World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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# Hepatoprotective potentials of *Bryophyllum pinnatum* against doxorubicin induced liver damage in Wistar rats.

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World Journal of Advanced Research and Reviews, 2025, 28(01), 943-956

Publication history: Received on 04 September 2025; revised on 10 October 2025; accepted on 13 October 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.1.3510

## Abstract

Doxorubicin (Dox), an antibiotic used in tumors treatment, has several substantial adverse effects that limit its clinical utilization. Dox administration impairs liver function, noting the vital role of liver in the body, it is pertinent to finding effective protective agents to combat Dox-induced liver damage. This study was therefore set to ascertain the hepatoprotective role of *Bryophyllum pinnatum* (*Crassulacaea*) against Dox-induced liver damage in rats. Five groups of 7 rats each were used for the study. The control received normal rat feed and drinking water. Group 2 (Dox only) received 50 mg/kg of Doxorubicin i.p. once daily. Group 3 (Extract group) took 600mg/kg of the ethanolic extract of *Bryophyllum pinnatum* orally once daily. Group 4 (Dox + extract) had Doxorubicin + extract and group 5 (Dox + Bis) received Doxorubicin + 5mg/kg of Bisoprolol orally once daily. The feeding regimens lasted 28 days. Blood samples and liver tissues were collected under ketamine (60 mg/kg body weight). Our results revealed that administration of induced liver damage in rats with a significant increase in Caspases-3 concentration and decrease in BCL-2 levels. Dox-only group also had significant ($p<0.05$) elevation of serum hepatic injury biomarkers (ALT, AST, ALP, total and direct bilirubin) as well as significant ($p<0.05$) reduction in albumin concentration. Furthermore, all the hepatic photomicrographs featured injuries to the hepatocytes. All these anomalies were ameliorated following treatment with ethanolic extract of *Bryophyllum pinnatum* or Bisoprolol. These findings reveal the hepatoprotective potentials of *Bryophyllum pinnatum* extract against doxorubicin-induced liver damage in rats.

**Keywords:** Doxorubicin; *Bryophyllum pinnatum*; Bisoprolol; Hepatoprotection; Caspases; BCl-2

## 1. Introduction

Doxorubicin (DOX) is a popular drug widely used to treat breast cancer, leukemia, bronchogenic carcinoma, gastric carcinoma, sarcomas and thyroid carcinomas as well as hematological malignancies [1]. Although DOX has a prominent antitumor activity, its adverse effects on non-cancerous cells limit its use [2]. It is metabolized by liver microsomal enzymes and cytoplasmic reductases, resulting in the accumulation of toxic and immunogenic intermediates that have been implicated in the induction of liver injury [3]. DOX toxicity is caused by a variety of processes including DNA damage in cancerous, preventing cell proliferation [4]. while in non-cancerous cells, it triggers oxidative stress and impairs the mitochondrial activity by building up reactive oxygen species (ROS) [5]. DOX could stimulate oxidative stress by inhibiting the transcription factor; nuclear factor (erythroid-derived 2)-like 2 (Nrf2) that synchronizes cellular redox homeostasis and controls the antioxidant and detoxification responses [6]. The pathway mediating DOX-induced impairment of redox homeostasis has been reported in numerous organs, like heart, kidneys and liver [7].

"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" [8]. Additionally, modern medicine utilizes active compounds isolated

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from plants and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and the traditional use. Bryophyllum pinnatum is a succulent, perennial plant about 1.0 m tall, with a fleshy cylindrical stem, a reddish colour (for the youngest), and potential health benefits [9]. Common names of *Bryophyllum pinnatum* include "life plant", "air plant", "African never die" and "Litana wa" in Nigeria, *Bryophyllum pinnatum* has been used to treat hypertension [10], rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcers, diabetes mellitus, microbial infections. Others include immune-modulatory, CNS depressant, analgesic, anti-inflammatory, antiulcer, insecticidal, anti-diabetic, anticonvulsant and antioxidant properties.

Caspases (cysteine aspartases, or cysteine-dependent aspartate-directed proteases, or cysteine-aspartic proteases), belonging to the family of proteases, are significant in the regulation of apoptosis and inflammatory processes and in development [11], as the intrinsic or extrinsic pathways of apoptosis both induce cell death by activating caspases [12]. It has been shown in several studies that BIS offers cardio-protective effects against apoptosis, autophagy, and oxidative stress through modulation of immune and inflammatory mediators. The BCl-2 family is a group of structurally related proteins that participate in the regulation of apoptosis, some as inhibitors and others as activators of this process [13]. Bcl-2, the first member of this family to be discovered, is an antagonist of apoptosis [14]. Additionally, BIS demonstrated a protective effect against myocardial ischaemia/reperfusion injury by reducing the size of an infarct. Bisoprolol was found to decrease serum levels of creatinine kinase and lactate dehydrogenase, lower the apoptotic index, and suppress tumour necrosis factor alpha (TNF-α) and interleukin 6 mediators, thus down regulating the caspase 3 and caspase 12 signaling pathways. When drugs are taken over long periods or in high doses could cause hepatotoxicity. This could be prevented through early intervention for better outcomes [15].

Despite the clinical efficacy of doxorubicin in cancer treatment, its use is often associated with cardiac toxicity, leading to adverse effects such as hepatotoxicity characterized by elevated bilirubin levels and disruption of hepatic cytoarchitecture. Recent research suggests potential therapeutic benefits of ethanolic leaf extract of *Bryophyllum pinnatum* (Crassulaceae) and bisoprolol in mitigating cardiac toxicity, hepatic functions and tissue integrity in doxorubicin-induced toxicity remain inadequately understood. Therefore, this research tends to evaluate the effect of ethanolic leaf extract of *Bryophyllum pinnatum* and bisoprolol on some liver function indices in doxorubicin induced liver damage in rats.

## 2. Materials and method

### 2.1. Collection and preparation of plant materials

The plant *Bryophyllum pinnatum (Ccrassulaceae)* (Africa never die) was obtained from Ugep local market, in Yakurr Local Government Area of Cross River State, on March, 2024. It was identified and authenticated in the Department of Botany, University of Calabar. Its ethanolic leaf extract was prepared in University of Cross River State (UNICROSS), Okuku Campus.

