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Effect of Ethanol Extract of *Anthocleista vogelii* leaves on the Liver Parameters of Albino Rats Exposed to Cadmium Oxide

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ABSTRACT

The present study was undertaken to examine the ameliorative potential of ethanol extract of *Anthocleista Vogelli* leaves on cadmium-induced hepatic damage in albino rats. The liver parameters; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin and Bilirubin were examined. Oral administered of cadmium (Cd) (5mg/kg Body weight) to induce toxicity was done once in a week. The extracts (200mg/kg, 400mg/kg and 600mg/kg Body Weight) to the rats were undertaken for 21 days after which liver parameters were measured in the serum using standard methods. The results showed a significant increase in the liver parameters of albino rats exposed to cadmium oxide. Treatments with (200mg/kg, 400mg/kg or 600 mg/kg Body weight) extract in cadmium induce toxicity in albino rats showed a significant ($P < 0.05$) reduction in the altered biochemical parameters. Hence, the ameliorative effect of *Anthocleista vogelii* leaves extract against cadmium oxide induced hepatic damage.

Keywords: *Anthocleista vogelii,* Liver, Cadmium oxide and Hepatotoxicity.

INTRODUCTION

Many scientific studies are being carried out to investigate heavy metals poisoning and toxicity, and how they affect our human existence; particularly our health. Cadmium oxide, a heavy metal compound is one of those heavy metals being studied due to its deleterious effects in the body [1, 2, 3, 4, 5, 6]. Some documented deleterious effect of cadmium toxicity includes; hepatocellular damage, testicular atrophy, hypertension, renal dysfunction, anemia and injury to the central nervous system [7, 8, 9, 10, 11]. In 2010, the World Health Organization (WHO) has published a list of 10 chemicals or groups of chemicals of concern for human health, which includes cadmium [12, 13, 14, 15]. Additionally, the US Agency for Toxic Substances and Disease Registry (ATSDR) ranked Cadmium in seventh place on the priority list of dangerous substances [16, 17, 18, 19, 20]. Due to its ubiquitous form in nature, cadmium oxide is widely distributed in the atmosphere, water and food, and when exposed to humans in low concentration can cause serious health problems and probably death [21, 22, 23]. Sources of cadmium exposure to humans include fossil fuels, iron and steel production, cement, nonferrous metals production, waste incineration, smoking, fertilizers, etc., and are supplied indirectly to the environment by activities like volcanic eruption, mining and use of phosphate fertilizers [24, 25, 26]. The mechanism of its excretion in humans is not particularly understood, hence it

bioaccumulates in various tissues especially kidneys, lungs, pancreas and liver [27, 28, 29]; causing dysfunction in various animal and human organs notably the kidney, brain, testes, heart and liver [30,31, 33].

In the liver, cadmium is taken into the hepatocytes where it binds to metallothioneins (MTs), glutathione (GSH) and other proteins or peptides and form new complexes. Hydroxyl radical is the most reactive and damaging radical formed; which initiates cellular damages and lipid peroxidation (LPO) even at extremely small concentrations [14, 17, 18, 19], thus the aetiology of hepatotoxicity is established. Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics [18]. Certain biomarkers used to monitor hepatoxicity include Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin [13].

The global burden of hepatotoxicity affects over fifty million people worldwide [20]. Medicinal plants are known to posses antioxidant properties because they contain appreciable minerals. Hence, in order to ameliorate cadmium induced hepatic injury; medicinal plants might be useful due to their relative availability, low cost and reduced side effects [21]. Various parts of medicinal plants have been used traditionally to mitigate and treat human ailments [25]. Meanwhile, researchers have studied plant extracts and isolated secondary metabolites to establish their pharmacological effects in *in-vivo, ex-vivo*, and *in-vitro* models [4, 7, 9, 10] for the treatment of various ailments.

