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Hepatoprotective effect of the ethanolic leaf extract of Carissa edulis (vahl) apocynaceae on carbon tetrachloride induced liver toxicity in rats

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ABSTRACT

Background: There are claims by herbalist and even indigenes of Plateau state on the efficient use of *Carissa edulis* in the management of liver diseases. This work was aimed at assessing these claims to determine the hepatoprotective effects of *Carissa edulis*.

Methods: The ethanolic leaf extract of *Carissa edulis* was scrutinized for hepatoprotective activity against Carbon tetrachloride (CCl_4) induced liver damage. Biochemical parameters were studied to assess the hepatoprotective activity of the ethanolic extract in serum. Total Bilirubin, Total Protein, Albumin, Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Total Cholesterol, Triglyceride and High-Density Lipoprotein were determined to assess the effect of the extract on CCl_4 induced hepatic damage. Other test conducted include, liver antioxidant test like glutathione, malondialdehyde, superoxide dismutase, catalase and histopathological assessment of liver sections..

Results: The markers in the animals treated with CCl_4 showed elevated concentration indicating severe hepatic damage by CCl_4. A significant decrease in serum markers was observed in rats treated with plant extract signifying the effect of the plant extract in restoring normal functional ability of the hepatocytes.

Conclusion: These results imply that the ethanolic leaf extract of Carissa edulis has a protective effect against CCl_4 induced hepato-cellular injury.

1. Introduction

The liver is essential for survival in humans, there is presently no way to reimburse for the absence of its function. This organ is also accountable for some biochemical reactions, including the synthesis and breakdown of small and complex molecule many of which are crucial for normal vital functions[1]

The liver plays a vital role in transforming and clearing chemicals and is vulnerable to toxicity from these agents. Certain medicinal agents when used in overdose and sometimes even within therapeutic range may injure the liver. Other chemical agents such as those in the laboratories and industries, natural chemicals are capable of also inducing Hepatotoxicity. More than 900 drugs have been implicated in causing liver injury[2] In the United States drugs induced liver injury is accountable for 5% of every hospital admission and 50% of all acute liver failure[3].

Many chemicals damage mitochondria, an intracellular organelle that generates energy. Its dysfunction discharges excessive amount of oxidants which in turn injures hepatic cell. Activation of some enzymes in the Cytochrome P_{450} system such as Cytochrome P_{450} 2E1 (CYP2E1) also produces oxidative stress[4].

Herbs-induced liver injury (HILI) occurs infrequently in only a few susceptible individuals[5,6] The clinical expression of HILI are like those of Drugs-induced liver injury (DILI)[5]. Similarly, 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 need metabolic breakdown to eliminate. However in the

course of metabolism, substances that are toxic to the kidney could be formed resulting to liver injury in vulnerable individuals[7].

Carissa edulis (CE) belongs to the family Apocynaceae; it was previously known as *C. pubescence*[8]. The plant is commonly known among Hausa people in Northern Nigeria as Cizaki and in Somalia as Adishawel. The English name of the plant is Arabic numnum[9]. The plant parts are employed in ethno medicine for a wide variety of illnesses such as, epilepsy, headache, chest complaint, gonorrhea, syphilis, rheumatism, rabies as well as diuretic[10]. Other folkloric applications of Carissa edulis include fever, sickle cell anemia and hernia [11]. *Carissa edulis* also find application as a form of dye[12-14].

Carissa edulis has been extensively employed as a traditional medicine in Kenya for the treatment of a variety of ailments without any reported side effects [15]. It was believed that this plant could virtually cure all forms of human ailments comprising Cancer, Diabetes and Human Immunodeficiency Virus- Acquired Immunodeficiency Syndrome (HIV-AIDS)[16].The petroleum ether, ethyl acetate and aqueous extract of *Carissa edulis* have a clear potency for lowering the blood pressure in rats at a dose dependent fashion [17]. Roots extracts of *Carissa edulis* (Forsk) Vahl (Apocynaceae) are used for the treatment of numerous pathological states and inflammatory disorders. Administration of *Carissa edulis* extract (30-300 mg/kg P.O) significantly inhibited carrageenan-induced foot Edemas with maximum inhibition of 62.7+9.1% and 66.4+ 7.8% respectively the extract also scavenged (DPPH) Diphenylpicrylhydrazine and prevented lipid per oxidation in rat brain homogenate. These outcomes imply that alcoholics extract of *Carissa edulis* exert in vivo inflammatory and antioxidant properties which could add to its activities[18].

