Available online on 15.07.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Evaluation of Antioxidant activities of Aqueous extract from *Bryophyllum pinnatum* on Carbon tetrachloride-induced albino rat tissues

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Article Info:

Article History:

Received 18 May 2021
Review Completed 23 June 2021
Accepted 02 July 2021
Available online 15 July 2021

Cite this article as:

Alli Smith YR, Aluko BT, Owolabi OV, Evaluation of Antioxidant activities of Aqueous extract from *Bryophyllum pinnatum* on Carbon tetrachloride-induced albino rat tissues, Journal of Drug Delivery and Therapeutics. 2021; 11(4):10-15

DOI: *http://dx.doi.org/10.22270/jddt.v11i4.4877*

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Abstract

Oxidative injury or cellular damage arising from free radicals or reactive oxygen species (ROS) has been implicated in the pathogenesis of many human diseases. These ROS are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals. Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. The aim of this study is to evaluate antioxidant activities of aqueous extract of *Bryophyllum pinnatum* using carbon tetrachloride-induced albino rats as model. An *in-vivo* antioxidant activity was carried out on the CCl_4 induced rats by administration of aqueous extract of *Bryophyllum pinnatum* at doses of 50, 100 and 200mg/Kg bw for 14 consecutive days, and one group was treated with Silymarin at a dose of 50 mg/Kg as a standard drug. The antioxidant activities were estimated by evaluating its effects on antioxidant parameters in the tissues of albino rats (Serum, Liver and Heart). The activity of catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and the levels of MDA and GSH were evaluated in tissue homogenates. Results showed that the rats fed with the higher concentrations of *Bryophyllum pinnatum* had significant increase ($p<0.05$) in glutathione content (GSH), GPx, SOD and catalase activities relative to normal and standard control. However, the level of MDA was decreased ($p<0.05$) for the treated groups (100 and 200mg/kg). These results suggest that aqueous extracts of *Bryophyllum pinnatum* has active substances contributing to varied degrees of potent antioxidant activity and could serve as important source of antioxidants in food, cosmetics and pharmaceutical industries.

Keywords: Oxidative injury, *Bryophyllum pinnatum*, antioxidant, Diseases, Tissues

1. INTRODUCTION

Reactive oxygen species (ROS) are produced by cells in moderate concentrations as part of their normal physiological function. When ROS levels increase, modification to cellular components occurs[1], triggering a change in the balance between ROS production and antioxidant production. This leads to an increase in oxidative stress, alteration of cellular homeostasis and inadequate functioning of various cellular pathways [2]. Oxidation of macromolecules such as proteins, lipids, carbohydrates and DNA is elevated in oxidative stress. Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells [3]. Reactive oxygen species can be eliminated by a number of enzymatic and non-enzymatic antioxidant mechanisms. The enzymatic antioxidant defenses including the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Non-enzymatic antioxidants include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), glutathione, carotenoids, flavonoids, and other antioxidants [4]. However, under oxidative stress conditions, enzymatic antioxidants may not be sufficient, and non-enzymatic antioxidants (dietary antioxidants) may be required to maintain optimal cellular functions [5]. Antioxidants may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress. The natural antioxidants, more recently, have attracted considerable attention of users and researchers largely on account of adverse toxicological reports on some synthetic antioxidants and growing awareness among consumers[6]. *Bryophyllum pinnatum* popularly known as Resurrection plant, air plant, cathedral bells, life plant, miracle leaf and Goethe plant is a perennial herb used in folkloric medicine in tropical Africa. It belongs to the family of *crassulaceae* and order of *saxifragales.* It is classified as weed and the plant flourishes throughout the southern part of Nigeria [7]. This study investigated the effect of *Bryophyllum pinnatum* on oxidative stress parameters, that is, activity levels of antioxidant enzymes (GPx, SOD and CAT) and the amount of Lipid peroxidation product (MDA) and Glutathione in the tissues of carbon tetrachloride induced albino rats.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh leaves of *Brytophyllum pinnatum* was collected from a local farm at Ora-Ekiti, Ekiti state. The plant leaves was

authenticated at the Herbarium section of Department of Plant science and Biotechnology, Ekiti State University (EKSU), Ado-Ekiti, Nigeria. A voucher specimen was deposited in the Department Herbarium (voucher Number: UHAE-2020/081). It was air dried at room temperature, pulverized with electric blender and then stored in a plastic container in the laboratory prior to analysis.