### 2.2. Preparation of *Crassulaceae* (africa never die) extract

*Bryophyllum pinnatum* (African never die) leaves were cleaned, washed to remove debris and dried. They were then grinded into powder using Allyson blender (model FY-999), after grinding. It was soaked in 98% ethanol solution (BDH chemical Ltd Poole England) for 24 hours and was then filtered, after 24 hours the supernatant was extracted, first using second time using white cellulose material, and then by Whatman number 1 filter paper, oven dried at 45$^{o}$C into paste form and stored at normal room temperature. The extraction gave a percentage yield of about 5.3%. The extract was stored at 4$^{o}$C till further use.

### 2.3. Experimental animal

Thirty-five (35) Wistar rats weighing between 150-250 were obtained from the animal house of physiology Department, faculty of Basic Medical Sciences, University of Cross River State (UNICROSS) Okuku campus. The animals were allowed to acclimatize for one week in the medical Physiology Department where the experiment was carried out. The animals were housed in well-ventilated cages. The beddings, feed and water were replaced every day, and kept under controlled environmental conditions (room temperature of about 27$^{o}$C and 12-hour light/dark cycle). The animals were given feed and water freely.

### 2.4. Experimental design

The animals were group into five group containing 7 rats in each group.

- Group 1 (Control): received normal rat chow and drinking water
- Group 2 (Extract fed): took 600 mg/kg body weight of *Crassuaceae* extract orally
- Group 3 (Dox only): Received 50 mg/kg body weight of doxorubicin
- Group 4 (Dox + Extract): took 50 mg/kg body weight of doxorubicin + 600 mg/kg body weight of *Crassuaceae* extract orally
- Group 5 (Dox + Bis): took 50 mg/kg body weight of doxorubicin + 5 mg/kg body weight of bisoprolol orally

Animals were also fed with normal salt and water *ad libitum*. Treatment lasted for 28 days.

### 2.5. Collection of blood samples

After 28 days of experimental regimens, the animals were sacrifice under 60 mg/kg body weight ketamine intraperitoneally. Blood was collected via cardiac puncture using a 5 ml syringe into plain capped sample bottles, which was then centrifuged at 3000 RPM for 10 minutes). Serum was extracted from the supernatant and taken for laboratory assay.

### 2.6. Harvesting of liver

The liver was harvested from each animal and placed in capped sample bottles containing 10 % formaldehyde for preservation, which would later be taken for further analysis to determine the cardiac biomarkers and histological study.

### 2.7. Determination of biochemical parameters

#### *2.7.1. Determination Caspase-3*

Caspase-3 and its inhibition can be determined by using the fluorescence correlation spectroscopy (FCS) technique. FCS is a highly sensitive method based on measuring the fluorescence fluctuations in a laser highly focused detection volume of less than 1.0 fL (10-15 L). This method is widely used for studying molecular diffusion and concentration, chemical kinetics, enzyme activity assay, and molecular interaction such as protein−protein interaction drug−protein in vitro and in vivo. However, FCS is limited for application in enzyme activity and molecule interactions since the difference in the molecule weight of the un-cleaved substrate and cleaved substrate should be more than 8 times.

FCS to determine caspase-3 and matrix metalloproteinase-9 activities by increasing the molecular weight of the substrates, Fluorescence cross-correlation spectroscopy (FCCS) is not dependent on molecular size changes, and applied FCCS to determine protease activity of caspase-3 in live cells.

#### *2.7.2. Determination of BCl-2*

The expression of BCl-2 has been detected in different types of hepatocytes in mono- and binucleated hepatocytes during chronic hepatitis B.

In animal cells, BCl-2 proteins have been found on the outer mitochondrial membrane, which is supposed to form a complex with the voltage-gated anion channel porin (VDAC). Interaction between BCl-2 with VDAC3- or VDAC1-derived peptides has been shown to protect against cell death by inhibiting cytochrome c release. Bcl-2 family proteins have also been detected in the perinuclear envelope and found to be broadly located in many tissues. The ability of the BCl-2 family proteins to form oligomeric pores in artificial lipid bilayers has been documented. When liberated from mitochondria, apoptogenic factors (Smac/Diablo homologue, cytochrome c homologue, etc.) activate caspases, which, in turn, induce programmed cell death.

#### *2.7.3. Determination of conjugated bilirubin*

Conjugated bilirubin, in the presence of a solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium. The intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically (546 nm).

#### *2.7.4. Determination of unconjugated bilirubin*

A 200-µl reaction mixture containing 150 µl UnaG solution (400 pmol) and 50 µl diluted bilirubin solution or serum (for a final UnaG concentration of 2 µM) was prepared, with 5 µl artificial unconjugated bilirubin solution or newborn serum added to 995 µl of 0.1 M phosphate-buffered saline (PBS; 200-fold dilution) as we have previously shown. A microplate reader SH-9000; Corona Electric Co., was used at 37 °C with fluorescence filters for excitation and emission wavelengths of 498 and 527 nm, respectively. Bilirubin concentrations ranging from 0.0 to 66.8 mg/dl were prepared using artificial

bilirubin standard solutions including albumin (Arrows Co., Osaka, Japan) and were measured to generate a standard calibration curve. Serum samples were diluted 200-fold with PBS and fluorescence intensity was measured. Serum concentrations of unconjugated bilirubin were extrapolated from the standard curve.

*2.7.5. Determination of alanine aminotransferase (ALT)*

Kinetic method according to the International Federation of Clinical Chemistry [16].

Assay Principle:

The series of the reaction involved in the assay system is as follows:

- The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.
- Pyruvate is reduced to lactate by lactate-dehydrogenase (LDH) present in the reagent with the simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH.
- Endogenous sample pyruvate is rapidly and completely reduced by LDH during the initial incubation period so that it does not interfere with the assay.

Procedure:

| | **Macro** | **Semi-micro** |
|---|---|---|
| Reagent R | 1.0mL | 500 µL |
| Specimen | 100µL | 50 µL |

It was mixed and initial absorbance read after 60 seconds and then the timer was started simultaneously. It was read again after 60, 120 and 180 seconds. The mean absorbance change per minute (ΔA/min) was thereafter determined.

Calculation:

The ALT activity formula:

$$U/l = 1746 \times \Delta A\ 340\ nm\ /min$$

*2.7.6. Determination of alkaline phosphatase (ALP)*

ALP – by Colorimetric method, [17].

Assay Principle

Colorimetric determination of alkaline Phosphatase activity according to the following reaction:

$$\text{Phenyl phosphate} \xrightarrow[\text{(at pH 10)}]{\text{Alkaline phosphatase}} \text{Phenol + Phosphate}$$

Phenol liberated is measured in the presence of 4-aminoantipyrine and Potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Calculation:

$$\frac{\text{Optical density of serum sample} - \text{Optical density of serum blank} \times (n)}{\text{Optical density of Standard}}$$

n = 20 (Kind and King IU/100 ml)

n = 142 (IU/L)

*2.7.7. Determination of aspartate aminotransferase (AST)*

This was done by colorimetric method [18].

