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Research Article

Curative and Protective Efficacy of *Cassio occidentalis* Ethanolic Leaf Extract on Carbon Tetrachloride Induced Liver Damage of Female Albino Wistar Rats

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Abstract

Medicinal plants have been widely used as therapeutic options for the treatment of many human diseases. *Cassia occidentalis* is used as diuretics, laxative, anti-bacteria, anti-inflammatory, anti-fungal, used in treatment of liver diseases and used externally applied on healing wounds, sores, itch, skin diseases, bone fracture, ringworm and throat infection. The study evaluated the hepato-protective and hepato-curative effect of *Cassio occidentalis* on carbon tetrachloride (CCl4) induced liver damage using albino rats. The albino rats were grouped into six (6) groups, group I and II served as normal control which received food and fluid only and negative control which received carbon tetrachloride, feed and water respectively, group III and IV served as the hepato-protective groups and group V and VI as hepato-curative groups. The rats at the end of 6 weeks were anaesthetised by chloroform in a close jar and blood samples were collected for biochemical, and the albino rats were sacrificed by cervical dislocation. The liver harvested for histopathological analysis and the relative organ weight was determined. The results of serum levels of liver enzyme markers, Alkaline Phosphate, Serum Aspartate Aminotransferase, and Alanine Aminotransferase revealed significant difference in the liver enzymes when compared with negative control group, the histology of the liver revealed curative and protective efficacy of *Cassia occidentalis* which is dose dependent. In conclusion, the treatment of liver related diseases may be protected and cured following the administration of *Cassia occidentalis* leaf extract.

Keywords: *Cassio occidentalis; Liver Enzymes; Protective; Curative; Carbon Tetrachloride*

Introduction

Liver diseases have been causing death among the adult population globally today [1]. Traditional medicine is considered as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [2]. Because of the absence of reliable drugs for the treatment and prevention of liver diseases in modern medicine [3], Natural medicinal remedies are increasingly gaining popularity and used worldwide as complementary alternative therapies [4] and based on the fact that the raw materials are available naturally and in abundance with an estimated record of 1062-63 potentially beneficial substances [2].

Silva., *et al*. [5] reported that *Cassia occidentalis* has been used as natural medicine in rainforests and tropical regions as laxative, analgesic, febrifuge, diuretic, hepato-protective, vermifuge and colagogo. Although well characterized toxic effects of the seed of *Cassia occidentali*s have been documented however, the toxicity of other parts of the plant is well determined [6]. Among such therapeutic preparations are plant-derived phytomedicines, nutraceuticals and cosmeceuticals (Drew, 2000). A research group has investigated the possible toxic effect of the stems of *Cassia occidentalis* in order to ensure its safe use [6]. Haraguchi., *et al*. [7] identified dianthrone, an anthraquinone-derived compound in *Cassia occidentalis* seeds and demonstrated that these substances could cause the characteristic

mitochondrial myopathy produced by this plant. Mechanism of *Cassia occidentalis* toxicity has been described as being due to impairment of mitochondrial function, including swelling, loss of mitochondrial matrix, fragmented mitochondrial cristae and glycogen depletion [8].

Liver damage in rats were induced by carbon tetrachloride [9]. Carbon tetrachloride tends to accumulate in fat [10-12]. Studies comparing uptake after gastric infusion and oral bolus doses in 10% Emulphor show that uptake and tissue levels were less after infusion than after a bolus dose [10]. Several studies have demonstrated that the metabolism of carbon tetrachloride, and hence carbon tetrachloride-induced effects, can be significantly influenced by the dosing vehicle (i.e. corn oil or aqueous emulsion), but there is no agreement as to the extent [13-16]. Atmospheric levels of carbon tetrachloride were around 0.5 - 1.0 μg/m^3 [11] many foodstuffs contained carbon tetrachloride at concentrations of a few μg/litre or μg/kg [11]. Foods often become contaminated by carbon tetrachloride when they are fumigated with it. However, carbon tetrachloride is now seldom used for this purpose.

