International Journal of Pharmacology and Toxicology, 9 (1) (2021) 41-49

International Journal of Pharmacology and Toxicology

Website: www.sciencepubco.com/index.php/IJPT

Research paper

The protective action of ethanolic stem bark extract of Carissa edulis (VAHL) Apocynaceae against carbon tetrachloride Hepatotoxicity in rats

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Abstract

Background: *Carissa edulis* is generally used for the treatment of diverse ailments, but little or no interest has been shown on its hepato-protective properties. This research work was aimed at evaluating the safety and claimed hepatoprotective activity of *Carissa edulis*.
Method: For the intent of data collection, the method of *Li et al.*, 2011 was used. In this method animals were treated with (250,500 and 1000mg/kg) of stem bark extract. The extract was given daily by gavage to the animals for 28 consecutive days. The 50% v/vCCl_4 and olive oil was gavaged through gastric tube twice a week. The tests conducted were liver function test, liver antioxidant enzymes test, lipid profile test as well as Histopathological assessment of the liver sections.
Results: Results of the study revealed that the markers in the animal treated with CCl_4 were significantly higher than the normal control at ($P<0.05$). While blood samples from animals treated with the stem bark extracts were significantly lower than the CCl_4 group at ($P<0.05$).
Conclusion: These results imply that the ethanolic stem bark extract of *Carissa edulis* have a protective effect against CCl_4 induced hepa-to - cellular injury.

Keywords: *Carissa edulis; Hepatoprotective Activity; Ethanolic Stem Bark Extract; Hepatic Damage; CCl_4.*

1. Introduction

1.1. Hepatotoxicity

The liver plays a vital role in transforming and clearing chemicals and is at risk of toxicity from these agents. Certain medicinal agents when taken in overdose and sometimes even in therapeutic range may damage the liver, other chemical agents such as those in the labor-atories and industries, natural chemicals can also stimulate Hepatotoxicity. More than 900 drugs have been associated with liver injury (Friedman *et al.*, 2003). Drug induced liver injury account for 5% of all hospital admission and 50% of all acute liver injury (Ostapowicz *et al.*, 2002, McNally & Peter 2006). The precise mechanism of drug induced liver injury remains basically unknown but it appears to involve two pathways namely, direct–Hepatotoxicity and adverse immune reaction.
Drug induced liver injury emanates from the bio activation of drugs to chemically reactive metabolites which have the ability to be inte-grated with cellular macromolecules such as protein, lipids and nucleic acid, resulting to protein dysfunction, lipid per oxidation, DNA damage and oxidative stress (Lee 2003, Deng *et al.*, 2009). Hepatocellular dysfunction and cell death also have the capability to initiate immunological reactions, as well as both innate and adaptive immune responses. It has been established that different inflammatory cy-tokines such as tumor necrosis factors (TNF) - interferon (IFN)-y, and interleukin (IL) B formed during liver injury are implicated in promoting tissue damage (Ishida *et al.*, 2002). Innate immune cells are also the main source of IL-6, IL-1O, and prostaglandins. All these cytokines have been discovered to posses' hepatoprotective roles (Masubuchi *et al.*, 2003).

1.2. Background and justification

Liver injury related with the consumption of herbal medicine is known as Herb-induced liver injury (HILI), which occurs infrequently in only a few susceptible individuals (Pantano *et al.*, 2006, Pantano *et al.*, 2017). The clinical presentation of HILI matches those of Drug-induced liver injury (DILI) (Pantano *et al.*, 2016). In addition HILI and DILI share common characteristics, as both cases are caused by chemical components that are formed either by natural or synthetic processes. These natural and synthetic chemicals are foreign to the body and have need of metabolic breakdown to eliminate. However, in the process of metabolism, substances that are toxic to the kidney could be formed (Frenzel &Teschke 2016). Herbs like Aloe vera, Black cohosh, Cascara, Chaparral, Comfrey, Ephendra or Kava could lead to toxic liver diseases (Marie 2015).
DILI incidence according to past published data was between 1 in 10,000 and 1 in 100,000. Nevertheless, more recent studies reported a higher incidence (Bjornsson 2015). There are numerous registries both in Western and Asian Countries which have provided valuable

information as regards the etiology, pathogenesis as well as the clinical presentation diagnosis and management of DILI. Some population studies in fact revealed an annual incidence of 19.1 cases per 100,000 inhabitants in Iceland (Bjornsson *et al.,* 2013) and 13.9 cases per 100,000 inhabitants in France with hospitalization of 12% and mortality of 6% 500 in deaths per annum in French widespread population (Saro *et al.,* 2000). Numerous drugs potentially cause DILI, but the most commonly involved are antibiotics which according to DILI network in the USA stand for about 46% of DILI cases (Chalassani *et al.,* 2008).

