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EFFECT OF METHANOL EXTRACT OF *ANARCARDIUM OCCIDENTALE* (CASHEW) STEM BARK ON SOME BIOCHEMICAL PARAMETERS OF CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT: Purpose: *Anacardium occidentale* (Cashew) has been used by Folk medicines as a remedy for many diseases. In this study, we investigated the ameliorative activity of methanolic stem bark extract of *Anacardium occidentale*. **Methods:** Blood samples of the rats from each group were collected by tail bleeding for baseline biochemical test to know the state of the animals' liver before the inducement of the injury. Thirty (30) Wistar rats were induced with CCl_4 (1.5 ml/kg, b.wt, 20 % CCl_4 / olive oil) for five days before treatment with the extract. After induction, elevated levels of the serum aminotransferases (ALT, AST), ALP and the decreased levels of ALB as compared to the enzyme markers levels before the induction of liver injury with CCl_4 indicated hepatocellular injury. **Results:** Administration of the methanolic extract of *Anacardium occidentale* of different concentration (250, 500 and 1000 mg/kg b.wt oral) for five days reversed the impact of CCl_4 toxicity significantly ($P<0.05$) on the serum markers ALT, AST and ALP of the damaged liver as compared to the negative control groups (groups administered with Normal saline). Treatment with the methanolic extract resulted in decreased levels of ALB in the blood of the rats. **Discussion:** The results of the study indicated that methanolic extract of *Anacardium occidentale* bark may possess a liver damaged curative property, which may be attributed to the presence of the phenolic compounds in the extract as shown in the phytochemical screening.

INTRODUCTION: Liver is the most important organ regulating homeostasis in the body [1]. It is required in biochemical pathways related to growth, fight against diseases and involved in energy provision.

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Besides its physiological functions, it also defends against the dangers of toxic drugs and other chemicals by metabolism. During metabolism, excessive free radicals are produced and may cause liver injury [2].

Free radicals such as hydroxide (·OH) and oxide (·O) are produced as a result of the activity of the metabolizing enzymes in the liver (cytochrome P_{450}) which readily attack the polyunsaturated fatty acid of the lipid membrane of hepatic cells, initiating a self-propagation chain reaction [3, 4]. This eventually leads to liver damage.

Liver injury is detectable by clinical signs (jaundice, swollen and tender liver), biochemical alterations (elevated levels of hepatic enzymes in the blood, loss of enzymic activities in the liver), or histological examination. Elevated levels of serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) may provide evidence of hepatocellular injury in the absence of clinical signs and histological examination [5, 6]. Medicinal plants have gained much attention as an alternative medicine useful for treatment, prevention or both of life-style related disorders [7]. They are used to treat a wide variety of diseases and are potential natural hepatoprotectives and antioxidant compounds in the treatment of liver diseases [8].

Anacardium occidentale (Cashew) is a tree in the family of Anacardiaceae. This family contains 73 genera and about 600 species of which cashew is by far the most important economically. It is a multipurpose tree that grows up to 15 mm high. This specie (*Anacardium occidentale*) has been in use as a cure for some diseases. Extracts from root, stem and fruit of *Anacardium occidentale* have been used by countries' folk medicines as a remedy for many diseases such as eczema, bronchitis, cough, veneral diseases, as well as impotence and syphilis-related skin diseases [7, 9]. *Anacardium occidentale* possess phytoconstituents such as saponins, tannins and flavonoids, which have been reported to exert antioxidant activities [10].

Liver damage can be induced by various factors and hepatotoxins such as carbon-tetrachloride (CCl_4), acetaminophen and ethanol [2]. Here we used carbon-tetrachloride (CCl_4) to induce liver injury in animal models to cause hepatic injury. In this study, *Anarcadium occidentale* was assessed *in vivo* for possible hepatoprotection on the carbon tetrachloride induced liver injury.

MATERIALS AND METHODS:

Preparation of the Plant Powder and Extraction: The stem bark of *A. occidentale* was obtained from Wenchi in the Brong-Ahafo Region of Ghana in the month of April, 2017. Botanical identification of the plant was done at the Department of Applied Biology, University for Development Studies, Navrongo campus. The stem bark of *Anacardium occidentale* was air dried at room temperature for two weeks and ground into powder form with pestle and mortar. Seventy (70)g of the air-dried ground bark of the plant was macerated with methanol (95%) at room temperature for 72 h. After filtration, the solution was concentrated to a paste form using rotary evaporator.

