Original Research Article

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

ABSTRACT:

Aims: To investigate the hepatoprotective potentials of ethanol leaf-extract of *Cajanus cajan (linn)* in ethanol-induced oxidative stress in albino 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 A, B, C, D, E, F with 6 rats in each group. Group A (Normal control) was administered normal saline only. Group B (standard control) was administered 5mg/kg body weight of standard drug (Silymarin); group C (positive control) was administered 3.7g/kg body weight of 99.7% ethanol only while rats in groups D, E and F (test groups) were administered graded doses of 200mg/kg, 400mg/kg and 600 mg/kg body weights of the ethanol leaf-extract of *Cajanus cajan* respectively. Groups B and D, E, F were administered 3.7g/kg body weight of 99.7% ethanol, once a day 3 hours after administration of standard drug and leaf-extract respectively for 14 days through oral intubation. Blood sample were collected from each group by femoral vein puncture. Liver marker enzymes were all determined using standard procedures.
Results: Activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) increased significantly ($P<0.05$) in positive control relative to the levels observed in other control groups. However, administration leaf-extract to the test groups showed a significant ($P<0.05$) reversal in the trend of these parameters to levels comparable to those observed in the standard and normal control groups.
Conclusion: We therefore conclude that ethanol leaf-extracts of *Cajanus cajan* contains principles which could augment hepatoprotective functions in albino rats.

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

1. INTRODUCTION

Alcoholic liver disease (ALD) is one of the most common causes of liver injury, accounting for 3.8% of all global deaths and 4.6% of global disability-adjusted life-years attributable to alcohol [1]. Consistent, excessive alcohol consumption has long been identified as an important risk factor for the development of liver disease [2]. Although the precise mechanisms and factors responsible for liver injury are not completely understood, many pathways have been suggested in which oxidative stress plays a key role in alcohol-induced liver injury [3] [4].The liver is the main organ responsible for metabolizing alcohol in the body, mainly through alcohol dehydrogenase, aldehyde dehydrogenase and cytochrome P450 (CYP2E1) [5]. Among the CYP450 family, cytochrome 2E1 (CYP2E1) has been identified as a key microsomal enzyme in alcoholic liver injury, since it is highly inducible with high catalytic activity against alcohol, which catalyzes the oxidation of exogenous and endogenous compounds, and it plays a significant role in the metabolism of alcohol by the liver. Thus, it is particularly relevant to the development of ALD caused by the generation of alcohol-induced reactive oxygen species (ROS) [6], which leads to lipid peroxidation in liver cells, and changes of alcohol metabolizing enzymes [7]. CYP2E1 has a specific metabolic effect on xenobiotics; alcohol itself is a strong inducer of CYP2El enzyme activity and expression [8]. In

addition, alcohol use results in the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which contributes to hepatocellular damage [9]. Previously, a large number of medicinal plants have been investigated for hepatoprotective effects against alcohol-induced liver damage.

Cajanus cajan is a tropical woody herb with yellow flowers and is a perennial plant belonging to the family leguminosae. It is commonly known as Pigeon pea in English, "Fio-fio in Igbo, "Waken turawa" in (Hausa) and "Otili" in Yoruba Nigeria. It is a highly nutritious seed of the tropical Pigeon pea plant [10]. It has been cultivated in ancient Egypt, Africa and Asia. This plant was introduced in America and in other several tropical countries and it is mainly produced in India [10]. It is a nutritious non-toxic edible tropical legume with several desirable characteristics, widely used in Indian folk medicine for the prevention of various liver disorders [10]. The pigeon pea leaf extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and healthy food industry [11].

Cajanus cajan preparations are often recommended for the treatment of liver disorders by most herbalists in Nigerian traditional societies and quite often claimed to offer significant relief to patients. In attempt to get more scientific evidence for this traditionally reported herbal drug and to increase the volume of information about the potential hepatoprotective properties of this plant leaves, our attentions were aroused to carry out this study to investigate the hepatoprotective potentials of ethanol leaf-extract of *Cajanus cajan* in ethanol-induced oxidative stress in albino rats.

2. METHODOLOGY

2.1 Collection and Authentication of Plant Materials

Fresh leaves of *Cajanus cajan* were collected from Ndegu-Obu village in Ameffia in Ohaukwu Local Government Area of Ebonyi State in the month of May, 2020 and was identified by Prof. S. S. Onyekwelu in the department of Applied Biology, Ebonyi State University Abakaliki, Ebonyi State, Nigeria.

2.2 Extraction of Plant Material.

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 material (350 g) was soaked in 1600ml of ethanol for 48 hours. The mixture was filtered with a muslin cloth. The filtrate was concentrated by evaporation to dryness using a rotary evaporator.

