International Journal of Biochemistry Research & Review

Volume 32, Issue 3, Page 10-15, 2023; Article no.IJBCRR.100189
ISSN: 2231-086X, NLM ID: 101654445

Hepatoprotective Activity of *Bryophyllum pinnatum* Leaves (Boiled Extract) on Albino Wistar Rats – *in vivo* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2023/v32i3803

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100189

Original Research Article

Received: 01/04/2023
Accepted: 01/06/2023
Published: 13/06/2023

ABSTRACT

Aim: The aim of this study is to determine the effects of boiled *Bryophyllum pinnatum* leaves on serum liver enzymes after the administration of alcohol-induced liver damage in Albino Wistar rats.
Study Design: Experimental.
Place and Duration of Study: Department of Biochemistry, University of Calabar (from June to August, 2021).
Methodology: Fresh *Bryophyllum pinnatum* leaves collected were washed thoroughly with clean water and allowed to dry at room temperature for 2 hours. The leaves were boiled at 100^{o}C to obtain a boiled extract from the fresh leaves. Alcohol and leaf extract of *Bryophyllum pinnatum* were administered orally for a period of one week each. The rats in group 2 and 3 were induced with sub-chronic liver damage orally using a commonly available alcoholic beverage, blended

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whiskey at a dose of 1.5 ml per body weight for a period of one week while the rats in group 3 were given boiled leaf extracts of *Bryophyllum pinnatum* for one week. At the end of the two weeks treatment period, the rats were weighed and sacrificed and their blood collected for analysis (liver function test).

Results: The animals administered with alcohol suffered mild hepatic damage. The negative control group which was administered only alcohol, had an increase in ALT when compared to the normal control. The group treated with the extract had a decrease in ALT, AST and ALP levels when compared to the negative control. This suggests that the *Bryophyllum pinnatum* leaves contain pharmacologically active compounds that can reduce alcohol-induced liver damage.

Conclusion and Recommendations: The results show minimal anti-hepatotoxic activities of *Bryophyllum pinnatum* leaves; the extract could be said to prevent liver damage induced by alcohol toxicity. Further study in the area of pharmacology is required in order to establish this natural product as an anti-hepatotoxic agent.

Keywords: Serum liver enzymes; alcohol-induced; Bryophyllum pinnatum; medicinal leaves; liver damage.

1. INTRODUCTION

The World Health Organization (WHO) defines traditional medicine as "including diverse health practices, approaches, knowledge and beliefs, incorporating plants, animals and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness." "Traditional medicine is still recognized as the preferred primary healthcare system in communities with over 60% of the world's population and about 80% in developing countries depending directly on medicinal plants for their medicinal purposes" [1]. "This is due to a number of reasons including affordability, accessibility and low cost. About 75% of the Nigerian population resides in rural areas and this percentage use herbs in one form or the other" [2].

"Plants and plant parts are being used to prevent as well as allay symptoms, or revert abnormalities. Most of the pharmaceutical products currently dispensed by physicians have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine" [3]. "Additionally, modern medicine utilizes active compounds isolated from plants and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and the traditional use. The non-nutritive bioactive chemical compounds (phytochemicals) in plants give them an evolutionary advantage and there exists a wide range 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 drugs and the content and known pharmacological activity of these substances is the scientific basis for their use in modern medicine" [4].

Bryophyllum pinnatum is a succulent, perennial plant about 1 m tall, with a fleshy cylindrical stem, a reddish colour (for the youngest), and potential health benefits [5]. Although native to Madagascar, it has been naturalized in several other regions, including the temperate regions of Asia, Australia and New Zealand. *Bryophyllum pinnatum* is known by some common names including "life plant", "air plant", and is locally called "never die" in Nigeria. *Bryophyllum pinnatum* has been recorded in Trinidad and Tobago for use as a traditional treatment for hypertension [6]. "Similarly, it has been used for the treatment of a variety of conditions in tropical America, India, China and including rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcers, diabetes mellitus, microbial infections" [7, 8,9]. Pharmacological studies on *Bryophyllum pinnatum* have reported several biological activities in the plant, some of which could authenticate the plant's traditional uses including immunodulatory [10], CNS depressant [11], analgesic, anti-inflammatory [12], antimicrobial antiulcer [13], insecticidal [14], anti-diabetic [15], anticonvulsant [16], and antioxidant properties [17].

