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Research Article

Hepatoprotective Potential of *Commiphora kerstingii* Engl. Stem Bark Against Carbon Tetrachloride-induced Acute Liver Injury

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ABSTRACT

Background and Objective: The stem bark of *Commiphora kerstingii* Engl. is used in northern Nigeria to treat jaundice and other liver diseases. In this study, *C. kerstingii* stem bark extract was investigated for its effect in acute liver injury induced by carbon tetrachloride (CCl_4). **Materials and Methods:** A limit test was employed to determine acute toxicity and median lethal dose. To determine its effect in acute liver injury, rats were pre-treated with *C. kerstingii* extract (50, 100, 200 mg kg^{-1}), distilled water (5 mL kg^{-1}) or silymarin (100 mg kg^{-1}), 1 hr before intraperitoneal injection of carbon tetrachloride and repeated once daily for 5 days. After 5-day pre-treatment and challenge, the liver and serum biochemical parameters were assessed. Liver tissue homogenate was assayed for antioxidant effect. **Results:** The extract was not acutely toxic and produced no mortality. Relative liver weight, alanine aminotransferase, total bilirubin changes in 200 mg kg^{-1} extract-treated groups were insignificant ($p>0.05$) relative to the unchallenged control group. Pathological changes observed in the CCl_4-challenged control group was mitigated in 200 mg kg^{-1} extract-treated groups. The liver homogenate of extract-treated groups showed an increase in antioxidant capacity relative to the normal and CCl_4-challenged control groups. In all the parameters studied, the effects of the extract were comparable to those produced by silymarin. **Conclusion:** *Commiphora kerstingii* stem bark extract is acutely safe when administered orally and possesses protective effects against acute liver injury, likely mediated by its ability to restore antioxidant defense.

KEYWORDS

Acute toxicity; antioxidant; *Commiphora kerstingii*; liver enzymes; liver injury; medicinal plant; silymarin

INTRODUCTION

Drug-induced hepatotoxicity represents a major clinical problem accounting for approximately 60% of all cases of acute liver failure[1]. Alcohol and hepatitis C are responsible for most of the liver diseases and can result in cirrhosis, hepatocellular carcinomas, organ failure and death in some cases[2]. Due to the burden of communicable and non-communicable diseases globally with an attendant increase in drug therapy for these diseases, drug-induced hepatotoxicity will likely be on the increase[3]. Experimentally, carbon tetrachloride (CCl_4) is widely used in *in vitro* and *in vivo* models of hepatotoxicity. It produces hepatotoxicity by the formation of the trichloromethyl radical (CCl_{3+}), which is highly reactive. CCl_4 is metabolized by cytochrome P450, mainly through the CYP2E1 isoform in the endoplasmic reticulum and mitochondria[4]. These radicals could infiltrate the organism's antioxidant defense system, react with proteins, attack unsaturated fatty acids, resulting in lipid peroxidation, eventual lowering of protein and accumulation of triglycerides (fatty liver), with an increase of hepatic enzymes in plasma[5]. The inhibition of the radical CCl_3 generation and/or improving antioxidant

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defense is a key point in the protection against free radical-induced damage. Based on this, this model is widely used for the evaluation of pharmaceuticals and natural products with hepatoprotective and antioxidant activity[6,7]. Currently, therapeutic liver-protective agents are very limited. Common examples like glycyrrhizin and silymarin were derived from licorice root and the milk thistle plant respectively, indicative of the potentials of medicinal plants as sources of new hepatoprotective therapies.