Carissa edulis has ethno botanical importance and has always been exploited by local people in search of remedies for various ailments. However its effects on the various ailments has not been scientifically studied or validated. It was against this background that this study was undertaken to scientifically test the hepatoprotective effects of the plants extracts in rats.

2. Methods

2.1 Collection Identification a Authentication of *Carissa edulis*

The leaves of *Carissa edulis* were collected from Fursum East, Jos East Local Government Area of Plateau state located at Latitude 9º 59′ 27′′ N. Longitude 9º 6′ 29′′E.(), in December 2017. 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.

The *Carissa edulis* leaves were removed from the whole plant and air dried at a temperature of about 20ºC and humidity of about 50%for fourteen (14) days. The dried parts were powdered using mortar and pestle and sieved with 29 mesh size. The powdered part was soxhlet extracted with 700mL 70% $^{V}/_{V}$ ethanol for 72 h. The resultant filtrate was evaporated to dryness on steam bath at 40^{0}C to give greenish extract that was stored in air tight containers and preserved at 4°C until required for use.

2.2 Animal study

48 Wistar albino rats (male) weighing between (120-220grams) acquired from experimental animal house of the Department of Pharmacology University of Jos Plateau State was used for the study. The rats were fed with standard animal pellets (Pfizer Feeds® Nigeria), given water *ad libitum* and sustained in a well-ventilated room; the temperature of the room was 30ºC. The cage floor was 70 inches while the height was 7 inches. The rats were kept by maintaining standard housing conditions and 12 hours light/darkness cycles. Ethical clearance was sort and obtained from the ethics committee of the University of Jos with reference number UJ/FPS/F17-00379.

2.3 Preliminary Phytochemical Studies of *Carissa edulis* Leaf Extract

Phytochemical evaluation of the ethanolic leaf extract of *Carissa edulis* was conducted to identify the presence or absence of secondary metabolites (alkaloids, anthraquinones, cardiac glycoside, carbohydrates, flavonoids, saponins, tannins, and steroid) using standard methods [19].

2.4 Determination of LD_{50} of Carissa Edulis

The acute toxicity study of the extract was determined for the extract using the Lorke's method. This was done in two phases. In the first phase, nine rats were separated at random into three groups of three rats' per-group and were given 10,100 and 1000 mg/kg of the extract of *Carissa edulis,* orally respectively. The rats were monitored for signs of adverse effects and death for 24 hours. Based on the outcome of the phase one study, the process was repeated via another set of three rats randomly separated into three groups of one rat each, given 1600, 2900 and 5000mg/kg body weight of the extracts respectively. For 14 days the rats were observed for signs of toxicity which include 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.

2.5 Hepatoprotective Study

The method of Li *et al*., [20] was adopted and modified. Thirty-six rats were randomly divided into 6 groups of 6 rats each. The groups were Group A normal control, Group B standard drug, Group C carbon tetrachloride treated group and the three test groups Group D, F and H (250, 500 and 1000mg/kg). The standard drug used was silymarin.

To study the protecting effect against carbon tetrachloride induced chronic hepatic injury *Carissa edulis* leaf extract (250, 500 and 1000mg/kg body weight) was given daily by oral gavage to the animals for 28 consecutive days (4 weeks). The 50% $^{V}/_{V}$ CCl_4 in olive oil was gavaged through the gastric tube twice a week on the third and seventh day of each week to the extract treated groups (D, F and H) for 4 weeks. The animals of Group A were administered distilled water 5mg/kg body weight for 28 days. Animals in Group C were given CCl_4 twice a week on the third and seventh day of each week for 4 weeks and with vehicle (Olive oil) on the rest of the days. While the animals in Group B were treated with standard drug silymarin in water at a dose of 50mg/kg daily for 28 consecutive days along with CCl_4 on the third and seventh day of each week for four weeks.

