Activities of aldehyde oxidase, Lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase in brain, heart and liver of rabbits exposed to cadmium and aqueous leaf extract of Bryophyllum pinnatum

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Abstract- This study examined the potency of aqueous leaf extract of *Bryophyllum pinnatum* on cadmium toxicity in the brain, heart and liver of rabbits through the assessment of the activities of aspartate aminotransferase and alanine aminotransferase for three weeks.

Fifteen male rabbits were used for this study. They were divided into three groups of five animals each. Rabbits in group I were administered 2mg/kg body weight of deionised water subcutaneously, rabbits in group two were administered 2mg/kg body weight of cadmium subcutaneously and rabbits in group three were administered 80mg/kg body weight aqueous leaf extract of *B. pinnatum*. At the end of three weeks of feeding, all the rabbits were weighed sacrificed and dissected. The brain, heart and liver tissues of each rabbit was excised and used for analysis.

The results obtained for the mean activities of aspartate aminotransferase and alanine aminotransferase showed a significant difference ($p < 0.05$) relative to the control in brain, liver and heart tissues. These results show the potency of *B. pinnatum* extract in reducing cadmium toxicity.

I. Introduction

Interest on the role of complementary and alternative drugs for the treatment of diseases has increased in recent time. Different types of herbs due to their high concentration of antioxidants have exhibited high potential for use in the treatment and management of certain clinical alteration. One of such herbs is *bryophyllum pinnatum* whose extract is known to be rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids (Marriage and Wilson, 1971; Costa et al, 1995). The leaves contain a group of chemicals called bufadienolides which are very active. Bufadienolides like bryotoxin A, B, C are very similar in structure and activity to two other cardiac glycosides, digoxin and digitoxin and possesses antibacterial, antitumorous, cancer preventative and insecticidal actions (Steyn, and Van Heerden, 1998; Rastogi, and Mehrotra, 1994). *Bryophyllum pinnatum* contain fatty acid fractions including palmitic acid (89.3%), stearic acid (10.7%), traces of arachidic and behenic acid (Takashi et al 1988, Almeida et al, 2000).The plant also contains HCN, oxalic acid (Siddiqui et al,1989), citric acid, isocitric acid, oxaloacetate (Almeida et al, 2000) , malic acid

(Sutton and Osmond,1972) and succinic acid. The plant is rich in vitamins and aminoacids; ascorbic acid, riboflavin, thiamine, niacin, pyridoxine, glycine, cysteine, casein hydrolysate, glutamic acid, protein hydrolysate, methionine, tyrosine, phenylalanine (Okwu, and Josiah, 2006). It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia (Kamboj and Saluja, 2009), classified as a weed (Oliver-Bever,1983) the plant flourishes throughout the Southern part of Nigeria (Gill, 1992). It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. *B. Pinnatum* has been extensively used in herbal medicine not only for their oxytoxic, analgesic, anti-inflammatory antimicrobial properties but also provide relief in the treatment of human gastrointestinal, hypermobility and pepetic ulceration (Shrivastava and Patel, 2007; Okwu and Iroabuchi, 2009). In traditional medicine, the leaves of the plant have been reported to possess antimicrobial (Akinpelu, 2000; Okwu and Uchenna 2009), anti-inflammatory, antifungal (Misra and Dixit, 1979), and anti-hypertensive (Ojewole, 2002) activities.

Cadmium is a lustrous, silver white ductile, very malleable heavy metal. It is soft enough to be cut with knife but tarnishes in air. (Cadmium Ass,1991). It is toxic at very low exposure levels and has acute and chronic effect on health and environment. It is extremely toxic to humans and in chronic exposure, it accumulates in the body, particularly in kidney and liver (Fasset, 1972). Acute poisoning from inhalation of fumes and ingestion of cadmium salt can result to death (Baldwin and Marshall 1999). Cadmium has been one of the most toxic and recognized environmental and industrial pollutant due to its ability to induce severe alterations in various organs by reducing the biosynthesis of enzymes of energy metabolism and also increasing the rate of oxidative destruction of membrane polyunsaturated fatty acid of these organs following either acute or chronic exposure. Because cadmium is a naturally occurring component of all soils, all food stuffs will contain some cadmium and therefore all humans are exposed to natural levels of cadmium. Therefore, the essence of this study is to ascertain the

potency of *bryophyllum pinnatum* extract on cadmium toxicity in various organs via the assessment of the activities of aspartate aminotransferase and alanine aminotransferase.