Principle**:**

The reaction involved in the assay system is as follows:

The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate

AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Calculation:

AST activity was obtained from the formula table in U/L

### 2.8. Measurement of total plasma proteins

The Biuret method was employed [19] based on the principle that the Cupric ions in alkaline solution react with peptide bonds in proteins producing a violet colour which is proportionate to the amount of protein present (Biuret reaction).

### 2.9. Measurement of serum albumin

Serum albumin was measured based on the principle that albumin binds with bromocresol green (BCG) at pH 4.2 causing a slight change in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin present when measured photometrically between 580 and 630 nm with maximum absorbance at 625 nm [20, 21].

Calculation:

Albumin (g/dl) = Absorbance of test × conc. of standard (g/dL)

Absorbance of standard (g/dL)

## 3. Results

### 3.1. Serum BCL-2 level in the different experimental groups

Result of the serum BCL-2 levels of control, Doxorubicin (Dox), Extract, Dox + Extract and Dox + Bisoprolol were 6.70 ±0.33, 1.40 ±0.15, 7.40 ±0.10, 5.50 ±0.12 and 4.50 ±0.14 ng/mL respectively. The result was significant lower ($p<0.01$) in Dox only when compared with the control, Dox + Extract and Dox + Bis groups. Values obtained for Dox + Extract and Dox + Biso were significantly ($p<0.05$) lower compared with the extract group, Figure 1.

### 3.2. Serum caspase-3 level in the different experimental groups

The result of the serum caspase-3 levels in of control, Doxorubicin (Dox), Extract, Dox + Extract and Dox + Bisoprolol were 2.50 ±0.13, 16.0 ±0.23, 3.50 ±0.07, 2.20 ±0.14 and 1.80 ±0.14 ng/mL respectively. The result showed a significantly higher ($p<0.01$) values in Dox only group when compared with the control. However, values obtained for other groups were significantly ($p<0.05$) lower compared with Dox only group, Figure 2.

### 3.3. Serum total proteins, albumin and globulin levels in the different experimental groups

The serum total proteins concentration (g/L) for the different experimental groups were: control (63.00 ±0.71), Dox (66.00 ±0.51), Extract (58.00 ±0.37), Dox + Extract (61.00 ±0.71) and Dox + Bis (68.00 ±0.68). It was significantly ($p<0.05$) higher in Dox only group compared with control. Values obtained for extract only group and Dox + Extract group were significantly ($p<0.05$) lower compared with control, Dox only and Dox + Bis groups, Table 1.

The serum albumin concentration (g/L) for the different experimental groups were: control (39.00 ±0.51), Dox (33.00 ±0.71), Extract (31.00 ±0.51), Dox + Extract (25.00 ±0.58) and Dox + Bis (30.00 ±0.81). It was significantly ($p<0.05$) lower in other experimental groups compared with control, Table 1.

The serum globulin concentration (g/L) for the different experimental groups were: control (24.00 ±0.45), Dox (33.00 ±0.68), Extract (27.00 ±0.37), Dox + Extract (35.00 ±0.66) and Dox + Bis (39.00 ±1.10). It was significantly ($p<0.05$) higher in Dox only, Dox + Extract and Dox + Bis groups compared with control and extract groups, Table 1.

### 3.4. Serum liver enzymes (AST, ALT and ALP) levels in the different experimental groups

Result of the serum AST levels (IU/L) of control, Doxorubicin (Dox), Extract, Dox + Extract and Dox + Bisoprolol were 22.00 ±0.71, 40.00 ±1.50, 26.00 ±0.71, 26.00 ±0.71 and 29.00 ±0.51 ng/mL respectively. It was significantly ($p<0.05$) higher in Dox only group compared with control and other experimental groups, Table 1.

The ALT concentration (IU/L) in the control group was (11.00 ±0.58), mean value obtained for Dox only group (23.00 ±0.97) was significantly ($p<0.05$) higher compared with control. The Extract only, Dox + Extract, and Dox + Bis groups had significantly ($p<0.05$) lower ALT levels compared with Dox only group, Table 1.

The ALP concentration (IU/L) in the Dox only group was significantly ($p<0.05$) higher compared with control group (that is 171.00 ±1.30 and 105.00 ±1.40 respectively). The mean values of ALP concentration for the Extract only, Dox + Extract, and Dox + Bis groups were significantly ($p<0.05$) lower compared with Dox only group, Table 1.

### 3.5. Serum total bilirubin level in the different experimental groups

The result of the serum total bilirubin levels (µmol/L) for the different groups were: control (5.10 ±0.33), Dox (9.90 ±0.59), Extract (3.5±0.093), Dox + Extract (8.0±0.60) and Dox + Bis (7.5±0.18). The result showed a significantly ($p<0.05$) higher level in Dox only group when compared with the control group. Values obtained for Dox + Extract and Dox + Bis groups were significantly ($p<0.05$) higher compared with other groups. The extract only groups had the lowest total bilirubin level compared with other groups, Table 2.

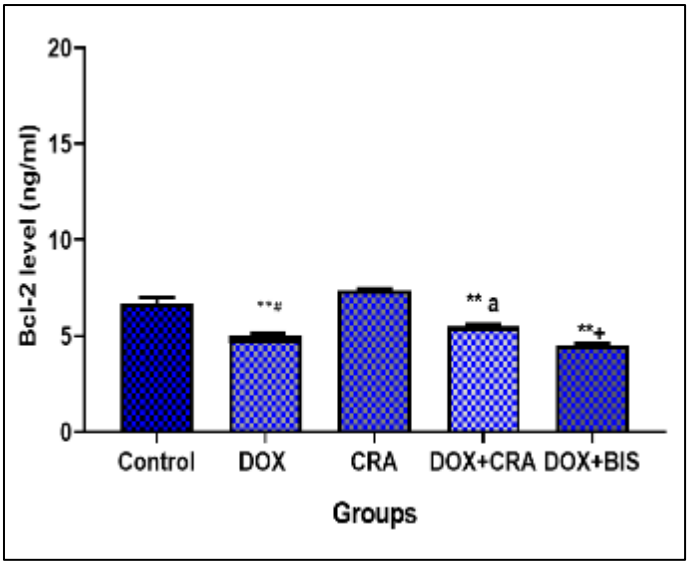
### 3.6. Serum conjugated and unconjugated bilirubin levels in the different experimental groups

The result of the serum conjugated bilirubin levels (µmol/L) were: control (2.4±0.071), Dox (5.0±0.071), extract (2.2±0.10), Dox + Extract (4.3±0.17) and Dox = Bis (3.7±0.14). Serum conjugated bilirubin levels were also significantly ($p<0.05$) higher in Dox only groups compared with control and other experimental groups. Values obtained for the extract only group was also significantly ($p<0.05$) lower compared with other groups. While values obtained for Dox + Extract and Dox + Bis groups were significantly ($p<0.05$) higher compared with other groups, Table 2.