Some medicinal plants and their components have been found useful for the treatment of liver diseases such as fatty liver, hepatitis, fibrosis, cirrhosis[8]. Thus, the exploration of these medicinal plants to ascertain their safety and efficacy is a promising strategy for further development of hepatoprotective agents to curb liver injury. The plant, *Commiphora kerstingi* Engl. (family Burseraceae) is a 10 m high softwood tree found in the Savanna from Togo to Nigeria[9]. The genus *Commiphora* is characterized by species that often grow as small trees or shrubs with spinescent branches, pale-gray bark and reddish-brown resinous bark exudates[10]. The Hausa of Northern Nigeria often cultivate *C. kerstingii*, locally called 'Dali' as a live fence and as a shade plant. Traditionally, it is believed that the tree is unlikely to burn easily due to its evergreen bark. The stem bark is sometimes used as an antidote to arrow poison and to treat fever, jaundice and has shown antimicrobial activity[11,12]. Previous studies on *C. kerstingii* report its antibacterial, antioxidant and anti-trypanosomal activities[9,13]. A literature search did not reveal any scientific report to validate the ethnomedicinal use of the plant against jaundice and liver disease. Hence, this study was undertaken to investigate the effect of *C. kerstingii* stem bark extract in carbon tetrachloride (CCl_4)-induced acute liver injury.

MATERIALS AND METHODS

Study area

The study was carried out in the Department of Pharmacology and Toxicology, Abuja from December, 2017 to February, 2018.

Drugs and reagents

Carbon tetrachloride (JHD®, China), silymarin (Silybon®, Micro Labs, India), ethanol, sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), formaldehyde, diphenyl picrylhydrazyl (DPPH) and dimethylsulfoxide (DMSO) were sourced from Sigma Aldrich (Mannheim, Germany) through a regional representative. Other reagents used were of analytical grade.

Plant material

Commiphora kerstingii stem bark was collected from a farm in Suleja, Niger State, Nigeria in August, 2017. A voucher specimen (NIPRD/H/6921) was prepared and deposited in the herbarium unit of the National Institute for Pharmaceutical Research and Development, Abuja. The plant material was washed in clean water and dried in a warm air oven maintained at 55-60°C for 1 week. The dried plant material was mechanically pulverized to a coarse powder and extracted by maceration in 70% v/v ethanol. After 24 hrs, the mixture was filtered and the filtrate was concentrated to constant weight on a water bath maintained at 55°C. The concentrate was then stored at 4°C in a refrigerator and freshly constituted before each use.

Animals

Adult Swiss albino mice and Wistar rats of either sex were used. They were maintained in the animal facility center of the National Institute for Pharmaceutical Research and Development (NIPRD) and acclimatized to laboratory conditions for two weeks before the study. They were fed standard rodent feed and allowed unrestricted access to clean drinking water during the entire study period. All applicable institutional and national guidelines for the care and use of animals were adhered to in the experiments[14].

Oral acute toxicity

A limit test was done according to the OECD guidelines for oral acute toxicity testing of chemicals[15]. Five mice were given 2000 mg kg^{-1} doses of extract, while five mice served as control and received equivalent volumes of the vehicle. After the extract was administered, food was withheld for a further 2 hrs. During this period, the mice were observed for signs of toxicity closely at 15 min, 30 min, 1 and 2 hrs; then at 4, 8 and 24 hrs. The mice were subsequently observed once daily for 14 days for signs of delayed toxicity and/or mortality. Observations were noted using the Hippocratic screening table for plant extracts[16].

Screening in acute CCl_4-induced liver injury

Experimental design

This assay was performed according to a method of Rubin *et al.*[17] that was recently applied in screening a plant extract and isolated compounds by Kang and Koppula[18]. Thirty-six rats of either sex were divided into groups of six rats per group. The extract and silymarin were dissolved in an aqueous vehicle containing 0.75% w/v tragacanth and administered orally. The rats were treated daily, 1 hr before CCl_4 administration for five days as follows: Group I received aqueous tragacanth vehicle orally (0.75% w/v,

10 mL kg^{-1}) and served as vehicle control. Groups II-VI received CCl_4 in liquid paraffin intra-peritoneally (1:1; 0.2 mL/100 g body weight) with the addition of aqueous tragacanth vehicle (0.75% w/v, 10 mL kg^{-1}) in Group II to serve as the CCl_4-challenged group. Groups III to V also received the ethanol extract of *C. kerstingii* at doses of 50, 100 and 200 mg kg^{-1} body weight per oral respectively. Group VI also received standard silymarin at a dose of 100 mg kg^{-1} orally.