Carissa edulis previously identified as *C. Pubescence* belongs to the family Apocynaceae, (Irvine 1961, Hutchinson & Dalziel 1963). The plant is usually known among Hausa people in Northern Nigeria as "Cizaki" and in Somalia as Adishawel. The English name of the plant is Arabic numnum. There are other common names such as: endelkoring-noeminaem (Africana), 'emir' (Arabic) muyonzo (Luganda), 'mianoa-mboo, (Swahili) and impambala myoloko, (Sofowara 1986).

The plant parts are used in ethno medicine for a large array of illnesses such as epilepsy, headache, chest complaint, gonorrhea, syphilis, rheumatism, rabies as well as diuretic. Other Folkloric indications of *Carissa edulis* include fever, sickle cell anemia and hernia (Ibrahim 1997). *Carissa edulis* is also being used as a source of dye (Oliver 1960, Irvine 1961, Burkil 1985, Banker & Verma 1987, Omino & Kokwaro1993).

Even though there are no adverse effect reports on *Carissa edulis* herbal medicine (Ahmad & Odin 2017, Hanan & Hassan 2017). It has been employed as a traditional medicine in Kenya for the treatment of different ailment without any reported side effect. (Adil *et al.,* 2003, Kabeinei *et al.,* 2011). It was alleged that this plant could virtually cure all forms of human ailment including cancer, diabetes and HIV-AIDS (Zephania 2011). The petroleum ether, ethyl acetate and aqueous extract of *Carissa edulis* have a marked potency for lowering the blood pressure in rats at a dose dependent manner (Alyoussef & Hassan 2010). Roots extracts of *Carissa edulis* are used for the treatment of numerous pathological states including inflammatory disorders. Oral administration of *Carissa edulis* extracts (30-300mg/kg P.O significantly inhibited carrageen an-induced foot edema through maximum inhibition of 62+9.1% and 66.4+.8% respectively (Woode *et al.,* 2007). The extract in addition scavenged DPPH (Diphenylpicryihydrazyl) and prevented lipid per oxidation in rat's brain homogenate. These results suggest that the alcoholic extract of *Carissa edulis* possibly exert in vivo inflammatory and antioxidant properties which may contribute to its activity.

Carissa edulis is widely used for the treatment of various ailments but little or no interest has been shown in its hepatoprotective properties. Hence the needs for a hepatoprotective study of this plant to ascertain its effect on the liver because the treatment and management of this organ results in more economic problems.

2. Method

2.1. Animals

Wistar albino rats (male) weighing between (120-220 grams) acquired from experimental animal house of the Department of Pharmacology University of Jos was used for the study. The rats were supplied with standard animal pellets (Pfizer Feeds Nigeria), given water *ad libitum* and maintained in a well ventilated room.

2.2. Collection identification and authentication of Carissa edulis

The stem bark of *Carissa edulis* was collected from Fursum East, Jos East Local Government Area of Plateau State, on the 7th December, 2016. The plant was identified and authenticated by Mr. J.J Azila of the Federal College of Forestry Jos. A voucher specimen number UJ/PCG/HSP/95A09 was issued and the specimen placed in the Faculty of Pharmaceutical Sciences University of Jos Herbarium for reference.

2.3. Preparation of Carissa edulis extract

The stem bark was detached from the whole plant and air dried at room temperature for fourteen (14) days. The dried part was powdered with mortar and pestle and sieved with 29 mesh size. The powder was soxhlet extracted with 700ml 70% v/v ethanol for 72 hours. The resultant filtrate was evaporated to dryness on steam bath at 40°C to give brownish extract that was stored up in air tight containers and preserved at 4°C until required for use.

2.4. Preliminary Phytochemical studies of Carissa edulis extract

Phytochemical evaluation of the ethanolic stem bark of *Carissa edulis* was carried out to identify the presence or absence of secondary metabolites (Alkaloids, Anthraquinones, Cardiac glycosides, Carbohydrates, Flavonoids, Saponins, Tannins and Steroids via standard methods (Trease & Evans 1989).

2.5. Determination of LD_{50} of Carissa edulis

The acute toxicity study of the stem bark extract was determined using the method of Lorke (1983). This was done in two phases. In the initial phase (phase 1), nine rats were randomly divided into three groups of three rats per-group and were administered 10, 100 and 1000mg/kg of the extract of *Carissa edulis* orally (via a cannula) respectively. The rats were observed for signs of adverse effects and death for 24hours. Based on the outcome of the phase one study, the procedures was repeated using another set of three rats randomly divided into three groups of one rat each, administered 1600, 2900 and 500mg/kg body weight of the extracts respectively for 14 days the rats were observed for signs of toxicity which consist of but not limited to paw ticking, salivation, stretching, rubbing of nose on the floor and wall of the cage, change in body weight and death.