The primary targets for carbon tetrachloride toxicity are liver and kidney. The severity of the effects on the liver depends on a number of factors, such as species susceptibility, route and mode of exposure, diet and co-exposure to other compounds, in particular ethanol. Furthermore, it appears that pre-treatment with various compounds, such as phenobarbital and vitamin A, enhances hepatotoxicity, while other compounds, such as vitamin E, reduce the hepatotoxic action of carbon tetrachloride [11]. Hepatotoxic effects (increased serum enzymes and histopathology) were observed in rats given carbon tetrachloride in corn oil by gavage at daily doses of 20 mg/kg of body weight and higher for 9 days. The same effects were observed in rats given oral doses of 10 mg/kg of body weight per day, 5 days per week, for 12 weeks. No measurable adverse effects were observed in rats given 1 mg/kg of body weight per day for 12 weeks [17].

Hepatotoxicity (increased serum enzymes, increased organ weight and pathological changes) was observed in male and female CD-1 mice given carbon tetrachloride in corn oil by gavage at doses of 625, 1250 or 2500 mg/kg of body weight per day for 14 consecutive days. After 90 days, hepatotoxic effects were observed in animals that had ingested 12, 120, 540 or 1200 mg/kg of body weight per day [18]. The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins [19]. It has been shown that CCl_4 induced lipid peroxidation can be obstructed by natural antioxidants [20]. The identification of naturally occurring inhibitors of peroxidation resulting in cell damage could therefore lead to important new strategies for disease prevention [20]. Carbon tetrachloride and its metabolites are excreted primarily in exhaled air and to a lesser extent in the urine and faeces [11]. Studies on the uptake, tissue distribution and elimination of carbon tetrachloride by mice, rats and hamsters have shown that rats are the least sensitive to the hepatotoxic effects of repeated inhaled carbon tetrachloride (32 - 770 mg/m^3) and are notably less sensitive than mice to the hepatocarcinogenic effects [12] and that this species sensitivity correlates with carbon tetrachloride equivalent dose to liver and with the ability to metabolize carbon tetrachloride [12,21]. Predictions obtained from physiologically based pharmacokinetic models suggest that metabolism of carbon tetrachloride in the average rate is greater than that in the average human [22]. Calore., *et al.* [8], in their studies demonstrated that oral treatment of pregnant rats with hydroalcoholic extracts of *Cassia occidentalis* stem and leaf in doses of 0.25 and 0.5 g/kg did not change most of the reproductive parameters [8]. However, it was observed that there an index of dead foetuses from pregnant rats treated with *Cassia occidentalis* 0.25 and 0.5 g/kg, suggesting a possible abortive effect of this extract [23].

Oral treatment of pregnant wistar rats with hydroalcoholic extract of *Cassia occidentalis* stem and leaf in doses of 0.25 - 0.5 g/kg showed that all hematological and biochemical parameters remained under the reference range for the species [24] and no statistically significant changes were observed between groups. Analysis of blood parameters is relevant for risk evaluation, as any changes in the hematological and biochemical systems have a higher predictive value for human toxicity, when data are translated from animal studies [25]. Since there was no effect on the levels of transaminases (ALT, AST) and creatinine, which are good indicators of liver and kidney functions, respectively, it is reasonable to suggest that the *Cassia occidentalis* leaf extract did not induce any damage to the liver and the kidneys. This is further confirmed by the histological assessment of these organs, and the fact that there was no effect on plasma cholesterol levels, suggesting a normal function of the liver [26]. On the other hand, studies of Muyibi., *et al.* [27] evaluated the effects of the oral subchronic treatment (42 days) with aqueous extract of *Cassia occidentalis* leaf on mean cell volume (MCV), hemoglobin concentration (HC), red and white blood cell count (RBC and WBC, respectively) and observed statistically significant reduction in the PCV, HC and RBC in the treated groups. However, although MCV and HC values were lower than control group values, they were within the reference range for the specie and may be considered physiologic values [24]. In contrast, RBC values in the group treated with *Cassia occidentalis* leaf 4 g/kg [26] was below the reference values, indicating anemia in these animals. On the other hand, recent studies of Ibrahim., *et al.* [28] demonstrated that *Cassia occidentalis* leaf extract improves the trypanosome-induced anemia.