The animals were sacrificed by the overdose of anesthetics followed by a secondary method of exsanguinations. On the 29th day each rat was anaesthetized with ethyl ether, the weight of the rats was 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 were:- (1) Liver Function Test which include, Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total protein (TP), Bilirubin (BIL) and Albumin (ALB). (2), Lipid Profile Test which include High Density Lipoprotein (HDL), Total Cholesterol (CHL) and Triglyceride (TRIG). The liver was harvested trimmed of adherent tissue and preserved in 10% formalin 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).

2.6 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

3.1 Phytochemical Screening of the Ethanolic Leaf Extract of *Carissa edulis*

The preliminary phytochemical test for the leaf extracts was positive for saponins, tannins, flavonoids carbohydrates and steroids. Tannins are more present while flavonoids are highly present in the extracts. Alkaloids, cardiac glycosides and anthraquinones were absent.

3.2 Determination of LD_{50} of the Leaf Extract of *Carissa edulis* in Rat

The extract did not cause mortality at a dose of 5000 mg/kg. The animals were calm, nonaggressive and did not exhibit decreased exploratory activities and stereotype behavior.

3.3 Effects of the Leaf Extracts of Carissa edulis on the Body Weight of Rats Treated with CCl_4

The normal control group showed there was a significant increase in body weight at day 14, (151.38 ± 4.58), day 21, (151.75 ± 4.63) and (172.50 ± 7.79) at day 28 from the initial weight of (134.98 ± 3.38 at day 0). In Group C, there was significant increase in weight from the initial (127.83 ± 2.26) to (140.88 ± 2.33) at day 14 to (149.90 ± 2.65) at day 21 and (158.0 ± 3.08) at day 28. In Group B there was no significant difference in the body weight from 137.40 ± 3.47 at day 0, to (136.71 ± 3.53) at day 7 to (135.18 ± 3.45) at day 14 to (132.40 ± 1.81) at day 21 and (128.53 ± 2.11) at day 28.

For Group D there was a significant increase in body weight from (136.06 ± 3.03) at day 0, to (150.85 ± 3.30) at day 14, (157.13 ± 4.07) at day 21 and (165.0 ± 4.23) at day 28.For Group F, there was a significant decrease from (145.98 ± 3.59) at day 0 to (119.30 ± 7.44) in day 28 at ($P < 0.05$).In Group H , there was a significant increase from (141.16 ± 3.96) at day 0, to (157.55 ± 5.08) at day 7 to (168.43 ± 4.09) at day 14 and (157. 21 ± 6.45)

Table 1: Effect of Leaf extract of *Carissa edulis* on Body weight of C_4Cl treated Rats

Time (days) Treatments	0	7	14	21	28
Normal Control	134.98 ±3.38	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[a]	140.88±2.33	149.90±2.65[b]	158.01±3.08[b]
250mg/Kg (Leaves) +CCL_4	136.06±3.03[bc]	143.95±3.30[b]	150.85±3.30[b]	157.13±4.07[b]	165.08±4.23[bc]
500mg/Kg (Leaves) +CCL_4	145.98±3.59[ac]	155.53±3.03[bc]	158.05±4.38[b]	138.50±16.28	119.30±7.44[ac]
1000mg/Kg (Leaves) +CCL_4	141.16±3.96[bc]	157.55±5.08[bc]	168.43±4.09[abc]	157.21±6.45[b]	136.54±6.86[ac]
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 $p<0.05$; n= ±SEM