Anthocleista vogelii is a medicinal plant that is used in treatment of diseases and swelling in the body [11]. It belongs to the family Gentiamuceae, an erect, cylindrical tube of about 20m tall [14]. Traditionally, the leaves and stem-bark are known for treating swellings in the body (anti-inflammatory) [8], while the root-bark and leaves are used in local medicine [9] Its potency as drug is revealed in phytochemical studies of the leaves carried out by [15]. Phytochemicals such as flavonoid, saponins, alkaloid, sterols, phenols, and terpenes were reported to be present. The presence of these secondary metabolites in them has been evaluated in different medicinal plants for treatment of ailments locally, thus making the plant species. a subject of therapeutic interest. This paper seeks to provide a review on the use of *Anthocleista vogelii* leaves extract in the treatment of cadmium oxide induced hepatotoxicity.

MATERIALS AND METHODS

Plant Collection

The fresh leaves of *Anthocleista vogelii* was collected in a nearby bush beside Presco campus, Ebonyi state University, Abakaliki. The leaves were identified by a botanist, Prof.E.Onyekweru of Applied Biology Department, Ebonyi State University Abakaliki.

Sample Processing

The sample was allowed to air-dry and turn crispy. The dried leaf sample was ground into power using mechanical grinder and sieved to remove chaff. The fine powders were packed into an airtight container ready for analytical use.

Preparation of Extract

Exactly, 250g of the powdered sample was weighed into 500ml of absolute ethanol in a plastic container. This was allowed to stand for 72hours with intermittent shaking. The mixture was filtered with Whatman No. 1 filter paper under reduced pressure. The resulting filtrate was evaporated using rotary evaporator. Packed in airtight container and stored in refrigerator at 4˚c pending further studies.

Preparation of Vitamin C

Vitamin C was obtained from Octovia Pharmarcy, Water Works Road Abakaliki Ebonyi State University. Exactly, 2g (5 tablets) of Vitamin C tablet was dissolved in 100ml of distilled water to obtain stock solution of 0.02g/ml.

Preparation of Cadmium oxide

Exactly 2g of cadmium oxide powder was dissolved in 100ml of distilled water to obtain a stock solution of 0.02g/ml.

Collection of animals

Adult albino rats were sourced from the animal farm of University of Nigeria Nsukka (UNN), weighing 200g-250g.They were kept in well ventilated and hygienic condition in the animal house of Biochemistry Department, Ebonyi State University, Abakaliki, Nigeria. During acclimatization, the animals had free access to animal grower feed and clean water.

Experimental Design

Thirty-six adult albino rats were obtained from the Animal house of the Department of Biochemistry, Faculty of Science, University of Nigeria Nsukka (UNN), weighing between 200 g and 250 g. The animals were allowed access to feed and water for a period of fourteen days, for their acclimatization prior to the commencement of the experiment. The animals were kept in well ventilated cages at room temperature (28˚ – 30 ºC), and under control lights. The rats were randomly distributed into six groups of six animals each. Group 1: served as the normal control and consisted of animals fed with rat pellet and water only. Group 2: consisted of animals fed with rat pellet and cadmium oxide only (positive control). Group 3: consisted of animals fed with rat pellet, cadmium oxide and Vitamin C (Standard control). Group 4: consisted of animals fed with rat pellet, cadmium and *A. Vogelii* extract (200 mg/kg). Group 5: consisted of animals fed with rat pellet, cadmium oxide and *A. Vogelii* extract (400 mg/kg), while Group 6: consisted of animals fed with rat pellet, cadmium oxide and *A. Vogelii* extract (600 mg/kg). *A. Vogelli* extracts (200, 400 and 600 mg/kg bw) was given orally by intubation for 21 days. Cadmium oxide was administered twice in a week (200 mg/l CdO) in the animals for three weeks to induce toxicity.

Collection of blood sample

After twenty-one (21) days, the rats were sacrificed and blood sample collected through venous puncture into a sterile bottle for analysis.

EXPERIMENTAL PROCEDURES

Quantitative estimation of serum alanine transaminase (ALT) and asapartate transaminase (AST): The method of Reitman and Frankel, (1975) was used.

Quantitative estimation of serum alkaline phosphatase (ALP) : Alkaline phosphatase in the serum was estimated by the end point colorimetric method (Englehardt, 1970).

Determination of albumin: Albumin concentration was determined in serum according to the method of Doumas (1971).