Serum biochemistry

On the 6th day, the rats were euthanized by chloroform inhalation. Blood samples were collected by carotid bleeding into plain serum tubes. After 1 hr the samples were centrifuged at 3000 rpm for 10 min to separate the serum for analysis. Parameters were determined using a Cobas C311® (Roche, Germany) auto analyzer and corresponding standard kits.

Organ: weight index and tissue histopathology

The liver of each rat was excised, blotted dry and weighed. The organ: weight index was determined as[19]:

$$\text{Weight index} = \frac{\text{Wet weight of organ (g)}}{\text{Body weight (g)}} \times 100$$

For the histological analysis, liver samples were fixed in phosphate-buffered formalin solution. Tissues were cut into thin slices of 5 mm×2 mm×1 mm, processed routinely and stained with hematoxylin/eosin.

Assay of antioxidant capacity of liver homogenate

Tissue homogenate preparation

A 100 mg quantity of liver tissue was weighed and rinsed in 1.15% KCl, placed in iced cold phosphate buffer (pH 7.4) and mechanically homogenized. The liver homogenate was centrifuged at 8,000×g for 15 min and the supernatant used for the antioxidant assay. This was performed as described by Janaszewska and Bartosz[20]. A 20 µL aliquot of the supernatant obtained from the liver homogenate was mixed with 480 µL of 10 mM sodium phosphate buffer (pH 7.4). To this, 500 µL of 0.1 mM solution of DPPH in methanol was added and the mixture was incubated in the dark at 21°C for 30 min. A sample tube containing 500 µL of buffer and 500 µL of DPPH was taken as reference, representing no antioxidant effect. Reaction mixture with low absorbance was considered to have high free radical scavenging activity.

Statistical analysis

Data were expressed as mean±standard error in mean (SEM). Differences between vehicle control and treated groups will be determined using one-way ANOVA with tukey's *post hoc* test (GraphPad Prism 5.0). The p-values less than 0.05 were considered statistically significant.

RESULTS

Acute toxicity

Administration of 2000 and 5000 mg kg^{-1} doses of extract did not produce any obvious signs of toxicity such as tremors and convulsions within the first four hours and no death was recorded up to 14 days after the initial administration of the extract.

Histopathology of the liver

The histopathology of the unchallenged liver showed extensive signs of liver injury, compared to the normal presentation in the unchallenged control group (Figure 1, Table 1). Edema, fatty deposit were common in all the CCl_4-challenged groups, but necrosis was not observed in the 200 mg kg^{-1} extract-treated group (Table 1). Tissue architecture was also preserved in this group compared to the untreated, challenged control group. The CCl_4-challenged control group showed hepatic cells that were edematous, necrotic and hemorrhagic with fatty deposit and loss of tissue architecture (Figure 2). Administration of the extract at the doses of 50, 100 and 200 mg kg^{-1} and silymarin (100 mg kg^{-1}) reduced the hepatic injury with the most remarkable reduction observed at 200 mg kg^{-1} group (Figure 3) and the silymarin-treated group (Figure 4).

Effect of extract on liver parameters in CCl_4-induced acute hepatic injury

The effects of the extract on liver enzyme parameters in CCl_4-induced hepatic injury are shown in Table 2. Challenge with CCl_4 resulted in a significant increase ($p<0.05$) in the liver enzymes AST, ALP and ALT as well as a rise in the liver: Body weight index. Administration of the ethanolic extract of *C. kerstingii* at three different dose levels attenuated the increased liver enzyme parameters produced by CCl_4, depicting a similar decrease to that caused by the silymarin treatment.

Effect of extract on antioxidant capacity of the liver homogenate

The hepatic injury induced by CCl_4 caused an increase in the absorbance values obtained as seen in group 2 animals signifying a decrease in the free radical scavenging ability (Table 2). This was subsequently attenuated by the administration of the 3 doses of the *C. kerstingii* extract and silymarin signifying an increase in the antioxidant capacity in the liver following the treatment.