3.4 Effect of the Ethanolic Leaf Extract of Carissa Edulis on the Liver Function Indices of Rats Treated with CCl_4

Table 2 shows that the three concentrations of the leaf extract (i.e. 250, 500 and 1000mg/kg body weight) of the leaf extract showed significance in measure of protection/healing 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 $P < 0.05$ (Table 2). In ALT, all the groups had lower levels of ALT compared to the CCl_4 group with ALT level of (363.60 ± 60.32). Groups F and H showed significant lower level of ALT compared to Group C. In AST, all the three concentrations of the leaf extracts were significantly lower than the Group C in a dose dependent manner, where Group H was the most sensitive treatment group with significantly lower AST level of (186.80 ± 12.39) which is similar or comparable to the normal control group. In ALP, all the three concentrations of the leaf extracts were significantly lower than Group C at ALP level 1275.66 ± 175.21).

In ALB, Group D, F and H were significantly lower than Group A. Group B was also significantly lower than Group A. It's only Group F that was significantly lower than Group C at ALB level (127.66 ± 175.21). In TP, Group A had the highest level of TP, but had no significant difference with Groups B and C. In BIL, the lowest level observed was in Group C followed by Group H and A. Group F was the only group significantly lower than Group C.

Table 2: Effect of the Ethanolic leaf Extract of *Carissa edulis* on Liver Function Indices of C_4CL treated rats.

Treatments	ALT	AST	ALB	ALP	TP	BIL
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}
D	188.10±27.65	327.10±18.67^{ac}	4.16±0.05^{ac}	569.33±61.78^{bc}	8.05±0.07	10.35±0.22^{b}
F	78.50±11.75^{c}	272.83±12.50^{c}	3.66±0.12^{bc}	635.05±70.49^{bc}	7.70±0.50	13.50±1.69^{ab}
H	72.10±0.72^{c}	186.80±12.39^{bc}	4.08±0.02^{a}	891.50±31.38^{c}	7.73±0.02	8.60±0.68^{b}
LSD	212.14	52.01	0.24	219.96	0.51	2.68

KEY:

Groups (A) = Normal Control (distilled water)
Groups (B) = Standard Drug (silymarin)
Groups (C) = $CCl_{4,}$
Groups (D) = 250mg/Kg (Leaves) +$CCl_{4,}$
Groups (F) = 500mg/Kg (Leaves) +$CCl_{4,}$
Groups (H) = 1000mg/Kg (Leaves) +CCl_4.

3.5 Effect of the Leaf Extract of *Carissa edulis* on Lipid Profile Parameters of Rats Treated with CCL_4

Table 3 shows that there was significant difference in TRIG, HDL and CHL levels between the treatment groups observed at $P \leq 0.05$ in each case. For TRIG, there was a significant increase in the value of TRIG, in Group C compared to Group A. TRIG levels in other groups were significantly lower than Group C. For HDL, the highest level was observed in Group C and H. The least values were observed in Group A (0.91 ± 0.15) and B (0.96 ± 0.19).For CHL, there was a significant increase between Group A and C. In Groups F and H there was a significant decrease in CHL level compared to Group C.

Table 3 Effect of the Ethanolic leaf extract of *Carissa edulis* on Lipid profile (TRIG, HDL and CHL) of C_4CL treated Rats

Treatments	TRIG	HDL	CHL
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}
D	0.83±0.01^{c}	1.06±0.02^{c}	1.64±0.01^{b}
F	0.60±0.08^{bc}	1.26±0.06^{abc}	1.39±0.02^{bc}
H	0.57±0.02^{abc}	1.30±0.05^{ab}	1.30±0.05^{abc}
LSD	0.12	0.27	0.24