2.6. Hepatoprotective studies

The method of (*Li et al.,* 2011) was adopted for the study. Thirty-six rats were randomly divided into 6 groups of 6 rats each. The groups were: Normal control, standard drug, carbon tetrachloride treated group and the three test groups (250, 500 and 1000mg/kg) for the stem bark extract. The standard drug used was Silymarin.

To study the protective effect against carbon tetrachloride induced chronic hepatic injury *Carissa edulis* stem bark extract (250, 500 and 1000/kg body weight) was given daily by oral gavage to animals for 28 consecutive days. The 50% v/vCCl_4 in olive oil was gavaged through gastric tube to the extract treated groups (250, 500 and 1000mg/kg body weight) twice a week on the third and seventh day of each week for four weeks (The duration of the 28days treatment). Then normal control group were administered distilled water 5ml/kg body weight daily for 28 days. Animals of the CCl_4 group were given CCl_4 twice a week on the third and seventh day of each week and with vehicle on rest of the days for 4 weeks. While the animals of the silymarin standard group were treated with the standard drug silymarin at a dose of 50mg/kg daily for 28 consecutive days alongside CCl_4 on the third and seventh day of each week through the gastric tube (Oral route).

On the 29th day each rat was anaesthetized with ethyl ether, the weights of the rats were recorded. The animals were then sacrificed and the blood sample collected from the jugular vein into plain tubes for biochemical evaluation. The biochemical test conducted include: (1) Liver function test which comprises, Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), Albumin (ALB), Alkaline Phosphatase(ALP),Total Protein (TP) and Bilirubin(BIL). (2) Lipid profile test which comprise of, (Total Cholesterol (CHL), High Density Lipoprotein (HDL) and Triglyceride (TRIG). The liver was harvested, trimmed of adherent tissue and preserved in 10% formaldehyde solution for succeeding histopathological examination. Part of the liver harvested was put into normal saline solution for measurement of Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Glutathione (GHS).

2.7. Statistical analysis

The results were expressed as Mean ± SEM where applicable. The data were subjected to ANOVA (Analysis of Variance) using SPSS software (version 20) and in each stage where ANOVA was significant a post-Hoc test (Fishers least Significant Difference) was carried out. The difference was taken to be statistically significant at $P<0.05$, after comparing treatment groups with negative control animals.

3. Results

Phytochemical screening of the Ethanolic Stem Bark Extracts of Carissa edulis

The preliminary phytochemical test for the stem bark extracts was positive for saponin tannins, flavonoids, carbohydrates, cardiac glycosides and steroids. Tannins, flavonoids, Carbohydrates and steroids were highly present while alkaloids and anthraquinones were absent.

Table 1: Phytochemical Screening of the Stem Bark of Carissa Edulis

Constituents	Bark Extract
Alkaloids	-
Saponins	+
Tannins	+++
Flavonoids	+++
Carbohydrates	+++
Cardiac glycosides	+
Steroids	+++
Anthraquinones	-

Keys:-Negative, + Present.

Determination of LD_{50} of the stem bark extracts of Carissa edulis in Rats

The extract did not cause mortality at a dose of 5000mg/kg. The animal was calm, non-aggressive, decreased exploratory activities and no stereotype behavior.

Table 2: Determination of LD_{50} of the Leaf Extract of Carissa Edulis

Dose mg/kg	Number of Rats Used	Number of Death	Behavioural Effect
10	3	0	Animals were calm, non-aggressive decreased exploratory activity no stereotyped behaviour.
100	3	0	Animals were calm, non-aggressive, decreased exploratory activities no stereotyped behaviour
1000	3	0	Animals were calm, non-aggressive, decreased exploratory activity no stereotyped behaviour
1600	1	0	Animal was calm, non-aggressive decreased exploratory activities, no stereotyped behavior
2900	1	0	Animal was calm, non-aggressive, decreased exploratory activities, no stereotyped behavior
5000	1	0	Animal was clam, non-aggressive, decreased exploratory activities no stereotyped behaviour

Effects of the Stem Bark Extract of Carissa edulis on the Body Weight of Rats Treated with CCl_4

The study revealed that in the normal control group, there was a significant increase in body weight at day 14(151.38±4.58), (155.7±4.63) at day 21 and (175.50±7.79) at day 28, from the initial weight (134.98±3.38) at day 0. In the CCl_4 group, there was a significant increase in weight from initial weight (127.83±2.26) to (140.88±2.33) at day 14 to (149.90±2.65) at day 21 and (158.01±3.08) at day 28.