However, the present study is aimed to evaluate the hepato-protective and hepato-curative effect of ethanolic leaf extracts of *Cassia occidentalis* on carbon tetrachloride induced liver damage of albino wistar rat and to relate the study as a substitute of herbal remedies to orthodox drugs.

Materials and Methods

Collection of plants materials

Fresh matured leaves of *Cassio occidentalis* plant was collected from a farm at Okofia Otolo Nnewi, Nnewi North Local Government Area, Anambra State. The plant material was identified in the Department of Botany, Nnamdi Azikwe University (NAU) by Tochukwu Egboka with a reference number of Nau/Bot/286.

Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethics Committee, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Extraction and preparation

The leaves of fresh *Cassia occidentalis* plant were shade dried under room temperature at (29^0C - 35^0C) for two (2) weeks, after which the leaves were pulverized into coarse form with a crestor high speed milling machine. 200g of coarse form was then macerated in absolute ethanol. This was left to stand for 24 hours. After that, the extract was filtered using muslin cloth on a plug glass wool in glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature which was between 40^0C and 45^0C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator (10^oC) until use.

Chemical

Carbon tetrachloride was obtained from Bridge Head, Onitsha Market, Anambra state.

Animal Care

All experimental investigations were done in compliance with "humane animal" as stated in the "Guide to the care and use of Laboratory Animals Resources" (NRC, 2011). A total of 30 male albino wistar rats were used for this study. Animals were acclimatized for two (2) weeks in the animal holding unit of the Anatomy Department, Faculty of Basic Medical Sciences, Nnamdi Azikwe University, Nnewi Campus. The animals were maintained under standard and good laboratory conditions of light (12 hours), temperature (23 ± 2°C), humidity (60% - 70%) and ventilation. They were given standard rat diet purchased from the same farm to avoid changes in dietary compositions and weight variability and adequate water ad libitum was given.

Experimental protocol and animal grouping

After acclimatization, animals were divided into six groups; A, B, C, D, E and F (n = 5). Group A, was the control group with animals receiving feeds (Grower's Mesh produced by flour mills Nigeria) and distilled water only. Group B is the negative control (administered CCL4 only), C, D, E and F were the treated group and treated as follows:

- Group B: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg daily for 2 weeks.
- Group C: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg for 2 weeks then CCL4 diluted with 'olive oil/kg + *Cassia occi dentalis* 1000 mg/kg for 4 weeks.
- Group D: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg for 2 weeks the CCL4 diluted with 'olive oil/kg + *Cassia occidentalis* 2000 mg/kg for 4 weeks.
- Group E: *Cassia occidentalis* 1000 mg/kg for 2weeks then carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg + *Cassia occidentalis* for 4 weeks
- Group F: *Cassia occidentalis* 2000 mg/kg for 2 weeks then carbon tetrachloride 0.2 mls, diluted with 'olive oil + *Cassia occidentalis* for 4 weeks.

Carbon tetrachloride and ethanolic leaf extract of *Cassia occidentalis* was done orally using 2 mls syringe and a cannula.

Animal sacrifice, organ harvesting and collection of blood samples

At the end of the experiment, animals were anesthetize with diethyl ether in a close jar, blood samples were collected directly from the apex of the heart (at the thoracic region) using 2 ml syringes and put into plain serum bottle, and then serum were separated by centrifugation and was stored in a refrigerator of temperature -18oC for biochemical analysis. Thereafter the animals were sacrificed with overdose of anesthetic (diethyl ether). Each animal was placed on the dissecting board, pinned to the board and dissecting set (sharp scalpel on scalpel holder for making incision; scissors for cutting and dissecting forceps for harvesting) were used to harvest the liver which was immediately weighed before transferring into 10% formal saline for proper fixing for histological sectioning and histochemical analysis.

Biochemical Analysis

Blood samples collected and centrifuged were used for liver function assessment i.e. analyzing the activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) as described by Reitman and Frankel (1957), Alkaline phosphatase (ALP) as described by Kind., *et al.* (1980) and total and direct bilirubin as described by Dangerfield and Finlayson (1953).

