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An In Vivo Study Of The Hepatoprotective Property Of *Bryophyllum pinnatum* Aqueous Leaf Extract On Alcohol Induced Liver Damage In Albino Wistar Rats

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ABSTRACT: Background: Plants and plant-derived substances have been utilized as herbal remedies to prevent, alleviate, or reverse various abnormalities. Pharmaceuticals such as opium, aspirin, digitalis, and quinine have a long history of being used as herbal remedies and are currently employed by physicians. Additionally, contemporary medicine often incorporates active chemicals derived from plants, with approximately 80% of these compounds showing a strong connection between their traditional use and modern therapeutic applications. *Bryophyllum pinnatum* leaf is known to contain bioactive components with antioxidant properties and potential pharmacological activity. Objectives: This study aimed to investigate the effects of an aqueous leaf extract of *Bryophyllum pinnatum* on serum liver enzymes in albino Wistar rats following alcohol-induced liver damage.
Methodology: Fifteen experimental albino Wistar rats weighing 110-223 g were divided into three groups of five rats each. Group 1 served as the standard control and received only rat food and water. Group 2 was administered alcohol at a rate of 1.5 ml/kg body weight, while Group 3 (the extract group) received a combination of alcohol (1.5 ml/kg body weight) and an aqueous leaf extract of *Bryophyllum pinnatum* (200 mg/kg body weight). After the treatment period, the experimental rats were sacrificed, and serum liver enzyme levels were measured. The laboratory results were analyzed using one-way ANOVA, followed by post-hoc testing.
Findings: Rats treated with alcohol showed a statistically significant decrease in the levels of tissue damage indicators, specifically AST and ALP, compared to the control group receiving a regular diet. However, no statistically significant difference in the reduction of ALT activity was observed between the experimental group and the conventional control group (Group 1). Notably, Group 3, which received post-treatment with the aqueous leaf extract at a dosage of 200 kg/kg body weight, exhibited a statistically significant reduction ($p < 0.05$) in AST and ALP levels compared to the rats in the standard control group.
Conclusion: The aqueous extract of *Bryophyllum pinnatum* leaves demonstrated a remarkable ability to protect the liver from toxicity-induced damage. Consequently, further research on the isolation and purification of active phytochemical compounds from the plant for potential use in the pharmacological industry is recommended.

INTRODUCTION

The perennial herb *Bryophyllum pinnatum* (seen in Figure 1), which belongs to the family of Crassulaceae and grows 3-5 feet tall, is commonly used traditionally as a medicine. Typically, this plant is an indigenous and exotic medicinal plant popularly used by

traditional healers to treat a variety of diseases, such as diabetes, liver diseases, renal calculi, hypertension, asthma, colds, abscesses, and bleeding disorders, amongst many others. Despite being native to Madagascar, it has spread to several other regions, including the temperate parts of Asia, Australia, and New Zealand. *Bryophyllum pinnatum* is often referred to as "Never die" in Nigeria. However, other common names may include "life plant" and "air plant" (Smith-Halle et al., 2012). The *Bryophyllum pinnatum* leaves are 10-20 cm long, opposite, and decussate, with the lower leaves being simple while the upper leaves are long-petioled with 3-7 foliate (Elufioye et al., 2022).

