Original Research Article

Hepatoprotective Potentials of Ethanolic Leaf-Extract of *Cajanus cajan* (*linn*) in Ethanol-evoked Oxidative Stress in Albino Rats.

ABSTRACT:

Aims: The hepatoprotective potentials of ethanolic leaf-extract of *Cajanus cajan (linn)* in ethanol-evoked oxidative stress was examined by checking the activities of liver function parameters such as Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) in rats.

Study design: Experimental Design.

Place and Duration of Study: This study was carried out in the Biochemistry laboratory and animal house of Ebonyi state University, Abakaliki Nigeria from May 2020 to December 2020.

Methodology: 36 albino rats were randomly assigned into 6 groups of 6 rats per group. Rats in Group A received 0.9% of NaCl solution only and served as normal control. Rats in group B received 5mg/kg of Silymarin and three hours later, was given 3.7g/kg of absolute ethanol, one time daily for two weeks and they served as standard control; rats in group C received 3.7g/kg of absolute ethanol only and served as positive control. Rats in groups D, E and F (test groups) received 200mg/kg, 400mg/kg and 600mg/kg body weights of the leaf-extract respectively and three hours later, all the rats in the test groups were given 3.7g/kg body weight of absolute ethanol, one time per day for two weeks. All the administrations were done through oral route. Collection of blood was done by puncturing the femoral veins of the rats in each group. Liver marker enzymes were all determined using standard procedures.

Results: ALT, AST and ALP activities increased significantly ($P<0.05$) in positive control group relative to the levels observed in other control groups. However, administration of leaf-extract to the test groups revealed a significant ($P<0.05$) decrease in the activities of these enzymes to levels similar to what was observed in the groups that were administered 0.9% of NaCl solution and silymarin.

Conclusion: It is therefore concluded that ethanol leaf-extracts of *Cajanus cajan* contains important antioxidants which could be hepatoprotective to albino rats.

Keywords: Alcohol, Albino rats, Alanine Transaminase, Aspartate Transaminase, Alkaline Phosphatase, Cajanus Cajan.

1. INTRODUCTION

Alcohol use disorder (AUD) results from uncontrolled consumption of alcoholic beverages, it is among the leading cause of liver diseases worldwide [1]. It accounts for about 3.8% deaths globally [1]. Acute alcoholic poisoning resulting from persistent intake of alcohol is one of the most important factor that has been long associated with liver pathology [2]. However, the exact factors that evoke liver injuries are not yet completely known. A lot of metabolic pathways have revealed reactive oxygen species as the major cause of alcohol-induced liver damage [3] [4].The human liver metabolizes alcohol, using either cytochrome P450 enzyme system, alcohol dehydrogenase or aldehyde dehydrogenase [5]. Cytochrome 2E1 (CYP2E1) is the most important microsomal enzyme during alcohol metabolism among the family of CYP450 system, because is an inducible enzyme that metabolizes alcohol by oxidation reactions. These oxidation reaction pathways play significant roles in generation of alcohol-induced oxidative stress [6]. Generally, oxidative stress due to consistent alcohol consumption could results from reactive oxygen species (ROS) and possible peroxidation of lipids in liver cells [7]. Also, CYP2E1 plays a specific metabolic role on metabolism of other xenobiotics [8]. Furthermore, acute alcoholic consumption can easily trigger the production of inflammatory cytokines, which could lead to liver cell injury [9].

Cajanus cajan is a leguminous plants and is a well-known herb with yellowish flowers. It is a woody plant popularly called pigeon pea in English and "Fio-fio" in Igbo language, Nigeria. *Cajanus cajan* has proteinous seed and has been grown in many parts of the world especially in Africa, America, India and Asia [10]. It is an edible legume with certain important phytochemicals, widely usedin folkloric medicine for treatment of liver diseases [10]. The leaf extracts of *Cajanus cajan* could be applied in medicine and modern food industries because it has natural antioxidants [11].

