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Protective Effect of *Bryophyllum pinnatum* (Lam.) Oken (Miracle Leaf) Extract on Butylglycol-Induced Hepatotoxicity

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ABSTRACT

The liver is an organ crucial for maintaining and regulating the body's homeostasis, however, this can be altered due to damage or dysfunction caused by environmental pollutants. The *Bryophyllum pinnatum* (Family: Crassulaceae) leaf has been traditionally used by the Yoruba people of South Western Nigeria to manage poisonings for many years. This study seeks to explore the protective effects of *Bryophyllum pinnatum* crude extracts against butylglycol- induced liver damage in albino rats. Twenty albino rats were divided into four groups of five. Group A received only distilled water (Negative control), Group B was given butyl glycol only (Positive control), Group C received the crude extract of *Bryophyllum pinnatum*, and Group D was administered both butyl glycol and the crude extract of *Bryophyllum pinnatum*. Phytochemical analysis, micronucleus assays, and enzyme assays (Alkaline Phosphatase (ALP) and Aspartate Amino Transferase (AST)) were conducted. The phytochemical analysis identified anthraquinone, saponin, terpenoid, tannin, alkaloid, flavonoid, cardiac glycoside, and reducing sugar in amounts of 10.5±0.40, 8.24±0.01, 0.13±0.03, 80.5±0.39, 13.6±0.01, 25.8±0.33, 14.8±0.66, and 20.8±0.27 mg/100g, respectively, while the micronucleus assay revealed a significantly elevated induction of micronucleated polychromatic erythrocytes in Group B (20.4±1.14 mPCEs/1000

PCEs) compared to Group A (1.0 ± 0.71 mPMs/1000 cells), $p < 0.05$. The highest levels of ALP and AST were found in Group B (531.81 ± 10.6 U/L; 175.83 ± 2.48 U/L), while Group A showed the lowest levels (174.28 ± 1.19 U/L; 61.29 ± 1.78 U/L), $p < 0.05$. In conclusion, the crude extract of *Bryophyllum pinnatum* may help to modulate and decrease butylglycol-induced hepatotoxicity in albino rats, supporting its traditional use for related ailments. However, it is important to establish and promote a dose-dependent relationship during administration.

Keywords: *Bryophyllum pinnatum*, Butylglycol, Hepatoprotective, Hepatotoxicity

1. INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body (Elias and Bengelsdorf, 2012). It is involved with almost all the biochemical pathways to growth, fight. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease (Guo, 2015).

The liver is the chief and the most significant metabolic organ of the body having an average weight of 1.5 kg for 70 kg body weight person, located in the upper right hand side of the abdomen. It is the centre of different metabolic reactions, which occur in the body. More than 75% of the hepatic parenchyma is made up of hepatocytes, which is the most accountable for maintaining each function of the liver and needs to support the body physiological function. It secures more than 50 significant function such as conversion of food compounds to critical blood compounds, storage if mineral and vitamin, manufacture of many vital plasma protein and minerals, maintenance of hormonal balance and metabolism and detoxification of toxic wastes of the body. It secretes biles that help in lipid digestion.

Furthermore, it is responsible for synthesizing the blood clotting factors prothrombin, fibrinogen and heparin which prevent the blood from clotting within the blood circulation (Saleem *et al.*, 2010). In addition, liver is noteworthy in functions such as metabolism of lipids, protein, and carbohydrates. The liver helps in the regulation of normal glucose concentration during fasting. It also plays a fundamental role in regulating glycogen metabolism, thereby, it clears insulin and suppress glucose production and enhances hepatic glucose production by glycogenolysis (Micheal *et al.*, 2010).

Bryophyllum pinnatum is a perennial succulent herb that belongs to the family Crassulaceae. It is native to Madagascar and widely cultivated in tropical and subtropical regions of the world. It is also known as the air plant, miracle leaf, or life plant, because of its ability to produce new plants from the margins of its leaves. *Bryophyllum pinnatum* has been used in traditional medicine for various ailments, such as kidney stones, respiratory disorders, wounds, menstrual cramps, skin conditions, digestive problems, and cardiovascular problems. Phytochemical analysis of the plant has revealed the presence of compounds such as flavonoids, phenols, tannins, saponins, terpenoids, and bufadienolides, which may be responsible for its pharmacological activities (Bagalkotkar *et al.*, 2016).