In the standard drug group silymarin there was no significant change in body weight. For 250mg/kg of the stem bark extract, there was a significant decrease in body weight from 148.85±5.40 at day 0 to 129.85±5.40 at day 28 ($P<0.05$). For 500mg/kg of the stem bark extract there was no significant difference in body weight compared with control ($P<0.05$).

For 1000mg/kg of the stem bark extract there was a significant increase in body weight from (133.83±1.51) at day 0 to (140.55±2.62) at day 7 to (150.26±2.53) at day 14, (153.01±2.54) at day 21 and (155.75±3.36) at day 28.

Table 3: Effect of Ethanolic Stem Bark Extract of Carissa Edulis on Body Weight (g) of Rats Treated with CCl_4

Treatment/ Days	0	7	14	21	28
Control	134.98±3	147.91±3.98	151.38±4.58	155.75±4.63	172.50±7.79
Standard Drug	137.40±3.47	136.71±3.53	135.18±3.45^a	132.40±1.81^a	128.53±2.11^a

CCl_4	127.83±2.26[b]	133.33±2.33[ab]	140.88±2.33	149.90±2.65[b]	158.01±3.08[b]
250mg/Kg (Bark) + CCL_4	148.06±5.06	161.00±5.89[bc]	163.05±5.06[abc]	148.31±5.77[b]	129.85±5.40[ac]
500mg/Kg (Bark) + CCL_4	128.55±2.87[b]	140.91±5.98[c]	140.98±5.30[b]	130.46±6.83[ac]	120.98±7.76[ac]
1000mg/Kg (Bark) + CCL_4	133.83±1.51[b]	140.55±2.62[c]	150.26±2.53[b]	153.01±2.54[b]	155.75±3.36[ab]
LSD(0.05)	10.90	11.87	11.42	14.07	15.86

Means tagged with superscript 'a' are significantly different compared to Normal Control at p<0.05
Means tagged with superscript 'b' are significantly different compared to Standard Drug at p<0.05
Means tagged with superscript 'c' are significantly different compared to CCl_4 at p<0.05
Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl_4 at p<0.05; value are Mean ±SEM

Effect of Ethanolic Stem Bark Extract of Carissa edulis on Liver Function Indices of Rats Treated with CCl_4.

The three concentrations of the stem bark extracts (250, 500 and 1000mg/kg) showed level of significance in measure of protection to the rats against CCl_4 liver damage with significant decrease in the level of the liver function indices compared to the CCl_4 group at P<0.05.
In ALT, all the groups (250, 500 and 1000mg/kg) had lower levels of ALT compared to the CCl_4 group at (363.60±60.32) but groups administered 500mg and 1000mg of the stem bark extract showed significantly lower level of ALT compared to the CCl_4 group.
In AST, all the three concentrations of the stem bark extracts were significantly lower than the CCl_4 group in a dose dependent manner. In ALP, all the three concentrations of the stem bark extracts were significantly lower than the CCl_4 group at (1275.66±175.21). In ALB, the treatment groups with the lowest concentrations were the normal control, 500 and 1000mg/kg of the bark extract having significantly lower level of (3.91±0.11). In TP, there was no significant difference between the normal control, the CCl_4 group and the three extract treated groups. In bilirubin, the lowest value was observed in the groups given 500mg of the stem bark extract followed by the CCl_4 group. The highest level was observed in the standard control group which is significantly higher than the other groups.

Table 4: Effect of the Ethanolic Stem Bark Extract of Carissa Edulis on Liver Function Indices of Rats Treated with CCl_4.

Treatments	ALT(U/L)	AST(U/L)	ALB(g/L)	ALP(U/L)	TP(g/L)	BIL(µmol/L)
A	90.80±0.21	322.90±7.30	3.62±0.14	719.93±85.25	7.90±0.06	9.38±0.91
B	139.93±13.01	322.53±27.80[a]	4.12±0.02[a]	1101.33±72.52[a]	7.90±0.12	17.30±1.73
C	363.60±60.32	527.66±42.18[ab]	3.91±0.11[a]	1275.66±175.21[a]	8.00±0.24	8.21±0.73[b]
E	351.25±23.15[a]	395.80±0.57[c]	3.91±0.01[ab]	660.00±1.15[bc]	7.50±0.44	10.70±0.05[b]
G	96.56±5.21[c]	273.13±19.48[c]	3.71±0.03[b]	686.00±27.73[bc]	7.10±0.85[a]	6.20±0.26[ab]
I	73.80±1.61[c]	256.35±3.92[bc]	3.59±0.10[b]	541.00±13.27[bc]	7.30±0.09	10.40±0.41[b]
LSD	212.14	52.01	0.24	219.96	0.51	2.68

