

Biochemical Assessment of Ethanol Leaf Extract of *Cnidoscolus aconitifolius* on Liver Integrity of Albino Rat Treated with Lead

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Abstract: Lead is a soft, grey-blue heavy metal which usually occurs in nature as oxide or salts. It accumulates in the environments, water bodies and eventually taken up by plants and animals where they effect their physiologic and hazardous damages. This research evaluates the effect of ethanol leaf extract of *Cnidoscolus aconitifolius* on AST, ALT, ALP, total protein and albumin in lead acetate-induced liver damage in albino rat. Twelve (12) albino rats weighing between 70- 175g (8-12 weeks old) were used. They were randomly distributed into four (4) groups (A, B, C and D) with three (3) animals each. The animals were acclimatized for two weeks (14 days) and placed on a regular feed (grower mash – Guinea feed). Clean water supply and proper sanitation were maintained in the animal house to ensure healthy and clean environment. The animals were administered with different doses of the extract for fourteen (14 days) depending on varying individual body weight. The result of this study shows a significant decrease ($P < 0.05$) in the serum total protein levels of the albino rats treated with lead acetate which when treated with ethanol leaves extract of *Cnidoscolus aconitifolius* showed a significant ($P < 0.05$) increase. The results also showed that albino rats treated with lead acetate had significant ($P < 0.05$) increase in serum ALT, AST and ALP levels which when treated with ethanol leaves extract of *Cnidoscolus aconitifolius* showed a significant ($P < 0.05$) decrease though dose dependent. This study suggests that ethanol leaf extract of *C. aconitifolius* may have a potent hepatoprotective effect against lead-induced liver damages.

Key words: AST • ALT • ALP • TP • ALB • *Cnidoscolus aconitifolius* • Lead toxicity

INTRODUCTION

Lead a soft, grey-blue heavy metal occurs in nature as oxide or salts, it is one of the most hazardous and cumulative environmental pollutants [1]. It is one of the most frequently reported causes of poisoning in both farm and domestic animals and humans throughout the world [2]. Lead poisoning is a type of metal poisoning and a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body. Lead is a poisonous metal, which occurs in both organic (tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in the environment [3]. Routes of exposure to lead include contaminated air, water, soil, food and consumer

products. Occupational exposure is a common cause of lead poisoning in adults.

The detrimental effects of lead poisoning have been well known since ancient times but some of the most severe consequence exposure to this metal has only been described recently. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the liver, heart, bones, intestines, kidneys and reproductive and nervous systems. Lead affects the higher function of the central nervous system and undermines brain growth, preventing the correct development of cognitive and behavioral function [4].

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion [5]. It is involved with almost all the biochemical pathways to

growth, fight against disease, nutrient supply, energy provision and reproduction [6]. Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are elevated in liver disease [7]. Liver diseases pose a serious challenge to international public health [8]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [9]. Moreover, there are still no specific treatments in modern medicine that give protection to the liver against damage or help to regenerate hepatic cells [10, 11].

Recently, the use of medicinal plants to cure various forms of liver diseases and dysfunctions is becoming increasingly popular and has received wide acceptance [12, 13]. Moreover, a large number of medicinal plants have been found to offer some hepatoprotection [14-16]. Some of the important medicinal plants used in Nigeria include; *C. rutidosperma*, *E. coccinea*, *E. heterophylla*, *P. bransilensis*, *Cnidoscopus aconitifolius*, *S. anthelmia*, *S. cyaenneusis*, *M. indica*. Notable among the plants named above is the vegetable, *Cnidoscopus aconitifolius*. Otherwise known as “obara ndu or akwukwo nri ohurun” in the igbo tribe of Nigeria. The plant is believed to possess various therapeutic [17] and nutritive value. Hence it is used by many tribes in Nigeria and Africa.

Cnidoscopus aconitifolius is commonly referred to as ‘Chaya’, ‘tree spinach’ in Mexico, “Iyana Ipaja” in Yoruba, ‘EfoIyana Ipaja’ or ‘Efo Jerusalem’ in Southwest Nigeria and ‘Hospital Too Far’ in Niger Delta areas of Nigeria belongs to the family of Euphorbiaceae. It is an ornamental, evergreen; drought deciduous shrub up to 6cm in height with alternate pinnate lobed leaves, milky sap and small flowers on dichotomously branched cymes [18]. The part of the plant mainly used for medicinal purposes is the leaf and shoot. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from improved digestion, stimulating lactation and strengthening of fingernails and to cure for alcoholism, insomnia, gout and vision improvement [19]. It has also been recommended traditionally in the treatment of diabetes, obesity, kidney stones, hemorrhoids and eye problems [20-22]. The nutritional analyses for Chaya leaves were found to contain high amounts of protein, crude fiber, calcium, vitamin C and carotene [23].