Determination of Total Bilirubin: Total bilirubin was determined according to the method by Schmidt and Eisenburg (1975).

RESULTS

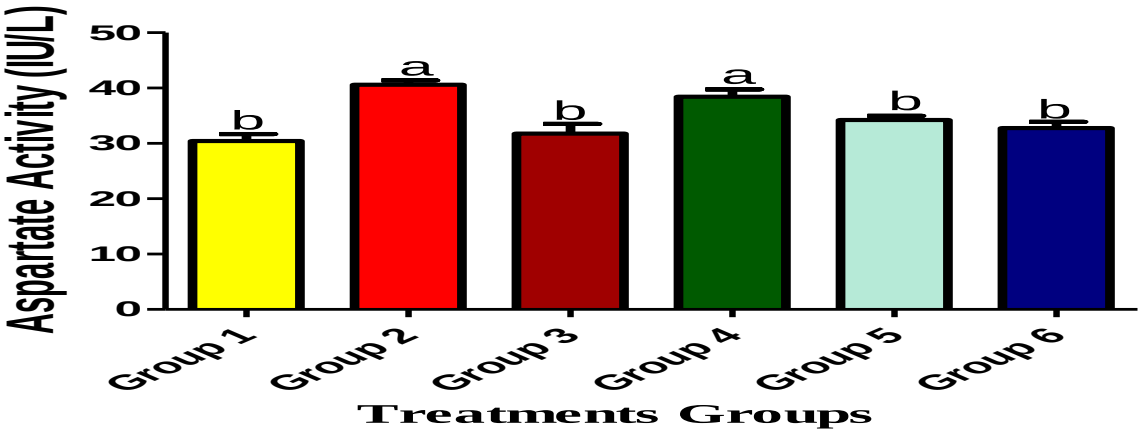

Figure 1. Plot of Aspartate level in different groups (**Group 1:** Control (Nothing given, only feed), **Group 2:** Heavy metal given, **Group 3:** Heavy metal + Vitamin C, **Group 4:** 200mg + Heavy metal, **Group 5:** 400mg+ Heavy metal, **Group 6:** 600mg + Heavy metal).

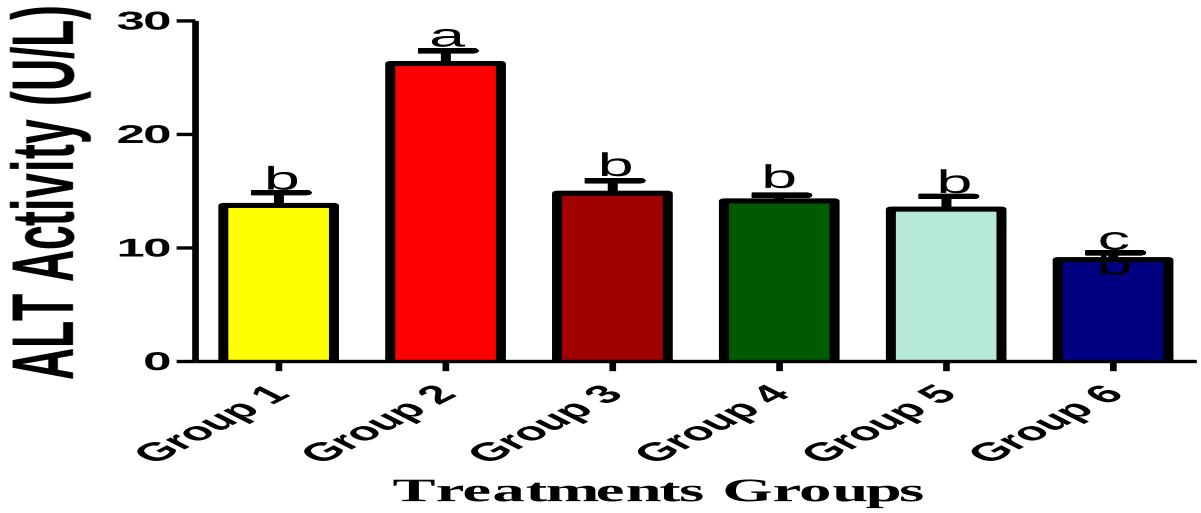

Figure 2. Plot of ALT level in different groups (**Group 1:** Control (Nothing given, only feed), **Group 2:** Heavy metal given, **Group 3:** Heavy metal + Vitamin C, **Group 4:** 200mg + Heavy metal, **Group 5:** 400mg+ Heavy metal, **Group 6:** 600mg + Heavy metal).