Means tagged with superscript 'a' are significantly different compared to Normal Control at p<0.05
Means tagged with superscript 'b' are significantly different compared to Standard Drug at p<0.05
Means tagged with superscript 'c' are significantly different compared to CCl_4 at p<0.05
Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl_4 at p<0.05; value are Mean ±SEM
KEY:
Groups (A) = Normal Control (distilled water)
Groups (B) = Standard Drug (silymarin)
Groups (C) = CCl_4,
Groups (E) = 250mg/Kg (Bark) + CCl_4,
Groups (G) = 500mg/Kg (Bark) + CCl_4,
Groups (I) = 1000mg/Kg (Bark) + CCl_4
LSD=Least Standard Deviation

Effect of the Stem Bark Extract of Carissa edulis on Lipid Profile Parameters of rats treated with CCl_4

There was significant difference in TRIG, HDL and CHL level between the treatment groups observed at P<0.05. For TRIG, there was significant increase in the value of TRIG, in CCl_4 group compared to the normal control group. The TRIG level in other groups were significantly lower than the CCl_4 group except 1000mg of the bark extract group which has the same level with CCl_4 group.
For HDL, the highest concentrations were observed in CCl_4 group and 1000mg of the bark extract group. The least values were observed in normal control group at (0.9±0.15) and silymarin group (0.96±0.19).
For CHL, there was a significant increase between the normal control group and the CCl_4 group. At doses of 500 and 1000mg/kg of stem bark extracts there was a significant decrease in CHL level compared to the CCl_4 group.

Table 5: Effect of the Ethanolic Stem Bark Extract of Carissa Edulis on Lipid Profile(TRIG, HDL and CHL) of Rats Treated with CCl_4

Treatments	TRIGmmol/L	HDLmmol/L	CHLmmol/L
A	0.77±0.05	0.91±0.15	1.57±0.09
B	0.82±0.07	0.96±0.19	1.91±0.21[a]
C	1.06±0.01[ab]	1.53±0.01[a]	1.82±0.03[a]
E	0.88±0.01[c]	1.17±0.04[c]	1.72±0.00
G	0.97±0.02[ab]	1.23±0.06[abc]	1.39±0.02[bc]
I	1.17±0.05[ab]	1.33±0.08[ab]	1.30±0.08[abc]
LSD	0.12	0.27	0.24

Means tagged with superscript 'a' are significantly different compared to Normal Control at p<0.05
Means tagged with superscript 'b' are significantly different compared to Standard Drug at p<0.05
Means tagged with superscript 'c' are significantly different compared to CCl_4 at p<0.05

Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl_4 at $p<0.05$; value are Mean ±SEM.

Effects of the Stem Bark Extracts of Carissa edulis on Liver Antioxidant Parameters of Rats Treated with CCl_4

There was a significant difference in all antioxidant parameters within treatment groups at ($P<0.05$). In malondialdehyde the highest value was observed in the CCl_4 group (11.8±0.11) compared to other groups. There was a significant decrease in malondialdehyde level in all the three concentrations of the stem bark extract in a dose dependent manner. For Glutathione, the highest level was observed in the normal control group (85.32±1.66) which was significantly higher than the other treatment group, the least value of glutathione was observed in the CCl_4 group (44.75±0.28). There was a significant increase in glutathione level in the three concentrations of the stem bark extract groups compared to the CCl_4 group.

For catalase the highest level was observed in the normal control group, while the least was observed in the CCl_4 group at (0.0013±0.000). There was a significant increase in catalase level in the animals treated with the three concentrations in each cases of the stem bark extract.

In superoxide dismutase, the least value was recorded in the CCl_4 group (1.9886±0.003). There was also a significant increase in superoxide dismutase level among animals treated with the three concentrations of the stem bark extracts.

Table 6: Effect of the Ethanolic Stem Bark Extract of Carissa Edulis on Liver Antioxidant Parameters of Rats treated with CCl_4

Treatment	Malondialdehydenmol/g	Glutathione mol/g	CatalaseU/g Hb	SuperoxideU/g Hb
A	8.52±0.07	88.33±1.66	0.0043±0.0011	1.9927±0.0005
B	$9.71±0.17^{a}$	$83.00±2.86^{a}$	0.0042±0.0031	1.9936±0.0013
C	$11.81±0.11^{ab}$	$44.75±0.28^{ab}$	$0.0013±0.0000^{ab}$	$1.9886±0.0003^{ab}$
E	$11.05±0.35^{abc}$	$57.00±0.44^{abc}$	$0.0029±0.0001^{abc}$	$1.9922±0.0019^{c}$
G	$9.18±0.19^{abc}$	$59.05±1.22^{abc}$	$0.0029±0.0003^{abc}$	$1.9942±0.0003^{c}$
I	$8.50±0.03^{bc}$	$76.70±1.93^{abc}$	$0.0051±0.0001^{abc}$	$1.9953±0.0007^{c}$
LSD	0.47	4.32	0.002	0.0039