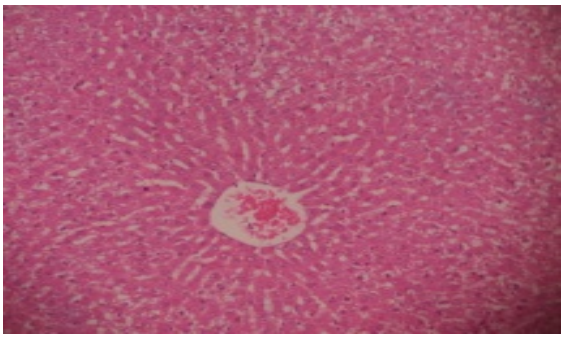

Figure 1: Normal rat liver histology shows a central vein containing erythrocytes (middle), normal hepatocytes with intact nuclei surrounded by intact cytoplasm and liver sinusoids represents as vehicle-treated, normal control group
Stain: Hematoxylin/eosin, Magnification: ×100

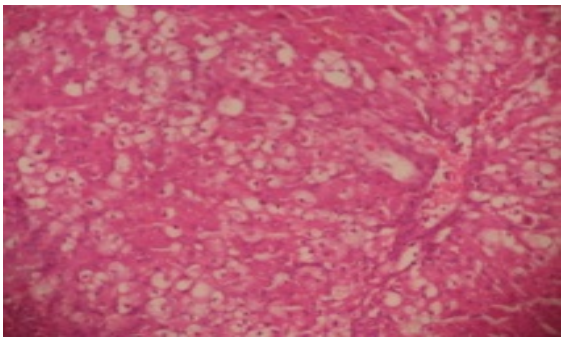

Figure 2: Liver of rat shows severe necrosis, fatty deposition and edema. There is complete loss of tissue architecture in some areas represents as vehicle+CCl_4 group
Stain: Hematoxylin/eosin, Magnification: ×100

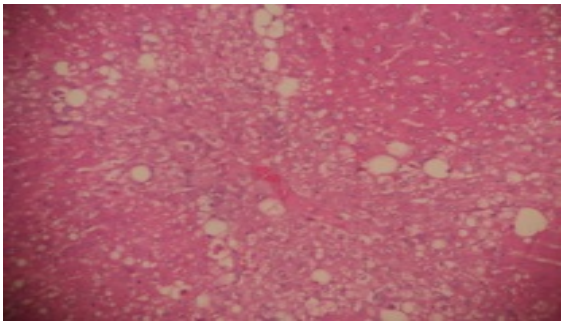

Figure 3: Liver of rat shows some fat deposits and edema, tissue morphology is modestly preserved represents as 200 mg kg^{-1} *C. kerstingii* extract-treated group
Stain: Hematoxylin/eosin, Magnification: ×100

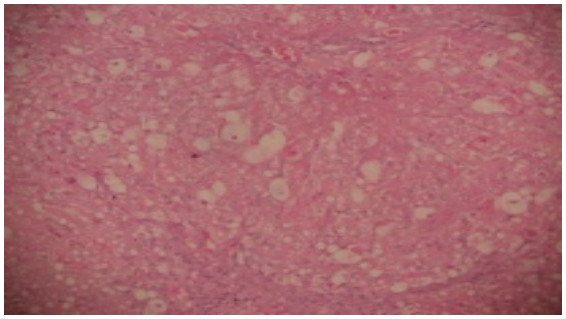

Figure 4: Liver of rat shows some loss of nuclear material, edema and fatty deposition with modest preservation of tissue architecture represents as 100 mg kg^{-1} silymarin-treated group
Stain: Hematoxylin/eosin, Magnification: ×100

Table 1: Effect of *C. kerstingii* extract on liver tissue histology in acute liver injury

Group	Dose (mg kg^{-1})	Edema	Necrosis	Fatty deposit	Hemorrhage	Loss of tissue architecture
Normal control	-	-	-	-	-	-
Vehicle+CCl_4	-	+++	+++	+++	+	++
Extract	50	+++	+++	++	+	++
	100	++	++	++	+	++
	200	++	+	++	+	-
Silymarin	100	++	+	++	++	-