Serum unconjugated bilirubin levels were also significantly ($p<0.05$) higher in Dox only, Dox + Extract and Dox + Bis groups compared with control and extract only groups. Values obtained for the extract only group was also significantly ($p<0.05$) lower compared with other groups, Table 2.

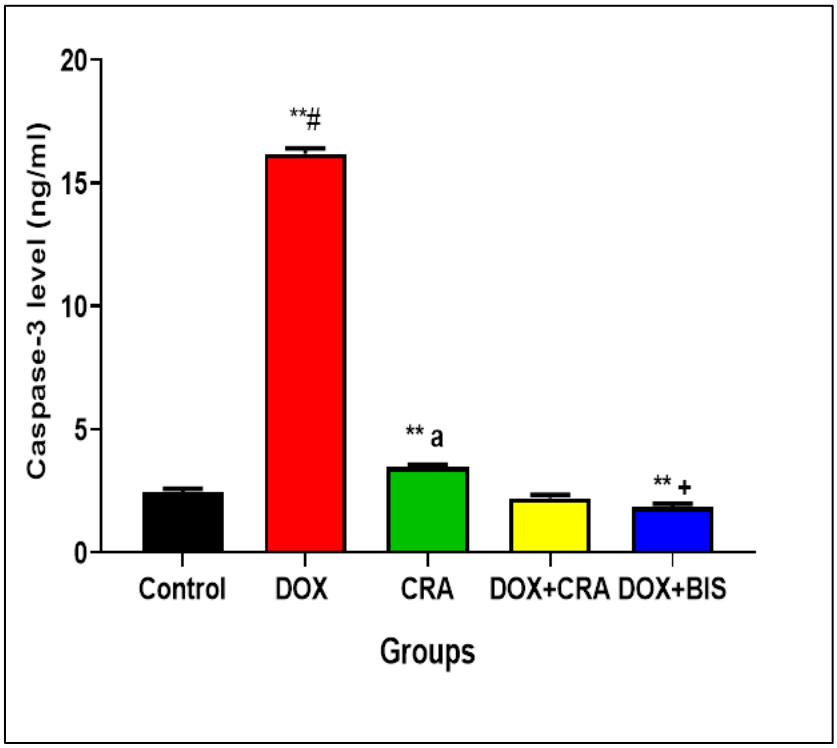
### 3.7. Histological study of the Liver

As shown in figures 3 to 6, the photomicrograph of the liver tissue in the control shows a central vein surrounded by hepatocytes (dark dotted cells) appearing normal. In the Dox group, the photomicrograph of the liver tissue shows a central vein surrounded by hepatocytes (dark dotted cells) marked alterations in the liver cytoarchitecture. Photomicrograph of the liver tissue in the Dox + Extract group shows a central vein surrounded by hepatocytes (dark dotted cells) appearing normal. In the Dox + Bis group, the photomicrograph of the liver tissue shows a central vein surrounded by hepatocytes (dark dotted cells) appearing normal.




Values are expressed as mean ±SEM, n = 7l * = p<0.01 compared with control; # = p<0.01 versus other treated groups; + = p<0.05 compared with Dox + Extract group; a =p<0.05 compared with Extract group

**Figure 1** Comparison of BCL-2 concentration in the different experimental groups




Values are expressed as mean ±SEM, n = 7; ** = p<0.01 compared with control; # = p<0.01 versus other treated groups; + = p<0.05 compared with DOX+CRA group, a =p<0.05 compared with DOX+CRA group

**Figure 2** Comparison of Caspases concentration in the different experimental groups

**Table 1** Serum proteins and liver enzymes concentration in the different experimental groups

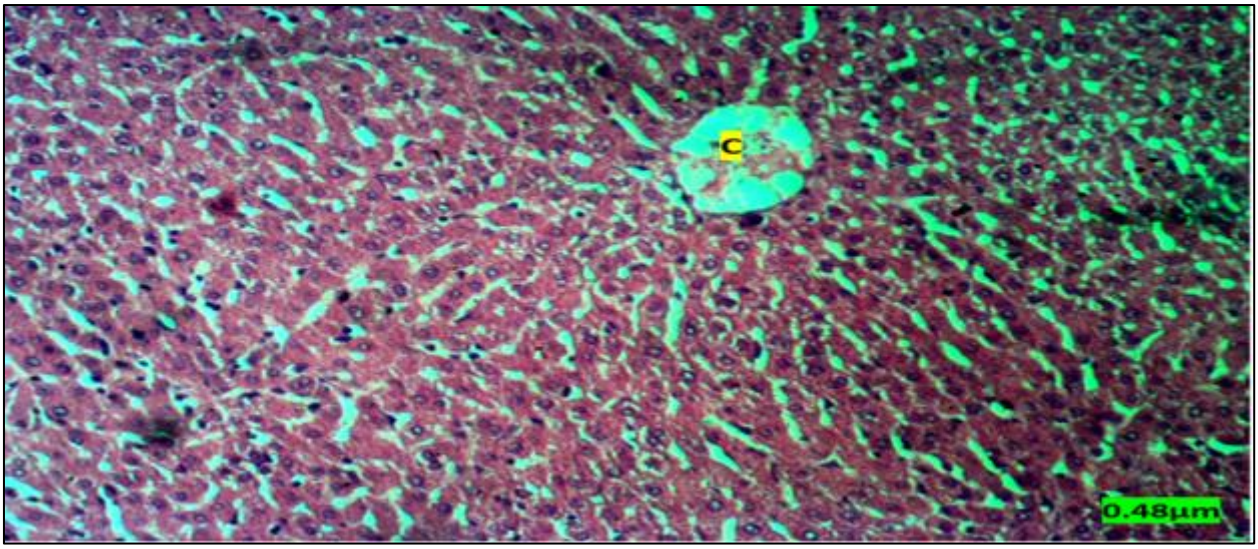
| | **Total protein (g/L)** | **Albumin (g/L)** | **Globulin (g/L)** | **AST (IU/L)** | **ALT (IU/L)** | **ALP (IU/L)** |
|---|---|---|---|---|---|---|
| Control | 63.00 | 39.00 | 24.00 | 22.00 | 11.00 | 105.00 |
| | ±0.71 | ±0.51 | ±0.45 | ±0.71 | ±0.58 | ±1.40 |
| | | | | | | |
| Dox | 66.00 | 33.00 | 33.00 | 40.00 | 23.00 | 171.00 |
| | ±0.51* | ±0.71* | ±0.68* | ±1.50* | ±0.97* | ±1.30* |
| | | | | | | |
| Extract | 58.00 | 31.00 | 27.00 | 26.00 | 17.00 | 89.00 |
| | ±0.37*,a | ±0.51* | ±0.37a | ±0.71*,a | ±0.51*,a | ±0.86*,a |
| | | | | | | |
| Dox+ Extract | 61.00 | 25.00 | 35.00 | 26.00 | 21.00 | 143.00 |
| | ±0.71a | ±0.588*,a | ±0.66*,b | ±0.71*,a | ±0.68*,b | ±0.75*,a,b |
| | | | | | | |
| Dox + Bis | 68.00 | 30.00 | 39.00 | 29.00 | 19.00 | 164.00 |
| | ±0.68b | ±0.81*,a,c | ±1.10*,a,b,c | ±0.51*,a | ±0.86*,a | ±0.71*,a,b,c |