Means tagged with superscript 'a' are significantly different compared to Normal Control at $p<0.05$

Means tagged with superscript 'b' are significantly different compared to Standard Drug at $p<0.05$

Means tagged with superscript 'c' are significantly different compared to CCl_4 at $p<0.05$

Means tagged with superscript a, b and 'c' are significantly different compared to Normal control, Standard Drug and CCl_4 at $p<0.05$; value are Mean.

Figure 1-6: Shows a histopathological observation of the liver after administering different doses of the extract in CCl_4 treated rats.

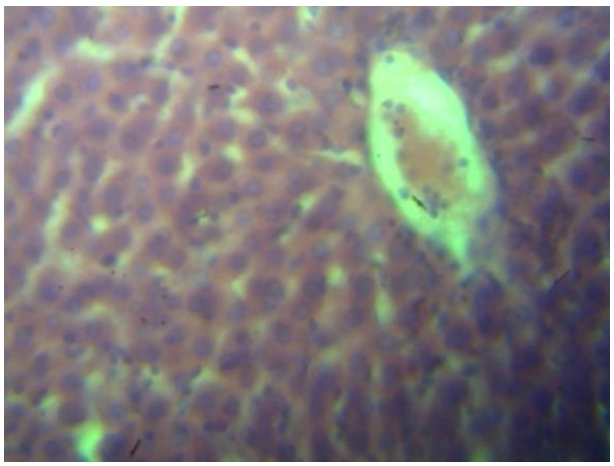

Fig.1: Cross Section of Normal Control.

Figure 1: Shows the liver section of a control rat's normal nuclei presence of Kupffer cells within the sinusoids and also normal radical arrangement of the hepatocyte.

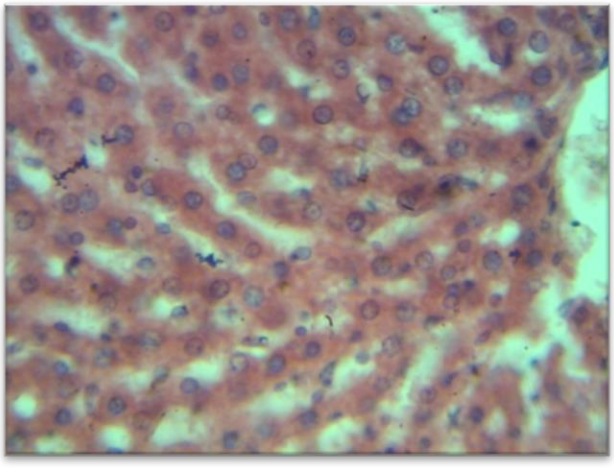

Fig. 2: Cross Section of the Rat Liver Treated with Silymarin.

Figure 2: Shows the liver section of a rat after the standard drug silymarin (50mg/kg) was administered with mild enlarged nuclei within the hepatocytes and a normal radial arrangement of hepatocytes with presence of Kupffer cells.

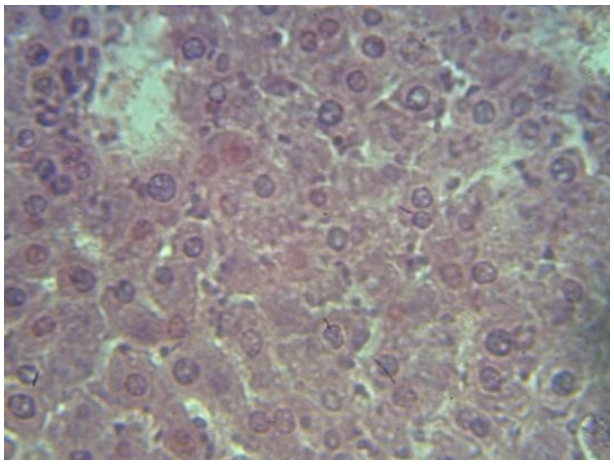

Fig.3: Cross Section of CCl_4 Group.

Figure 3: shows the section of the liver of CCl_4 group of animals which exhibited necrosis, intense congestion, and massive enlargement of the nuclei with alteration of the radial arrangement of hepatocytes.