Phytochemical Analysis: A qualitative phytochemical analysis of the crude extract of *Anacardium occidentale* bark was carried out to determine the phyto constituents present in the extract using standard methods [11, 12]. Secondary metabolites tested included alkaloids, Reducing sugars, triterpenoids, flavonoids, phenolics, saponins, Polyuronides and steroids. The methods are as follows:

Test for Saponins: To 2 ml of the extract, 2 ml of distilled water was added in a test tube. The solution was shaken and observed for 15 minutes, the absence of a stable persistent foam for 15 min indicated the absence of saponin.

Test for Reducing Sugars: The methanol extract (1 ml) was diluted with water (2 ml) and 1 ml of Fehling's solution was added and heated. A brick red precipitate denoted the presence of reducing sugars.

Test for Polyuronides: The extract, 2 ml, was added dropwise to 10 ml of acetone in a test tube. A thick precipitate was formed denoting the presence of polyuronides.

Test for Phenolic Compounds: Drops of 5% Ferric chloride was added to 3 ml of extract. A sudden change in colour to black showed the presence of phenolic compounds.

Test for Flavonoids: Extracts were evaporated to dryness and the residue was dissolved using 50% methanol in a test tube. A piece of magnesium was dropped into the mixture, followed by 3 drops of concentrated HCl with subsequent appearance of orange color indicative of the presence of flavonoids

Test for Triterpenes: The methanol extract (10ml) was evaporated to dryness. The residue was then dissolved successively in acetic anhydride (0.5 ml) and chloroform (0.5 ml). The solutions were then

transferred to a dry test tube. Concentrated sulphuric acid (2 ml) (Liebermann-Burchard's reaction) was then added at the bottom. A reddish-brown coloration at the interface was not observed which indicated a negative result for triterpenes.

Test for Alkaloids: The plant extract (0.5 g) was added to 5 ml of dilute sulphuric acid H_2SO_4 (1%) on a steam bath. The solution was filtered, and the filtrate was treated with a few drops of Dragendroffs reagent. Reddish brown turbidity or precipitate was not observed which indicated a negative result for alkaloids.

Animals: A total of 30 Wistar rats (both male and female) weighing 160 - 301 g were obtained from the Center for Plant Medicine Research, Mampong, Ghana. The animals were maintained in an air-conditioned house at a temperature of 23 ± 2 °C. The animals were provided with standard feed and drinking water was also made available. All the experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

Experimental Design: The animals were randomly divided into five groups (I to V) with six rats each in (each group). Groups I and II served as positive and negative control groups respectively, and groups III, IV and V served as treatment groups with different doses of the methanolic plant extract. Blood samples of the rats from each group were collected by tail bleeding for baseline biochemical test to know the state of the animals' liver before the inducement of the injury. Liver injury was then induced in all the experimental animals by the administration of carbon tetrachloride (1.5 ml/Kg of 20% CCl_4 / olive oil) *via* subcutaneous injection for 5 days. Blood samples were collected on the 5^{th} day, two (2) h after the administration of the CCl_4 to test for liver enzyme markers to confirm liver damage.

Group I rats were administered with silymarin (100 mg/kg b.wt) as the reference drug or positive control for next five (5) days whereas group II rats were given normal saline (as negative control). Rats in group III, IV and V were administered with 250 mg/kg b.wt, 500 mg/kg b.wt and 1000 mg/kg b.wt of methanolic cashew stem bark crude extract respectively for five days continuously.

Determination of Serum Biochemical Parameters: At the end of the treatment period, blood samples were collected by tail bleeding from all animals. Sera were separated out by centrifugation at 3000 rpm for 5 min and stored at a temperature of 20 ºC until analysis. Liver damage was assessed by the estimation of serum activities of ALT, AST, ALP and total albumin using commercially available test kits. The results were expressed as unit / liter (U/L).

RESULTS AND DISCUSSION:

Results:

Yield of Extracts: Seventy grams (70) g of the raw material was macerated in methanol (95%) and gave 19.72 g of methanolic stem bark crude extract of the plant. The percentage yield was 28.17%.