It is worthy to note that studies have reported a wide range of active phytochemicals in *Bryophyllum pinnatum which had been* considered to be responsible for the plant's diverse pharmacological activities. These include alkaloids, triterpenes, glycosides [18], flavonoids [16,19], and steroids [12] amongst many others. "Although folklore claims many herbal remedies are yet to be authenticated scientifically,

Bryophyllum pinnatum has been adequately studied with justification of most of the claims. This has enhanced the promotion of the use of *Bryophyllum pinnatum* and other plants either as alternatives or as complements of orthodox medicine. In many cases, *Bryophyllum pinnatum* leaves were soaked in cold water overnight, boiled, squeezed or roasted and the extract obtained used to treat a number of disease conditions. In addition to the aforementioned fact, *Bryophyllum pinnatum* is a popular herbal medicine due to local belief that natural extracts of the herb are free of adverse effect" [20]. This present study is thus aimed at investigating the effects of *Bryophyllum pinnatum* leaves on serum liver enzymes following the administration of alcohol induced liver damage in Albino Wistar rats.

"Alcoholic liver disease is the major cause of liver diseases in Western countries with significant negative health effects including risk of death and cancer" [21]. "More than 90% of all heavy drinkers develop fatty liver while about 25% develop the more severe alcoholic hepatitis and 15% cirrhosis" [22]. "Alcoholism causes development of large fatty globules throughout the liver and can begin to occur after a few days of heavy drinking" [23]. The aim of this study is to determine the effects of boiled *Bryophyllum pinnatum* leaves on serum liver enzymes after the administration of alcohol-induced liver damage in Albino Wistar rats.

2. MATERIALS AND METHODS

Fresh samples of *Bryophyllum pinnatum* leaves were harvested from the garden at satellite town in Calabar, Cross River State and identified by a taxonomist in Department of Botany, University of Calabar, Cross River State. Fresh *Bryophyllum pinnatum* leaves collected were washed thoroughly with clean water and allowed to dry at room temperature for 2 hours. An extraction process with cold water obtained aqueous extract from the fresh leaves. The extraction was carried out by boiling fresh leaves using a hot plate at 100^{o}C for 1 hour with intermittent stirring. The resulting extract was filtered into a sterilized conical flask first using a sieve and subsequently with filter papers. The filtrate was then collected into a sterilized sample bottle and stored in the refrigerator for use.

2.1 Experimental Procedure

Fifteen (15) albino Wistar rats weighing 100-120 g obtained from the animal house, College of Medical Sciences, University of Calabar, Calabar were used. The animals were placed in a well-ventilated room at temperature 22 ± 3^{o}C, acclimatized for two (2) weeks with standard rodent pellets and water. The rats were divided into three groups of five rats each.

Alcohol and leaf extract (5 ml) of *Bryophyllum pinnatum* were administered orally with the aid of a studded needle and syringe for a period of one week each. The rats in group 2 and 3 were induced with sub-chronic liver damage orally using a commonly available alcoholic beverage, blended whiskey at a dose of 1.5 ml per body weight (110-120g) for a period of one week while the rats in group 3 were given boiled leaf extracts of *Bryophyllum pinnatum* for one week. At the end of the two weeks treatment period, the rats were weighed and sacrificed and blood was collected for analysis.

At the end of the treatment period, the animals were anaesthetized with chloroform. They were then dissected and their blood collected with sterile syringes by cardiac puncture into sterile labeled plain vials. They were then centrifuged at 4000 g for 10 minutes to allow for separation of serum from cells. The serum was then precipitated into a well-labeled plain sample tube for liver function test.

2.2 Statistical Analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc (Duncan test) for significant values. Statistical Package for Social Sciences software (SPSS) version 20 was used for statistical analysis and the charts were plotted using Microsoft Excel application. Data were expressed as mean ± SEM.

3. RESULTS

3.1 Effect on Alanine Transaminase

As seen from the table, there was no statistically significant difference among the groups when compared to the normal control, NC (35.00 ± 4.01). There was no statistically significant difference ($p < 0.05$) between the normal control group and the exact control group (28.25 ± 2.69). The negative control had the highest alanine transaminase (ALT) mean concentration (42.00 ± 3.46) but no statistically significant difference was found when compared with every other group. There was no statistically significant difference between the negative group and the normal group.

