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Bryophyllum Pinnatum: A Potential Attenuator of Cadmium-Induced Oxidative Stress in Rabbits

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Abstract: *Cadmium has been famously implicated in the stimulation of free radical production in biosystems resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals. This study therefore, examined the antidotal and ameliorative capacity of crude ethanolic extract of Bryophyllum pinnatum on cadmium-induced oxidative stress using rabbit models. A total of fifteen rabbits (1.30±0.05kg) were used for the study. After two weeks of acclimatization, the rabbits were randomly rifted into three experimental groups- (N, CD & CB) with five animals per group. The control group (N) was injected normal saline intraperitoneally (3mg/kg body weight) and the test groups (CD & CB) were administered cadmium once daily by subcutaneous injection (3mg/kg body weight). The ethanolic extract of the plant was orally administered once daily at a dose of 100mg/kg body weight. The oxidative and antioxidative stress parameters were assessed in tissues. The results showed significant difference ($p< 0.05$)in treated groups relative to the control group with the exception of glutathione peroxidase activity in leg muscles. Therefore, the results obtained in this study confirmed the potency of the plant to annihilate cadmium toxicity in animals.*
Keywords: *antioxidative stress parameters, Bryophyllum pinnatum, cadmium toxicity, oxidative stress*

I. Introduction

Cadmium (Cd) is a metal with no known beneficial properties that support life- there is no evidence that it is either biologically necessary or beneficial [1, 2]. At low concentrations, it is toxic to all life, including plants, fish, birds, mammals especially humans, and microorganisms such that it causes cancer, birth defect and genetic mutation [1, 3]. In one comparative acute toxicity testing of sixty-three heavy metals, cadmium was the most toxic metal [4, 5]. The most common uses of Cd include electroplating, some industrial paints and some types of batteries. The human population is mostly exposed to cadmium mainly through food and cigarette smoking [6, 7]. Cadmium has been shown to be toxic due to its ability to induce severe alterations in various organs by generating free radicals, which cause oxidative destruction of membrane polyunsaturated fatty acids of these organs and thus lipid peroxidation [8-10]. The tissues in which this effect has been reported include the brain, liver, kidney, testes, heart, eye and intestine [11-14].

Oxidative stress results from the imbalance of reactive oxygen species (ROS) and defense mechanisms which results in cell damage. The enhanced production of free radicals and oxidative stress can also be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics. Also, available reports indicate that cadmium-induced toxicity results from generation of free radicals, which lead to lipid peroxidation, causing damage to many biosystems and organs, and a change in their functions and structures [15-17]. The liver and kidney, however, represent the major target of cadmium toxicity [18]. When cadmium enters the body, it is transported to the liver bound to metallothionein (a low molecular weight protein rich in cysteine residues) and mainly distributed from the liver and kidney, where it bioaccumulates and causes damage to these tissues[19].

Medicinal plants which contain substances that could be used for therapeutic purposes and precursors for the synthesis of useful drugs have been employed in the treatment of illnesses within local or regional practices [20, 21]. Most developing countries of the world, rural and urban dwellers, literates and illiterates depend heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine [20, 22]. In Nigeria today, traditional and herbal healing systems play an important role in healthcare delivery and about 70-80% of the population depend on traditional healers for most of their ailments [23]. One of such plants used in the treatment of a wide range of ailments is Bryophyllum pinnatum (B. Pinnatun). The plant belongs to the family of crassulaceae, classified as a weed and its common names include: African never die, Resurrection plant, Love plant, Life plant, Air plant, etc. It is a fleshy herb, about 60-120cm tall, which branches from the base 10cm long and 5.6cm broad respectively. The margins are notched with irregular blunt or rounded teeth, which sometimes being bulbils in their axes [24]. Previous research revealed that the leaves of B. Pinnatum have been used in diverse ways: as an anti-ulcer agent, anti-fungal, anti-inflammatory, anti-

hypertensive and an analgesic [23]. The plant is also needed for the treatment of earache and in ophthalmologic preparations [7, 25].

The numerous medicinal properties and uses of the plant extract coupled with the paucity of information in the ameliorative potentials of the crude plant extract in science literature makes it vital to investigate the fate of cadmium-induced oxidative stress in rabbits administereded with crude ethanolic extract of B. Pinnatum.

II. Materials and Methods

2.1 **Chemicals:**

All chemicals and reagents used were of analytical grade and were obtained from internationally reputed suppliers such as Sigma (UK) and BDH (UK). Only freshly prepared solutions and reagents were used.