2.2 Experimental animals

A total of 30 male albino mice, (150-200g) were purchased from the Laboratory Animal house Facility of College of Medicine, Ekiti State University, Ado Ekiti. They were housed in clean metal cages, with free access to water and feed. Animals were handled according to the standard guidelines of the committee on Care and Use of experimental Animal Resources which is in compliance with the National Institute of Health Guidelines for care and use of laboratory animals (Pub No. 85-23, revised 1985).

2.3 Preparation of *Brytophyllum pinnatum*e aqueous extract

100g of the powdery form was dissolved in 500ml of distilled water for 24hours. The crude extract was filtered with Whatman filter paper and evaporated under pressure at 60°C using a rotary evaporator[8].

2.4 Experimental Design

A total of 30 male albino rats were randomized into 6 groups of 5 animals each. They were treated as follows: Group 1 (Normal control) received only distilled water and rat pellets, Group 2 (Untreated control) received 3ml/kg body weight. Of CCl_4, Group 3, 4 and 5 received 3ml/kg body wt. Of Ccl4 and treated with 50, 100 and 200mg/kg of aqueous extract of *Bryophyllum pinnatum* respectively, Group 6 received 3ml/kg body wt. Of CCl_4 and treated 50mg/kg bwt of Silymarin a standard hepatoprotective drug for 14days.

2.5 Collection of blood and organs

At the end of the experimental period, animals were anesthetized (using diethyl ether) and blood and organ samples were collected. The whole blood of each animal were collected via cardiac puncture and immediately preserved on ice cold until further processing. The blood samples were centrifuged at 3000 rpm for 10 min, serum from each blood sample were separated and preserved at -30 °C for further analysis. The organs needed were extracted from each animal, washed with normal saline, wiped with filter paper, weighed and preserved at -30 °C until subsequent analysis.

2.6 Preparation of Tissues Homogenates

The tissues were homogenized separately in appropriate Tris-HCl buffer (100 Mm, pH 7.4) (1/5 w/v). The homogenates were centrifuged for 10 min at 3000 rpm to yield a pellet that was discarded and the supernatants (homogenate) used for the various biochemical assays.

2.7 Total Protein Determination

The protein concentrations in the serum of the animals will be assayed, using Biuret reagent as described by[9]. The principle was based on the formation of purple coloured complex of cupric ion with peptide bond. An aliquot of sample (1ml) was put into an empty bottle. 4ml of biuret reagent is added. The test-tube was shaken properly and left at room temperature for 30mins for colour development. The absorbance was read against blank at 540nm.

Total protein conc.= abs sample/abs standard×concentration of standard

2.8 Estimation of lipid peroxidation Malondialdehyde (MDA)

MDA was determined by spectrophotometry of the pink coloured product of thiobarbituric acid (TBA) reactive substances complex. Briefly, 0.1ml of the test sample was mixed with 0.5ml of 10% TCA, and 0.5ml of 75% TBA was then added to it. The mixture was then placed in a water bath at 80oC for 45minutes.The absorbance of the resulting pink solution was measured against a reference blank of distilled water at 532nm.The test sample was calibrated using the MDA as a standard and the results was expressed as the amount of free MDA produced. The MDA level was calculated according to the method of[10]. Lipid peroxidation in units/mg protein or gram tissue will be computed with a molar extinction coefficient of 1.56 x 105 M^{-1}/cm.

2.9 Determination of Glutathione (GSH)

Reduced glutathione was determined by the method of Ellman [11].The assay is based on the oxidation of GSH by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid (TNB) which has yellow color. Therefore, GSH concentration can be determined by measuring absorbance at 412 nm. For this assay, 50 µL of the tissue homogenate was diluted in 10 mL of phosphate buffer (0.1 M, pH 8). To 3 mL of the mixture of dilution, 20 µL of DTNB (0.01 M) was added. Absorbance is read at 412 nm against a blank prepared under the same conditions