3.6 Effect of the Leaf Extract of *Carissa edulis* on Liver Antioxidant Parameters of Rats Treated with CCl_4

There was a significant decrease in all the antioxidant parameters within the treatment groups at ($P < 0.05$).In malondialdehyde, the highest value was observed in Group C (11.8 ± 0.11) compared to the other groups. There was a significant decrease in malondialdehyde level in all the three concentrations of the leaf extract in a dose dependent manner. For Glutathione, the highest level was observed in Group A (85.32 ± 1.66) which was significantly higher than the other groups. The least value of glutathione was observed in Group C (44.75 ± 0.28). There was also a significant increase in glutathione level in Groups D, F and H compared to Group C. In the case of Catalase, the highest level was observed in Group A while the least was observed in Group C at (0.0013 ± 0.000). There was a significant increase in Catalase level in the animals treated with the three concentration of the leaf extract. In superoxide dismutase, the least value was recorded in Group C (1.9886 ± 0.003). There was also a significant increase in superoxide dismutase level among animals treated with the three concentrations of the leaf extract (Groups D, F and H).

Table 4 Effect of leaf Extract of *Carissa edulis* on liver antioxidant parameters of CCl_4treated Rats

Treatments	Malondialdehyde	Glutathione	Catalase	Superoxide
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}
D	9.86±0.12^{ac}	60.93±1.87^{abc}	0.0023±0.0000^{abc}	1.9941±0.0003^{c}
F	8.32±0.09^{bc}	66.25±0.51^{abc}	0.0037±0.0002^{abc}	1.9964±0.0009^{abc}
H	7.96±0.04^{abc}	70.34±0.78^{abc}	0.0037±0.0002^{c}	1.9962±0.0007^{abc}
LSD	0.47	4.32	0.002	0.0039

3.7 Histopathological observation of the liver after administering different doses of the extracts in CCl_4treated Rats

The histopathological studies showed a distinct recovery from the structural damage caused by CCl_4 in the extract treated groups (Groups D, F and H), this result is consistent with the biochemical analysis and liver antioxidant status.

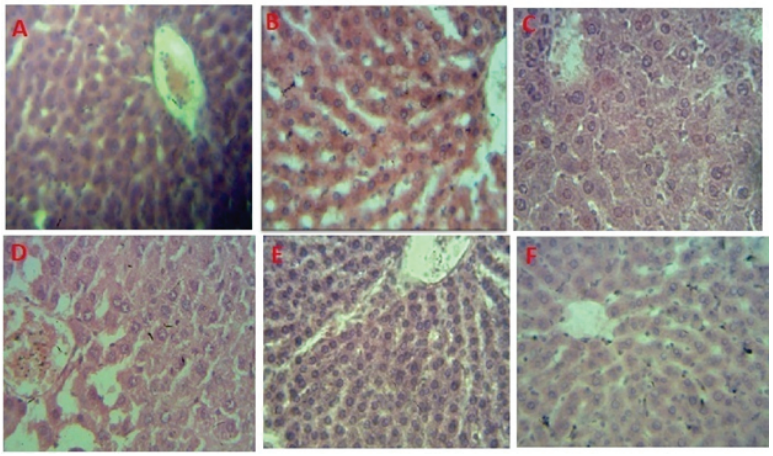

Figure 1. (A) Cross section of normal control, (B) Cross section of rats treated with silymarin© Cross section of rats treated with CCL_4, (D) Cross section of rats treated with 250 mg/kg of leaf extract (E) Cross section of rats treated with 500 mg/kg of leaf extract and (F) Cross section of rats treated with 1000 mg/kg of leaf extract

4. Discussion

The presence of flavonoids, saponins and tannins in the ethanolic leaf extract of *Carissa edulis* was established by phytochemical analysis, and these compounds are reported to possess antioxidant properties [21]. A potential of hepatoprotective property underlying *Carissa edulis* may be ascribed to the anti-oxidative constituents. The plants most regularly used to treat liver disorders are *Curcuma longa* (turmeric), *Glycyrrhiza glabra* (licorice) and *Camellia sinensis* (green tea), and they are all reported to be hepatoprotective owing to the strong antioxidative properties The mechanism of the antioxidant action of *Carissa edulis* extract was been stipulated to be due to the reduction of free radicals along with the scavenging of reactive oxygen species and other free radicals The LD_{50} of the leaf extract was found to be greater than 5000 mg/kg following oral administration in rats and according to the Hodge and Sterner scale, the ethanolic leaf extract of *Carissa edulis* plant is non-toxic.