II. MATERIALS AND METHODS.

EXPERIMENTAL ANIMALS

Fifteen male Rabbits of 1.31± 0.05kg and about six to seven months old purchased from Aduwawa cattle market Benin-city, Edo-State, were used for this study. The rabbits were weighed and assigned comparable weights in all groups (± 0.05kg). The animals were acclimatized with their respective diets for a period of one week after which the animals were weighed again and then divided into three groups of five (5) rabbits each: group 1, the control (N); and two test groups tagged cadmium group which is the group 2 (CD) and *Bryophyllum* group which is the group 3 (B). Rabbits in group I were administered 2mg/kg body weight of deionised water subcutaneously, rabbits in group two were administered 2mg/kg body weight of cadmium subcutaneously and rabbits in group three were administered 80mg/kg body weight aqueous leaf extract of *B. pinnatum* subcutaneously**.** The rabbit were fed with pellet finishers from Top feeds and tap water supplied by Uniben water board. The weights of the animals were taken at the end of every week for the period of three weeks and on the day the animal were sacrificed. The cages housing the rabbits were kept in an environment with free supply of air and light. Water and feed were changed daily.

PREPARATION OF BRYOPHYLLIUM PINNATUM EXTRACT

The leaves of *Bryophyllum pinnatum* were obtained from Santua garden, Ugbowo, Benin City, Edo state and identified at botany department, Ambrose Ali University, Ekpoma. They were washed with distilled water to remove dust and other particles and allowed to dry for about ten minutes. The leaves were blended into paste without adding water. Thereafter, 4 g of the paste was dissolved in 20 ml of distilled water to give *Bryophyllum pinnatum* with 0.2 mg/ml concentration.

COLLECTION OF SAMPLE

At the end of three weeks of feeding, all the rabbits were weighed sacrificed and dissected. The liver, kidney and brain tissues of each rabbit was excised and kept separately in sample bottles (10ml) and then stored at -4^0C for analysis.

PREPARATION OF TISSUE SAMPLE

1g of the brain, heart and liver tissue was homogenized separately in 5ml of 0.9% normal saline for 10 seconds. The cytosolic fraction was obtained from the homogenate by successive centrifugation at 9000*g* for 20 min and 105,000*g* for 60 min. The supernatant was used as source of sample.

Biochemical assay

ALANINE AMINO TRANSFERASE (ALT)

PRINCIPLE: ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The colour intensity was measured against the blank at 546nm (Reitman and frankel, 1957).

METHOD: The blank and sample test tubes were set up in triplicates. To these were added 0.25ml buffer solution containing phosphate buffer, L- alanine and alpha ketoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30minutes at 37ºC and pH 7.4. Exactly 0.25ml of reagent containing 2,4-dinitrophenylhydrazine was later added to all tubes while 0.25ml of sample was added to sample blank tube. These tubes were mixed thoroughly and allowed to stand for exactly 20 minutes at 20ºC to 25ºC. Exactly 0.025ml of sodium hydroxide solution was then added to all tubes and mixed. The absorbance was read against the blank after 5 minutes at 546nm.

ASPARTATE AMINO TRANSFERASE

PRINCIPLE: Aspartate amino tranferase was assayed spectrophotometrically in a coupled reaction with malate dehydrogenase in the presence of NADH (Karmen et al,1955, Amador and Wacker, 1963). The reagent mixture contained 134mM of aspartate, 5.64mM of 2-oxoglutarate, 0.24 mM of NADH, 5u/ml of lactate dehydrogenase, 1.25u/ml of malate dehydrogenase and 50mM of phosphate buffer at pH 7.4 The enzyme was diluted by dissolving the enzyme at a concentration of 1mg/ml in 0.1 M potassium phosphate of pH 7.4. and then diluted further in the buffer to a concentration of 0.05 to 0.25 u/ml.
METHOD: The spectrophotometer was adjusted to 340nm at 25ºC. Exactly 2.9ml of the reagent mixture was put into cuvettte and incubated for four minutes. To this, 0.1ml of the appropriately diluted enzyme was added at zero time and the decrease in absorbance at 340nm for five minutes was recorded.
CALCULATION

$$\frac{\text{Change in absorbance at 340nm/minute}}{62 \times \text{mg enzyme in reaction mixture}}$$

III. STATISTICAL ANALYSIS

Statistical analysis was carried out using the mean, standard deviation and standard error of mean respectively. The data obtained were subjected to analysis of variance (ANOVA). The mean differences were separated by Duncan's multiple range test at 0.05 level of significance (Sokal and Rohlf, 1969).

