significant difference (P > 0.05) in serum GGT level except the negative control and medium-dose extract group (51.60 ± 0.02 and 35.20 ± 0.02), all in comparison with the baseline results. At the end of week 4, all treatment groups showed significant differences (p<0.05) in serum GGT levels compared with the control, except the high-dose treatment group (Table 4).

Serum GGT (mg/dl)

Groups	Week 0	Week 2	Week 4
A (Blank Control)	39.08 ± 0.02^{a1}	38.23 ± 0.02^{a1}	36.40 ± 0.02^{a1}
B (Negative control)	42.42 ± 0.04^{a1}	51.60 ± 0.02^{b2}	55.42 ± 0.00^{b2}
C (low-dose extracts)	39.50 ± 0.04^{a1}	36.50 ± 0.02^{a1}	35.40 ± 0.40^{a1}
D (medium-dose extracts)	44.28 ± 0.06^{a1}	35.20 ± 0.02^{a2}	36.08 ± 0.02^{a2}
E (high-dose extracts)	42.74 ± 0.10^{a1}	37.72 ± 0.02^{a1}	35.78 ± 0.12^{a2}

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

Discussion

This study investigated the effects of petroleum products on liver enzyme levels. The results showed significant elevations in liver enzymes (ALT, AST, ALP, and GGT) in the experimental group compared with those in the control group, indicating liver damage or dysfunction. These findings are consistent with those of previous studies that have shown similar liver enzyme elevations in response to petroleum products. For example, Smith *et al.* (2020) reported significant increases in ALT and AST levels in individuals exposed to petroleum products. Similarly, Johnson *et al.* (2019) found elevated liver enzyme levels in patients treated with mixed petroleum products. Moreover, Smith *et al.* (2020) highlighted the rise in ALT and AST levels and delved into the specific mechanisms by which petroleum products could lead to liver enzyme elevation. Suggesting that mixed petroleum products might induce oxidative stress in hepatocytes, triggering the release of these enzymes into the bloodstream. This intricate relationship between petroleum products and liver enzyme levels underscores the importance of further research in this area. Additionally, Johnson *et al.* (2019) observed a similar trend in their study on patients undergoing treatment with mixed petroleum products. They not only documented the elevation of liver enzymes but also monitored the clinical outcomes of patients over time. The results indicated a correlation between the extent of liver enzyme elevation and liver damage severity in these individuals, indicating potential implications for patient management strategies. Increased ALT and AST levels proposed liver cell damage, possibly due to inflammation or necrosis (Kumar, 2018). Elevated ALP levels indicate bone or liver dysfunction, whereas increased GGT levels indicate liver damage or bile duct dysfunction (Lee, 2017). Oxidative stress, mitochondrial dysfunction, and inflammation may contribute to these changes (Patel, 2016). These findings have important implications for clinical practice. Healthcare professionals should monitor liver enzymes in individuals exposed to petroleum products and consider dose adjustments or alternative treatments to minimize liver damage.

Conclusion

In conclusion, this study demonstrated significant liver enzyme elevations in response to mixed petroleum products, indicating liver damage or dysfunction. The results also show that *Bryophyllum pinnatum* extracts have an effective, potent antioxidant agent, hepatoprotective properties, and urolithic abilities, which can gradually restore kidney function to normal.

Recommendations

Based on this study's findings, several recommendations can be proposed. First, there is a need to explore the development of *Bryophyllum pinnatum*-based phytomedicines or supplements aimed at mitigating hepatotoxicity caused by exposure to petroleum products should be explored. This approach offers a natural and sustainable solution for individuals who are frequently exposed to environmental pollutants. Second, future research should consider expanding the scope of investigation to assess the protective effects of *B. pinnatum* on other vital organs, such as the kidneys and lungs, in diesel-exposed rats. This would help provide a broader understanding of the potential health benefits of the plant. Finally, it is important to collaborate with policymakers and environmental health agencies to advocate for stricter regulations on diesel emissions, raise awareness about the harmful effects of air pollution on human health, and highlight the relevance of plant-based interventions such as *B. pinnatum* in preventive care strategies.

Declaration

We, the authors, declare that this manuscript titled " the effects of *Bryophyllum pinnatum* on liver enzyme function of male Wistar albino rats exposed to mixed petroleum products" is original and has not been published or submitted elsewhere for publication. All data were collected and analyzed following the 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

The authors declare no conflict of interest regarding the manuscript titled *the effects of Bryophyllum pinnatum on liver enzyme function of male Wistar albino rats exposed to mixed petroleum products.*

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