In certain tribes in Nigeria like Ndegu-Obu Ameffia village of Ebonyi State, Igbo, Nigeria, pigeon pea leaf-extracts are usually administered by herbalists for treatment of several liver injuries. Over the years, this leaf-extract has proved to be very efficacious in the management and treatment of most liver diseases. Consequently, this study was carried out to help provide more scientific explanations to why this leaf extract is used in Nigerian folk medicine for treatment of liver disorders and to make available more knowledge on the hepatoprotective functions of the pigeon pea plants which could possibly be applied in pharmaceutical and food industries.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

Pigeon pea plant leaves were plucked from Ndegu-Obu Ameffia village of Ebonyi State Nigeria in May, 2020 and was identified by Prof. S. S. Onyekwelu in Applied Biology department of Ebonyi State University Abakaliki, Ebonyi State, Nigeria.

2.2 Materials.

The leaves were dried at room temperature (about 20-25 ^{0}C) for four weeks and ground into fine powder with electrical blending machine sterilized using ethanol. The powdered plant materials (350 g) were soaked in 1600ml of ethanol for 2 days. Filtration of the mixture was done with a muslin cloth. Concentration of the filtrate was carried out using a rotary evaporator. This was done as described by [15]

2.3 Experimental Design

36 albino rats were randomly assigned into 6 groups of 6 rats per group. They were allowed to acclimatize for a week. Group A represented the Normal control, group B represented the standard control, group C represented the positive control and groups D,E and F represented the tests groups. Rats in Group A received 0.9% of NaCl solution only. Rats in group B received the dose of 5mg/kg body weight of Silymarin and three hours later, received 3.7g/kg body weight of absolute ethanol, one time daily for two weeks; rats in group C received 3.7g/kg of absolute ethanol. Rats in groups D, E and F (test groups) received 200mg/kg, 400mg/kg and 600mg/kg body weights of the leaf-extract respectively and three hours later, all the rats in the test groups were given 3.7g/kg body weight of absolute ethanol, one time per day for two weeks. All the administrations were done through oral route. Standard feeds (growers' marsh) were used to feed the rats for 2 weeks and water was made available throughout the period of this study.

2.4 Induction of Oxidative Stress and Liver Damage

Oxidative stress and liver damage were induced in the albino rats by administration of 3.7g/kg body weight of absolute ethanol to the rats without treatment according to the method of [12].

2.5 Collection of blood sample

On the 14th day of administration, chloroform was used to anesthetize the rats, blood sample collection was done by puncturing the rats' femoral veins following the method described by [23].

2.6 Determination of liver function parameters

Liver Function Parameters such as the activities of ALT and AST were determined according to [13] method while ALP level was determined using [14] method.

2.7 Statistical Analysis

The data obtained are presented as mean value ± standard deviation (SD) and data was subjected to One-way Analyses of Variance (ANOVA). Significant difference were obtained at $P<0.05$. This analysis was estimated using computer software known as graph pad prism 5.0.

3. RESULTS AND DISCUSSION

3.1 Liver Function Parameters (ALT, AST and ALP)

Liver marker enzymes such as ALT, AST and ALP increased significantly ($P<0.05$) in the positive control group relative to groups that received 0.9% of NaCl solution and normal saline but the groups that received the leaf extracts (test groups) presented significant ($P>0.05$) decreased in the activities of these enzymes as shown in (Fig.1, fig.2 and fig.3). [15]

reported a noticeable (P>0.05) decrease in ALT, AST and ALP activities in albino rats treated with *Cajanus cajan* ethanol leaf-extract after inducing them with diabetes using alloxan while a noticeable (P>0.05) decrease was also observed in dose dependent manner in rats groups treated with *Moringa oleifera* ethanol leaf extract. Similarly, [16] investigated the anticardiotoxicity and chemoprotective effects of pigeon pea (*Cajanus cajan*) sprout on anthracycline induced cardiotoxicity in organs of wistar albino rats, their findings showed that pigeon pea sprouts extract progressively lowered the activities of cardiac tissue enzymes in the plasma ALT, ALP and AST. Also, [17] investigated the hepatoprotective activity of ethanol extract of *Cajanus cajan* leaves and the data obtained showed that ethanol leaf- extract of *Cajanus cajan* and silymarin produced significant (P<0.05) hepatoprotective effects by decreasing the activities of ALT, AST and ALP. However, [18] reported a noticeable (P>0.05) decrease in the activities of ALT, AST but there was no noticeable change in ALT activity among the groups treated with leaf-extract of *Cajanus cajan*. This slight variation seen between the studies of [18] with our present study could be as a result the difference in methodology or doses of the leaf-extract used.