2.10 Determination Superoxide dismutase (SOD) activity

The levels of SOD activity was determined by the method of [12]. This involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in a UV vial spectrophotometer. For the determination of specific activity of SOD in homogenate sample of stomach tissue, the rate of autoxidation of epinephrine was noted at 30 seconds intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

Calculation

SOD activity(U/mg protein)= SOD activity (U/ml)/conc protein(mg/ml)

2.11 Determination Catalase activity

This experiment was carried out using the method described by[13]. 0.2ml of sample was mixed with 0.8ml distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2mL of solution (800µmol) and 2.5mL of phosphate buffer in a 10mL flat bottom flask. 0.5mL of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1mL portion of the reaction mixture was withdrawn and blown into 1mL dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above. The mononuclear velocity constant, K, for the decomposition of H_2O_2 by catalase was determined by using the equation for a first-order reaction: $K = 1/t \log S_0/S$, **w**here S_0 is the initial concentration of H_2O_2 and S is the concentration of the peroxide at t min. The values of the K are plotted against time in minutes and the velocity constant of catalase $K_{(0)}$ at 0 min determined by extrapolation. The catalase contents of the enzyme preparation were expressed in terms of Katalasefeiahigkeit or 'Katf' according to von Euler and Josephson (1927).

Calculation

$$ko = \frac{\text{change in abs of sample}}{\text{change in abs of blnk}}$$

$$\text{Kat. f} = K_{(0)} / \text{mg protein/ml}$$

2.12 Assay for Glutathione Peroxidise (GPx) Activity

Glutathione peroxidase (GPx) was measured by the method described by[14] Briefly, the reaction mixture contained 0.2 ml 0.4 M phosphate buffer (P^H 7.0), 0.1ml 10 mM sodium azide,0.2 ml tissue homogenized in 0.4M phosphate buffer PH 7.0. 0.2 ml tissue homogenized in 0.4 M, phosphate buffer ,PH 7.0, 0.2 ml reduced glutathione , 0.1 ml 0.2 mM hydrogen peroxide. The contents were incubated for 10 min at 37o c,0.4 ml 10% TCA was added to stop the reaction and centrifuged at 3200 × g for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% sodium nitrate). The activities were expressed as µg of GSH consumed/ min/mg protein.

2.13 Statistical analysis

Results were expressed as means of triplicate ± SD. Data were statistically analyzed with Graph Pad Prism® version 6.00 by the help of turkey multiple comparison test using one way anova. Differences are considered significant when $P < 0.05$.

3. RESULTS

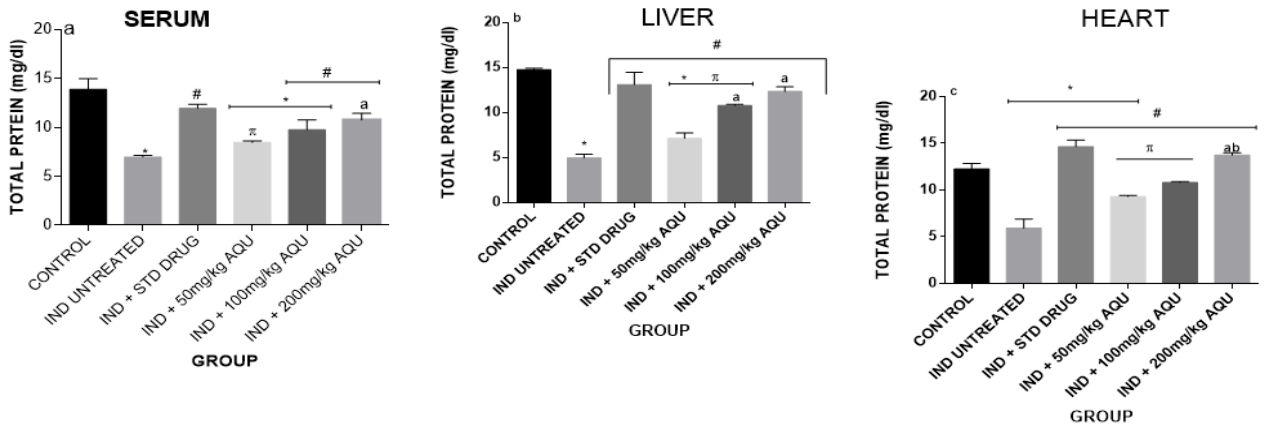

Figure 1: **Effects of Aqueous extract of *Bryophyllum pinnatum* on Total Protein levels in the tissues (a) Serum, (b) Liver,(c)Heart of Carbon tetrachloride -induced albino rats.** All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group, bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group.IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum.*