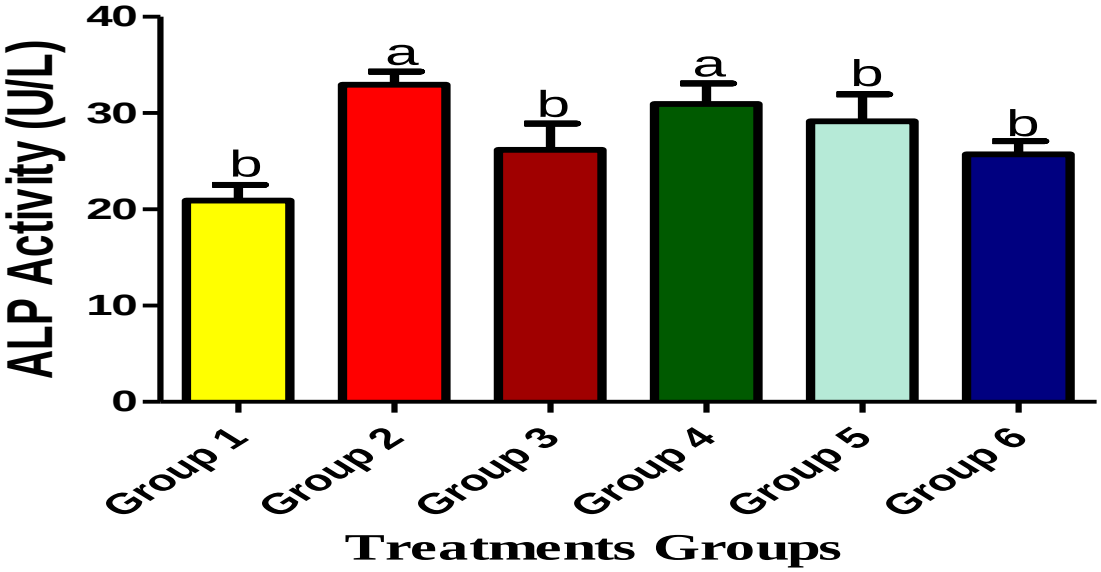

Figure 3. Plot of ALP level in different groups (**Group 1:** Control (Nothing given, only feed), **Group 2:** Heavy metal given, **Group 3:** Heavy metal + Vitamin C, **Group 4:** 200mg + Heavy metal, **Group 5:** 400mg+ Heavy metal, **Group 6:** 600mg + Heavy metal).