Values are expressed as mean ±SEM, n = 7. * = p<0.05 vs control; a = p<0.05 vs Dox; b = p<0.05 vs Extract; c = p<0.05vs Dox + Extract

**Table 2** Serum bilirubin concentrations and platelet count in the different experimental groups

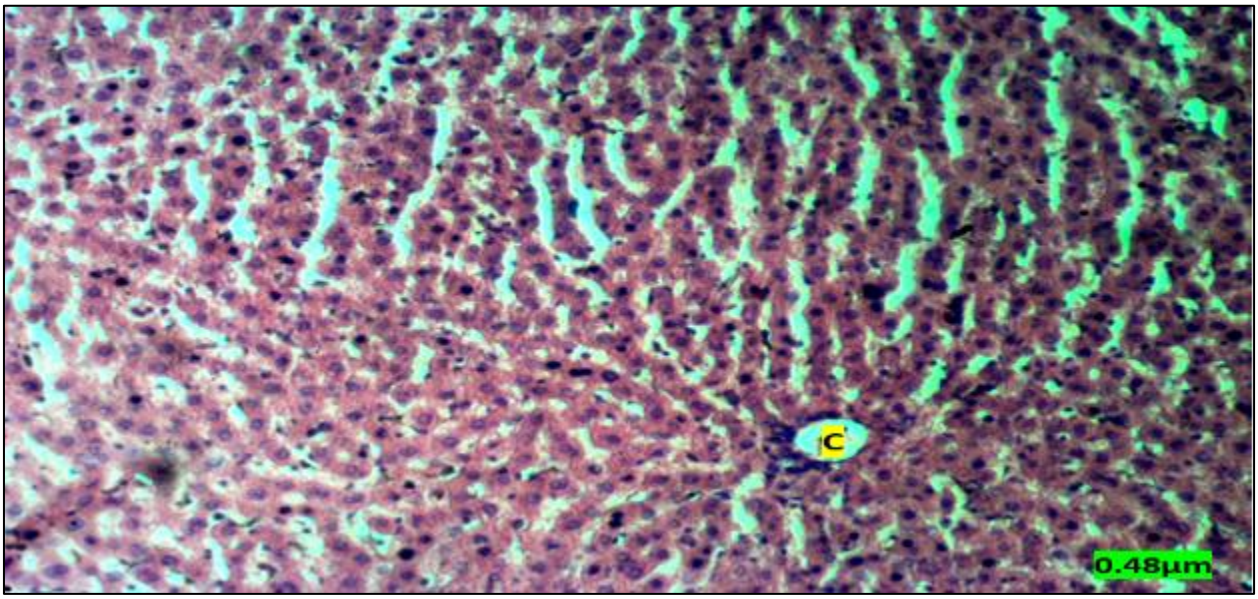
| | **Total bilirubin (µmol/L)** | **Conjugated bilirubin (µmol/L)** | **Unconjugated bilirubin (µmol/L)** |
|---|---|---|---|
| Control | 5.10 | 2.40 | 2.70 |
| | ±0.33 | ±0.07 | ±0.20 |
| | | | |
| Dox | 9.90 | 5.00 | 4.90 |
| | ±0.598 | ±0.07* | ±0.33* |
| | | | |
| Extract | 3.50 | 2.20 | 1.30 |
| | ±0.09*,a | ±0.10a | ±0.10*,a |
| | | | |
| Dox+ Extract | 14.00 | 9.10 | 4.90 |
| | ±0.60*,a,b | ±0.17*,a,b | ±0.39*,b |
| | | | |
| Dox + Bis | 14.00 | 8.70 | 5.30 |
| | ±0.18*,a,b | ±0.14*,a,b | ±0.16*,b |

Values are expressed as mean ±SEM, n = 7. * = p<0.05 vs control; a = p<0.05 vs Dox; b = p<0.05 vs Extract; c = p<0.05vs Dox + Extract