Persistent alcohol consumption can damage the hepatocytes because the liver is one of the primary organs that is seriously affected during alcohol metabolism. Once the liver cells are damaged, the activities of the liver enzymes like the transaminases get elevated in the system [19]. These transaminase enzymes could leak out from the injured hepatocytes into the blood. However, *Cajanus cajan* leaf-extracts as shown in figures 1, 2 and 3 of this present study contains valuable natural antioxidant sources that could repair damages caused to the parenchymal liver cells. In spite of the fact that alcoholic liver disease is a major cause of death among adults with alcoholic addiction disorder, herbs that can stand to protect the liver cells from alcoholic damage are still very scarce. In the present study, we have shown how certain liver enzyme activities are affected as a result of consistent alcohol consumption. This study has also helped us to have a deeper understanding of how ethanol leaf-extract of pigeon pea serves to protect the liver cells from the effect of alcohol evoked injuries. Interestingly, this leaf-extract showed significant protective effect to the liver when compared to the effects observed in standard drug used for liver treatment. In fact, ethanol leaf-extract from *Cajanus cajan* extract could be considered to have a greater protective effects against alcohol induced liver damage because, the leaf-extract is a mixture of uncharacterized phytochemicals whereas silymarin is a characterized compound. Our major findings in this study also agreed with the observations made by [20] [21] [22].

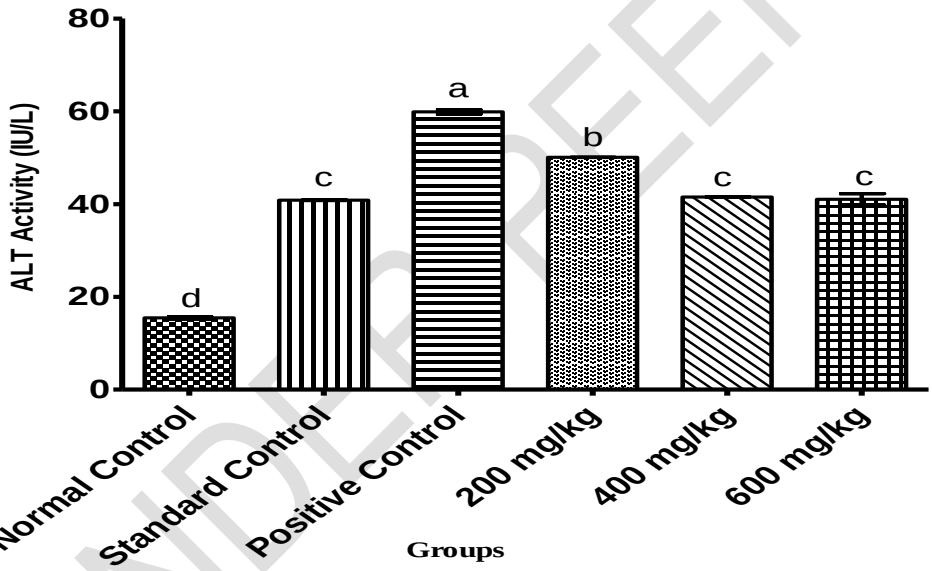

Fig. 1: Alanine Transaminase Activities in Albino Rats Treated with Ethanol-Leaf Extract of *Cajanus cajan*. Data are presented in bar charts as Mean value ± Standard Deviation (n=6). Mean values bearing different letters showed noticeable difference at *P<0.05* in (fig.1) above.