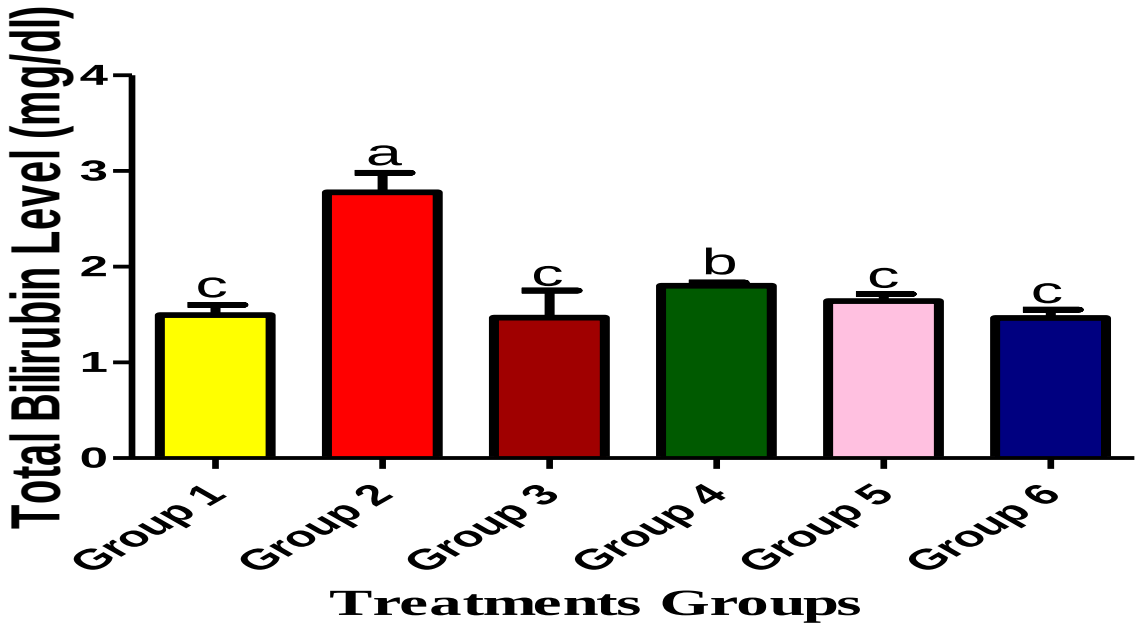

Figure 4. Plot of Total Bilirubin level in different groups (**Group 1:** Control (Nothing given, only feed), **Group 2:** Heavy metal given, **Group 3:** Heavy metal + Vitamin C,

Group 4: 200mg + Heavy metal, **Group 5:** 400mg+ Heavy metal, **Group 6:** 600mg + Heavy metal).

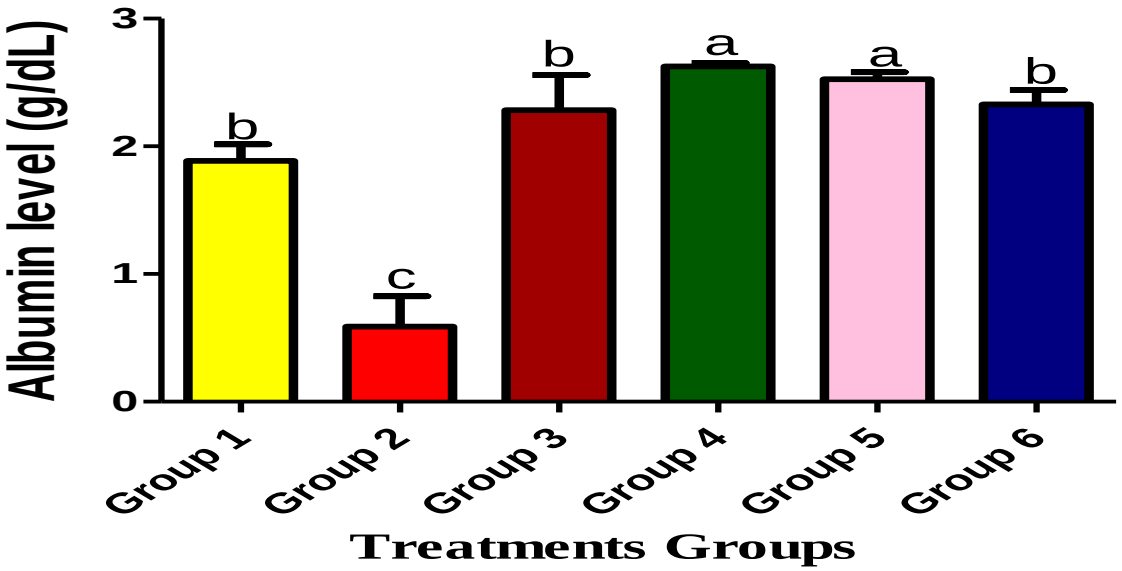

Figure 5. Plot of Bilirubin level in different groups (**Group 1:** Control (Nothing given, only feed), **Group 2:** Heavy metal given, **Group 3:** Heavy metal + Vitamin C, **Group 4:** 200mg + Heavy metal, **Group 5:** 400mg+ Heavy metal, **Group 6:** 600mg + Heavy metal).

