Effects of *Anacardium Occidentale* Extract on Carbon Tetrachloride-Induced Hepatotoxicity on Rats

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Abstract: **This work aimed at investigating the effects of *Anacardium occidentale* ethanolic-leaf extract on rats induced hepatotoxicity by carbon tetrachloride. Forty male-Albino Wister rats were grouped into eight groups; group A (Positive control-those that received water and feed only), group B (Negative control- carbon tetrachloride only for 7 d), group C (Rats that were given carbon tetrachloride for 7 d + 150 mg/kgbw of the extract for 28 d, group D (Rats that received carbon tetrachloride only for 7 d + 300 mg/kgbw of the extract for 28 d and group E (Rats that received carbon tetrachloride for 7 d + 600 mg/kgbw of the extract for 28 d. Activities of hepatic enzymes (Serum aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase), and the level of bilirubin were determined using an Ultraviolet-Visible spectrophotometer (Model 752G, LabTech, China). Serum enzyme activities and bilirubin levels decreased significantly ($P<0.05$) in rats in groups C, D, and E when compared with those of rats in the negative control group. The liver weights of rats in groups C, D, and E increased significantly ($P<0.05$) against negative control. *Anacardium occidentale* ethanolic-leaf extract therefore, had hepatocurative effects on rats induced hepatotoxicity by CCl_4 and this is dependent on dose. The extract was therefore found to have curative effects on the rats induced hepatotoxicity.**

Keywords: **Anacardium, Bilirubin, Carbon tetrachloride, Control groups, Liver function test, Rats.**

I. INTRODUCTION

Background

The liver is an important organ in the body were most metabolic activities take place [1]-[2] . Metabolism of biochemical molecules such as carbohydrates, proteins, lipids, bilirubin, minerals, and vitamins takes place in the liver [3]-[5] . The global increase in liver diseases is alarming due to increased exposure to xenobiotic substances such as carbon tetrachloride (CCl_4), industrial chemicals, environmental pollutants, drugs, and constituents from plants. Carbon tetrachloride is a well-known model used by many researchers in inducing liver toxicity in experimental animals . [6] This is as a result of oxidative damage caused by CCl_4.

Plants have been used as the basis for the production of orthodox drugs [7]. This validates a lot of claims by traditional medicine practitioners concerning the value of traditional drugs in health care delivery [4] ,[8]-[9]. Traditional cures have been in place over a long period and it is older than synthetic drugs.

Anacardium occidentale (Cashew) is one of the species of plants found in many parts of Africa including Nigeria, Ghana, Togo, Ivory Coast, Republic of Congo, and Gambia [9]. Its fruits are eaten raw while the seeds are eaten when dried or roasted by the people of the South-Eastern part of Nigeria (Ibos).

A lot of claims have been made by many traditional medicine practitioners from Nnewi, Anambra State, Nigeria, on the use of *Anacardium occidentale* in treating liver injuries without any scientific backup-up. In this work, therefore, we aimed at investigating the effects of an ethanolic-leaf extract of *Anacardium occidentale* on CCl_4-induced hepatotoxicity on male-Albino rats. This is to know whether the extract has curative potentials against CCl_4-induced hepatotoxicity.

II. MATERIALS AND METHODS

A. Materials

All chemicals used in this work were of analytical grade and purchased from British Drug House (BDH) Ltd., Pool, England via her sales representative in Ikeja, Lagos State, Nigeria. Vital top feed grower was obtained from UAC Foods Ltd., Jos, Plateau State, Nigeria. All reagent kits used were purchased from Randox Laboratories Ltd., United Kingdom via her sales representative in Ikeja, Lagos State, Nigeria.

B. Methods

1" plant collection and preparation:

Anacardium occidentale leaves were obtained from Nnewi, Anambra State, Nigeria. It was identified at the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, and issued with herbarium number NAU75G. Plant leaves were prepared according to the method described by [10].

2" animal care and handling:

This study was conducted according to rules and regulations approved by the Ethical Committee of Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University (NAU), Nnewi Campus, Anambra State, Nigeria, and ethical approval granted. The ethical certificate number is NAU/NC/126. The care and use of laboratory animals' guidelines were complied with. Animal suffering was minimized to its bearest minimum.