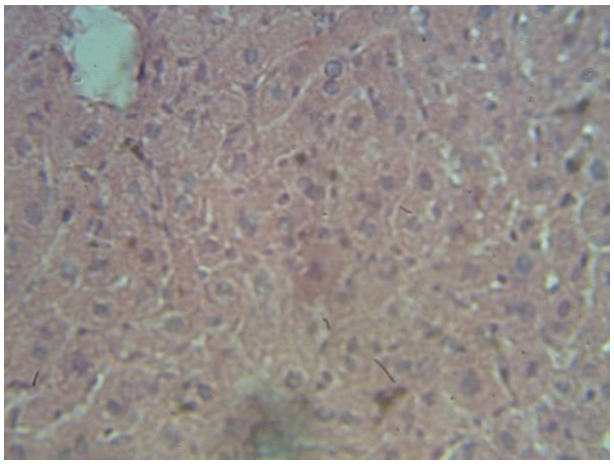

Fig. 4: Cross Section of 250mg per kg Stem Bark Extract.

Figure 4: Shows the section of a rat's liver after 250mg/kg of the bark extract was administered with massive alteration of radial arrangement of hepatocyte and karyorrhexis of the nuclei.

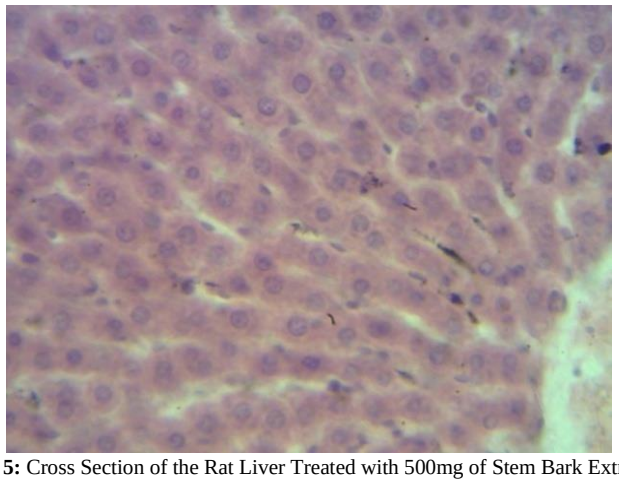

Fig. 5: Cross Section of the Rat Liver Treated with 500mg of Stem Bark Extract.

Figure 5: Shows the liver section of a rat after administering 500mg/kg of the bark extract with massive enlargement of nuclei, karyoclasia and distortion of the radial arrangement of hepatocyte with few Kupffer cells within the sinusoids.

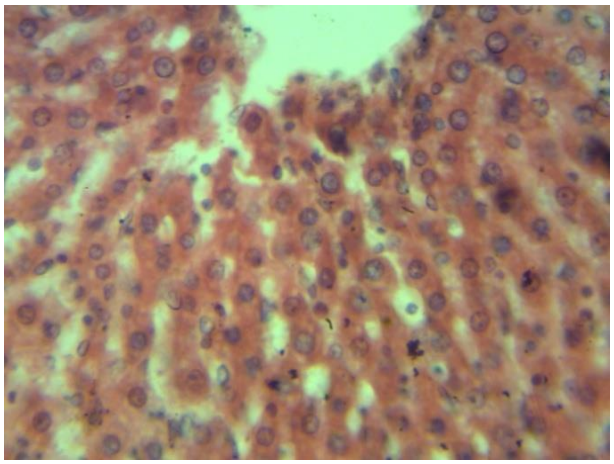

Fig. 6: Cross Section of the Rat Liver Treated with 1000mg of Stem Bark Extract.

Figure 6: Shows the liver section of a rat after administrating 1000mg/kg of the bark extract with massive enlargement of nuclei within the hepatocytes and mild distortion of the radial arrangement of hepatocytes with presence of Kupffer cell.

4. Discussion

The presence of flavonoids, saponins and tannins in the ethanolic bark extracts of *Carissa edulis* was established by phytochemical analysis, and these compounds are reported to have antioxidant properties (Shankar *et al*., 2005).

The possible hepatoprotective property underlying *Carissa edulis* may be attributed to the antioxidant constituents, the plants most frequently used to treat liver disorders are *Curcuma longa* (turmeric), *Glycyrrhizin glabra* (Licorice) and *Camellia sinesis* (green tea), and they are all reported to be hepatoprotective due to the dominant antioxidative properties (Donatus *et al.*, 1990, Soni *et al.*, 1992, Wang & Han 1993, Miyagawa *et al.*, 1997).

The mechanism of the antioxidant property of *Carissa edulis* extracts has been stipulated to be due to the reduction of free radicals and also scavenging of reactive oxygen species and other free radicals (Woode *et al.*, 2007).