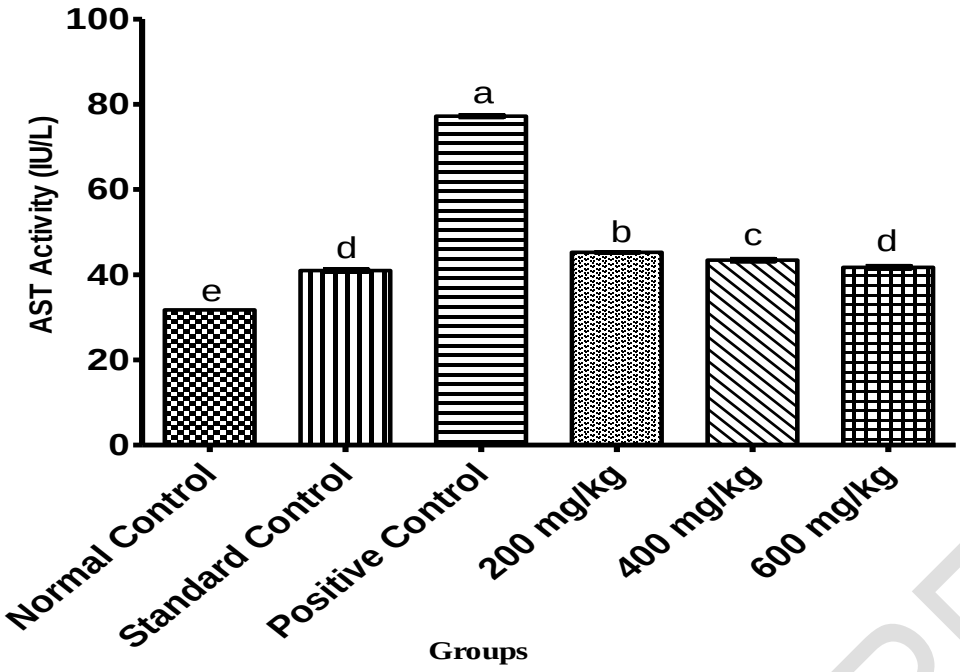

Fig. 2: Aspartate Transaminase Activities in Albino Rats Treated With Ethanol-Leaf Extract of *Cajanus cajan*. Data are shown in bar charts as Mean ± Standard Deviation (n=6). Mean values bearing different letters showed significant difference at *P<0.05* in (fig.2) above.

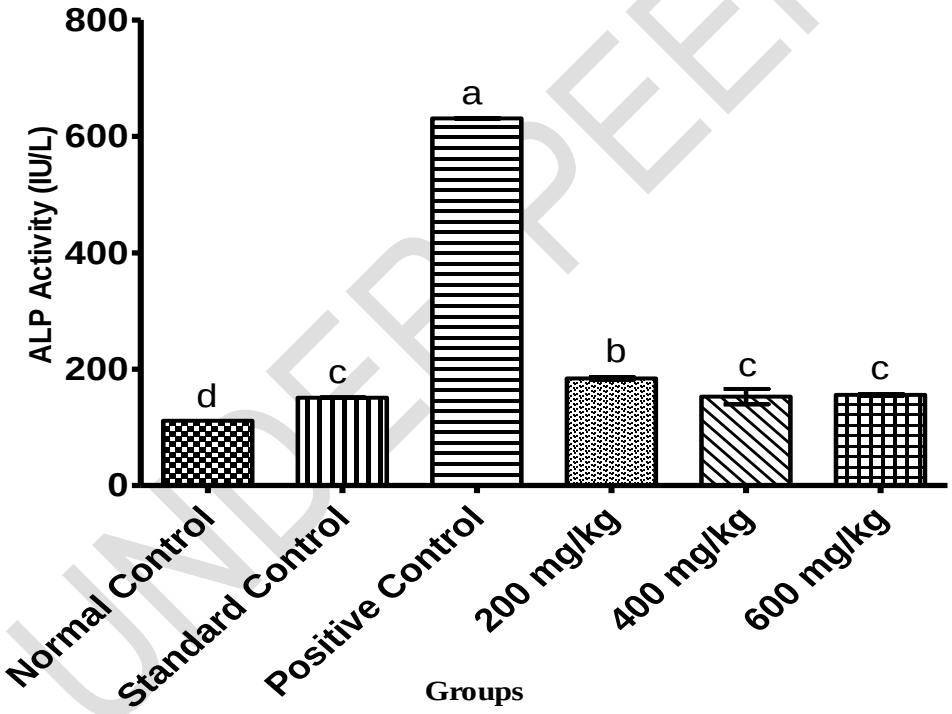

Fig. 3: Alkaline Phosphatase Activities in Albino Rats Treated With Ethanol-Leaf Extract of *Cajanus Cajan*. Data are presented in bar charts as Mean ± Standard Deviation (n=6). Mean values bearing different letters showed significant difference at *P<0.05* in (fig.3) above

4. CONCLUSION

It is therefore concluded that ethanol leaf-extracts of *Cajanus cajan* contains important antioxidants which could be hepatoprotective to albino rats by lowering the activities of liver function parameters such as ALT, AST, ALP. Also, the findings from this study further explain why the leaf-extract is used by herbalists in management and treatment of liver diseases in Nigerian traditional societies.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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