Statistical Analysis

Data were expressed as Mean ± Standard Deviation (SD) using tables. The differences between the groups were compared for statistical significance by using ANOVA and student '' test. $P < 0.05$ was taken as the significant level.

Results

		Mean	± SEM	P-Value	T-Value
Group A	Initial	120.00	± 5.77		
	Final	200.00	± 24.03	0.088	-3.150
Group B	Initial	170.00	± 15.27		
	Final	130.00	± 5.77	0.057	4.000
Group C	Initial	123.33	± 8.81		
	Final	163.33	± 8.81	0.067	-3.671
Group D	Initial	130.00	± 10.00		
	Final	183.00	± 8.81	0.067	-5.400
Group E	Initial	116.66	± 6.66		
	Final	180.00	± 11.54	0.011*	-9.500
Group F	Initial	126.66	± 12.01		
	Final	166.66	± 6.66	0.020*	-6.928

Table 1: *Showed the body weight.*

Table 1 Showed bodyweight changes for test compared with the control group compared. Data were analyzed using Student dependent T-test and values were considered significant at $P < 0.05$. *$P < 0.05$ means significant, $P > 0.05$ means not significant.

		Mean	± SEM	P-value	F-value
Relative Liver weight (g)	Group B	3.00	± 0.50		
	Group A	1.15	± 0.25	0.017*	
	Group C	1.25	± 0.65	0.021*	3.592
	Group D	1.05	± 0.25	0.013*	
	Group E	1.15	± 0.30	0.017*	
	Group F	1.20	± 0.23	0.019*	

Table 2: *Relative organ weight of the liver.*

Table 2 Showed relative organ weight for test and control group compared with -ve control. Data were analyzed data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at $P < 0.05$. *$P < 0.05$ means significant and $P > 0.05$ means not significant.

		Mean	± SEM	P-value	F-value
Direct Bilirubin	Group B	58.00	± 2.70		
	Group A	17.65	± 2.55	0.000*	
	Group C	42.55	± 0.45	0.001*	68.732
	Group D	37.85	± 0.65	0.000*	
	Group E	35.80	± 0.10	0.000*	
	Group F	30.00	± 0.50	0.000*	
Total Bilirubin	Group B	59.00	± 6.20		
	Group A	14.60	± 0.60	0.000*	
	Group C	37.85	± 2.35	0.003*	27.410
	Group D	23.15	± 2.65	0.000*	
	Group E	29.35	± 1.15	0.000*	
	Group F	21.50	± 1.50	0.000*	

Table 3: *Showed liver function test.*

Table 3 Showed liver function tests for test and control group compared with -ve control. Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at $P < 0.05$. *$P > 0.05$ means significant and $P < 0.05$ means not significant. There is a significant decrease across the group when compared with -ve control.

		Mean	± SEM	P-value	F-value
Alanine Transaminase	Group B	71.55	± 3.45		
	Group A	27.70	± 1.70	0.000*	
	Group C	43.15	± 3.15	0.000*	48.532
	Group D	32.90	± 0.10	0.000*	
	Group E	29.45	± 1.45	0.000*	
	Group F	27.10	± 3.10	0.000*	
Alkaline Phosphatase	Group B	78.55	± 6.45		
	Group A	22.10	± 1.10	0.000*	
	Group C	55.10	± 3.90	0.000*	37.910
	Group D	33.35	± 1.65	0.000*	
	Group E	29.10	± 1.10	0.000*	
	Group F	24.85	± 3.85	0.000*	
Aspartate Transaminase	Group B	78.20	± 2.50		
	Group A	40.95	± 2.88	0.000*	
	Group C	43.10	± 1.67	0.000*	10.817
	Group D	46.30	± 1.66	0.000*	
	Group E	43.33	± 1.85	0.000*	
	Group F	37.60	± 3.50	0.000*	

Table 4: *Liver function test.*

Table 4 showed liver function tests for test and control group compared with -ve control. Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparison, and data were considered significant at $P < 0.05$. *$P < 0.05$ means significant and $P > 0.05$ means not significant. There is a significant decrease across the groups when compared with -ve control.