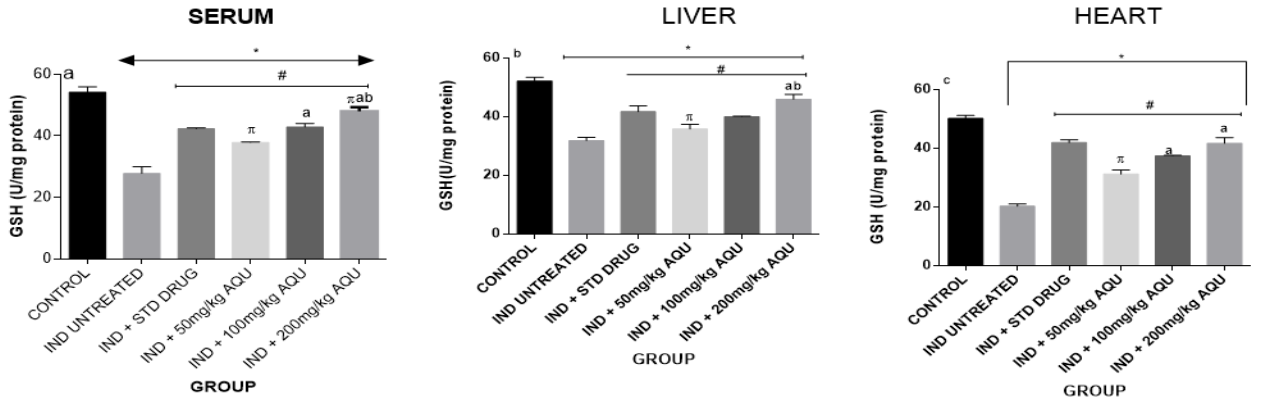

Figure 2: **Effects of Aqueous extract of *Bryophyllum pinnatum* on GSH levels in the tissues (a) Serum, (b) Liver, (c)Heart of Carbon tetrachloride -induced albino rats.** All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group, bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group.IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum*; GSH: Glutathione.

The induction of the rats with CCl_4 led to a significant decrease ($P < 0.05$) in the concentration of total protein and GSH in all the tissues when compared with the control as shown in (Figure 1and 2). Treatment with different concentrations of the extract ameliorates the effect significantly with 100 and 200mg/kg competing favorably with a significant increase ($P < 0.05$) with the standard drug .

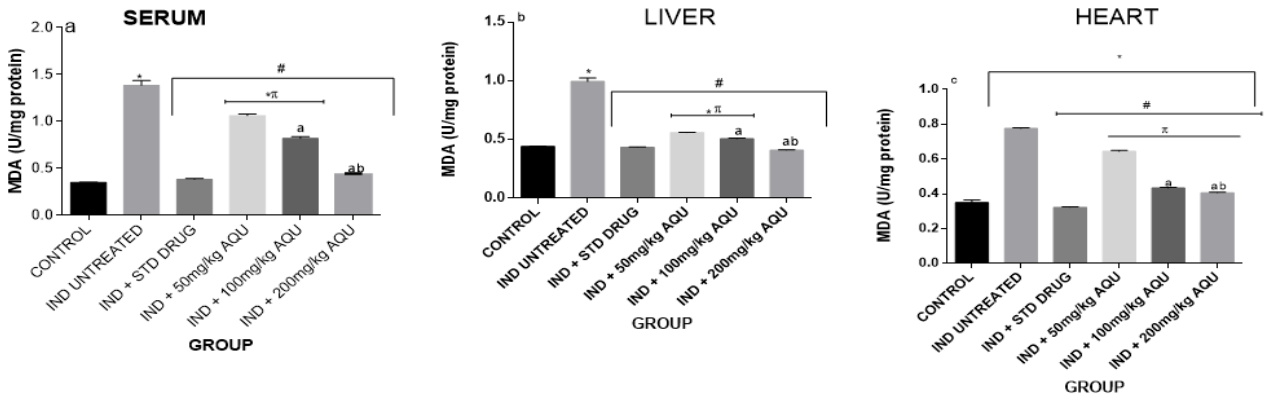

Figure 3: **Effects of Aqueous extract of *Bryophyllum pinnatum* on MDA levels in the tissues (a) Serum, (b) Liver, (c)Heart of Carbon tetrachloride -induced albino rats**. All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group, bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group. IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum*; MDA: Malonydialdehyde.