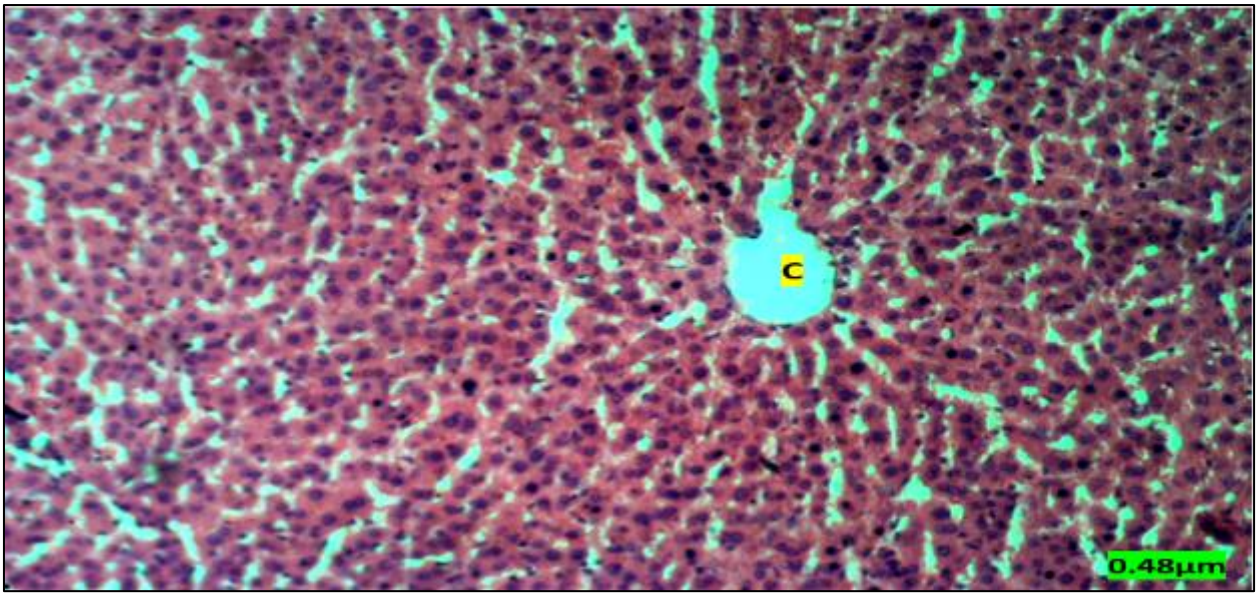

H & E. x 300

**Figure 3** Control group



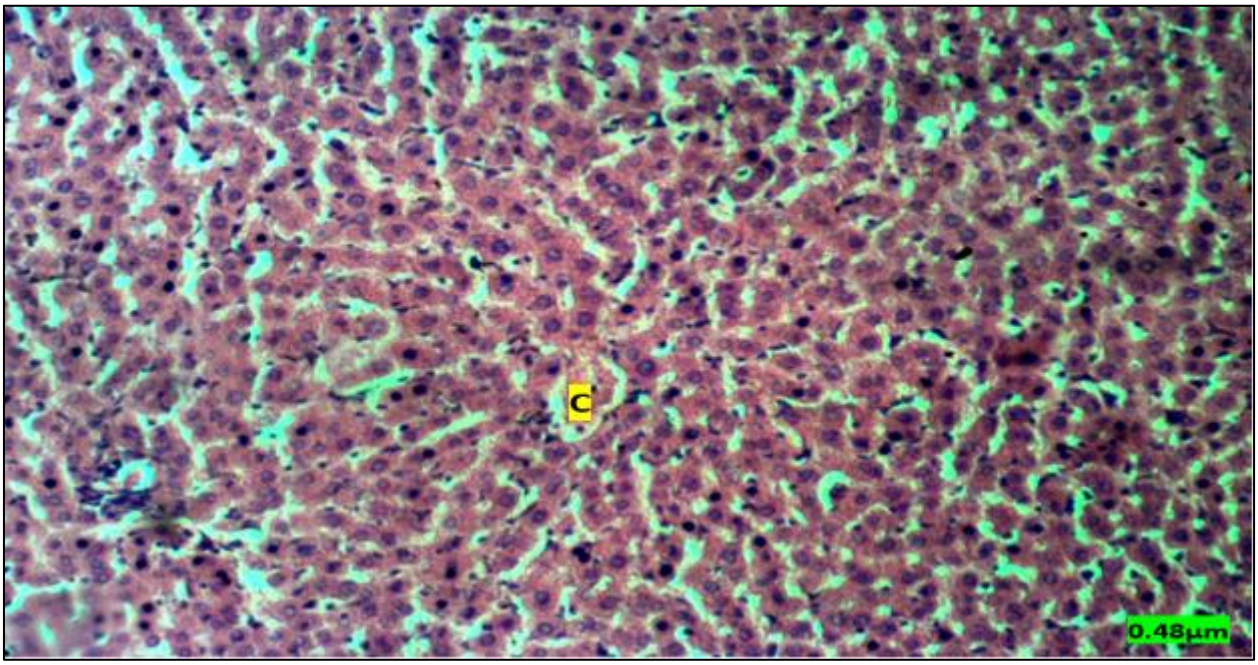

H & E stain x 300

**Figure 4** Dox only group




H & E stain x 300

**Figure 5** Dox + Extract group




H & E stain x 300

**Figure 6** Dox & Bis group

## 4. Discussion

This research work was targeted at evaluating the effect of ethanolic leaf extract of *Bryophyllum pinnatum* (*Crassulaceae*) and bisoprolol on apoptotic makers (BCL-2, Caspase-3) and liver function biomarkers in doxorubicin induced liver toxicity.

The apoptotic makers like (caspase-3 and BCL-2) are used to determine and ascertain the deleterious effect of doxorubicin treatment on liver cells, these biomarkers are indices of cell death or cell survival (proliferation) respectively [6,22-25].

Liver function biomarkers like total bilirubin, conjugated and unconjugated bilirubin, AST, ALT, ALP and serum proteins concentrations are indicators of the functionality to the liver [26,27].

The results from this present study indicate that doxorubicin significantly decreased serum Bcl-2 levels, as expected, due to its role in inducing apoptosis compared to normal control. The reduced Bcl-2 level correlates with increased cell death, particularly through the apoptotic pathway. Previous studies [6], have shown that doxorubicin's cytotoxic effect reduces anti-apoptotic proteins like Bcl-2. However, treatment with Crassulaceae and bisoprolol restored Bcl-2 levels respectively, suggesting a protective effect against apoptosis. These results are consistent with previous work [28,29], which demonstrated that plant extracts rich in bioactive compounds could attenuate doxorubicin-induced oxidative stress and apoptosis by upregulating Bcl-2 expression. The higher Bcl-2 levels observed in the Cr-only group indicate that the extract, alone or in combination with bisoprolol, may enhance cellular defense mechanisms and reduce apoptosis.

Caspase-3, a key executioner of apoptosis, was markedly elevated in the doxorubicin-only group, confirming the pro-apoptotic effects of doxorubicin. This finding is supported by prior research [30,31], which linked doxorubicin administration to an increase in caspase-3 activity and apoptosis in cardiac and liver tissues. Administration of Crassulaceae and bisoprolol, however, significantly reduced caspase-3 levels respectively, suggesting that both agents mitigated the apoptotic effects of doxorubicin. The lower caspase-3 levels in the treatment groups are in line with the findings [6], who reported that bioactive plant compounds and beta-blockers such as bisoprolol may reduce apoptosis by suppressing caspase-3 activity. The ability of *Crassulaceae* to reduce caspase-3 levels emphasizes its potential as an anti-apoptotic agent, likely due to its antioxidative and anti-inflammatory properties.