An increase in body weight through the treatment period is a sign of protection against hepatic injury

whereas a decrease signifies Hepatotoxicity [22]. The study revealed that there was no clear pattern on the impact of the extract on body weight (see Table 1). Under this circumstance, it will be difficult to know the exact impact of the treatment on the body weight. Hence the need for further research on this area. On the liver function indices, the experimental data showed a significant increase in the activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) following exposure to CCl_4 at $P < 0.05$ in rats (see Table 2). This result is consistent with many authors who reported that these enzymes levels are significantly elevated following CCl_4 exposure[23,17]

Treatment with the ethanolic leaf extracts of *Carissa edulis* demonstrated a dose dependent and significant protection to the rats against CCl_4 induced liver injury at $P < 0.05$ (see Table Bilirubin is a metabolic waste product produced from the breakdown of erythrocytes[24]. Studies have revealed that CCl_4 administration causes an increase in bilirubin level owing to its toxicity However the result of this study reveals that CCl_4 did not produce an increase in bilirubin level as expected (see Table 2) probably because of the environmental factors, age and nutritional factors For total protein, studies revealed that there is a decrease in the level of total protein after hepatic intoxication Also an increase in the protein level signifies hepatoprotective activity as it speed up the renewal and production of liver cells [25]. Contrary to this expectation, the induction of liver injury using CCl_4 did not produce a reduction in the level of total protein (Table2) probably owing to factors like; the animal state of hydration, chronic infection and inflammation or humoral immunodeficiency[26].

Past experimental researches reported that CCl_4 decrease albumin level [3, 26, 27]. Administration of CCl_4 cause hepatic changes that lead to quick loss of the capability of the liver to synthesize albumin since albumin is produced on a polysome bound to the endoplasmic reticulum [23, 28]. The result of this study showed significant increase in Albumin level in about 50% of the extract treated group compared to the normal control and no significant difference in the remaining group 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 $P \leq 0.05$

The value of Triglyceride, for all the treated groups were lower than the CCl_4 group, this may imply stabilization of the endoplasmic, reticulum resulting to protein synthesis due to the extract, .For CHL, the levels in the extract treated groups were lower than the CCl_4 group with a significant reduction at 500mg and 1000mg/kg of the leaf extract groups. An increase in membrane Cholesterol (CHL) shows a relationship with a decrease in membrane functions including changes in membrane receptors, enzyme accessibility and their activation[8]. The ability of the extract to reverse the anomaly may suggest that the extract has the possibility to decrease serum CHL in hypercholesterolemia.

For High density lipoprotein (HDL), 500 and 1000mg of the leaf 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 influenced by genetic factors than other lipoproteins[29].For liver antioxidant parameters, a reduction in the level of antioxidant enzymes and increase in lipid per oxidation level were observed following CCl_4 administration[30]. These findings revealed the effectiveness of *Carissa edulis* extract in preventing CCl_4 Hepatotoxicity by enhancing the activity of the liver Superoxide dismutase (SOD), Catalase (CAT) and glutathione (GHS) enzymes while reducing the Malondialdehyde enzymes (MDA) content (see Table 4). This may be ascribed to the presence of the several compounds with high antioxidant activities that scavenge the produced radicals [11]. The result of this study may signify that the extract have a strong scavenging effect against active oxygen radicals by reducing the level of MDA an indicator of lipid per oxidation and increasing the activities of SOD, Catalase and Glutathione. The result from the histopathological studies also presented important evidence supporting the biochemical analysis and liver antioxidant status. At a dose of 250mg/kg of the leaf extract (Figure 1D), there was karyoclasia, massive enlargement of the nuclei within the hepatocyte and distortion of the radial arrangement of hepatocyte with a few kupffer cells. At a

dose of 500mg/kg of the leaf extract, (Figure 1E) there was a slight improvement in the radial arrangement of the hepatocytes and the nuclei within the hepatocytes. At a dose of 1000mg of the leaf extract (Figure 1F), there was a distinctive recovery from the structural damage caused by CCl_4 (Figure 1C) which is comparable to the standard drug silymarin (Figure B) and the normal control (Figure 1A). This indicates pronounced protection to the liver.