Any clinical defects or conditions which rise to impairment of liver are known as liver diseases. The diseases can be chronic or acute, chronic liver disease causes periodical

destruction and regeneration of liver. Parenchyma generates fibrosis and cirrhosis of the liver. Eventually, it causes an extreme degree of inflammation of the liver producing chronic hepatitis, cirrhosis and liver carcinoma. Liver damage is very likely since it has to detoxicate a lot of toxic substances that are ingested via food. Most of the hepatotoxic chemicals damage hepatic cells by producing reactive species.

Due to excessive exposure to hepatotoxic chemicals, sometimes the free radicals generated are so high that they over power the liver and cause jaundice, cirrhosis and fatty liver. Production of reactive species manifests in tissues thiol depletion, lipid peroxidation, plasma membrane damage etc. Resulting in severe hepatic injury. A large number of plant and formulation have been to claim to have hepatoprotective activity. Nearly, 160 constituents from the plants have been claimed to possess liver protecting activity (Handa *et al.*, 2016).

Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model.

2. MATERIALS AND METHODS

2. 1. Collection and Preparation of Crude Extract

Bryophyllum pinnatum leaves were collected from the botanical garden of Moshood Abiola Polytechnics, Abeokuta, Ogun state, Nigeria. To avoid contaminations, the leave was thoroughly washed in running tap water then rinsed properly in a distilled water (Umoren, 2021). The crude extract was carried out using method described by Akinwummi *et al.* (2019). Then leaves was pulverized in a blender and extract was obtained by squeezing, then the mixture was passed through Whatman No 1 filter paper to get a clear extract.

2. 2. Animal Maintenance

Albino male rats (7-9 weeks) weighing 201- 250g were used in this study. Rats were reared in the animal house of science and laboratory Technology, Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria. Water and food in the form of pellets were given *ad libitum* to the rats. The bedding was changed every day to maintain clean environment and to reduce the risk of infection.

2. 3. Phytochemical Screening

Phytochemical screening was carried out on hot water and ethanol extracts of *Bryophyllum pinnatum* leaves using standard procedures to identify the constituents.

Phenols

The quantity of phenols was determined using the spectrophotometer method. The plant sample was boiled with 50 ml of diethyl ether for 15 min. 5 ml of the boiled sample was then taken into 50 ml flask, and 10 ml of distilled water was added. After the addition of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol is added to the mixture. The sample was made up to the mark and left for 30 min to react for colour

development and measured at 505 nm wavelengths using a spectrophotometer (Molyneux *et al.*, 2017).

Alkaloids

5 g of the plant sample were prepared in a beaker and 201 ml of 10% acetic acid in ethanol was added to the plant sample. The mixture was covered and allowed to stand for 4 hours. The mixture then filtered and the extract was allowed to become concentrated in a water bath till it reaches $\frac{1}{4}$ of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which was then dried and weighed.

Tannins

Quantity of tannin was determined by using the spectrophotometer method. 0.5 g of plant sample was weighed into a 50 ml plastic bottle. 50 ml of distilled was added and stirred for 1 h. The sample was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtered sample was then pipette out into test tube and mixed with 2 ml of 0.1 M ferric chloride in 0.1 M hydrochloric acid and 0.008 M potassium ferrocynide. The absorbance of the sample was measured with a spectrophotometer at 395 nm wavelength within 10 min.

Saponins

The plant samples were ground and 20 g of each plant sample was put into a conical flask and 100 ml of 20% ethanol was added to the plant sample. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was then filtered and the residue re-extracted with another 201 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90 °C. The concentrated was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and vigorously shaken.

The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process was repeated. 60 ml of n-butyl alcohol was added and the combined n-butyl alcohol extracts was washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

Flavonoids

Exactly, 10 g of plant sample is repeatedly extracted 10g of plant sample is repeatedly extracted with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was then filtered through filter paper and the filtrate was later transferred into a water bath and the solution was evaporated into dryness. The sample was then weighed until a constant weight.