Doxorubicin-treated animals exhibited significantly elevated serum total bilirubin levels, indicative of liver damage and compromised bilirubin metabolism. This observation is in agreement with previous findings [6], who also reported elevated bilirubin levels in doxorubicin-induced liver toxicity. In contrast, the *Crassulaceae* and bisoprolol-treated groups showed a decrease in total respectively. The reduction in bilirubin levels in these groups highlights the hepatoprotective effects of *Crassulaceae* and bisoprolol, which likely mitigated the oxidative damage to hepatocytes. Earlier studies [32], have demonstrated that plant extracts with antioxidative properties, such as those found in *Crassulaceae*, can protect the liver from drug-induced damage and improve bilirubin metabolism. The conjugated bilirubin levels were also significantly elevated in the doxorubicin-treated group, further confirming hepatic damage. This elevation is consistent with findings from previous studies [33], where doxorubicin disrupted hepatic function and impaired bilirubin conjugation. Treatment with Crassulaceae and bisoprolol significantly reduced conjugated bilirubin levels respectively, indicating improved liver function and reduced hepatocellular damage. This reduction is supported by an earlier work [34], who demonstrated that antioxidants and beta-blockers could help maintain normal hepatic function by protecting hepatocytes from oxidative stress.

Our present study revealed increase levels of liver enzymes in doxorubicin treated groups. These findings come in accordance with previous observations on DOX-induced hepato-toxicity and apoptosis in subjects possessing some sort of liver damage characterized by elevated liver enzymes (AST and ALT) and increased direct and total bilirubin concentrations upon receiving DOX [35,36]. The mechanisms underlying DOX-prompted hepatotoxicity are complex and caused by a variety of processes. Recent research has identified oxidative stress as a significant mechanism of oxidation- induced hepatotoxicity [26]. DOX has been shown to cause redox homeostasis imbalance, which is defined by an elevation in ROS and a reduction in antioxidant defenses, resulting in oxidization of DNA and other macromolecules including lipids leading to liver damage [35]. Oxidative stress along with hepatocyte apoptosis evidenced by caspase-3 expression [35,36].

Our study shows reductions in albumin concentrations following doxorubicin administration. This is consistent with previous study that shows reduction in serum total protein, albumin and globulin levels by doxorubicin administration [37,38].

Treatment with the extract of *B. pinnetum* ameliorated this deleterious effect of Dox on liver enzymes and serum proteins concentrations, by reducing the levels near control levels. *Crassulaceae* extract has been shown to possess hepatoprotective protective effects in previous studies [29,39].

Histological analysis revealed marked alterations in the liver cytoarchitecture of doxorubicin-treated animals, including hepatocellular necrosis, vacuolization, and inflammation. These findings are consistent with earlier research [33], which described similar pathological changes in the liver following doxorubicin treatment. However, the administration of Crassulaceae and bisoprolol ameliorated these alterations, restoring the liver's structural integrity. The hepatoprotective effects of Crassulaceae have been previously documented [29, 33,40].

## 5. Conclusion

In conclusion, doxorubicin destroys liver cells and impairs liver functions. Treatment with ethanolic leaf extract of *Crassulaceae* or bisoprolol prevents liver damage, and improves liver functions by stabilizing the levels of caspases, liver bilirubin, AST, ALT, ALP, serum proteins and cytoarchitecture of the liver in the face of doxorubicin.

## Compliance with ethical standards

### *Acknowledgement*

The authors of this article wish to acknowledge all those who assisted to make this research a success. We appreciate the staff of Chemical Pathology, University of Calabar Teaching Hospital, Calabar, Nigeria, especially Dr. Iya Bassey and Dr. Bassey Icha for their assistance to run the biochemical assay for this study. Mr Ededet Umoh is also appreciated for sourcing and providing the rats used for this study.

### *Disclosure of conflict of interest*

The author of this research paper declares no conflict of interest

### *Statement of Ethical Approval*

Ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethical Committee with ethical approval number No: 024PHY20323.

### *Statement of Informed Consent:*

The research was carried on experimental animals and not humans, hence informed consent was not necessary.

## References

[1] Srivastava A, Amreddy N, Babu A, Panneerselvam J, Mehta M, Muralidharan R, Ramesh R. Nanosomes carrying doxorubicin exhibit potent anticancer activity against human lung cancer cells. Scientific reports, 2016; 6(1):38541.

[2] Sohail M, Sun Z, Li Y, Gu X, Xu H. Research progress in strategies to improve the efficacy and safety of doxorubicin for cancer chemotherapy. Expert Review Anticancer Therapy, 2021;21(12):1385-1398.

[3] Gu X, Manautou JE. (2012). Molecular mechanisms underlying chemical liver injury. Expert reviews in molecular medicine, 2012; 14:e4.

[4] Rawat PS, Jaiswal A, Khurana A, Bhatti JS, Navik U. Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. Biomedicine & Pharmacotherapy, 2021;139: 111708.

[5] Boese AC, Kang S. Mitochondrial metabolism-mediated redox regulation in cancer progression. Redox Biology, 2021;42:101870.

[6] Saleh DO, Mahmoud SS, Hassan A, Sanad EF. Doxorubicin-induced hepatic toxicity in rats: Mechanistic protective role of Omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK-3b axis modulation. Saudi J Biol Sci, 2022;29:103308.

[7] Renu K Pureti LP, Vellingiri B, Gopalakrishnan AV. Toxic effects and molecular mechanism of doxorubicin on different organs–an update. Toxin Reviews, 2022;41(2), 650-674.

[8] Matole V, Thorat Y, Ghurghure S, Ingle S, Birajdar A, Nangare G, Sakhare A. A brief review on herbal medicines. Research J Pharmacognosy Phytochemistry, 2021;13(2): 101-102.

[9] Gbolade A, Adedokun O, Igbinomwanhia E, Alli M. Lethality of *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) Leaf Methanol Extract and Fractions against Naja nigricollis and Bitis arietans Venoms. American Journal of Essential Oils and Natural Products, 2020;8(2):06-11.

[10] Clement YN, Baksh-Comeau YS, Seaforth CE. An ethnobotanical survey of medicinal plants in Trinidad. Journal Ethnobiology Ethnomedicine, 2015;11:1-28.

[11] Sahoo G, Samal D, Khandayataray P, Murthy MK. A review on caspases: key regulators of biological activities and apoptosis. Molecular Neurobiology, 2023;60(10):5805-5837.