2.2 Collection of Plant Materials

The leaves of the plant were obtained from Agbor, Delta State, Nigeria, and authenticated by the Department of Botany, University of Benin,Benin City, Nigeria, and a voucher specimen was kept in the herbarium for future reference.

2.3 Animal Housing

Fifteen male rabbits of about six to seven months old were purchased and housed in the animal house of the Department of Biochemistry, University of Benin, Nigeria, where they were allowed to acclimatize for two weeks. They were fed with commercially available pellet feeds and allowed access to clean deionised water.

2.4 Preparation of Plant Extract

The leaves of B. Pinnatum were washed, shade-dried and macerated by means of warring blender at the Pharmacognosy unit of the institution to obtain a fine powder for the extraction. About 250g of the powder were extracted exhaustively with ethanol in a soxhlet extractor and the mixture sieved. The remaining ethanol in the crude extract was evaporated by means of rotary evaporator to get a consistent concentrated viscous liquid of the crude extract.The extract was freeze-dried for subsequent laboratory use.

2.5 Experimental Design

After two weeks of acclimatization to standard laboratory conditions, fifteen (15) male adult rabbits were randomly divided into three groups of five rabbits per group, labelled N, CD and CB respectively. The control (N) group was injected 3mg/kg body weight normal saline subcutaneously. The tests groups known as cadmium (CD) and cadmium-bryophullum (CB) groups were administered 3mg/kg body weight cadmium (as $CdSO_4.8H_2O$) subcutaneously. Concurrently, the CB group was administered 100mg/kg body weight of the crude ethanolic extract orally by means of a gavage.

All treatments were carried out once daily; in accordance with the principles of laboratory animal care (NIH Publication No. 85-93, Revised 1985). At the end of four weeks experimental treatment, tissues of interest were excised from the rabbits and kept in chilled conditions prior to assay.

2.6 Collection and Preparation of Tissues

After an overnight fast, the rabbits were sacrificed and dissected. The liver, kidney, leg and head muscles were excised and kept in chilled condition to maintain the physiology of enzymes located in each of the respective organs. Prior to assay of oxidative and antioxidative stress indicators, a weighed portion of each organ was washed several times with ice-cold normal saline solution, homogenised in 10ml of 0.9% sodium chloride solution using pre-chilled mortar and pestle. The homogenates were centrifuged at 4000g for 30mins and the supernatants were subsequently used to assay for the activities of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and the estimation of lipid peroxidation and total protein respectively.

2.7 Biochemical Assessment

Lipid peroxidation was determined by the measurement of thiobarbituric acid reactive substances (TBARS) in tissues using the method of Yagi [26]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm. Total protein was estimated by the method of Lowry et al [27].

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of Kakkar et al. [28] based on the 50% inhibition of the formation of NADH-phenazine methosulfatenitroblue tetrazolium formazan at 520 nm. Catalase (CAT;EC 1.11.1.6) activity was assayed by the method of Sinha [21]. Hemolysate was treated with H_2O_2 (0.2 mol/l) and the reaction was arrested after 60s by the addition of dichromate–acetic acid reagent, cooled and the intensity of color read at 620 nm. Various aliquots of H_2O_2 were used as the standard. A system devoid of the two enzymes served as control.

The activity of glutathione peroxidase (GPx;EC1.11.1.9) was assayed by following the oxidation of NADPH at 340 nm with t-butyl-hydroperoxide by Tamura et al [30]. All enzyme activities were calculated per milligram of protein

2.8 Statistical Analysis

The data for biochemical analyses were expressed as Mean±SD. Statistical comparisons were performed by one factor analysis of variance (ANOVA: LSD, DUNCAN and SNK tests) using the statistical package for social science version 20.0 (SPSS Inc, Chicago II, USA). Results designated by different letters along each column were considered significant ($p< 0.05$).