2.3 Experimental Design

A total of thirty-six (36) albino rats were used in this study. The rats were randomly assigned into six (6) experimental groups of A, B, C, D, E and F with six (6) rats in each group after acclimatization for 7 days as: Group A (normal control) was given normal saline only. Group B (standard control) was administered standard drug (silymarin) at the dose of 5mg/kg body weight and co-administered absolute ethanol (3.7g/kg body weight) 3 hours later. Group C (positive control) was administered absolute ethanol of 3.7g/kg body weight of the rats only. Groups D, E and F (Test groups) were administered graded doses of 200, 400 and 600 mg/kg body weight of ethanol leaf extract of *Cajanus cajan* respectively and absolute ethanol of 3.7g/kg body weight, 3 hours later. This administration lasted for 14 days through oral intubation. All the animals were all fed with grower's marsh and water *ad libitum.*

2.4 Induction of Oxidative Stress and Liver Damage

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

2.5 Collection of blood sample

After 14 days of administration, the rats were mildly anesthetized using chloroform, blood sample were collected from the albino rat by femoral vein puncture method.

2.6 Methods of determination of liver function parameters

Liver Function Parameters such as the activities of Alanine transaminase (ALT) and Aspartate transaminase (AST) were assayed according to the method of [13] while Alkaline phosphatase (ALP) activity was assayed according to the method of [14].

2.7 Statistical Analysis

The results were expressed as mean ± 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 compared to normal and standard control groups but the groups administered ethanol leaf extract of *Cajanus cajan* at the doses of 200, 400 and 600mg/kg body weight of the rats showed significant (P<0.05) decreased in the activities of these enzymes as shown in (Fig.1, fig.2 and fig.3) respectively. [15] reported a significant (P<0.05) decrease in ALT, AST and ALP activities in alloxan induced diabetic albino rats treated with *Cajanus cajan* ethanol leaf-extract when compared to those treated with *Moringa oleifera* ethanol leaf extract which also showed a significant (P<0.05) decrease in dose dependent manner. Similarly, [16] investigated the anticardiotoxicity and chemoprotective effect of pigeon pea (*Cajanus cajan*) sprout on anthracycline induced cardiotoxicity in the organs of wistar albino rats which showed that pigeon pea significantly (P<0.05) and 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* (L.) Millsp. (Pigeon pea) leaves and the results showed that ethanol 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 significant (P< 0.05) decrease in the activities of ALT, AST but no significant change in ALT activity in the treatment of leaf-extract *Cajanus cajan*. This slight variation seen with the study of [18] with our present study may be attributed to the methodology, experimental model or doses of the extract used.

The liver is one of the major alcohol target organs known to be severely damaged due to chronic alcohol intake [19]. Any damage to the parenchymal liver cells will result in elevations in these transaminases [19]. During liver damage, these enzymes leak out from the injured hepatocytes into the blood. However, *Cajanus cajan* leaf extracts as shown in the chats generated from this present study contains valuable natural antioxidant sources that could repair damages caused to the parenchymal liver cells. Although liver disease due to alcohol consumption is a common cause of death in adults, medicinal support to alcohol-induced liver dysfunction is indeed very limited. In this investigation, we have demonstrated some of the important biochemical parameters such as liver marker enzymes and oxidative stress indices in rats liver that are adversely affected due to chronic alcohol intake. This has enabled us to verify the potentiality of ethanol leaf-extract of *Cajanus cajan* in protecting the defect. Interestingly, this leaf-extract showed significant liver damage protection effects when compared to the effects of the standard drug (silymarin), used in the case of human liver impairment treatment. In fact, ethanol leaf-extract from *Cajanus cajan* effects should be considered to have a greater protection effects against alcohol induced liver damage because, the leaf-extract is a mixture of uncharacterized chemical compounds whereas silymarin is a pure chemical compound. We have observed that alcohol-induced liver injury is mainly caused by oxidative stress which corroborate earlier observations of [20] [21] [22].

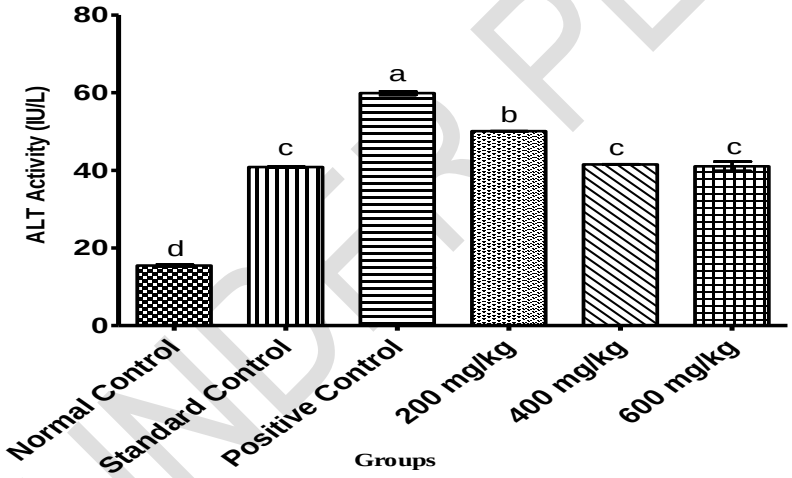

Fig. 1: ALT Activities in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus cajan*. Data are shown in bar charts as Mean ± Standard Deviation (n=6). Mean values with different alphabets showed significant difference at *P*=.05 in (fig.1) above.