[12] Lossi L. The concept of intrinsic versus extrinsic apoptosis. Biochemical Journal, 2022;479(3), 357-384.

[13] Czabotar PE, Garcia-Saez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nature reviews Molecular cell biology, 2023;24(10):732-748

[14] Ashkenazi A, Fairbrother WJ, Leverson JD, Souers AJ. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nature reviews drug discovery, 2017;16(4), 273-284.

[15] Cusi K, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, Younossi Z. American Association of Clinical Endocrinology clinical practice guideline for the diagnosis and management of nonalcoholic fatty liver disease in primary care and endocrinology clinical settings: co-sponsored by the American Association for the Study of Liver Diseases (AASLD). Endocrine Practice, 2022;28(5), 528-562.

[16] IFCC expert panel on enzymes part 3. J Clin Chem Clin Biochem. 1986;24:481-95

[17] Belfield A, Goldberg DM. Enzyme 1971;12:561

[18] Reitman S, Frankel S. Am J.Clin.Path, 1975;28:65.

[19] Reinhold JG. Total protein, albumin and globulin. In: Reiner, M. (Ed.), Standard Methods of Clinical Chemistry, 1. Academic Press, New York, pp. 88-120:1953.

[20] Basil T, Doumas W, Watson A, Homer GB. Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta 1997;258:21–30.

[21] Ofem OE, Eno AE, Nku CO, Antai AB. Viscum album (mistletoe) extract prevents changes in levels of red blood cells, PCV, Hb, serum proteins and ESR in high salt-fed rats. J Ethnopharmacology, 2009;126:421-426

[22] Hussar P. Apoptosis regulators bcl-2 and caspase-3. Encyclopedia, 2022;2(4):1624-1636.

[23] Orabi SH, Abd Eldaium D, Hassan A, Sabagh HSE, Abd Eldaim MA. Allicin modulates diclofenac sodium induced hepatonephro toxicity in rats via reducing oxidative stress and caspase 3 protein expression. Environ. Toxicol. Pharmacol. 2020;74:103306

[24] Fan H, Yang J, Xing J, Guo B, Wang W, Hu Y, Wang YZ. Apoptosis-Induced Wnt2/β-Catenin Signaling Triggers Astrocytic Dedifferentiation and Facilitates Neurogenesis in Ischemic Cortex. 2020.

[25] Jiao C, Zhang H, Li H, Fu X, Lin Y, Cao C, Li P. Caspase-3/GSDME mediated apoptosis: A potential pathway for sepsis. Inter Immunopharm, 2023;124:111022.

[26] Tan BL, Norhaizan ME, Liew WPP, Rahman, HS Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front. Pharmacol, 2018;9:1162.

[27] Hyun J, Han J, Lee C, Yoon M, Jung Y. Pathophysiological aspects of alcohol metabolism in the liver. International Journal of Molecular Sciences. 2021;22(11):5717.

[28] Bassey I, Udo E, Adesite S. Effect of crude aqueous leaves extract of *Bryophyllum pinnatum* on antioxidant status, blood glucose, lipid profile, liver and renal function indices in albino rats. Global Journal of Pure and Applied Sciences. 2021;27:231-241.

[29] Nneoyi-Egbe AF, Onyenweaku E.E, Akpanukoh A. 2023). Hepatoprotective Activity of *Bryophyllum pinnatum* Leaves (Boiled Extract) on Albino Wistar Rats in-vivo Study. International Journal of Biochemistry Research & Review. 32(3)10-15.

[30] Afsar T, Razak S, Almajwal A, Al-Disi D. (2020). Doxorubicin-induced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; Improvement by Acacia hydaspica tannin-rich ethyl acetate fraction. Saudi J Biol Sci, 27 (2020) 2251–2260.

[31] Gabellini C, Trisciuoglio D, Del Bufalo D. Non-canonical roles of Bcl-2 and Bcl-xL proteins: relevance of BH4 domain. Carcinogenesis, 2017;38(6):579-587.

[32] Arya R. The role of medicinal plants in development of modern medicine. 2016; 4210.4727.

[33] Song S, Chu L, Liang H, Chen J, Liang J, Huang Z, Zhang B, Chen X. Protective effects of dioscin against doxorubicin-induced hepatotoxicity via regulation of Sirt1/FOXO1/NF-jb signal. Front. Pharmacol. 2019;10:1030.

[34] Pereira MF, K., de Carvalho C, A., Veiga, TAM, Melgoza A., Hernández RB, dos Santos GS, Guo S. The psychoactive effects of *Bryophyllum pinnatum* (Lam.) Oken leaves in young zebrafish. Plos one, 2022;17(3), e0264987.

[35] AlAsmari AF, Alharbi M, Alqahtani F, Alasmari F, AlSwayyed M, Alzarea, SI, Al-Alallah, IA, Alghamdi A, Hakami HM, Alyousef MK, Sari Y, Ali N. Diosmin Alleviates Doxorubicin-Induced Liver Injury via Modulation of Oxidative Stress-Mediated Hepatic Inflammation and Apoptosis via NfkB and MAPK Pathway: A Preclinical Study. Antioxidants 2021;10 (12):1998.

[36] Barakat BM, Ahmed HI, Bahr HI, Elbahaie AM. Protective effect of boswellic acids against doxorubicin-induced hepatotoxicity: impact on Nrf2/HO-1 defense pathway. Oxid. Med. Cell Longev. 2018;1–10.

[37] Sindhur A, Kumar A, Chaudhary AR, Tiwari DK, Prabha N. Effect of doxorubicin on haematological and blood biochemical profile of healthy dogs. Haryana Vet. 2019;58(1), 11-14.

[38] Grant MK, Seelig DM, Sharkey LC, Choi WS, Abdelgawad IY, Zordoky BN. Sexual dimorphism of acute doxorubicin-induced nephrotoxicity in C57Bl/6 mice. PloS One 2019;14:e0212486.

[39] Andrade AWL, Guerra GC.B, de Souza Araújo DF, de Araújo Júnior RF, de Araújo AA, de Carvalho TG, Zucolotto SM. Anti-inflammatory and chemopreventive effects of *Bryophyllum pinnatum* (Lamarck) leaf extract in experimental colitis models in rodents. Frontiers in pharmacology, 2020;11:998.

[40] Elufioye TO, Oyedeji AO, Habtemariam SA review of the traditional uses, phytochemistry and pharmacology of *Bryophyllum pinnatum* (Lam.) (Crassulaceae). Journal of Biologically Active Products from Nature, 2022;12(3): 190-222.