Steroids

Exactly, 1 ml of the extract was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml) were added, followed by potassium hex cyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water bath maintained at 70 ±20 °C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

2. 4. Treatment Groups

The acclimatized rats were divided randomly into four groups; each contain 5 rats. Animals in 1st group serve as negative control. The 2nd group received Butyl glycol (24 mg/kg orally for 21days) dissolved in distilled water. The 3rd group received both Butyl glycol + ethanol extract of *Bryophyllum pinnatum* leaves (8.5 mg/kg orally for 21d ays). The 4th group received *Bryophyllum pinnatum* only.

2. 5. Necropsy of Experimental Animals

Prior to the sacrifice, the animals were starved overnight. Thus, the experimental animals were sacrifice on the thirty sixty (36th) day; twenty-four (24) hours after the last dosage of the test substances were administered. The rats were anaesthesia. Blood was then drawn by cardiac puncture to determine the alkaline phosphatase (ALP) activity.

2. 6. Determination Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase (ALP) activity was also assayed in the plasma using the reconstituted ALP reagent. Essentially, 2.5 ml of the reagent at 25 °C was mixed with 0.05 ml of the sample. The mixture was then incubated at 25 °C and the absorbance of the mixture was read twice at a minute interval at 405 nm. The change in absorbance per minute was then estimated.

2. 7. Micronucleus Assay

Essentially, the femur of each mouse was freed and stripped clean of muscles. The iliac end of the femur was carefully shortened until a small opening to the marrow became visible. Subsequently, 1 ml syringe with Fetal Bovine Serum (FBS) was passed through the opening to flush out the bone marrow cells.

This was thoroughly mixed and then transferred into different ependorff tubes. The mixture was then centrifuged at 2010 rpm for 5minutes. The pellet obtained was re-suspended in FBS and centrifuged again at 2010 rpm for 5 minutes. Thereafter, 0.5 ml of FBS was added to the pellet in the ependorff tubes and mixed thoroughly.

The homogenous suspension obtained was dropped on a pre-cleaned pre-labelled slide and smeared. The slides were air dried and fixed in absolute methanol for two minutes and further air dried to remove the methanol.

The dried slides were stained. Briefly, the slides were placed vertically in a coupling jar containing 0.4 % of May-Grunwald stain for 3-4 minutes and immediately transferred into another coupling jar containing 1:1 May-Grunwald and distilled water for another 3-4 minutes.

The slides were removed and rinsed in distilled water and allowed to dry. The dried slides were then stained in 5 % Giemsa stain that was initially dissolved in 0.01M phosphate buffer pH 6.8.

They were thereafter rinsed in distilled water, air dried, mounted in DPX (BDH) and covered with cover glass smeared with xylene. The stained and mounted slides were coded and scored using an Olympus XSZ 107 BN microscope for the presence of micronucleated polychromatic erythrocytes (Akinwunmi *et al.*, 2018). Data were expressed as Mean \pm SE.

It was determined by using SPSS 14.0.

3. RESULTS

Table 1, shows the qualitative phytochemical screening of crude extract of *Bryophyllum pinnatum*. The table shows that the crude extracts of the plant contained anthraquinone, saponin, terpenoid, tannin, alkaloid, flavonoid, cardiac glycosides, reducing sugar while phlobatannin and ascorbic acid were observed to be absent in the extract.

Table 1. Qualitative Analysis of the Crude extract of *Bryophyllum pinnatum*.

Phytochemical	Sign
Anthraquinone	+
Saponin	+
Terpenoid	+
Tannin	+
Alkaloid	+
Phlobatannin	-
Ascorbic Acid	-
Flavonoid	+
Cardiac glycosides	+
Reducing Sugar	+

Key: - Absent, + Present

Table 2 shows the quantitative analyses of the phytochemicals present in the crude extract of *Bryophyllum pinnatum*. Tannin had the highest concentrations (80.54 ± 0.39 mg/100g), followed by flavonoids (25.78 ± 0.33 mg/100g), reducing sugar (20.79 ± 0.27 mg/10g) while terpenoid had the least concentration of 0.125 ± 0.025 mg/100g.