Fig. 1: *Cnidoscopus aconitifolius*

MATERIALS AND METHODS

Chemicals and Reagents: The chemical and reagents used were of standard analytical grades. The reagents were all purchased from Randox, UK.

Collection and Extraction of Plant Material: The fresh leaves of *Cnidoscopus aconitifolius* were collected from an uncultivated farm land, beside Ebonyi State University Abakaliki, Ebonyi State South-East region of Nigeria. The plant was identified by the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The leaves were washed and shade dried under room temperature. The dried leaves were then pulverized into fine granules using manual grinder and sieved using 0.25 mm sieve. The powdered leaves weighing 100 g were soaked in 500 ml of ethanol at room temperature for 24 hours. After 24 hours, the extract obtained was filtered using sieve cloth. The filtrates was evaporated and the residue weighed.

Animal Model and Experimental Design: Twenty (24) albino rats weighing between 70- 175 g (8-12 weeks old) were purchased from a farm at Awka in Anambra State, Nigeria and housed at the animal farm of the Department of Biochemistry, Ebonyi State University, Abakaliki. They were randomly distributed into four (4) groups (A, B, C and D) with six (6) animals each labeled. The animals were acclimatized for two weeks (14 days) and placed on a regular feed (grower mash – Guinea feed), clean water was also provided daily. Proper sanitation was maintained in the animal house to ensure healthy and clean environment. The animals were administered different doses of the extract for fourteen (14 days) base on varying individual body weight. Illustrated further as;

Group A: Control (Normal, Untreated), received distilled water without any treatment.

Group B: Lead acetate treated group, received freshly dissolved 5 g of lead acetate in 50 ml distilled water for 14 days depending on their body weight.

Group C: Received 250 mg/kg of extract + lead acetate.

Group D: Received 500 mg/kg of extract + lead acetate

In this study, all the animal experimentations were carried out following strictly the guidelines for the care and use of laboratory animals obtained from the Institutional Animal Ethics Committee of Ebonyi State University (IAEC) in line with the specifications outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health.

Administration of Lead Acetate, Extracts and Collection of Blood Sample: The different animal groups received their respective doses orally using 1ml syringe, once daily (10 am), for a period of 14 days. After which the animals were sacrificed and the blood samples of the animals were collected through vein puncture technique. Their blood were collected into labeled plain specimen bottles and centrifuged at 5000 g for 10 minutes to separate the serum which was used for the biochemical analysis.

Determination of Biochemical Parameters:

Determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine phosphatase (ALP) activities, albumin (ALB), total protein (TP) were done using (Reitman and Frankel, 1957) methods.

Statistical Analysis: Results are expressed as mean \pm standard deviation. The differences among mean were analyzed by one-way ANOVA to check the level of relationship between the treated and control variables. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

The effect of ethanol leaves extract of *C. aconifolius* on total proteins (TP), albumin (ALB), Aspartate transaminase (AST) alanine aminotransaminase (ALT), alkaline phosphatase (ALP) of albino rats treated with lead acetate are presented in Figures 1,2,3,4 and 5 respectively. The result of this study showed a significant decrease ($P < 0.05$) in the plasma total protein levels of the albino rats treated with lead acetate which when treated with ethanol leaves extract of *C. aconifolius* showed a significant ($P < 0.05$) increase. The result of this study showed that albino rats treated with lead acetate had