Phytochemical Analysis: Phytochemical analysis of methanolic stem bark extract of *Anacardium occidentale* showed the presence of reducing sugars, phenolic compounds, flavonoids and polyuronides however, saponins, triterpenes and alkaloids were absent as shown in **Table 1** below.

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC STEM BARK EXTRACT OF *A. OCCIDENTALE*

Phytochemicals	Methanolic extract of *A. occidentale*
Saponins	-
Reducing sugar	+
Polyuronides	+
Phenolic compounds	+
Flavonoids	+
Triterpenes	-
Alkaloids	-

Key (+) = Present, (-) = Absent

Biochemical Analysis: The subcutaneous administration of CCl_4 (20% CCl_4 / olive oil, 1.5 ml/kg b.wt) gave a significant ($P<0.05$) elevation of the serum ALT **Fig. 1**, AST **Fig. 2** and ALP **Fig. 3**, when compared to their respective baseline (day 0) values. Also, decreased levels of ALB **Fig. 4** were observed after the injury was induced with 1.5 ml/kg/b.wt of 20 % CCl_4 / olive oil. The levels of ALT, AST and ALP reduced significantly ($p<0.05$) when treated with silymarin and the methanolic stem bark extract of *Anarcadium occidentale* as shown in **Fig. 1** through to **Fig. 4**. There was also a significant ($p<0.05$) increase in the levels of ALB **Fig. 4**.

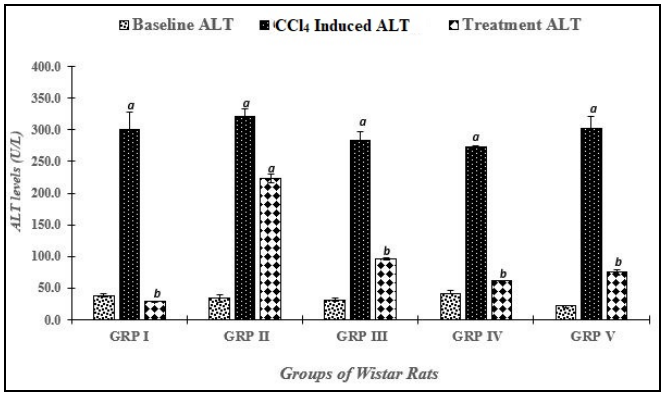

FIG. 1: EFFECT OF METHANOLIC STEM BARK EXTRACT OF *ANACARDIUM OCCIDENTALE* (MBEAO) ON ALT LEVEL AGAINST 1.5 ml/kg OF 20% CCl_4 / OLIVE OIL - INDUCED LIVER TOXICITY IN ADULT WISTAR RATS $^{a}P< 0.05$; significant difference when compared with the Baseline ALT level; $^{b}P < 0.05$; significant difference when compared with 1.5 ml/Kg of 20 % CCl_4 / olive oil liver injury induction ALT: Alanine Aminotransferase; CCl_4: Carbon tetrachloride, GRP I: Treated with silymarin, GRP II: Treated with Normal saline, GRP III: Treated with 250 mg/kg of MBEAO, GRP IV: Treated with 500 mg/kg of MBEAO and GRP V: Treated with 1000 mg/kg of MBEAO.

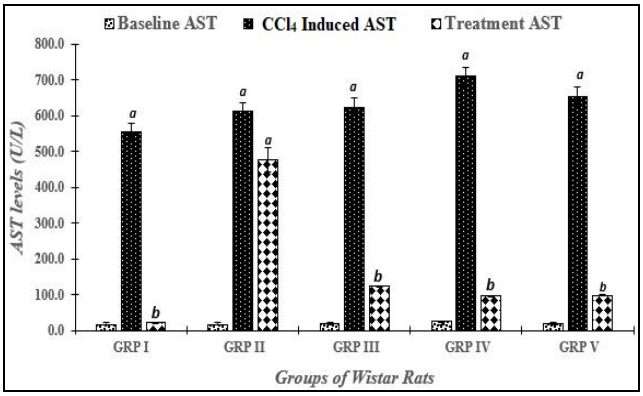

FIG. 2: EFFECT OF METHANOLIC STEM BARK EXTRACT OF *ANACARDIUM OCCIDENTALE* (MBEAO) ON AST LEVEL AGAINST 1.5 ml/kg OF 20% CCl_4 / OLIVE OIL - INDUCED LIVER TOXICITY IN ADULT WISTAR RATS $^{a}P< 0.05$; significant difference when compared with the Baseline AST level; $^{b}P < 0.05$; significant difference when compared with 1.5 ml/Kg of 20 % CCl_4 / olive oil liver injury induction.AST: Aspartate Aminotransferase; CCl_4: Carbon tetrachloride, GRP I: Treated with silymarin, GRP II: Treated with Normal saline, GRP III: Treated with 250 mg/kg of MBEAO, GRP IV: Treated with 500 mg/kg of MBEAO and GRP V: Treated with 1000 mg/kg of MBEAO.