IV. RESULTS

Results are as presented in table 1. Results showed that *B. pinnatum* significantly ($P<0.05$) increased the activities of aspartate aminotransferase and alanine aminotransferase when compared with the control in brain, liver and heart tissues.

Table 1: The effect of cadmium and aqueous leaf extract of *Bryophyllum pinnatum* administration on the activities of aspartate aminotransferase and alanine aminotransferase in rabbits.

Tissue	Groups	Aspartate aminotransferase (u/l)	Alanine aminotransferase (u/l)
BRAIN	**Control**	16.60±1.12	32.60±6.31
	Cadmium	15.40±0.60	31.00±9.95
	Bryophyllum	17.80±1.74	45.20±3.88
HEART	**Control**	49.73±14.19	35.00±.87
	Cadmium	26.80±6.71	21.40+9.16
	Bryophyllum	70.75±18.25	53.20±1.72
Liver	**Control**	42.00±6.81	6.00±1.16
	Cadmium	13.20±5.89	4.00±0.00
	Bryophyllum	43.20±9.74	7.00±1.00

V. DISCUSSION

Cadmium is extremely toxic to humans and in chronic exposure, it accumulates in the body, particularly in kidney and liver (Fasset, 1 972) and adversely affect the functions of these organs (Yang and Shu, 2015). Enzymes play vital roles in the existence and functioning of various organs and tissues. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two widely used and well-established enzymes to detect diseases of the liver, heart, skeletal muscle, brain, and kidney as well as damage caused by toxicants to these organs (Lum, 1995). AST catalyzes the transfer of amino group of aspartic acid to α-ketoglutarate which eventually results in the formation of oxaloacetic acid and glutamic acid respectively. ALT catalyzes the transfer of anamino group from alanine to alpha ketoglutarate to yield glutamate and pyruvate as a part of amino acid metabolism.

Aspartate aminotransferase and alanine aminotransferase activities are used as indicators of hepatocyte damage. In earlier stages of liver damage, these enzymes of hepatocyte penetrate the cells and enter the blood stream (Kallies et al, 1964). In this present study, treatment of rabbits with cadmium significantly ($P<0.05$) decreased the activities of aspartate aminotransferase and alanine aminotransferase in the brain, heart and liver. The reduction in these enzymes may be due to oxidative injury in liver causing these enzymes to leak out of the liver into the blood. This is in agreement with report by Renugadevi, and Prabu who found

that the levels of serum hepatospecific enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and the level of total bilirubin were significantly increased in cadmium treated rats. The decrease in the activities of these enzymes following the treatment of rabbits with cadmium was highest in the liver and least in the brain showing that the liver is most susceptible to cadmium damage compared to the heart and brain while the brain is the least susceptible.

Herbal medicine has been explored and proven to be effective in the management of health requirement locally and internationally (WHO, 2002).The activities of plant extracts in effecting any therapeutic or biological changes in ailing or diseased animals or living tissues are direct functions of the chemical constituents present in them after extraction (Ayinde and Agbakwuru, 2010).The extract of *bryophyllum pinnatum* is known to be rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids (Marriage and Wilson, 1971; Costa et al, 1995). Which are known for their antioxidant activities. Therefore the finding from this study that *bryophyllum pinnatum* significantly ($P<0.05$) increased the activities of aspartate aminotransferase and alanine aminotransferase when compared with the control in brain, liver and heart tissues may be as a result of its rich phytochemicals which may have caused an increase in the activity of the antioxidant-defense system there by having a protective effect on hepatic tissues.Thus, the results suggest that *bryophyllum pinnatum* extract acts as a potent hepatoprotective agent against cadmium induced hepatotoxicity in rabbits.

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