Table 2. Quantitative Analysis of Crude extract of *Bryophyllum pinnatum*.

Phytochemicals	Concentration (mg/100g)
Anthraquinone	10.5 ± 0.40
Saponin	8.24 ± 0.01
Terpenoid	0.125 ± 0.025

Tannin	80.54±0.39
Alkaloid	13.63±0.01
Flavonoid	25.78±0.33
Cardiac glycosides	14.76±0.66
Reducing Sugar	20.79±0.27

Table 3 shows the total number of micronucleated polychromatic erythrocytes cells (MPCEs) in the rats treated with crude extract of *Bryophyllum pinnatum* leaves and Butyl glycol. It shows that Group A that was treated with only distilled water had the least MPCEs of 1.0±0.71 per 1000 cells while Group B that was treated with only Butyl glycol had the highest MPCEs of 20.4±1.14 per 1000cells. Group D which was treated with Butyl glycol and crude extract of *Bryophyllum pinnatum* leaves had 10.80±0.84 MPCEs/1000cells.

Table 3. Micronucleus Assay

Treatment Group	Mean (n=5)	Standard Deviation
Group A	1.00 ^a	0.71
Group B	20.40 ^d	1.14
Group C	5.20 ^b	0.84
Group D	10.80 ^c	0.84

Mean in the same column with different superscript are significant at p<0.05

Key: Group A = Distilled water only, Group B = Butyl glycol only, Group C = Crude extract of *Bryophyllum pinnatum* leaves, Group D = Butyl glycol and Crude extract of *Bryophyllum pinnatum* leaves

The alkaline phosphatase (ALP) activity of the rats treated with crude extract of *Bryophyllum pinnatum* leaves and Butyl glycol is as presented in Table 4, It shows that Group A that was treated with only water had the least ALP activity of 174.28±1.19 U/l while Group B which was treated with only Butyl glycol had the highest ALP activity of 531.81±10.60 U/l. Comparing with group B, Group C which was treated with Butyl glycol and crude extract of *Bryophyllum pinnatum* leaves had lower ALP activity of 349.86±1.63 U/l. There are significant differences between the ALP activities of the groups.

The Aspartate Amino Transferase (AST) activity of the rats treated with crude extract of *Bryophyllum pinnatum* leaves and Butyl glycol is as presented in Table 5. It shows that Group A that was treated with only water had the least AST activity of 61.29±1.63 U/l while Group B which was treated with only Butyl glycol had the highest AST activity of 175.83±2.47 U/l.

Comparing with group B, Group C which was treated with Butyl glycol and crude extract of *Bryophyllum pinnatum* leaves had lower AST activity of 109.58 ± 1.10 U/l. There are significant differences between the AST activities of the groups.

Table 4. Alkaline Phosphatase (ALP) Activity

Treatment Group	Mean (n=5)	Standard Deviation
Group A	174.28 ^a	1.19
Group B	531.81 ^d	10.60
Group C	245.97 ^b	3.16
Group D	349.86 ^c	1.63

Mean in the same column with different superscript are significant at $p < 0.05$

Key: Group A = Distilled water only, Group B = Butyl glycol only, Group C = Crude extract of *Bryophyllum pinnatum* leaves, Group D = Butyl glycol and Crude extract of *Bryophyllum pinnatum* leaves

Table 5. Aspartate Amino Transferase Activity

Treatment Group	Mean (n=5)	Standard Deviation
Group A	61.29 ^a	1.78
Group B	175.83 ^d	2.47
Group C	109.58 ^b	1.10
Group D	160.49 ^c	8.47

Mean in the same column with different superscript are significant at $p < 0.05$

Key: Group A = Distilled water only, Group B = Butyl glycol only, Group C = Crude extract of *Bryophyllum pinnatum* leaves, Group D = Butyl glycol and Crude extract of *Bryophyllum pinnatum* leaves

4. DISCUSSION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 2016). In this study, the plant extract has tannin, phenol, alkaloid, reducing sugar, flavonoid and terpenoid while quantitative analysis showed their concentration in the following order tannin (80.54 ± 0.39 mg/100g) > flavonoids