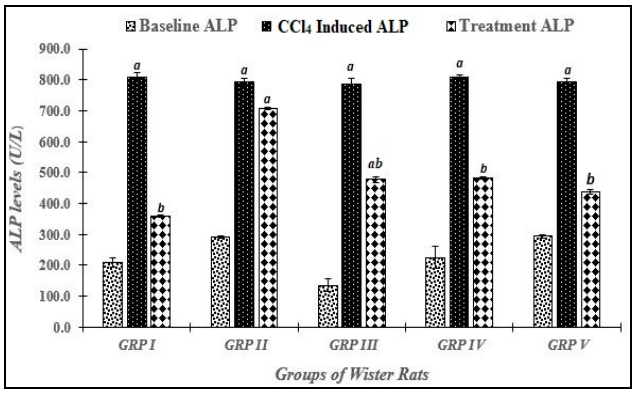

FIG. 3: EFFECT OF ETHANOLIC STEM BARK EXTRACT OF *ANACARDIUM OCCIDENTALE* (MBEAO) ON ALP LEVEL AGAINST 1.5 ml/kg OF 20% CCl_4 / OLIVE OIL - INDUCED LIVER TOXICITY IN ADULT WISTAR RATS $^{a}P<0.05$; significant difference when compared with the Baseline ALP level; $^{b}P<0.05$; significant difference when compared with 1.5 ml/Kg of 20 % CCl_4 / olive oil liver injury induction.ALP: Alkaline Phosphatase; CCl_4: Carbon tetrachloride, GRP I: Treated with silymarin, GRP II: Treated with Normal saline, GRP III: Treated with 250 mg/kg of MBEAO, GRP IV: Treated with 500 mg/kg of MBEAO and GRP V: Treated with 1000 mg/kg of MBEAO.

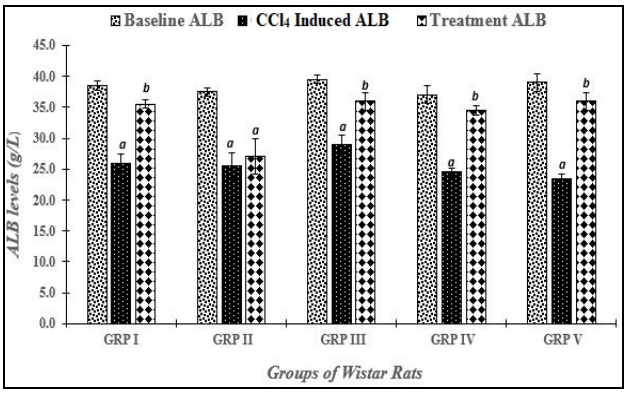

FIG. 4: EFFECT OF ETHANOLIC STEM BARK EXTRACT OF *ANACARDIUM OCCIDENTALE* (MBEAO) ON ALB LEVEL AGAINST 1.5 ml/kg OF 20% CCl_4 / OLIVE OIL - INDUCED LIVER TOXICITY IN ADULT WISTAR RATS $^{a}P< 0.05$; significant difference when compared with the Baseline ALB level; $^{b}P < 0.05$; significant difference when compared with 1.5 ml/Kg of 20 % CCl_4 / olive oil liver injury induction. ALB: Albumin; CCl_4: Carbon tetrachloride, GRP I: Treated with silymarin, GRP II: Treated with Normal saline, GRP III: Treated with 250 mg/kg of MBEAO, GRP IV: Treated with 500 mg/kg of MBEAO and GRP V: Treated with 1000 mg/kg of MBEAO.

DISCUSSION: The yield of our extract was 47.01 % with 95 % methanol and jelly nature of the extract was observed. Phytochemical screening of the methanolic extracts of cashew stem bark revealed the presence of phenolic compounds, flavonoids, reducing sugars and polyuronides and the absence of saponins, alkaloids and triterpenes.