3" oral acute toxicity test (LD_{50}):

The LD_{50} of *Anacardium occidentale* ethanolic-leaf extract was determined using a new method for determining acute toxicity in animal models as described by Enegide *et al*., [12]. This was determined to be 3,000 mg/kgbw. Selection of doses were based on the outcome of LD_{50}. High dose= 3000 divided by 5= 600 mg/kgbw, Moderate dose= 3000 divided by 10 = 300 mg/kgbw, Low dose= 3000 divided by 20=150 mg/kgbw.

4" experimental design:

Forty male-Albino Wistar rats weighing between 150-300 g were used for this study. Male rats were used because they are more reliable in terms of getting data. The rats were allowed to acclimatize for a period of 14 d, after which they were grouped into 5 groups of 8 rats each.

Group A: Positive control (those that received distilled water and feed only)

Group B: Negative control (those that received carbon tetrachloride (CCl_4) only)

Group C: Rats that received CCl_4 for 7 d + 150 mg/kgbw of ethanolic extract of *Anacardium occidentale* leaf for 28 d

Group D: Rats that received CCl_4 for 7 d + 300 mg/kgbw of ethanolic extract of *Anacardium occidentale* leaf for 28 d

Group E: Rats that received CCl_4 for 7 d + 600 mg/kgbw of ethanolic extract of *Anacardium occidentale* leaf for 28 d

4. induction of hepatotoxicity:

Hepatotoxicity was induced using CCl_4 according to the method of [11], by injecting 1 ml of CCl_4 and olive oil in the ratio of 1:1 v/v intraperitoneally for 7 d.

5" animal sample collection:

The animals were anesthetized with chloroform (BDH, Poole, England) in an enclosed container, after 24 h of the last administered dose of an ethanolic-leaf extract of *Anacardium occidentale* leaf. Blood samples were collected via an orbital sinus. The livers were harvested and stored in 10 % formalin for histopathological examination. Serum was separated using a centrifuge machine (Alpine Medical, model 905 A, England) at 3000 (revolution per minute (rpm) for 20 min at room temperature and analyzed for liver function.

6" liver function test (LFT):

a. determination of serum enzyme activities
   Activities of aspartate aminotransaminase(AST), alanine aminotransaminase(ALT), and alkaline phosphatase (ALP) were determined according to the method described by [13] by using spectrophotometer .
b. estimation of serum bilirubin
   This was done according to the method described by [14].
c. histological examination
   This was carried out according to the method of [15], by using haematoxylin and eosin staining technique.
d. statistical analysis of results

Data were analyzed using Statistical Package for Social Sciences (SPSS version 25). The results were expressed as mean ± SD. Data for alkaline phosphatase, alanine aminotransaminase, aspartate aminotransaminase, and bilirubins were analyzed using a one-way analysis of variance (ANOVA), followed by Post hoc least significant difference (LSD) while body weight was analyzed using Students' dependent T-test. Values were considered significant at $P<0.05$.

III. RESULTS

Table I shows the results of Liver weights of rats induced hepatotoxicity and given Anacardium occidentale ethanolic-leaf extract. It was observed that liver weights of all the groups(groups B, C and E) were significantly higher($P< 0.05$) than that of group A. There were significant increase in liver weights of rats in groups C,D and E when compared to those in group B ($P < 0.050$).

Table II shows the results of serum enzyme activities of rats injected CCl_4 and given *Anacardium occidentale* ethanolic-leaf extract. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats injected CCl_4 only (group B) were significantly higher ($P< 0.05$) than other groups. The AST and ALT activities of rats given 150 mg/kgbw (group C), 300 mg/kgbw (group D), and 600 mg/kgbw (group E) were higher than those of rats given feed and water only (group A) but not significant. The alkaline phosphatase (ALP) activities in rats in group B were significantly higher ($P <0.05$) than in other groups (groups A, C, D, and E). Those in groups C, D, and E (treatment groups) were higher than that of group A (positive control) but not significant ($P<0.05$). The activities of ALP were higher in group E than in groups C and D but not significant.

Table III shows the results of serum bilirubins of rats injected CCl_4 and given *Anacardium occidentale* ethanolic-leaf extract. Serum total bilirubin levels of rats in group B were significantly higher ($P<0.05$) than those in groups A, C, D, and E. Those in groups C, D, and E were significantly higher when compared with group A. Serum total bilirubin levels of rats in groups D and E were significantly higher when compared with that in group C. The same trend was maintained for serum direct (conjugated bilirubin) and indirect (unconjugated bilirubin). See tables 1 and 2 on pages 8-9 after the list of references.