III. Results

Table 1: Lipid peroxidation and activities of antioxidant enzymes in tissues of rabbits treated with cadmium and B. Pinnatum

Tissues	Groups	MDA(mmoles/mg protein)	CAT(U/mg protein)	SOD (U/mg protein)	GPx(U/L
LIVER	N	1.43 ± 0.11^{a}	190.01 ± 7.0^{a}	2.40 ± 0.10^{a}	1.57 ± 0.07^{a}
	CD	1.69 ± 0.02^{b}	136.15 ± 8.0^{b}	2.14 ± 0.20^{b}	0.89 ± 0.03^{b}
	CB	1.57 ± 0.10^{c}	152.90 ± 7.0^{c}	2.51 ± 0.10^{c}	2.10 ± 0.05^{c}
KIDNEY	N	1.91 ± 0.08^{a}	7.34 ± 0.03^{a}	1.84 ± 0.06^{a}	0.62 ± 0.10^{a}
	CD	2.86 ± 0.01^{b}	5.80 ± 0.02^{b}	1.76 ± 0.21^{b}	0.45 ± 0.20^{b}
	CB	2.36 ± 0.04^{c}	7.50 ± 0.03^{c}	1.78 ± 0.01^{c}	0.74 ± 0.20^{c}
LEG MUSCLE	N	0.71 ± 0.03^{a}	1.35 ± 0.16^{a}	3.38 ± 0.11^{a}	1.03 ± 0.22^{a}
	CD	0.72 ± 0.02^{b}	0.45 ± 0.01^{b}	3.17 ± 0.10^{b}	1.04 ± 0.03^{a}
	CB	0.64 ± 0.03^{c}	1.23 ± 0.02^{c}	3.61 ± 0.03^{c}	1.10 ± 0.02^{a}
HEAD MUSCLE	N	2.97 ± 1.00^{a}	2.09 ± 0.02^{a}	13.66 ± 1.20^{a}	6.10 ± 0.21^{a}
	CD	5.78 ± 1.36^{b}	1.73 ± 0.04^{b}	6.30 ± 0.24^{b}	3.44 ± 0.67^{b}
	CB	2.10 ± 0.80^{c}	1.95 ± 0.03^{c}	11.89 ± 0.54^{c}	3.25 ± 0.60^{c}

*Expermental data were expressed as mean ± S.D of five rabbits in each group. $p<0.05$ was considered significant. Biochemical parameters marked with the same letters across the groups per column designate no significant difference ($P>0.05$) relative to control. "N" as control group, "CD" as cadmium treated groups and "CB" as cadmium and extract of B, pinnatum treated groups.

TABLE 1 shows the effect of B. Pinnatum and cadmium administration on oxidative and antioxidative stress parameters in respective tissues of rabbits. Lipid peroxidation as assessed by TRARS levels was significantly lower in CB group than CD group relative to the control vehicle in various tissues. The activities of antioxidant enzymes such as SOD, CAT and GPx showed significant increase ($p<0.05$) in CB group than CD group as compared with the control group in liver tissues, kidney tissues, leg and head muscles respectively.

However, the activity of GPx showed no significant difference ($p>0.05$) in the groups in leg muscles of rabbits