The induction of the rats with CCl_4 significant increased the generation of lipid peroxidation (P < 0.05) in all the tissues (Figure 3) when compared with the control as shown in figure 2. Treatment with different concentrations of the extract inhibited lipid peroxidation as there was a significant reduction in the level of MDA towards the control values. 200mg/kg significantly decreased (P < 0.05) the level at the same rate as the standard drug.

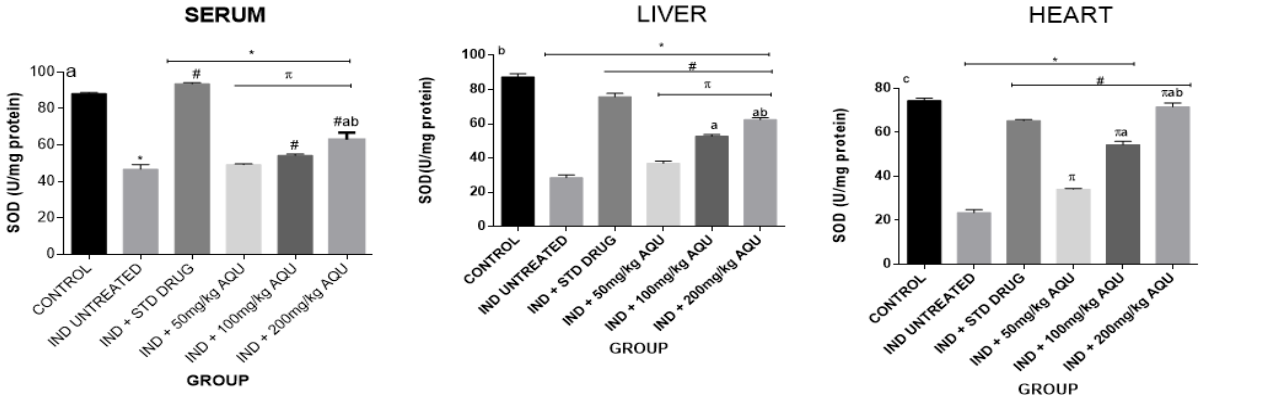

Figure 4: **Effects of Aqueous extract of *Bryophyllum pinnatum* on the activity of SOD in the tissues (a) Serum, (b) Liver, (c)Heart of Carbon tetrachloride -induced albino rats**. All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group, bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group.IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum*; SOD: Superoxide dismutase.

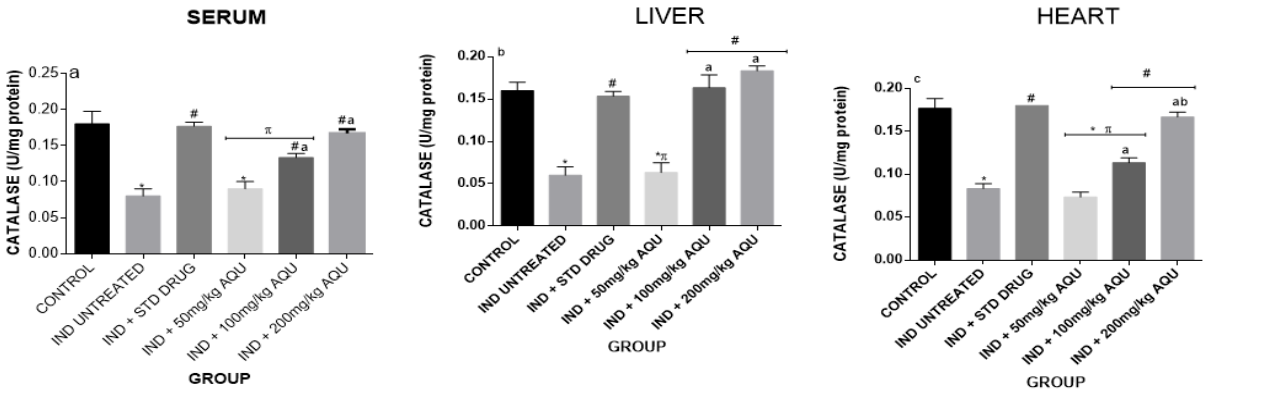

Figure 5: **Effects of Aqueous extract of *Bryophyllum pinnatum* on the activity of Catalase in the tissues (a) Serum, (b) Liver, (c)Heart of Carbon tetrachloride -induced albino rats**. All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group, bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group.IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum.*