-: Absent, +: Minimal, ++: Moderate, +++: Extensively observed

Table 2: Effect of *C. kerstingii* extract on some liver parameters in acute liver injury

Group	Liver: Body weight index	ALP	ALT	AST	Antioxidant capacity (absorbance)
Vehicle control	3.98±0.17	297.60±29.62	125.30±7.04	249.30±39.72	0.462±0.01
Vehicle+CCl_4	6.04±0.36**	390.50±51.19	566.00±139.7*	660.70±353.00	0.481±0.02
50 mg kg^{-1} extract+CCl_4	5.34±0.36*	374.00±68.15	361.30±67.10	427.30±134.70	0.438±0.03
100 mg kg^{-1} extract+CCl_4	5.39±0.33*	373.70±56.83	540.70±145.40*	452.40±168.80	0.424±0.01
200 mg kg^{-1} extract+CCl_4	5.15±0.37	393.50±40.71	188.00±57.74	561.00±207.50	0.475±0.01
100 mg kg^{-1} silymarin+CCl_4	6.13±0.12**	318.20±43.03	404.50±135.50	418.50±163.90	0.471±0.01

Data presented as means of six measurements and standard error of the mean. Level of significance taken at $p<0.05$, compared to the vehicle control group, *$p<0.05$, **$p<0.01$. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Table 3: Effect of *C. kerstingii* extract on bilirubin and lipid parameters in acute liver injury

Group	Direct bilirubin	Total bilirubin	Cholesterol	HDL	Triglycerides
Vehicle control	0.95±0.16	1.6±0.16	73.17±7.30	55.67±3.69	59.17±4.99
Vehicle+CCl_4	6.35±1.46*	10.75±2.25*	75.00±7.13	27.75±2.14***	80.00±10.46
50 mg kg^{-1} extract+CCl_4	3.33±0.61	4.33±1.05	60.67±10.90	35.25±4.39*	44.33±9.33
100 mg kg^{-1} extract+CCl_4	3.63±0.68	7.55±2.32	73.17±10.02	36.17±3.95**	59.00±11.92
200 mg kg^{-1} extract+CCl_4	7.10±2.83*	10.17±4.13	73.33±6.57	32.25±5.78**	57.00±6.11
100 mg kg^{-1} silymarin+CCl_4	8.33±1.80**	10.35±2.01*	83.50±2.25	42.20±2.75	66.33±9.21

Data presented as means of six measurements and standard error of the mean. Level of significance taken at $p<0.05$, compared to the vehicle control group, *$p<0.05$, **$p<0.01$, ***$p<0.001$. HDL: High density lipoprotein

Effect of extract on serum biochemical parameters in CCl_4-induced acute hepatic injury

The effect of *C. kerstingii* extract on some serum biochemical parameters in the liver of the CCl_4-challenged rats is shown in Table 3. Administration of CCl_4 caused a significant rise ($p<0.05$) indirect bilirubin, total bilirubin, cholesterol and triglycerides. The levels of these serum parameters were attenuated with the administration of the extract with the highest attenuation observed at 50 mg kg^{-1}. For HDL, all the extract and silymarin treated groups showed decreased levels compared to that of the vehicle-treated group.

DISCUSSION

The ethanol stem bark extract of *Commiphora kerstingii* showed protective effects against acute liver assault and this finding correlates with its traditional use in the treatment of liver disease in northern Nigeria. The liver is the largest and major metabolic organ in the body. The primary role of the liver is to metabolize nutrients, synthesize glucose and lipids and detoxify drugs and plant products (xenobiotics)[21,22]. Liver injury can result from the administration of drugs and toxic chemicals such as volatile anesthetic agents, alcohol, CCl_4 and acetaminophen resulting in signs such as necrosis, edema and loss of hepatic cell integrity as observed in the histological samples of both the normal and injured hepatocytes[23,24].