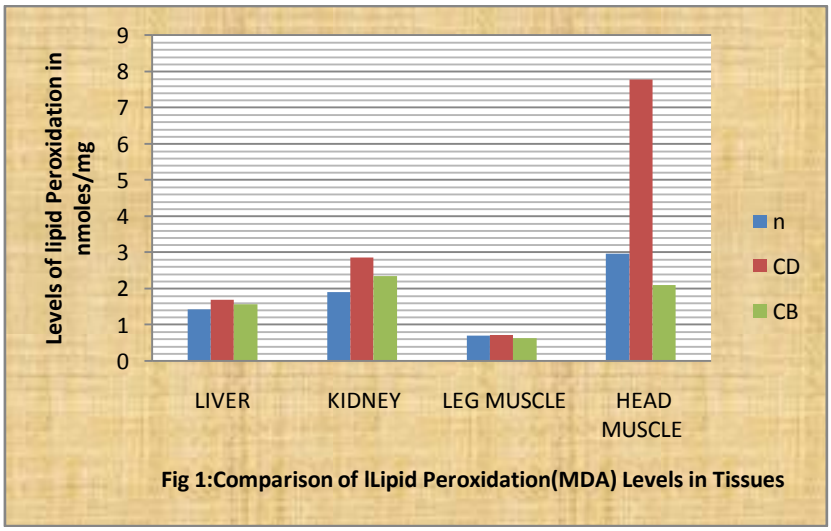

Fig 1:Comparison of lLipid Peroxidation(MDA) Levels in Tissues

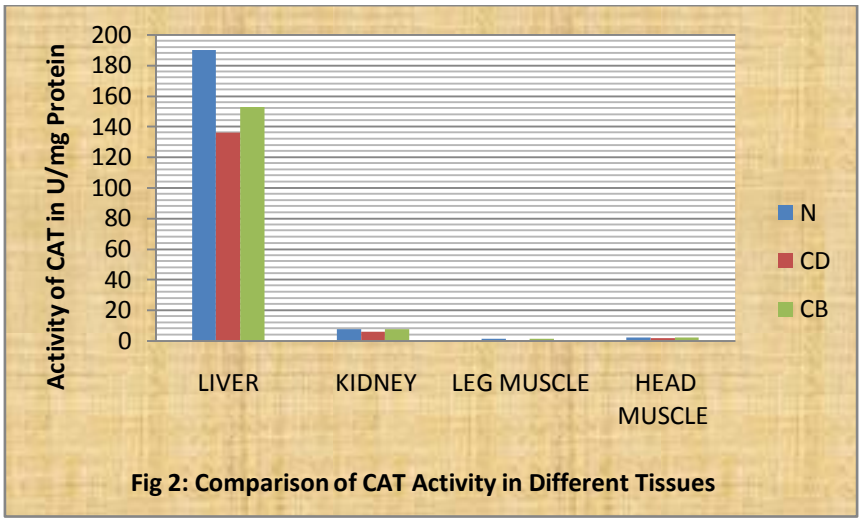

Fig 2: Comparison of CAT Activity in Different Tissues

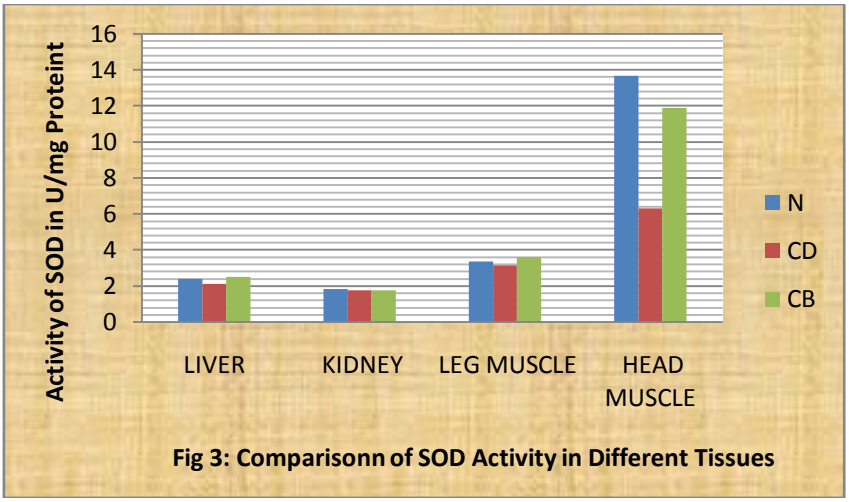

Fig 3: Comparisonn of SOD Activity in Different Tissues

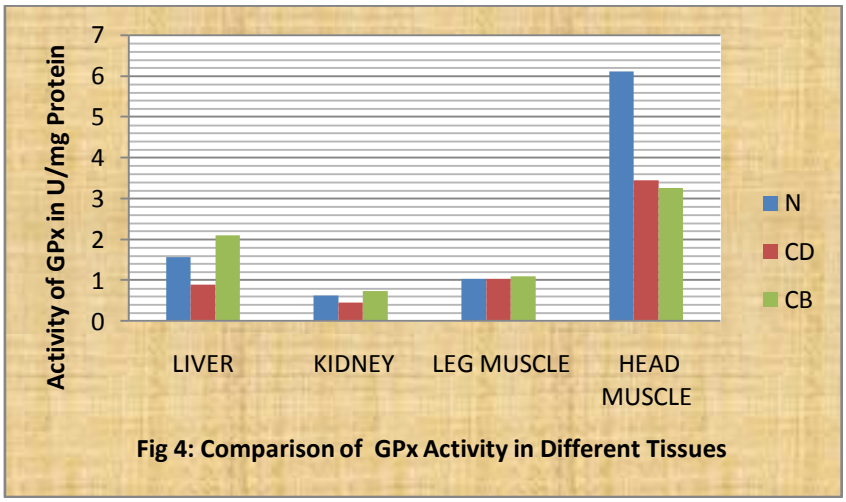

Fig 4: Comparison of GPx Activity in Different Tissues

IV. Discussion

One of the biochemical changes occurring in plants and animals subjected to heavy metal stress conditions such as cadmium is the production of reactive oxygen species (ROS) like superoxide radical, hydroperoxide, singlet oxygen and hydroxyl radicals [31]. The ROS are strong oxidizing agents that cause oxidative damage to biomolecules such as lipids, proteins and nucleic acids, and eventually leads to cell death [32]. Mechanisms for the generation of ROS in biological systems are represented by both enzymatic and non-enzymatic reactions. Therefore, oxidative stress is due to a disturbance in the balance between the production of ROS and the deficiency of the antioxidant defense systems. In other words, oxidative stress results if excessive