Figure 1: *Bryophyllum pinnatum* plant
Source: www.medicinalplantanduses.com

It is worthy to note that over 60% of the world's population and about 80% in developing countries still rely primarily on medicinal plants for their medicinal purposes, making traditional medicine the preferred primary healthcare system in these areas (Oteng-Mintah et al., 2019). This is due to a variety of factors, including cost-effectiveness, accessibility, and affordability. Typically, about 75% of Nigerians live in rural areas, and this proportion uses herbs in some capacity (Agbodike, 2011). Plants and plant parts are used to prevent, alleviate symptoms or reverse abnormalities. Opium, aspirin, digitalis, and quinine are just a few with a long history of use as herbal remedies (Arya, 2016). Additionally, active chemicals derived from plants are used in contemporary medicine. About 80% of these active components show an impressive link between their therapeutic usage in modern medicine and their traditional use. The non-nutritive bioactive chemical compounds (phytochemicals) in plants give them an evolutionary advantage, and there exists a wide range of them in herbs, fruits, vegetables, nuts, etc. Although levels may vary because of various factors like variety, processing, and growing conditions, these phytochemicals have the potential to be used as medications, and the content and identified pharmacological activity of these substances form the scientific basis for their use in contemporary medicine (Ahn, 2017).

One of the most severe health challenges in the world is liver disease, which can be brought on by a variety of factors, including alcohol, medicines, toxicants, chemicals, and pathogenic microbes (bacteria and viruses, including HCV and HBV). The term "alcohol liver disease" refers to the manifestation of excessive alcohol use. According to O'Shea et al. (2010), there are three types of alcohol-induced liver damage: fatty liver, alcoholic hepatitis, and chronic hepatitis with liver fibrosis or cirrhosis. These conditions cause functional and structural impairment to the liver, resulting in the leakage of enzymes compartmentalized in the hepatocytes. Plasma produced in the liver, such as albumin, decreases in the plasma, hence causing damage to the liver, diagnosed by performing enzyme assays or evaluating total plasma proteins (Vasudevan et al., 2016). In Western nations, this is the primary cause of liver diseases. According to Basra and Sarpreet (2011), more than 90% of all heavy drinkers experience fatty liver, about 25% experience the more severe alcoholic hepatitis, and 15% experience cirrhosis.

According to Lans (2006), *Bryophyllum pinnatum* is used in Trinidad and Tobago as a traditional remedy for hypertension. Similarly, it has been used as a cure for a variety of conditions in tropical America, India, and China. This includes microbiological infections, rheumatism, bodily pain, arthritis, heartburn, skin ulcers, peptic ulcers, as well as diabetes mellitus (Ghasi et al., 2011; Chopra et al., 2002; Okafor and Ham, 1996). The biological activity of *Bryophyllum pinnatum* has been

reported in various pharmacological research, some of which could support the plant's traditional applications as a CNS depressant, for example, while other uses could include analgesic, anti-inflammatory, antimicrobial, antiulcer, and anti-diabetic (Salahdeen and Yemitan, 2006; Afzal et al., 2012; Pal and Chaudhuri, 1991; Ojewole, 2005). In addition to the aforementioned fact, *Bryophyllum pinnatum* is a popular herbal medicine due to the local belief that natural extracts are free of adverse effects (Sarpreet, 2011). Different parts of *Bryophyllum pinnatum*, such as the leaves, bark, and juice, have found application in the management of various diseases. The leaves have been used both internally as a tonic, carminative, and as an astringent (Elufioye et al., 2022).

The study aims to ascertain the impact of an aqueous leaf extract of *Bryophyllum pinnatum* on the serum liver enzymes following the administration of alcohol-induced liver damage in albino Wistar rats.

MATERIALS AND METHODS

Experimental Setup and Equipment

The laboratory equipment utilized in this study includes a grinding machine, an electronic weighing scale, beakers, test tubes, plastic buckets, a measuring cylinder, a refrigerator, a thermo-regulated water bath, syringes, studded needles, a spectrophotometer, pipettes, cuvettes, and a centrifuge.

Reagents/materials

In this study, the materials used included *Bryophyllum pinnatum* leaves, plastic buckets, Whatman's filter paper No. 1, a sieve, a basket, a chess cloth, palletized rat feed, a branded whiskey alcoholic beverage, water bottles, sawdust, polycarbonate cages, hand gloves, chloroform, distilled water, and reagent kits for the determination of aspartate transaminase, alanine transaminase, and alkaline phosphate. The purchase of alcohol occurred at Bez Pharmaceutical shops, namely from the brand Blended Whiskey (48%), located in Etta Agbo, Calabar, Cross River State.