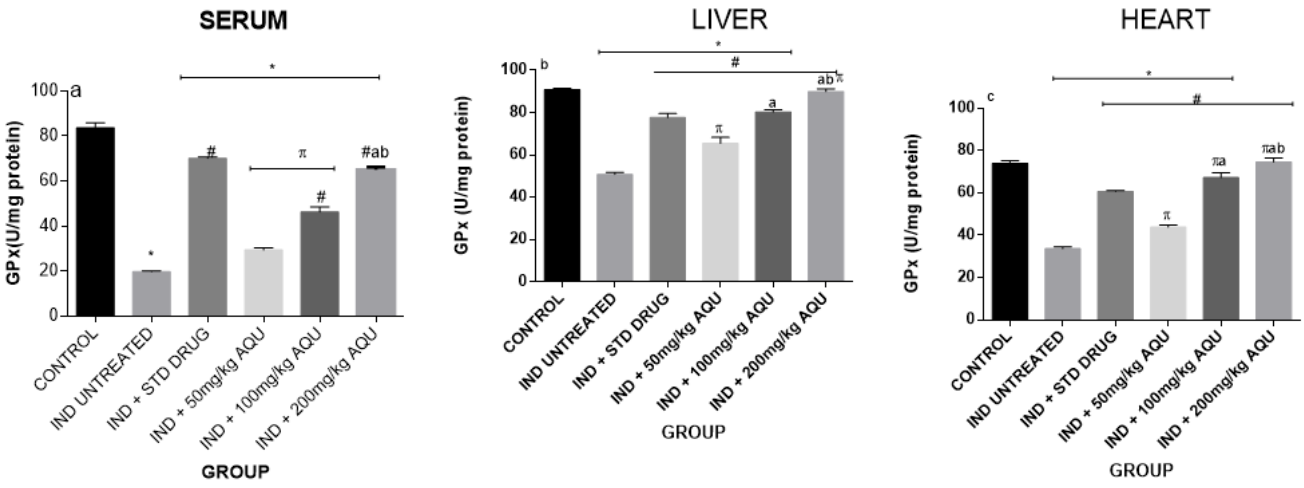

Figure 6: **Effects of Aqueous extract of *Bryophyllum pinnatum* on the activity of GPx in the tissues (a) Serum, (b) Liver, (c) Heart of Carbon tetrachloride -induced albino rats**. All values are expressed as mean ± standard deviation of mean (n = 3 in each group). *P < 0.05, vs control group, #P < 0.05, vs CCl4 untreated group, πP < 0.05, vs CCl4 ind + standard drug group, aP < 0.05, vs CCl4 ind + 50mg/kg aqueous extract group,bP < 0.05, vs CCl4 ind + 100mg/kg aqueous extract group group.IND:CCl4 induced; AQU: Aqueous extract of *Bryophyllum pinnatum;* GPx: Glutathione peroxidase

The results of SOD, catalase and GPx (Figure4,5,6) revealed a significant increase (P < 0.05) in the serum, liver and heart tissue for the groups treated with 100 and 200 mg/kg aqueous extract of *Bryophyllum pinnatum* when compared with the CCl4 induced untreated group.

4. DISCUSSSION

All aerobic organisms have defense mechanisms to protect against oxidative damage. Antioxidants are, by definition, compounds that have the ability to inhibit or prevent oxidation by electron donation[15,2]. Some of the actions of antioxidants include prevention or repair of the damage caused by ROS and RNS, as well as the elimination of free radicals[15].The body has evolved a complex defence strategy to minimize the damaging effects of various oxidants. Central to this defence are the non-enzymatic and enzymatic antioxidants. These include reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) respectively, which act in concert to protect the organism from oxidative damage[16]. Thus, this study evaluated the effect of the extracts on oxidative stress parameters MDA, GSH and CAT, SOD and GPx in albino rat tissues (serum, liver and Heart) where oxidative stress appears to play a crucial role.