In this study, the administration of carbon tetrachloride alone resulted in severe acute liver damage. Carbon tetrachloride is a powerful environmental toxin released into the atmosphere as a result of industrial activities. It causes liver injury due to the release of free radicals[25]. Carbon tetrachloride is usually utilized in animal models to induce acute hepatic injury. This is because the breakdown of CCl_4 results in the formation of lipid peroxides and low-pressure reactive oxygen species. These radicals cause the deterioration of the hepatocyte membranes and organelles, degeneration and eventual death of the liver cells resulting in the release of enzymes such as AST, ALT and ALP into the blood. Thus, hepatic damage is assessed by the increased level of these enzymes in circulation[26]. Although the administration of the *C. kerstingii* extract did not exhibit complete protection against the injurious effects of CCl_4, it offered significant protection against it, evidenced by the prevention of an increase in levels of liver enzymes, bilirubin, lipid markers and confirmed by the histological results.

Bilirubin is a product of heme catabolism, it is a tetrapyrrole majorly obtained from hemoglobin degradation and sometimes from other heme proteins[27]. Direct bilirubin is the water-soluble form of bilirubin and it usually reacts with assay reagents. It consists largely of conjugated bilirubin, however, some unconjugated bilirubin still forms part of direct bilirubin[26]. The CCl_4-induced untreated group had significantly elevated direct and total bilirubin levels, suggesting either decreased hepatic clearance or overproduction. This also affects lipid metabolism, evidenced as fatty liver as seen in histological studies[28]. These changes were attenuated by the extract and since CCl_4 damage is free-radical mediated, the activity of the extract likely involves modulation of antioxidant defense in the liver. Other species of the genus *Commiphora* have demonstrated hepatoprotective action against drug and chemical-induced hepatic injury, mediated mainly through antioxidant mechanisms[29]. Pre-treatment with the resin of *C. opobalsamum* replenished the non-protein sulfhydryl of the liver caused by CCl_4-induced liver damage[30]. Also, the Methanol bark extract of *C. berryi* attenuated the increased levels of AST, ALT, ALP and serum bilirubin, activated SOD, catalase and glutathione peroxidase and reduced the fatty degeneration and necrosis in CCl_4-induced hepatic injury model in rats[26]. Previous studies have also supported the antioxidant property of *C. kerstingii* stem bark and attributed it to the presence of phenolic compounds such as tannins.

Liver damage is majorly attributed to oxidative stress which refers to the imbalance between free radicals/ reactive oxygen species and endogenous antioxidants in the liver. High levels of these reactive oxygen species from cell metabolism react with biomolecules and DNA to exert damage to the hepatic cells[31]. Antioxidants, on the other hand, reduce free radicals by releasing an electron to stabilize free radical thus minimizing the deleterious effects generated by these radicals in the cell. The activity of the extract may rely on its ability to restore the balance between free radicals/reactive oxygen species and endogenous antioxidant capacity. The human body produces endogenous antioxidants such as reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxide (GPx) and catalase which aid in preventing oxidative stress. Antioxidant/pro-oxidant balance is however subject to aberration, creating the need for exogenous antioxidants to curb disease. This implies that the extract may be useful when administered for other pathologies that arise from redox imbalance. A limitation of the study was the short duration of extract administration during CCl_4 challenge. The study duration can be extended in further studies to ascertain the potential of the extract to promote liver healing after the withdrawal of the toxicant. Also, long term safety studies of the extract to determine its effect on other body organs and toxicity markers are also warranted before its possible clinical application.

CONCLUSION

At the doses used, the extract showed a protective effect against acute liver injury induced by carbon tetrachloride and provides some justification for its traditional use against liver disease. More studies are necessary to reveal its effect in chronic liver injury.

SIGNIFICANCE STATEMENT

This study highlights for the first time, the hepatoprotective effect of *C. kerstingii* bark against acute liver injury caused by a chemical toxicant. The plant, therefore, has prospective use for protection of the liver from injury of different etiologies, attributed to its ability to boost antioxidant defenses.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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DISCLAIMERS

The opinions expressed in this article are the authors' personal views and do not represent that of their affiliated organizations, employers, or associations.

DATA AVAILABILITY STATEMENT

Not Applicable

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