(25.78±0.33 mg/100g) > reducing sugar (20.79±0.27 mg/10g) > Terpenoid (0.125±0.025 mg/100g). The presence of these phytochemicals in plants is known to show therapeutic effects as well as exhibiting physiological activity. Flavonoids are known to have antioxidant effects and have been shown to inhibit the spread of reactive oxygen species that causes cancer and inflammation of the kidney (Kim *et al.*, 2018). This explains its medicinal importance and its use in the traditional treatment of various ailments. The findings of this study are in agreement with Nakweti *et al.* (2013) and Adebisi *et al.* (2021) who reports the presence of tannin, phenol, alkaloid, flavonoid and terpenoid in *Bryophyllum pinnatum*. Meanwhile, the presence of anthraquinone and steroid in the plant was also reported (Adebisi *et al.*, 2021). This difference in the phytochemical composition could be as a result of the use of different solvents for extraction in the study.

Micronuclei are extra-nuclear bodies that contain damaged chromosome fragments as a result of being exposed to genotoxic substances (Akinwumi *et al.*, 2016). As found in this study, the frequency of occurrence of micronucleated polychromatic erythrocytes cells (MPCs) in the rats in the distilled water treated group (1.0±0.71 MPCs/1000 cells) which is minute could be an exposure to genotoxic substances from the environment. The increase in the MPCs observed in the Butylglycol group and the Butylglycol and *Bryophyllum pinnatum* crude extract group respectively shows that Butylglycol has genotoxic potential which is in agreement with the report made by Akinwunmi *et al.* (2018) however, the *Crude extract of Bryophyllum pinnatum leaves treated group* has a toxic effect at the dose of administration but far less than that of the Butylglycol group. The reduction of MPCs in the Butyl glycol and *Bryophyllum pinnatum* crude extract group when compared with that of the Butylglycol group, indicated that the plant extract had a protective effect on the toxic effects of the Butyl glycol. This supports the report made by Hassan *et al.* (2015) that *Bryophyllum pinnatum* extract had protective effects.

An alkaline phosphatase (ALP) test measures the amount of the enzyme ALP in the blood. Very high levels of ALP can be caused by liver problems, such as hepatitis, blockage of the bile ducts (obstructive jaundice) gallstones, liver cancer or cancer that has spread (metastasized) to the liver from another part of the body. High ALP levels can be caused by bone diseases, such as Paget's disease, rickets, bone tumor, heart failure (Abdel-Misih and Bloomston, 2015).

The ALP level for the Butylglycol and crude extract treated group from the study showed a reduction in the activities of Butylglycol hepatotoxicity. The Butylglycol and crude extract treated group has the lower AST activity (349.86±1.63 U/l), suggesting the anti-tumorigenic and anti-proliferative activities of the crude extract of *Bryophyllum pinnatum* leaves on the growth of liver diseases. Furthermore, it suggests the therapeutic benefits of the crude extract of *Bryophyllum pinnatum* leaves to protect the toxicity of Butylglycol can be associated with the phytochemical compounds which are antioxidants and free radical scavengers in nature (Olugbami *et al.*, 2014). This is in agreement with the report of Shamasundar *et al.* (2015) who reported the presence of protective effects of *Bryophyllum pinnatum* extracts.

5. CONCLUSION AND RECOMMENDATIONS

Based on the findings from this study, the crude extract of *Bryophyllum pinnatum* leaves reduced the toxicity of Butyl glycol induced hepatotoxicity in the rats probably due to the presence of phytochemicals such as tannin, phenol, alkaloid, reducing sugar, flavonoid and

terpenoid which possess modulatory and therapeutic efficacy. The following recommendations are suggested.

- i. Crude extract of *Bryophyllum pinnatum* leaves can be recommended for the folklore management of liver related diseases. However, their indiscriminate consumption and sales at all outlets should be discouraged.
- ii. There should be public awareness on the use of crude extract of *Bryophyllum pinnatum* leaves for the treatment of liver related diseases but self-medication should be discouraged.
- iii. All herbal concoction used by herbal healers should be regulated by the government regulatory bodies such as National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organisation of Nigeria (SON).

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