Plates 1-5 represent photomicrographs of liver sections of rats in groups A, B, C, D, and E. The liver architecture of rats in group A is intact. The liver architecture of rat in group B (negative control) was altered marked by degeneration of hepatic cells, severe hepatic inflammation, and severe fibrosis. Liver sections of rats in groups C, D, and E showed improved liver architecture. See plates 1-5 on page 10. After a list of references.

IV. DISCUSSION

This study has so far investigated the effect of *Anacardium occidentale* ethanolic-leaf extract on the liver of male Albino-Wistar rats induced hepatotoxicity by carbon tetrachloride (CCl_4).

Findings from this study showed a significant increase in the relative liver weight in-group C, D and E when compared to group B, but a significant reduction in the relative weight of the liver in-group B, which was administered CCL4 only. The physiology behind the significant reduction in the liver weight in-group B could be attributed to necrosis and apoptosis caused by free radicals generation as noticed in CCl4 toxicity [11]. This suggests that the administration of the extract attenuated the changes in the weight of liver in group C, D, and E when compared to group B; this is attributed to the antioxidant activities of the *Anacardium Occidentale*, which has potency of reverting damages caused by CCL4. This agrees with the similar work reported by [11] on " Carbon tetrachloride-induced hepatic damage on experimental Srague Dawley rats: Antioxidant potential of *Xylopia aethiopica*".

The results of the enzyme assay carried out in this work showed that ethanolic-extract of *Anacardium occidentale* leaf reduced serum AST, ALT, and ALP activities significantly ($P<0.05$) while rats injected CCl_4 only(negative control group) showed increased activities of AST, ALT, and ALP. The decreased activities of the above liver enzymes in groups of rats given an ethanolic extract of *Anacardium occidentale* leaf after injecting CCl_4, as witnessed in this work, maybe suggesting a reversal of liver injury induced by CCl_4. This agrees with a similar report of [16], who reported that ethanolic stem bark extract of *Anacardium occidentale* reversed the impact of CCl_4 toxicity on serum markers of ALT, AST, and ALP of the damaged liver. Toxicity causes liver enzymes to move to serum, thereby increasing their activities in the serum [2].

It was equally observed in this work that ethanolic-leaf extract of *Anacardium occidentale* caused a significant reduction in total bilirubin, direct bilirubin(conjugated), and indirect bilirubin(unconjugated) in the treatment groups (groups C, D, and E) when compared with group B which received CCl_4 only. This suggests overproduction of bilirubin caused by the toxicity of CCl_4 may have been reversed by the extract. This is in line with the work reported by [6] on "The effects of *Sida corymbosa* leaf extract against abnormal bilirubin and total protein on Albino-Wistar rats". The reason for the reduction of serum bilirubin levels by *Anacardium occidentale* maybe because of the presence of some antioxidants such as vitamin C, anthocyanin, flavonoids, and zinc. Konan and Bacchi [17] reported the presence of flavonoids, vitamin C, and zinc during the phytochemical analysis of *Anacardium occidentale*-leaf extract. It was equally observed from the findings of this work that much decrease in serum bilirubin levels was witnessed in rats given the highest dose of 600 mg/kgbw of the extract (group E), followed by those given 300 mg/kgbw of the extract for 28 d after injecting CCl_4 for 7 d (group D). This again suggests that the treatment with ethanolic-leaf extract of *Anacardium occidentale* may be dependent on dose with 600 mg/kgbw being the best dose followed by 300 mg/kgbw. Histological findings of this work confirmed the results of biochemical findings that Anacardium occidentale-ethanolic leaf extract may have reversed liver damage induced by CCl_4 toxicity. This is because histological examination carried out revealed regeneration of hepatic cells in liver tissues of rats given *Anacardium occidentale*-ethanolic leaf extract after injecting CCl_4 (groups C, D, and E).

V. CONCLUSION

Anacardium occidentale ethanolic-leaf extract may therefore have curative effects against CCl_4-induced hepatotoxicity on male-Albino Wistar rats, since the liver functions of rats induced hepatotoxicity and treated with *Anacardium occidentale* ethanolic-leaf extract improved. This treatment may be dependent on the dose with 600 mg/kgbw of the extract being the best dose for the treatment.