Carbon tetrachloride (CCl4) is a colorless, volatile, non-inflammable liquid that is produced by the mixture of chlorine with chloroform in the presence of light. CCl4 toxicity develops not due to the CCl4 itself but for the generation of free radical CCl3 and other metabolites produced by cytochrome P450. Ultimately they lead to cellular damage by alteration of cellular structure through lipid peroxidation and in some other pathways. Severe conditions may develop through multiple organ dysfunction by these free radicals[17]. The observed increase in lipid peroxidation(Figure 3) is this study was significantly reduced (P < 0.05)upon treatment with aqueous extract of *Bryohyllum pinnatum.* Plants synthesize several beneficial compounds via metabolism. The primary products of plant metabolism are proteins and cellulose. Secondary metabolic components include phenolic compounds, terpenoids, and nitrogen containing compounds[18]. Studies have shown the role of antioxidants (drug and plant-derived antioxidant) in scavenging free radicals and ultimately preventing oxidative stress resulting from the effects of pro-oxidants on the cells, thereby abrogating the disease-causing potentials of free radicals in the cells[19,20].

Reduced glutathione (GSH) is a tripeptide, non enzymatic biological antioxidant present in the liver which helps to donate reducing equivalent to NADP+ and oxidant molecules. Decreased GSH level is associated with reduced antioxidant status in the living system. This reduction is as well linked to various diseases related to oxidative stress such are atherosclerosis, diabetes mellitus, carcinogenesis and others. The observed elevated GSH level (Figure 2) upon administration of the plant extract may depict its ability in boosting the antioxidant defence of biological system thereby preventing diseases associated with oxidative stress[21].

The activity of Catalase is one of the indices of antioxidant status of the body. Decrease in the activity of this enzyme may lead to deleterious effects as a result of superoxide and hydrogen peroxide assimilation[22]. Increased catalase (P < 0.05)activity observed in the present study (Figure 5)in all the tissues could be an indication of chemopreventive potential of the plant in the pathogenesis of oxidative stress-related diseases which may be associated to the presence of various bioactive principles in the plant such as alkaloids, flavonoids, saponins, tannins, carbohydrates, cardioactive glycosides, steroids, phenols and reducing sugars [23]. This is in consonant with the report given by[20], in a study on the effect of *Annona muricata* in rats.

Also, elevated activities of superoxide dismutase (SOD) and Glutathione peroxidase (GPx) in groups administered 100mg/kg and 200mg/kg (Figure 4,6) could depict that the extract at these doses may be effective in combating rapid generation of free radicals in the body system. GPx plays a pivotal role in H_2O_2 catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH. Decreased activity of GPx may result from radical induced inactivation and glycation of the enzymes [24]. In the rats treated with *Bryophyllum pinnatum* extract, significant increase (P < 0.05)in GPx was observed. This might reflect the antioxidant potency of the extract by preventing glycation and inactivation of GPx. Similar effect to the control group in the tissues may be indicative of the

safety intake of *Bryophyllum pinnatum* extract at such concentrations

CONCLUSION

The present study was carried out to evaluate the Antioxidant activity of aqueous extracts prepared from the leaves of *Bryophyllum pinnatum*, this activity was tested *in vivo* using *Albino Wistar* rats. The results showed that oral administration of extracts(50, 100 and 200mg/kg body weight) led to increased antioxidant capacity in the tissues and caused amelioration in the liver and heart antioxidant status, by decreasing the MDA concentration and increasing the rate of reduced glutathione (GSH), Superoxide dismutase(SOD), Catalase(CAT) and Glutathione peroxidase activity. It could be deduced that the aqueous leaf extract of *Bryophyllum pinnatum* possess significant antioxidant capacity which could be good scavenger of free radicals and invariably useful in the prevention of oxidative stress related diseases. It could serve as an excellent antioxidant agent.

Conflicts of Interest:

The Authors declare that there is no conflict of interest.

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