5. CONCLUSION

This study demonstrated the protective effect of *Carissa edulis* ethanolic leaf extract against CCl_4 induced liver injury in rats. This is most likely due to the presence of some endogenous substances with antioxidant and detoxifying properties. The active compound responsible for the hepatoprotective activity has not yet been identified in the study. The mechanism of action also remained unproven. Further investigation is therefore required to identify the constituents of the tested plant responsible for the hepatoprotective effect.

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References

1. Althnaian TI, AlbokhadalmEI, Bathr SM (2013). Biochemical and histopathological study in rats intoxicated with carbon tetrachloride and treated with camel milk. Springer Plus 2(1): 57 – 64 https://doi.org/10.1186/2193-1801-2-57
2. Camp L, Benilde B, Anna RG, Robert JA, Jones, LM, Claudia O, Senthil Kumar, S (2004). (Eds). Reports of the thematic strategy for sill protection volume introduction and executive summary, EUR 2131 EN1.
3. Khan AA & Alzohairy M (2011). Hepatoprotective effect of Camel milk against CCl_4induced Hepatotoxicity in rats. *Asian Journal of Biochemistry*, 6(2), 171 – 180.https://doi.org/10.3923/ajb.2011.171.180
4. Donatus IA, Sardjoko TA, Verneulen NP (1990). Cytotoxic and Cytoprotective activities of Curcumin. Effects of Paracetamol induced cytotoxicity, lipid per oxidation and glutathione depletion in Rats hepatocytes. *Biochemical Pharmacology* 39, 1869 – 1875.5.
5. Li R, Gou N, Nfu Z, Ding G, Zou Y & Wang Z (2011). Hepatoprotective actions of *Radix paenial rubra* aqueous extract against CCl4 induced hepatic damage. *Molecules*, 8684-8693. https://doi.org/10.3390/molecules16108684
6. Kyszejko B &Lyzzywe G (1999). Effect of sub lethal concentration of Deltametrin on biochemical parameters of blood serum of carp *(Cyprinus carprol*). Acta Ichtyol EF *Piscatorial*, 292, 109 – 116.
7. Buyakasik Y, Goker H, Buyakasik NS, Sayinalp N, Hasnedaroglu IC, & Ozebe OI (2008) . Effect of platelet count on secretion capacity, formularization and use of formulae for evaluation of platelets secretion in thrombocytopenic patients. *Blood Coagulation* Fibrinolysis 19: 633 –637.
8. Emerole GO, Thabrew M (1983). Changes in some rat hepatic microsomal Component induced by prolonged administration of chloroquine. *Biochemical Pharmacology*, 32(20), 3005 –3007.https://doi.org/10.1016/0742-8413(85)90104-5
9. Sofowara A (1986).*The state of medicinal plant research in Nigeria 1st* Edition University Press Limited, Ife Nigeria page 145-148.
10. Jaeschke H, Gores GJ, Cederbaun AI, Hinson JA, Pessayre D & Lemaster JJ (2002). Mechanism of Hepatotoxicity. *Toxicological Sciences*. 65(2), 166 – 176.
11. Dasgupta A, Ramsey KL, Smith JS, Auble DT (2004). Sir antagonist 1 (San 1) is a ubiquitanligage. *Journal of Biological Chemistry*, 278(26), 26830 –26938. https://doi.org/10.1074/jbc.M400894200
12. Ciszewski TS, Ralston S, Acheson D, Wasi, S, Strong SJ (1993). Protein level and Plasma-pheresis Intensity. *Transfusion Medicine*, 3, 59 – 65. http://doi.org10.1111/j.1365-3148.1993.tb00105.x.
13. Anthea MH, Jean MC, William J, Susan W, Maryonna Q, Laitart D, Wright JD (1993). Human biology and health (3rdedn). New Jersey: Eaglewood Cliffs Prentice Hall
14. Fahim FA, Esmat AY, Fadel HM, Hassan KF (1999). Allied studies on the effect of *Rosmarinus officinalis* (Linn) on experimental Hepatotoxicity and mutagenesis. *International Journal of Food Science and Nutrition*, 50, 413-427. https://doi.org/10.1080/096374899100987
15. Adil M, Allahverdiyev N, Olga, NO (2013). Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and their component. Science Direct 17(1): 245-259.
16. Ostapowicz G, Fontana RJ, Scheldt FV, Larson A, Davern TJ, Han SH & Lee WM (2002). Acute liver failure study group results of prospective study of acute liver failure at 17 tertiary care centers in the United States. *Annals of Internal Medicine*, 137(9), 947-954.https://doi.org/10.7326/0003-4819-137-12-200212170-00007
17. Alyoussef H, Hassan W (2010). Phytochemical and biological studies of the aerial parts of *Carissa edulis* growing in Saudi Arabia. Biosciences Biotechnology Research Asia 7(2): 635-46. https://doi.org/10.13005/bpj/314
18. Nedi T, Mekonnen N & Urqa K (2004). Diuretic