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Table I: The Effect of Ethanolic-leaf Extract of *Anacardium occidentale* on Liver Weights of Albino Rats Induced Hepatotoxicity by Carbon Tetrachloride

Groups		MEAN	±SEM	P-Value	F-Value
Relative liver weight (g)	Group A (Control)	3.36	±0.32	0.00*	
	Group B (CCl4 Only)	3.71	±0.05		
	Group C (CCl4) one week and 150mg/kg of A.O 3weeks)	3.83	±0.02	0.01*	14.95
	Group D (CCl4) one week and 300mg/kg of A.O 3weeks)	4.82	±0.01	0.02*	
	Group E (CCl4) for one week and treated with 600mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 3 weeks)	3.88	±0.00	0.01*	

Values are means of triplicate results ± SD. Data was analyzed using t-test and values were considered significant at $p<0.05$.

WD: Weight difference, CCl4= Carbon tetrachloride, A.O= *Anacardium occidentale*.

Table II: Results of Serum Enzyme Activities of Rats Injected Carbon Tetrachloride and Given *Anacardium occidentale* Ethanolic-leaf extract

Enzyme	Groups	Activity (U/l)	P-value	F-value
Aspartate transaminase	(Group A (Control)	33.83	±2.92	0.03*
	Group B (CCl_4 Only)	54.00	±1.53	
	Group C (CCl_4 one week and 150mg/kg of extract for 21 d)	34.67	±0.66	0.01* 5.02
	Group D (CCl_4 one week and 300mg/kg of extract for 21 d)	38.33	±1.76	0.05*
	Group E (CCl_4 for 7 d and treated with 600 mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	39.33	±1.45	1.00
Alanine Transaminase	(Group A (Control)	12.67	±0.88	0.00*
	Group B (CCl_4 Only)	48.33	±1.20	
	Group C (CCl_4 for 7 d and 150mg/kg of A.O 3weeks)	13.67	±0.33	0.01* 8.79
	Group D (CCl_4 for 7 d and 300mg/kg of extract for 21 d 3)	14.00	±0.57	0.02*
	Group E (CCl_4 for 7 d and treated with 600mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	13.00	±0.57	0.04*
Alkaline phosphatase	(Group A (Control)	124.26	±0.78	0.00*
	Group B (CCl_4 Only)	148.22	±0.04	
	Group C (CCl_4 for 7 d and 150mg/kg of A.O 3weeks)	125.50	±0.29	0.01* 171.5
	Group D (CCl_4 for 7 d and 300mg/kg of extract for 21 d 3)	125.74	±0.37	0.02*
	Group E (CCl_4 for 7 d and treated with 600mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	132.73	±1.45	0.04*

Values are means of triplicate results ± SD. Results were analyzed using ANOVA followed by Post Hoc LSD multiple comparison and data were considered significant at $p<0.05$. *= comparison of serum enzyme activities of rats in group B with other groups.

Table III: Results of Serum Bilirubin Levels of Rats Injected Carbon Tetrachloride and Given *Anacardium occidentale* Ethanolic Leaf-extract

Groups		Levels (mg/dl)	P-value	F-value
Total bilirubin	Group A (Positive control)	3..83	± ± 0.19	0.01*
	Group B (CCl_4 Only-negative control)	4.4.00	±0.01	
	Group C (CCl_4 one week and 150mg/kg of extract for 21 d)	3.4.67	±0.13	0.02* 17.14
	Group D (CCl_4 one week and 300mg/kg of extract for 21 d)	3.8.33	±0.08	0.01*

	Group E (CCl_4 for 7 d and treated with 600 mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	3.9.33	±0.01	0.03*
Conjugated bilirubin	Group A (Positive control)	2.65	±0.00	0.00*
	Group B (CCl_4 Only-negative control)	3.53	±0.03	
	Group C (CCl_4 for 7 d and 150mg/kg of A.O 3weeks)	3.04	±0.00	0.02* 362.7
	Group D (CCl_4 for 7 d and 300mg/kg of extract for 21 d 3)	2.75	±0.01	0.04*
	Group E (CCl_4 for 7 d and treated with 600mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	2.66	±0.03	0.05*
Unconjugated bilirubin	Group A (Positive control)	0.18	±0.00	0.01*
	Group B (CCl_4 Only-negative control)	0.26	±0.00	
	Group C (CCl_4 for 7 d and 150mg/kg of A.O 3weeks)	0.16	±0.00	0.04* 1827.9
	Group D (CCl_4 for 7 d and 300mg/kg of extract for 21 d 3)	0.27	±0.00	0.03*
	Group E (CCl_4 for 7 d and treated with 600mg/kg of ethanolic extract of *Anacardium occidentale* leaf for 21 d	0.21	±0.00	0.02*

Values are means of triplicate results ± SD. Results were analyzed using ANOVA followed by Post Hoc. LSD multiple comparison and data were considered significant at $p<0.05$. *= comparison of serum enzyme activities of rats in group B with other groups.