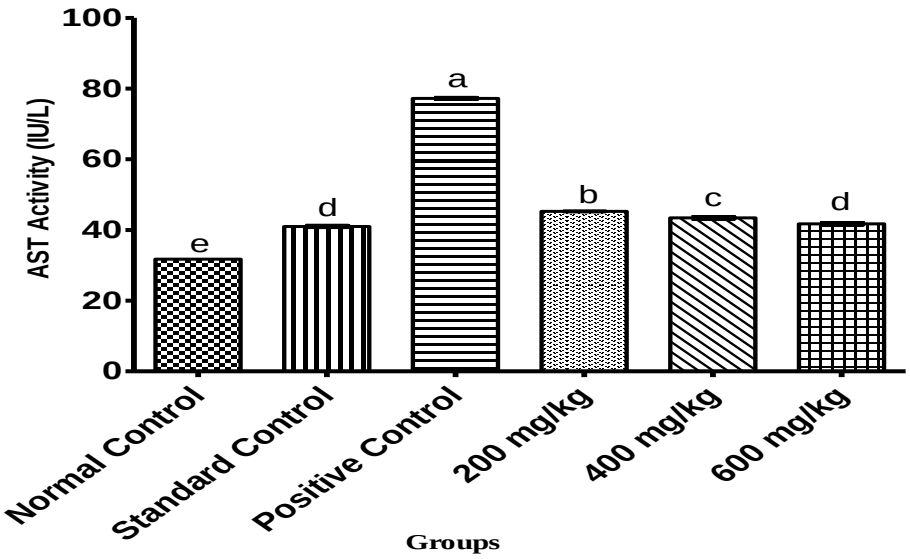

Fig. 2: AST Activities in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus cajan*. Data are shown in bar charts as Mean ± Standard Deviation (n=6). Mean values with different alphabets showed significant difference at *P*=.05 in (fig.2) above.

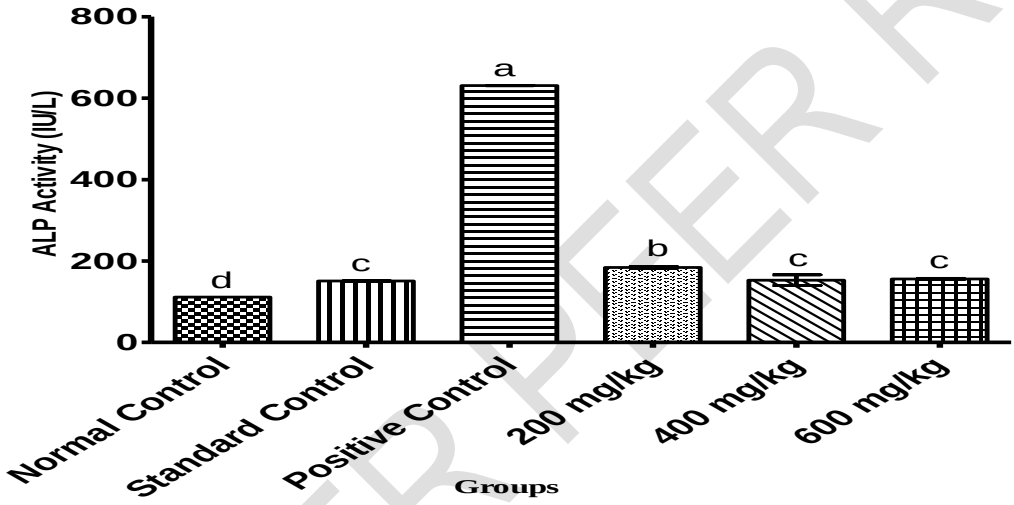

Fig. 3: ALP Activities in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus Cajan*. Data are shown in bar charts as Mean ± Standard Deviation (n=6). Mean values with different alphabets showed significant difference at *P*=.05 in (fig.3) above

4. CONCLUSION

We therefore conclude, that the ethanol-leaf extract of *Cajanus cajan* is capable of lowering the activities of liver function parameters such as ALT, AST, ALP. From the result it showed that extract of *Cajanus cajan* could be hepatic protective. This may partially explain the use of the leaves by herbalists in management and treatment of liver diseases in Nigerian traditional societies.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

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