Histological findings

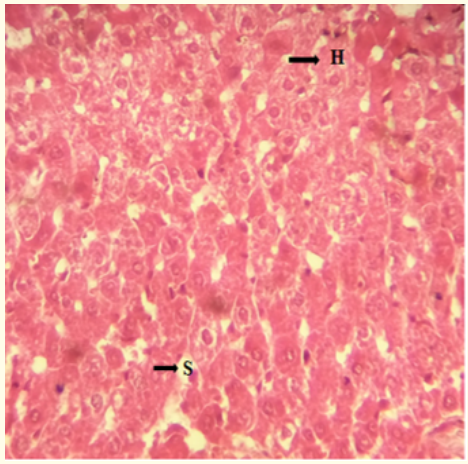

Plate 1: *Showed a photomicrograph of the liver of albino rat from group A (control) with a normal typical liver histo-architectural features with no visible lesion, showing Hepatocytes (H) and Sinusoids (S) of normal histology [H&E×400].*

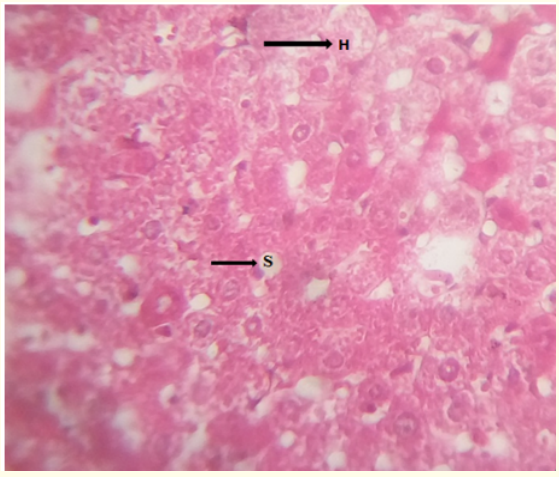

Plate 2: *Showed the photomicrograph of liver of group B (-VE CONTROL) of albino rat, showing Hepatocytes (H) degenerated, containing numerous intra-cytoplasmic fat vacuoles evidenced by cytoplasmic clearing (fatty change or steatosis), sinusoid (S) with periportal*

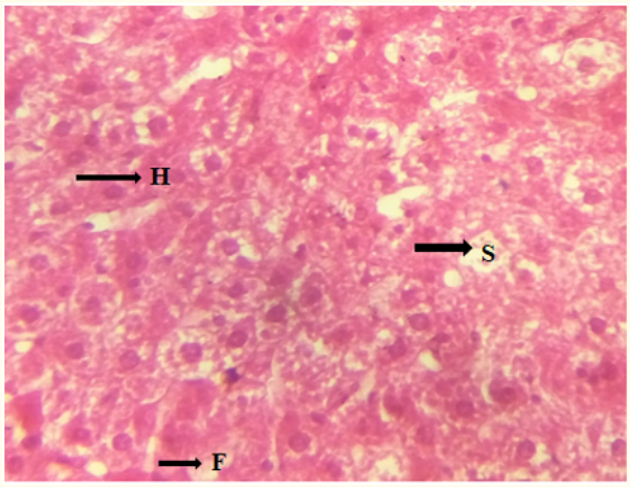

Plate 3: *Showed the Photomicrograph of liver of group C of albino rat, showing Sinusoid (S) and (F) with a mild portal congestion and peri-portal cellular infiltration by mononuclear cells and hepatocytes (H) diffusely vacuolated histology [H&E×400].*

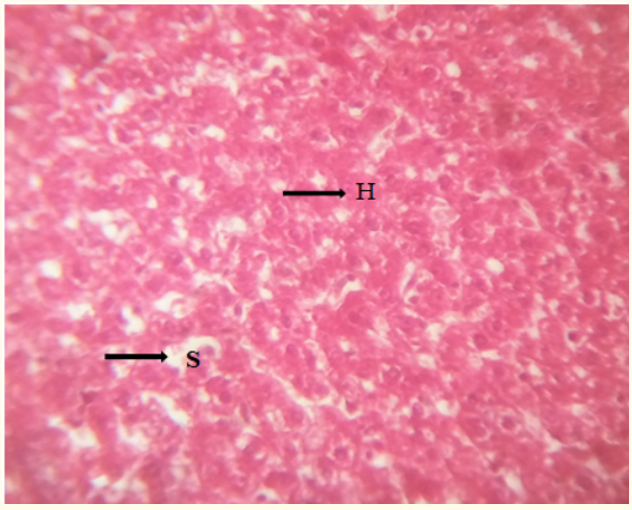

Plate 4: *Showed the Photomicrograph of liver of group D of albino rat, showing Sinusoid (S) with less fatty change/steatosis and the hepatocytes (H) clearly seen [H&E×400].*