The presence of phenolic compounds and flavonoids were also observed in [13, 14]. Phenolic compounds have antioxidant activity [15]. This activity is believed to be mainly due to their redox properties, which plays an important role in absorbing and neutralizing free radical [15, 16] as oxidative stress is a crucial factor in liver diseases [17]. The presence of phenolic compounds in our extract may be responsible to provide antioxidant activity and reports on anti-inflammatory properties of the *Anarcardium occidentale* stem bark [18 - 20] and thus its hepatoprotective activity observed in this study.

Liver injury marker enzymes levels (Baseline values) before the induction of liver injury indicated that the animals were without liver injury but the serum liver injury marker enzymes (ALT, AST and ALP) (**Fig. 1, 2, 3** and **4**) increased and a decreased albumin level after administration of 1.5 ml/kg of 20 % CCl_4/olive oil were observed. It is well evidenced that acute hepatotoxicity can be induced by carbon tetrachloride (CCl_4) [21]. Carbon tetrachloride (CCl_4) is a highly reactive halogenated molecule that has been used in experimental models of drug-induced hepato-cellular injury [22].

CCl_4 is metabolized by the cytochrome P_{450} family in the hepatic endoplasmic reticulum to form a highly reactive and unstable trichloromethyl radicals ($\cdot CCl_3$) which binds to the unsaturated fatty acids of the membrane lipid covalently, resulting in the formation of lipid radicals, thus initiating the chain reaction of lipid peroxidation within and outside the hepatic cells and cause liver injury [2, 23].

In this study, liver injury was induced when 1.5 ml/kg of 20% CCl_4/olive oil solution was administered to all experimental rats subcutan-eously. This dose induced a significant increase of the liver enzymes such as ALT, AST and ALP **Fig. 1, 2** and **3** when compared to the respective baseline values. Also, the decreased amount of total ALB levels **Fig. 4** induced by CCl_4 is a further indication of liver damage, decrease in the number of hepatocytes which in turn may result in a decrease in hepatic capacity to synthesize albumin [24]. Therefore decreased levels of albumin as recorded in CCl_4-induced rats revealed liver injury and may be attributed to the damage produced and localized in the endoplasmic reticulum leading to decrease in protein synthesis [25]. Silymarin is a well-established hepatoprotective drug, which is used as a reference drug for comparison of hepatoprotective activity of plants extracts [26, 27, 28, 29]. The levels of the elevated biochemical parameters (ALT, AST, ALP) because of CCl_4-induced hepatotoxicity, reduced by the administration of silymarin as seen in all figures, when compared to the baseline levels of the enzyme markers.

Concentration of 250, 500 and 1000 mg/kg of methanolic stem back extracts were administered to the rats because Okonkwo and his colleagues reported that the methanolic extract of the plant under study has no toxic effect to the liver unless the concentration is above 2900 mg/kg [30]. The administration of methanolic stem bark of cashew extract reduced the levels of the liver marker enzymes (ALT, AST and ALP) when compared to their levels when the liver was injured, the dose related fashion reduction in ALT in **Fig. 1**, indicated that the 1000 mg/kg was more effective against ALT than the 500 mg/kg and 250 mg/kg dose levels.

A reduction in the values of ALT and AST in the groups given only normal saline (negative control group) was observed, but not as much reduction as the groups treated with the extract and silymarin. This reduction may be attributed to normal healing of the animal liver. Both ALT and AST are commonly thought of as liver enzymes because of their high concentration in hepatocytes, however, ALT is marked specific for liver function.

ALT is specifically produced in the hepatocytes, making it relatively more specific to hepatic function compared to AST, which is present in variety of tissues such as liver, muscles and red blood cells [31].

CONCLUSION: The investigation on the hepatoprotective activity of *A. occidentale* against CCl_4 - induced hepatocellular damage revealed that the administration of the methanolic extract has reduced the impact of CCl_4 toxicity on serum biochemical liver marker enzymes (ALT, AST, ALP).

Thus, the results of the study indicated that methanolic extract of *Anacardium occidentale* bark may possess a hepatoprotective and a curative activity, which may be attributed to the presence of the phenolic compounds in the extract and can be explore for liver disease management.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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