Effect of the Crude Extract of *Carissa edulis* in Rats. Journal of Ethno pharmacology 95: 57-61. https://doi.org/10.1016/j.jep.2004.06.017

19. Mahheswari M & Rao PGM (2005). Antihepatoprotective effects of Grape seed in Rats. *Indian Journal of Pharmacology* 37 (3), 179.https://doi.org/10.4103/0253-7613.16216

20. Frenzel C, Teschke R (2016). Herbal Hepatotoxicity: Clinical Characteristics and Listing Compilation. *International Journal of Molecular Sciences*. 1 (7), 50-88. https://doi.org/10.3390/ijms17050588.

21. Shankar MB, Parikh, JR. Geetha M, Mehta RS, & Saluja, AK (2005). Hepatoprotective activity of benzopyrone from *Tephrosia purpunea* pers. *Journal of Natural Remedies* 5(2), 115 –120.

22. Lorke D (1983). A new approach to acute toxicity testing, Arch Toxicology 54(4) 275-287.

23. Hicks LE, Langham RA, Takenaka J (1969). Formation on Membrane – bound Polyribosome. *American Journal of Public Health*. 2(1), 202 – 777.

24. Canadian Centre for Occupational Health and Safety (2013) what is LD_{50} and LC_{50}. Retrieved October 8, 2015, from Canadian centre for occupational health and safety website: http://www.ccohs.ca/oshanswer/chemicals/id50.html.

25. Hale JM (1983). *The action of hydrogen sulfide on polarographic oxygen sensors, Aquatic and physiological applications*. Gnaiger E, Forstner H (Eds) Springer, Berlin, Heidelberg, New York; 73 – 75.

26. Banker GJ, Verma SK (1987). Preliminary studies of flowering and fruiting in *Carissa edulis*. Journal of Natural Product 4(7): 1056-1057.

27. Burkil HM (1985). The useful plant of West Africa, Second Edition Volume/Families. A-D. Royal Botanical Gardens. London: Kew 40(2): 176

28. Ibrahim H (1997). Pharmacognostic and biological analgesic activity studies of *Carissa edulis* Vahl. PhD Ahmadu Bello University Zaria, Nigeria. 157-60, 235-45, 306-307.

29. Omino FA & Kokwaro JO (1993). Ethno botanical of Apocynaceae Species in Kenya. *Journal of Ethno Pharmacology* 4: 167-180.

30. Mehmetcik G, Ozdemirier G, Kocak-Toker N, Cevikbas U & Uysal M (2008). Effect of Pretreatment with artichoke extract on carbon tetrachloride induced liver injury and oxidative stress. *Experimental toxicology pathology*, 60(6), 475 – 480.