production of ROS overwhelms the antioxidant defense or when there is a significant decrease or lack of antioxidant defense system [33, 34]. The epoxides generated due to increased oxidative stress may spontaneously react with nucleophilic centres in the cell thereby covalently binding to DNA, RNA and proteins.

Such reactions may lead to cytotoxicity and carcinogenicity depending on the properties of the epoxides [35]. Moreover, severe oxidative stress is not only known to cause DNA damage and mutations of tumour suppressor genes, which are initial events in carcinogenesis [33], but can play an important role in the promotion of aging and cardiovascular diseases [36].

Lipids especially polyunsaturated fatty acids (PUFA) are very susceptible to free radical attack, which can initiate lipid peroxidation. The end product of lipid peroxidation, malondialdehyde (MDA), due to its high cytotoxicity and inhibitory action on protective enzymes, it is suggested to act as tumour promoter and an initiator of many pathological conditions [37]. Therefore, studies have shown that cadmium can promote the generation of ROS, inhibit or stimulate the activities of antioxidant enzymes such as CAT, SOD and GPx respectively [38].

Reports on preliminary phytochemical investigation of different parts of plant extract of B. Pinnatum revealed the presence of alkaloids, phenols, flavonoids, saponins, carotenoids, glycosides [39]. Therefore, traditional medicine has a long history and there is increasingly wide acceptability of the practice in the treatment of a number of ailments due to the presence of bioactive compounds in such plants. This practice mainly uses plants and this presupposes the efficacy and safety of the plant materials used. B. Pinnatum has been employed in the treatment of a number of ailments [7, 40].

The antioxidant enzymes; CAT, SOD and GPx are widely distributed in all tissues, and during cadmium toxicity, reports showed that the liver and kidney are the prime target of cadmium bioaccumulation [13, 18]. The levels of lipid peroxidation (MDA) and its comparative concentrations in various tissues are shown in TABLE 1 and Fig. 1 respectively. There was marked significant difference ($p<0.05$), between the treated (CD & CB) groups relative to the control group in liver, kidney and muscle tissues. The induction of high levels of lipid peroxidation in CD group in various tissues conforms to the report of Eriyamremu et al [13] and Sarkal et al [15]. Although there is paucity in literature regarding the ameliorative capacity of the crude ethanolic extract of B. Pinnatum on cadmium-induced generation of ROS in the respective tissues, the low levels of MDA may be attributed to the synergistic effect of the antioxidant capacity of the plant itself and the defense system of the animal model.

SOD protects cells against O_2^- by dismutation of the highly reactive superoxide anion to O_2 and to a less reactive species, H_2O_2. CAT and GPx in turn protect the cell from H_2O_2 generated by various reactions [15, 18]. In our studies, (TABLE 1 & Fig. 2-4), we observed a marked significant difference ($p<0.05$) between the treated groups as compared with the control group, with the exception of GPx activity showing no significant difference ($p>0.05$) relative to control in leg muscle. The observed increase in MDA levels in respective tissues of CD group correlates with the decline in CAT, SOD and GPx activities in that group. However, the slight increase in CAT, SOD and GPx activities in CB group as compared with the CD and control groups may be attributed to phytochemicals present in the crude ethanolic extract of B. Pinnatum, as reported by Muhammad et al [39] and Nayana et al [40]. In view of its protective role against cadmium toxicity, the plant could be used as an anti-hepatotoxic and anti-nephrotoxic agents thereby maintaining tissue integrity.

V. Conclusion

The observed changes on antioxidant parameters are indications that the crude ethanolic leaf extract of B. Pinnatum may be used to attenuate cadmium toxicity to a considerable degree when administered subcutaneously. There is, however, need for crude leaf extract of the plant to be fractionated by HPLC so as to characterize the active biochemical that is possibly causing the observed effects on the antioxidant parameters in the rabbit models. In conclusion, there is room for further research to consolidate the potentials of the plant as an attenuator of cadmium toxicity through consideration of route of administration and dosage of the plant extract.

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