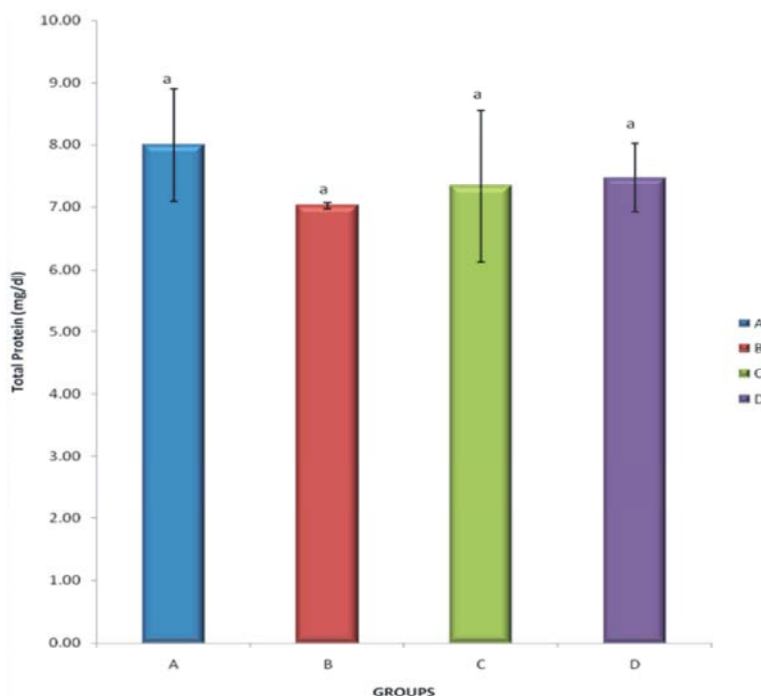


Fig. 1: Effect of ethanol leaves extract of *C. aconifolius* on total proteins of albino rats treated with lead acetate. Data are presented as mean \pm SD of four rats. Bars with different letter are significant ($P < 0.05$).

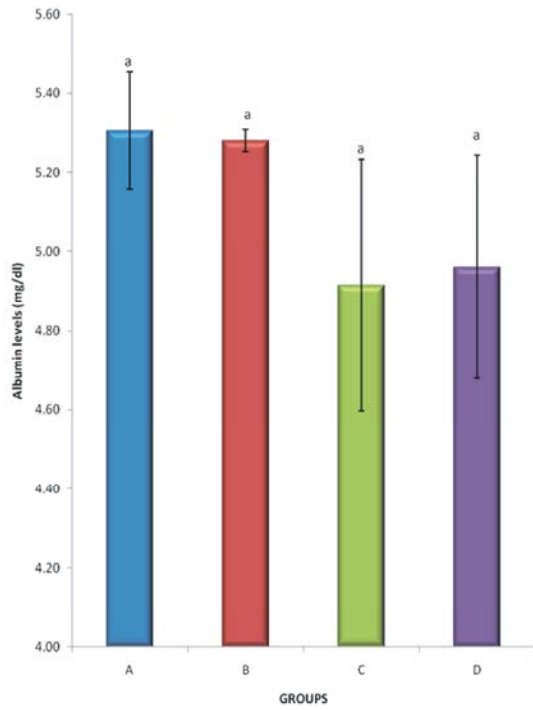


Fig. 2: Effect of ethanol leaves extract of *C. aconifolius* on Albumin (ALB) of albino rats treated with lead acetate.

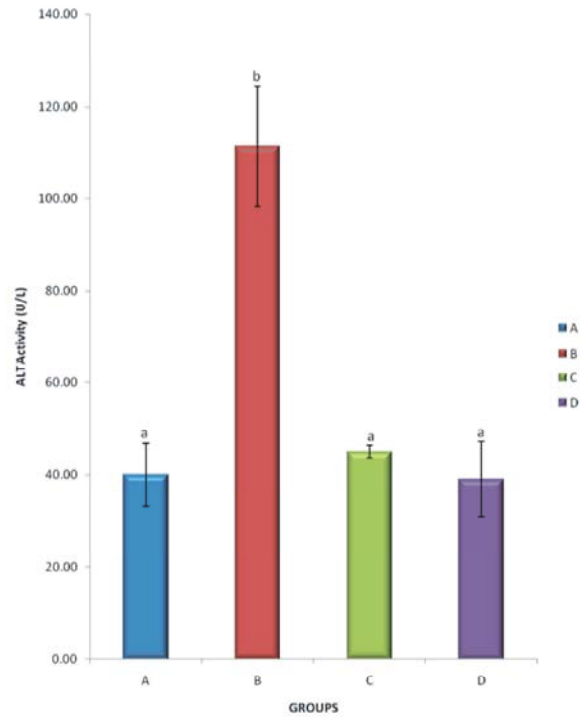


Fig. 4: Effect of ethanol leaves extract of *C. aconifolius* on ALT activity of albino rats treated with lead acetate.

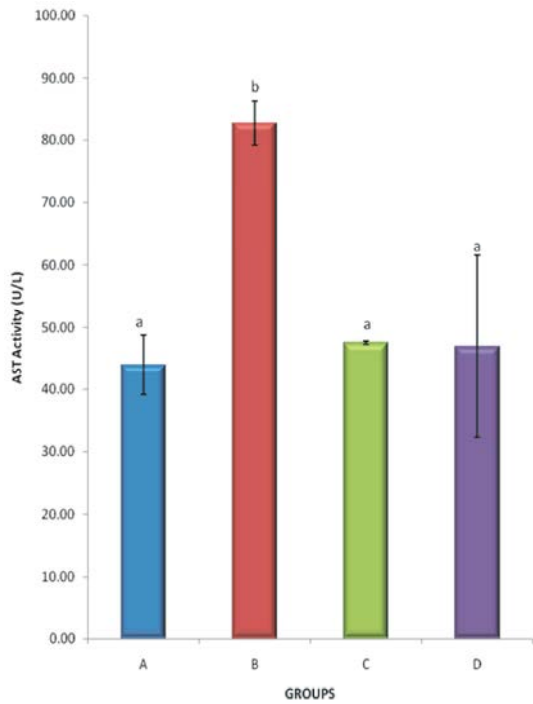


Fig. 3: Effect of ethanol leaves extract of *C. aconifolius* on AST activity of albino rats treated with lead acetate.