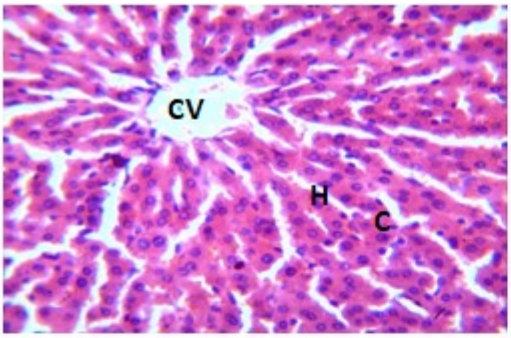

Plate I: A photomicrograph of the liver section of rats in group A .Central vein(CV) Cytoplasm,. (C) well intact and hepatocytes (H) appearing normal.Stained by H &E(x400).

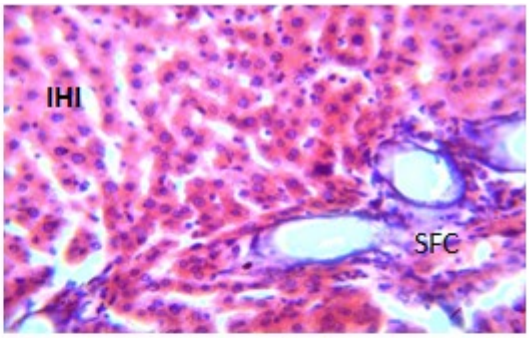

Plate: A photomicrograph of the liver section of rat in group B. There was severe degeneration of liver tissue with severe intrahepatic inflammation (IHI), severe fatty changes(SFC) (x400).

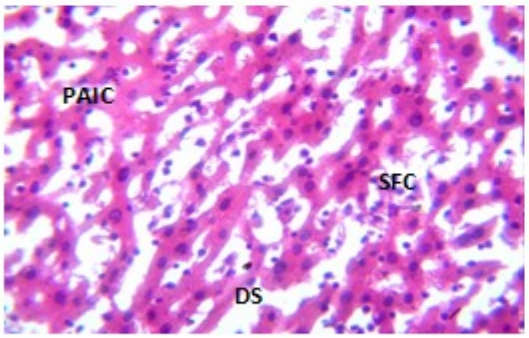

Plate 3: A photomicrograph of the liver section of rat in group C. There was a mild regeneration of hepatic tissue with a moderate portal aggregate of inflammatory cells (PAIC), severe fatty changes (SFC), and severe dilation of the sinusoid (DS) (x400).

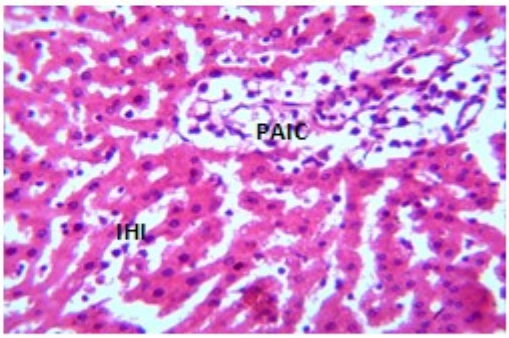

Plate 4: A photomicrograph of the liver section of rat in group D. There was mild regeneration of hepatic tissue with a severe portal aggregate of inflammatory cells (PAIC). Intra hepatic inflammation (IHI)(x400).

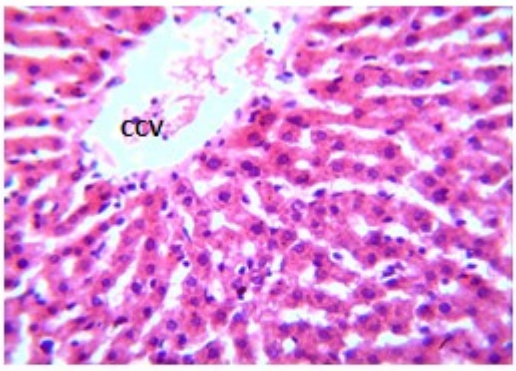

Plate 5:photomicrograph of the liver section of rat in group E.This shows a mild effect on the tissue and congestion of the central vein (CCV) (x400).