Sample Collection, Identification, and Preparation

The *Bryophyllum pinnatum* leaves were collected from the garden located at Road 2, Satellite Town in Calabar, Cross River State, Nigeria. Subsequently, a taxonomist from the Department of Botany at the University of Calabar conducted the identification process. The collected leaves underwent a thorough washing process using tap water in order to remove any dirt and physical contaminants. The leaves were ground using a manual grinding mill and thereafter stored in a sterile, airtight container to prevent any potential contamination.

The present study focuses on the preparation of an extract using the cold maceration technique of extraction.

A freshly obtained sample of *Bryophyllum pinnatum* leaves was pulverized using a manual grinding machine. Subsequently, the resulting ground sample, which exhibited natural concentration, was subjected to filtration using a sieve basket followed by a cheesecloth. Subsequently, the resulting filtrate underwent an additional filtration process employing Whatman filter paper No. 1. The obtained extract was thereafter transferred into plastic vials and kept at a temperature of 4°C in a refrigerator for future experimental use.

Methodology

A total of fifteen (15) albino Wistar rats, with weights ranging from 110-223 kg, were acquired from the animal house located in the Department of Biochemistry at the University of Calabar in Calabar, Cross River State, Nigeria. The animals were subjected to a one-week acclimatization period within the designated animal housing facility. During this time, they were given with a diet consisting of conventional rat pellets and a total of 10 liters of water. The animals were kept under controlled conditions of temperature and humidity. The rats were subsequently separated into three groups, each consisting of five.

Table 1: Experimental design

Group	Designation	Treatment	Dose
1	NC	Water	10 litres
2	ALC	Alcohol	1.5 ml per body weight
3	FBP	Alcohol and FBP	1.5 ml per body weight + 200 mg of FBP

Key:
NC – Normal Control Group
ALC – Negative Control Group
FBP – Group administered with alcohol and extract (n=1)

Administration of alcohol and leaf extracts

Oral administration of alcohol and leaf extract of *Bryophyllum pinnatum* was conducted using a studded needle and syringe for a duration of one week for each substance. Rats in groups 2 and 3 were orally administered a commonly available alcoholic beverage, Branded Whiskey (45%), at a dosage of 1.5 ml per body weight for a duration of one week to induce sub-chronic liver

damage. Following this period, rats in group 2 (referred to as Alcohol only, ALC) were euthanized to evaluate the extent of liver damage. Following this, on the penultimate day, rats in group 3 were administered an aqueous leaf extract of *Bryophyllum pinnatum* immediately after alcohol consumption. Upon the conclusion of the treatment period, the rats were subjected to a further weighing procedure, followed by their euthanization.

Collection and preparation of blood for analysis

At the end of the 14-day treatment period, the animals were anaesthetized with chloroform and were then dissected, their blood collected with sterile syringes by cardiac puncture into sterile labeled plain vials. And were allowed to clot for about 2 hours, they were then centrifuged at 4000 rpm for 15 minutes to allow for separation of serum from cells. The serum was then precipitated into a plain and well-labeled vial for liver function analysis.

Assay procedure for liver enzyme parameters

Determination of Aspartate Amino Transaminase (AST) activity using the Randox Colorimetric kit, which is based on the methodology established by Reitman and Frankel in 1957.

$$\alpha\text{-ketoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

Pyruvate production depends on the amount of L-alanine transaminated, and hence the activity of ALT is measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenylhydrazine at 546 nm. 100 µl of serum was added to 1ml working reagent. After mixing, tubes were incubated for 1 minute at 37^0C. The changes in absorbance per minute during 3 minutes were recorded against blank at 340 nm.

Estimation of Aspartate Amino Transaminase (AST) activity using Randox Colorimetric kit based on Reitman and Frankel (1957)

The reaction involving α-ketoglutarate and L-alanine, catalyzed by alanine aminotransferase (ALT), results in the formation of L-glutamate and oxaloacetate.