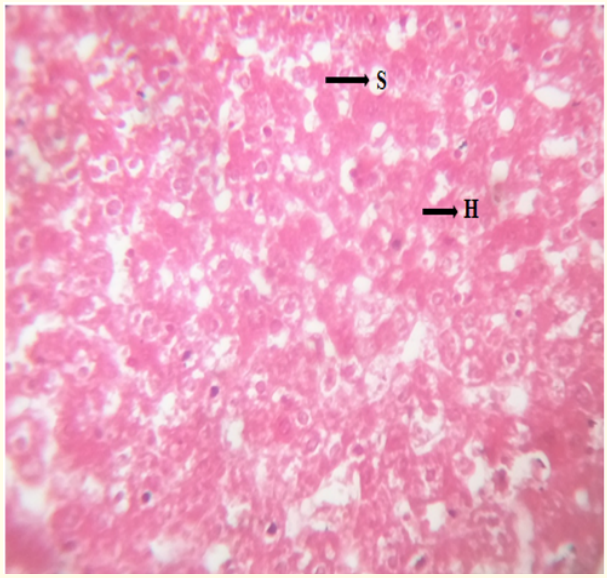

Plate 5: *Showed the photomicrograph of liver of group E of albino rat, showing sinusoid (S) with mild periportal cellular infiltration, the hepatocytes (H) seen mildly vacuolated [H&E×400].*

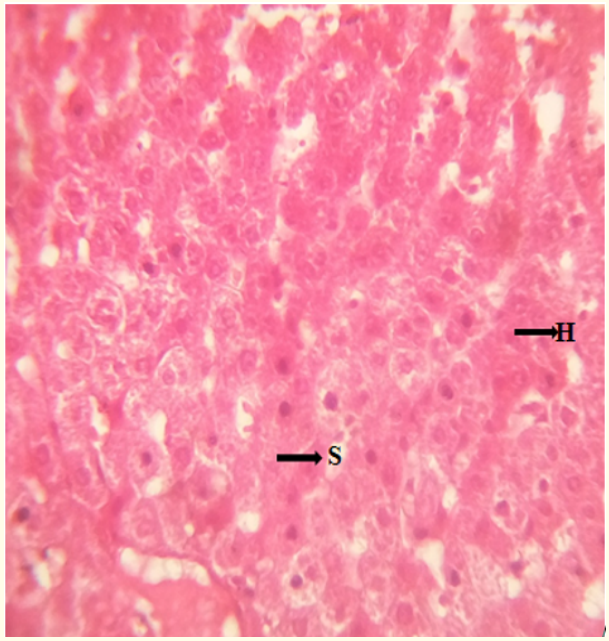

Plate 6: *Showed the Photomicrograph of liver of group F of albino rat, showing Sinusoid (S) with less fatty change/steatosis and the hepatocytes (H) clearly seen with mild cellular infiltration [H&E×400].*

Discussion

High rate of liver disease has been causing death among the adult population globally today [1] because of the absence of reliable drugs for the treatment and prevention of liver diseases in modern medicine [3]. *Cassio occidentalis* is used as a herbal remedy for treatment of liver diseases as reported by different researchers [18,20,25-28]. Body weight is normally investigated as a sensitive indicator of chemically induced changes to organs. The comparison between the ratio-metric differences of organ: body weight of the control group and the treated groups has been used to evaluate the toxic effect of the test substance [29]. The result of this study shows that following the administration of carbon tetrachloride (CCL4) that the body weight was significantly affected when compared with the control, concurrently group c and d while E and F were not significantly affected, it may be attributed to pre-treatment and post- treatment of leaf extract of *Cassia occidentalis* which agrees with Donfack*., et al.* [30] that the impact of the leaf extract of *Cassia occidentalis* on the body weight may be attributed to the multiple physiological effects of the micronutrients and phytochemical composition present in the plant.