Table 1. Serum liver enzyme activities in experimental animals (U/L)

	ALT	AST	ALP
NC	35.00 ± 4.01	102.00 ± 0.95	361.94 ± 23.73
ALC	42.00 ± 3.46	52.33 ± 5.61[a]	83.80 ± 7.81[a]
BBP	28.25 ± 2.69	95.25 ± 2.39[b]	298.00 ± 27.44[b]

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities (U/L). a = p < 0.05 vs. NC, b = p < 0.05 vs. ALC. Values expressed as mean ± SEM, n=5
Key: NC = Normal control group; ALC = Alcohol only (negative control group);
BBP = Boiled leaf extract of Bryophyllum pinnatum (extract control group)

3.2 Effect on Aspartate Transaminase

There was a statistically significant increase in aspartate transaminase (AST) concentration in all the groups except the negative control group when compared to the normal control (102.00 ± 0.95). The increase in the extract control group (95.25 ± 2.39) was not statistically significant different from the normal group. The normal control recorded the highest mean AST concentration (102.00 ± 0.95). The extract control group value (95.25 ± 2.39) was statistically higher ($p < 0.05$) than the negative control group (52.33 ± 5.61).

3.3 Effect on Alkaline Phosphatase

There was a significant increase ($p<0.05$) in alkaline phosphatase (ALP) concentration in all the groups except the negative control group (83.80 ± 7.81). The normal control had the highest mean ALP concentration (361.94 ± 23.73) which showed statistically significant difference from the groups. The extract control was significantly different from the negative control.

4. DISCUSSION

"The liver is a prime target organ of any form of toxicity. This is because various studies corroborate the view that the liver plays a critical role in the biotransformation of chemical substances and facilitates their elimination from the body" [24,25]. "Studies have shown that alcohol is a chemical hepatotoxin known for inducing characteristic features similar to those seen in acute hepatitis in humans" [26,27]. "The liver is highly susceptible to alcohol damage because it is the primary site of alcohol metabolism. Most of the liver damage induced by excessive alcohol consumption are attributed to alcohol metabolism and its by-products" [28,29]. "Assessment of liver damage was determined by serum concentrations of ALT, AST and ALP. These liver transaminases AST and ALT provide information about the state and integrity of the liver. Although serum levels of AST and ALP are measured clinically as bio markers for liver health, an increase in ALT activity is more pronounced" [30]. "When body tissues or an organ such as liver is damaged, variety of enzymes usually found in the cytosol are released into the bloodstream causing the level of the enzymes to rise. The amount of these enzymes in the blood is directly related to the extent of damage to the liver" [30]. The negative control group which were administered only alcohol had an increase in ALT when compared to the normal control although not statistically significant. The animals administered with alcohol suffered mild hepatic damage. *Bryophyllum pinnatum* contains many phytochemicals with pharmacological actions such as alkaloids, triterpenes, glycosides [18], flavonoids [16,19], steroids [12], bufadienolides [31], lipids and organic acids [32].

Previous studies have also shown that some of the phytochemical constituents such as bufadienoides, steroids, cardienolides and flavonoids of the crude leaf extract of *Bryophyllum pinnatum* may possess analgesic, cardiac protective and antidiabetic properties [33, 34]. In a similar study [33], results of oral administration of crude aqueous leaf extract of *Bryophyllum pinnatum* (CALEBP) at various dosages, showed no significant variation in AST and ALT activities among the groups. This may be as a result of the fact that they did not induce alcoholic liver damage on the experimental rats before administering the CALEBP; hence the albino wistar rats still had normal, not damaged livers. The difference in concentrations of the extract administered may also contribute to this.

In this study, the group treated with the extract had a decrease in ALT, AST and ALP levels when compared to the negative control. This suggests that the *Bryophyllum pinnatum* leaves contain phytochemicals which can reduce alcohol-induced liver damage.

5. CONCLUSION

The results obtained from this present study have shown minimal anti-hepatotoxic activities of *Bryophyllum pinnatum* leaves. The extract of *Bryophyllum pinnatum* leaves could be said to prevent liver damage induced by alcohol toxicity. Therefore, further extensive study in the areas of pharmacology and ethno-medicine is essential to establish this natural product as an anti-hepatotoxic agent.

ACKNOWLEDGEMENTS

The efforts of students who assisted in carrying out the experimental design of this work is hereby appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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