The concentration of oxaloacetic acid, which is directly proportional to the amount of aspartate eaten by the enzyme, is used to evaluate the activity of AST. This is achieved by measuring the concentration of oxaloacetate hydrazine generated during the reaction with 2,4-dinitrophenylhydrazine. Subsequently, a volume of 100 µl of serum was introduced into 1ml of the working reagent. Following thorough mixing, the tubes were subjected to an incubation period of 1 minute at a temperature of 37°C. The alteration in absorbance was measured relative to the blank at a wavelength of 340 nm. The void was filled with distilled water.

Estimation of Alkaline phosphate (ALP) activity using Randox Colorimetric kit based on Rec. GSCC (DGKC), 1974

$$\text{P-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{Phosphate} + \text{p-Nitrophenol}$$

Alkaline phosphatase (ALP) in serum catalyzes the hydrolysis of p-nitrophenol phosphate to phosphate and p-Nitrophenol. The rate of formation of p-Nitrophenol is measured as an increase in the absorbance which is proportional to the ALP activity in the sample. 20 µl of serum was added to 1 ml of working reagent. The tubes were incubated for 1 minute at 37^0C. The change in absorbance per minute during 3 minutes was recorded against blank at 405 nm.

Statistical analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA) followed with post hoc (Tukey) test for significant values. P-value < 0.05 was considered statistically significant. Graph pad Prism Version 7 (Statistical Package) was used for statistical analysis and Microsoft Excel Application Software 08. Data were expressed as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSIONS

Effect on serum aspartate aminotransaminase

Toxicity Table 2 shows the results for the serum liver enzymes on both the control group and the experimental rats. Decrease in AST activity (µl) was observed in all the experimental group when compared to the normal control (NC) group (102.00 ± 0.95). However, the alcohol (ALC) group (52.33 ± 5.61) showed the lowest ($p < 0.05$) AST activity when compared to NC and fresh leaf extract (FBP) groups.

Effect on serum alanine aminotransferase activity

There was no statistically significant difference ($p < 0.05$) in ALT activity (µl) in all the groups. The increase in ALT activity in the alcohol (ALC) group (42.00 ± 3.46) is not statistically significantly different from the normal control (NC) group.

There was no significant differencebetween the NC group (35.00 ± 4.01) andthe fresh leaf extract (FBP) group (30.20 ± 1.72). The increase in ALT activity in the (ALC) group (42.00 ± 3.46) is not significantly($p < 0.05$) difference the increase in ALT activity in the extract group (30.20 ± 1.72).

Effect on serum alkaline phosphatase activity

Decrease in ALP activity (µl) was observed in all the experimental groups when compared to normal control (NC) group (361.94 ± 23.73). However, the alcohol (ALC) group (83.80 ± 7.81[a]) showed the lowest ($p < 0.05$) ALP activity when compared to the NC and fresh leaf extract (FBP) groups.

DISCUSSION

The impact of aqueous leaf extract of *Bryophyllum pinnatum* on serum liver enzymes was examined in the present study. The liver is a primary target organ of any form of toxicity. This is because various studies corroborate the view that the liver is essential to the biotransformation of chemicals and to the body's ability to eliminate them (Torruellas *et al.,* 2014; O'Shea *et al.,* 2010). According to studies, alcohol is a type of chemical hepatotoxin that causes symptoms comparable to those of acute hepatitis in humans (Hyun *et al.,* 2021; Maher, 1997). Due to the fact that it is the primary site for alcohol metabolism, the liver is extremely vulnerable to alcohol-related damages.