The relative organ weight (ratios of liver: body weight) where compared with the negative control, the result showed a significant difference; this may be that the defensive mechanism of the animal has not been overcome and/or may be that the dose has not accumulated sufficiently to manifest any significant change, such that the weight change of the liver of group B (-ve control) was significantly decreased when compared with normal control and experimental groups. However, the significant decrease of the ratio of liver: body weight following the administration of the carbon tetrachloride (CCL4) may be attributed to tissue degeneration and shrinking resulting in necrosis; this may be due to the role of the liver in detoxification, excretion and following the administration of *Cassia occidentalis* the organs were restored.

The activity of AST, ALT and ALP are normally found in the cytoplasm [31], which are released into the blood circulation after cellular damage (Sallie., *et al.* 1991). In this study, treatment of animals with single dose of CCl4 caused significant ($P < 0.05$) elevation in the levels of serum ALT, AST, ALP, unconjugated bilirubin and conjugated bilirubin as well as significant decrease in level of serum total protein and albumin compared to the negative control. This current study showed a similar rise in the levels of ALT, AST, ALP and bilirubin oral administration of CCl_4 to induce liver injury as early reported by Bahar., *et al*. [32] and Kwalle., *et al*. [2], a marked elevation in the serum levels of ALT, AST and ALP in CCl_4 treated animals compared to that of the normal control animals. This findings also showed a significant elevation in serum values of ALT and AST in rats exposed to a single toxic non-fatal dose of CCl_4 as early reported by El-Dosuky., *et al*. [33], Anupam., *et al.* [34] and all this changes is due to free radicals which been reported as the predominant mechanism of hepatotoxicity [35]. The decreased in serum total protein and albumin serum level may be due to the interaction of CCl_4 with protein molecules leading to an impairment of cellular processes. The same observation was earlier suggested by Chung., *et al* [36]. The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins [16].

The liver cells damage was evident by the significant increase ($p < 0.05$) in the level of unconjugated bilirubin in the serum of the group treated with only CCl_4 when compared with the normal control group and other experimental groups. Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced [37], this result from bilirubin being the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile [38].

Subsequently the normalization of these enzymes suggests that *Cassia occidentalis* extract root methanol was capable of regeneration parenchymal cells, thus protecting against membrane fragility consequently and minimizing the leakage of liver enzymes into blood circulation. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [39]. It is therefore, a clear manifestation of hepato protective effect of the extracts which may be due antioxidant activity of phenolic and Flavonoids present in extracts [40]. Significant ($p < 0.05$) decrease in conjugated bilirubin and unconjugated bilirubin levels in animals treated with 200 and 300 mg/kg body weight compared to CCL_4 control group was observed and this reduction of the conjugated and unconjugated bilirubin levels by the methanol root extract suggests that the extract might have activated the Constitutive Androstane Receptor (CAR) which is a key regulator in bilirubin clearance in the liver [41]. The primary function of CAR in the bilirubin clearance pathway is to direct a coordinate response to elevated levels of bilirubin by increasing the hepatic expression of each component of the pathway [41].

The results from the photomicrograph revealed curative and protective effect across the tested groups when compared with the group B which is the negative control. The histology interpretations of the sides revealed that the groups that took high dose of *Cassia occidentalis* ethanolic leaf extract presented more curative and protective effect. See plate 6 and 4 respectively [42,43].

Conclusion

The curative and protective potentials of *Cassia occidentalis* ethanolic leaf extract cannot be neglected, which revealed the efficacy of *Cassio occidentalis* on carbon tetrachloride (CCL_4) induced liver damage of female albino rats. The research showed that the leaf extract may have curative and protective effect and also, may be dose dependent. However, it may be inferred that liver related diseases can be protected and cured following the administration of *Cassia occidentalis* leaf extract in human beings.

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