DISCUSSION

The therapeutic effect of *Anthocleista vogelii* leaves extract on hepatotoxicity induced by Cadmium oxide was studied. The present study established that administration of aqueous extract of *A. Vogelli* (200,400 or 600 mg/kg BW) significantly protect the liver against toxicity exerted by cadmium oxide. The synergetic effects of the bioactive constituents present in the extract may be responsible for the observed results. The results showed a significant therapeutic influence on liver parameters in treatment groups administered with the extract. The leaves of *A. vogelli* have been widely used by natives in treating swellings and generally as a local medicine [8]. Hepatotoxicity induced by cadmium oxide was studied using the following liver biomarkers; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total bilirubin and Albumin. Significant elevations ($p \leq 0.05$) of AST and ALT levels (Fig. 1 & 2) in rats exposed to Cadmium oxide only were observed when compared with the control group. Upon administration of the leaf extract, a significant reduction ($p \leq 0.05$) in AST and ALT levels were observed in the treatment groups (Group 4, 5 & 6) administered with the extract in a dose dependent manner (200 mg/kg, 400mg/kg and 600 mg/kg dose). Both aminotransferases (AST and ALT) are mainly concentrated in the liver; ALT is localized solely in the cytoplasm, whereas AST is present both in the cytosol and mitochondria of hepatocytes [16]. According to [17], Alanine aminotransferase is the most frequently relied biomarker of hepatotoxicity. It is a liver enzyme that plays an important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate. Elevated level of this enzyme

is released during liver damage. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver [12, 14]. AST levels however, though useful in detecting hepatocellular necrosis, are considered a less specific biomarker enzyme for hepatocellular injury [16].

Bilirubin is an endogenous anion derived from the regular degradation of haemoglobin from the red blood cells and excreted from the liver in the bile. It is a chemical normally present in the blood in small amounts and used by the liver to produce bile. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a build-up of bilirubin in the blood and extracellular (outside the cells) fluid. Serum bilirubin could be elevated if its levels build up in blood circulation. Increased levels of bilirubin in the blood may occur due to hepatic damage and can lead to jaundice and other hepatotoxicity symptoms [15]. A significant ($p \leq 0.05$) increase in total bilirubin levels were recorded in rats exposed to Cadmium oxide only. The increased serum level of total bilirubin in the present study is a clear indication of hepatic dysfunction but, upon administration of *A. Vogelli* extract a significant reversal effect was recorded in group 4, 5 and 6. This was in agreement with the result from similar studies carried out by [18, 19]. In acute human hepatic injury, total bilirubin measured is seen as a better indicator of disease severity compared to ALT [21]. The ability of *A. Vogelli* extracts to ameliorate the elevated levels of total bilirubin, also confirms its protective role. High levels of serum alkaline phosphatase (ALP) are also linked to the condition and function of liver cells. An elevated serum alkaline phosphatase is associated with liver damage (Renugadevi and Prabu, 2010). Administration of aqueous extract of *A. Vogelli* (200 mg/kg, 400 mg/kg and 600mg/kg bw) mitigated cadmium-induced hepatotoxicity as revealed by the decreased levels of ALP, AST and ALT compared with the cadmium oxide exposed in group: G2. [16] reports that ALP plays an important role in deciding the type of liver damage by hepatotoxins. Albumin, which is manufactured by the liver, is a major protein that circulates in the blood stream. Albumin concentration is a biochemical parameter for monitoring liver function in the blood [16]. Abnormal levels of this protein have been reported to be associated with liver damage [20] Albumin concentration in the serum of albino rats exposed to cadmium oxide were significantly lower ($p<0.05$) in comparison to the treatment groups. In the present study, the significant decrease in the serum albumin of animals exposed to cadmium oxide can be attributed to impairment in hepatocyte functions causing decreased cytochrome P-450 activity and inhibition in protein metabolism in the liver [15].

CONCLUSION

The outcome of this study clearly indicates that the ethanol extract of *Anthocleista vogelii* leaves ameliorated the altered liver function parameters triggered by cadmium oxide-induced hepatotoxicity in the biochemical parameters. The plant therefore could serve as a possible potent herbal drug for the correction of liver malfunction.

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