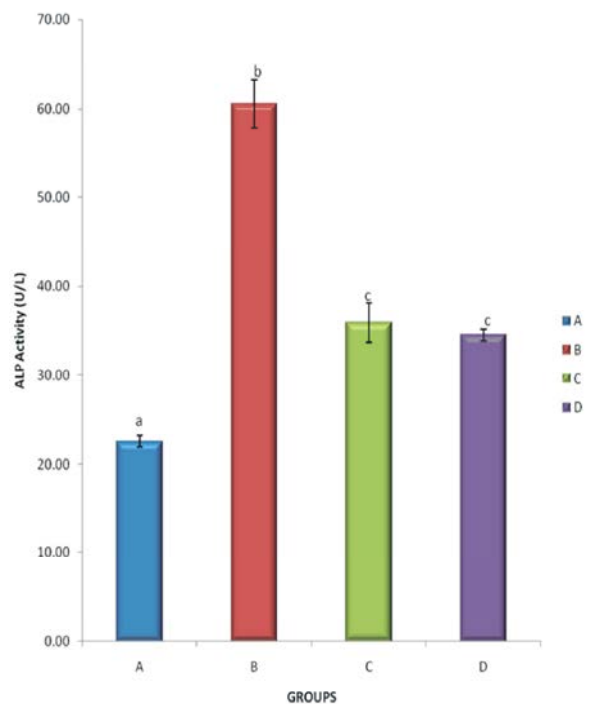


Fig. 5: Effect of ethanol leaves extract of *C. aconifolius* on ALP activity of albino rats treated with lead acetate.

significant ($P < 0.05$) increase in serum ALT, AST and ALP levels which when treated with ethanol leaves extract of *C. aconifolius* showed a significant ($P < 0.05$) decrease that were dose dependent (Figures 3, 4 and 5).

DISCUSSION

In developing countries, the indigenous populations largely depend on traditional systems of medicine [23, 24]. Plants have been used for therapeutic purposes and many of the currently available drugs are directly or indirectly derived from plants [25, 26].

Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into plasma indicates the sign of hepatic tissue damage [27, 28]. Generally, measurement of ALT, AST and ALP are used as important diagnostic markers to indicate liver injury due to hepatotoxins [29].

The result of the present study showed significant ($P < 0.05$) decrease in plasma total protein levels of the albino rats treated with lead acetate which when treated with ethanol leaves extract of *C. aconifolius* showed a significant ($P < 0.05$) increase (Figure 1). The reduction of protein may be due to proteolysis, increased metabolism under toxicant stress [25] as well as reduction in protein synthesis due to damages to hepatocytes responsible for liver protein synthesis.

Aminotransferases are the most frequently used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis. The result of this study showed that albino rats treated with lead acetate had dramatic increase in serum ALT, AST and ALP levels which when treated with ethanol leaves extract of *C. aconifolia* showed a significant ($P < 0.05$) decrease that were dose dependent (Figures 3,4 and 5). ALT, AST and ALP are metabolic enzymes involved in amino acid metabolism [23]. The observed general increase of these enzymes in the plasma indicates an underlying liver injury [11, 14, 18]. Also, it has been reported that alterations in enzymes activities in the plasma directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture [7], a signal of underlying pathological process [8].

ALP in the cellular external membrane plays the major role in phosphate metabolism and it prevents the external membrane from being damaged [15]. Increase in plasma level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. Significant elevation of serum ALP is an indication of cholestasis. It has also been reported that increase in the serum levels

of ALP indicate the extent of cellular damage on the liver [16]; its increase in activity is associated to necrosis of the liver and kidney [18].

CONCLUSION

In conclusions this results revealed that exposure to lead toxicity leads to impairment of liver function. It further demonstrates that *C. aconifolius* has significant healing effect on lead induced liver damage.

Recommendation: The results of this study show that ethanol leaf extract of *C. aconifolia* might have a potent hepatoprotective action against lead-induced liver damage (lead toxicity). However, further study on the detailed chemical constituents, toxicity and pharmacological effects of the extracts is recommended.

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