One of the most frequently used indicators for liver toxicity, serum concentrations of AST, ALT and ALP, were used to examine liver damage. Several enzymes that are typically found in the cytosol of liver cells are released into the bloodstream when the plasma membrane of the liver cell is disrupted. Their amount in the serum is a valuable quantitative indicator of the degree and nature of hepatocellular damage. In comparison to rats in Group 1 (the standard control group), all of the rats in Group 2 (alcohol only, ALC) and Group 3 (fresh leaf extract of *Bryophyllum pinnatum*, FBP), showed decreased serum enzyme activity after alcohol administration. However, there was no statistically significant difference between Group 1 and the alcohol Group 3 (alcohol only ALC) regarding the rise in ALT activity. Findings from this study demonstrate a significant decrease in AST and ALT values following the administration of a hepatotoxicant like alcohol, but not a significant decrease in ALT activity. It is worthy to note that while a similar research by Nadro et al. (2006) had similar results to this study, on the other hand, a similar study by Bassey and Adesite (2021), reported results of oral administration of crude aqueous leaf extract of *Bryophyllum pinnatum* at various dosages showed no significant variation in AST and ALT activities among the groups. A possible explanation for this result may be the fact that their study did not induce alcoholic liver damage on the experimental rats before administering the *Bryophyllum pinnatum* aqueous extract, leaving the albino wistar rats' livers intact and undamaged. Additionally, the difference in concentrations of the extract administered may also contribute to this.

Given that *Bryophyllum pinnatum* has several applications in traditional medicine in different parts of the world, it is also called the "miracle plant" (Elufioye *et al.,* 2022). In the Southeastern part of Nigeria, it is used to ease the dropping of the placenta of a newly born baby and for managing childhood illness (Afzal *et al.,* 2012; Agoha, 1974). The leaves are included in common herbal remedies for several diseases such as hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches, and body pains in many West African countries, including Nigeria.In a different study, the juice of the leaves and the ethanolic extract were investigated against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats, the results showed that levels of serum glutamyl oxaloacetic acid transaminase (SGOT), serum glutamyl pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and serum bilirubin (SBLN) were reduced significantly ($P < 0.001$) after treatment of rats with the juice and ethanolic extract along with the toxicant - CCl_4 (Yadav and Dixit, 2003).

Summarily, *Bryophyllum pinnatum* has been demonstrated in previous studies to contain antioxidants and other bioactive components with pharmacological effects. It is also significant to note that phytochemicals are abundant in every part of the plant. *Bryophyllum pinnatum* is known to contain a large variety of active phytochemicals considered the basis for the plant's diverse pharmacological activities. Among them include alkaloids, triterpenes, glycosides, flavonoids and steroids amongst many others (Saad, 2006; Okwu and Josiah, 2006; Griswold *et al.,* 2018; Afzal *et al.,* 2012). Though many herbal cures associated with folklore claims are yet to be authenticated scientifically, *Bryophyllum pinnatum* has undergone sufficient research, supporting the majority of the claims. The findings of this study suggest that the leaves of *Bryophyllum pinnatum* contain phytochemicals which can reduce alcohol-induced liver damage.

CONTRIBUTION TO KNOWLEDGE

The results from this study have shown that the aqueous extract of the *Bryophyllum pinnatum* leaves contain phytochemicals which have hepatoprotective properties. This is seen by the reduction in concentration of certain serum liver enzymes after the administration of the extract to the liver damaged rats. This suggests that the *Bryophyllum pinnatum*leaves may be useful in the prevention, treatment of liver damage.

CONCLUSION

The investigation of the effect that the aqueous extract of *Bryophyllum pinnatum*leaves has on serum liver enzyme activity in albino wistar rats with alcohol-induced liver damage, showthat the leaves have an appreciable ability to prevent hepatotoxicity. The combined administration of alcohol and the extract was found to protect the liver from acute alcohol-induced damage. Further studies on particular phytochemicals with pharmacological activities and their isolation/purification for use in drug and supplement synthesis, will go a long way in promoting the use of natural plants in medicine.

CONFLICT OF INTEREST

As the authors of this manuscript, we certify that we have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in the manuscript.

COMPETING INTERESTS

None: There is no competing interests.

ETHICS COMMITTEE

None.

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