The LD_{50} of the plant stem bark extract was established to be greater than 5000 mg/kg following oral administration in rats and according to the Hodge and Sterner Scale (CCOHS 2013). The ethanolic stem bark extract of *Carissa edulis* plant is said to be practically non-toxic.

An increase in body weight in the duration of the treatment period is indications of protection against hepatic injury while a decrease implies Hepatotoxicity (Pingale *et al.*, 2008). There was no clear pattern on the impact of the extract on body weight; under this circumstance it will be difficult to know the exact impact of the treatment on body weight hence the need for further research on this area.

On liver function indices, the data showed a significant increase in the activities of Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) after exposure to CCl_4 at $P<0.05$ in rats. This outcome is consistent with many authors who reported that these enzymes levels are significantly elevated after CCl_4 administration (Mehmetcik *et al.*, 2008, Aric & Cetin 2011, Tin *et al.*, 2011). Treatment with the ethanolic stem bark extract showed a dose dependent & significant protection to the rats against CCl_4 induced liver injury at $P<0.05$.

Studies have revealed that CCl_4 administration causes an elevation in bilirubin level owing to its toxicity (Recknagal 1967). However, the result of this study revealed that CCl_4 did not produce an increase in bilirubin level as expected. This is probably due to environmental factors, age and nutritional factors (Hale 1983, Schwetner 1994, Buyakasik *et al.*, 2008).

For total protein, studies have shown that, there is a decrease in the level of total protein due to hepatic intoxication (Recknagal 1967). Also an increase in the protein level signifies hepatoprotective activity as it speeds up the regeneration and production of liver cells (Mahheswari & Rao 2005). Contrary to this expectation, the induction of liver injury using CCl_4 did not produce a reduction in the level of total protein probably due to factors like, the animal state of hydration, chronic infection and inflammation or humoral immunodeficiency (Ciszewski *et al.*, 1993).

Previous studies reported that CCl_4 causes reduction in albumin level (Fahim *et al.*, 1999, Khan & Alzohairy 2011, Althnaian 2013). Administration of CCl_4 causes hepatic changes which leads to rapid loss of the capability of the liver to synthesize albumin since albumin is produced on a polysome bound to the endoplasmic reticulum (Redman 1969, Hicks *et al.*, 1969). The result of this study showed significant increase in albumin level in about 50% of the extract treated groups, this shows the ability of the extract to stimulate the synthesis of albumin.

There was a significant difference in the lipid profile (HDL, CHL, and TRIG) levels between the treatment groups observed at $P<0.05$.

The values of Triglyceride for all the groups were lower than the CCl_4 group with significant reduction at 500mg and 1000mg/kg of the stem barks extract groups. An increase in membrane Cholesterol is associated with a decrease in membrane functions as well as changes in membrane receptor, enzyme accessibility and their activation (Emerole & Thabrew 1983). The ability of the extracts to reverse the abnormality may suggest that the extract has the potential to decrease serum CHL in hypercholesterolemia.

In the case of High Density Lipoprotein (HDL), 500 and 1000mg of the stem bark extract produced a significant increase in HDL level compared to the normal control group. However, the highest level of HDL was observed in the CCl_4 group, this elevation is not consistent with most findings probably because HDL is more strongly controlled by genetic factors than other lipoproteins Yamashita *et al.*, 2000).

For liver antioxidant parameters, a decrease in the level of antioxidant enzymes and increase in lipid per oxidation level were noticed after the CCl_4 administration (Camp *et al.*, 2003). This finding demonstrated the effectiveness of *Carissa edulis* extract in averting CCl_4 Hepatotoxicity by enhancing the activity of the liver SOP, Catalase and Glutathione enzymes while reducing the liver MDA content. This may be ascribed to the presence of the several compounds which posses high antioxidant activities that scavenge the product radicals (Daspupta *et al.*, 2004).

The histopathological studies also provided important evidence supporting the biochemical analysis and liver antioxidant status. At doses of 500mg and 1000mg of the extracts there was a distinct recovery from the structural damage caused by CCl_4 which is comparable to the standard drug silymarin. This indicates pronounced protection to the liver.

5. Conclusion

This study demonstrated the protective effect of Carissa edulis ethanolic stem bark extract against CCl_4 induced liver injury in rats. This may be due to the presence of some endogenous substance with antioxidant and detoxifying properties. The active compounds responsible for the hepatoprotective activity have not been identified in this study. The mechanism of action also remained unproven. Hence the need for further research to identify the constituents of the tested plant responsible for the hepatoprotective effect.

Acknowledgement

Professor John C. Aguyi, Mentoring, guidance and social support Mr. Luka Wazoh, Mr. Azi Sunday and Mr. DA Bulus technical support

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