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Influence of aqueous leafy stem extract of *Cochlospermum tinctorium* A. Rich. (Cochlospermaceae) on liver injury induced by subacute exposure of rats to carbon tetrachloride

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ABSTRACT

Objectives: Liver disease is a serious public health problem. There are many causes of liver disease and the liver is a vital organ in the body, so when it is damaged, its function can be affected. *Cochlospermum tinctorium* is a plant commonly used by Central African populations to relieve liver-related ailments such as jaundice and hepatitis. This study aimed to assess the hepatoprotective activity of aqueous leafy stem extract of *C. tinctorium* against liver injury induced by subacute exposure of rats to carbon tetrachloride (CCl_4).

Material and Methods: Thirty rats were distributed into six groups including control (H_2O), healthy control (H_2O), positive control (silymarin 25 mg/kg), extract control (aqueous leafy stem extract 50 mg/kg), and tests (aqueous leafy stem extract 50 or 25 mg/kg). Liver injury was induced by CCl_4 (0.5 mL/kg) on the 4th and 11th days of the treatment. Rats were sacrificed on the 15th day, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GT) activity, and serum levels of total bilirubin, creatinine, and tissue oxidative stress markers (malondialdehyde, glutathione, catalase, and superoxide dismutase) were evaluated. Histological examinations of the liver and kidney were performed. A phytochemical study of *C. tinctorium* aqueous leafy stem extract was done.

Results: This study showed that *C. tinctorium* aqueous leafy stem extract (50 or 25 mg/kg) significantly reduced ($P < 0.01$) ALT (94.79 ± 14.99 U/L) and γ-GT (10.08 ± 5.40 U/L) activity, and decreased the serum total bilirubin level compared to control. The aqueous leafy stem extract significantly diminished ($P < 0.01$) tissue MDA level (2.67 ± 0.05 µmol/mg protein), increased glutathione level (347.08 ± 10.81 nmol/mg protein), catalase (131.03 ± 6.99 µmol/min/mg protein), and SOD activity (86.0 ±1.50 U/mg). Liver microphotography showed hepatic parenchyma with almost no leukocyte infiltration in the portal and perisinusoidal spaces, and an important reduction of cell necrosis following treatment with the aqueous leafy stem extract compared to the control.

Conclusion: These results demonstrate that the hepatoprotective activity of the aqueous leafy stem extract of *C. tinctorium* may be due to its antioxidant and anti-inflammatory properties and may, therefore, justify the use of this plant as a candidate for complementary study to proceed with the development of medicine against liver diseases.

Keywords: *Cochlospermum tinctorium*, Carbon tetrachloride, Liver injury, Anti-inflammatory effect, Hepatoprotective activity, Oxidative stress

INTRODUCTION

Liver diseases are one of the leading causes of morbidity and mortality worldwide.[1] According to the World Health Organization, approximately 325 million people live with chronic liver diseases, which cause the death of more than 1 million people each year.[1] In Africa, liver diseases affect 6.1% of the population, which corresponds to approximately 60 million people, and constitute a real public health problem, since they can be classified into both communicable and non-communicable diseases.[1] Hepatitis is the cause of 10,000 deaths per year in Cameroon.[2] The term "hepatitis" refers to the inflammatory reaction of the liver mesenchyme that is observed during liver diseases and that causes denaturation of the hepatic parenchyma.[3] According to the different causes of liver diseases, a distinction is made between infectious hepatitis, linked to infectious agents (viruses, bacteria, and parasites), and toxic and iatrogenic hepatitis due to hepatic intoxication by natural, synthetic, or industrial products such as phalloides, aflatoxins, lipid, alcohol, solvents such as carbon tetrachloride (CCl_4), cleaning products, lead, and medicines.[3] These substances cause alteration or destruction of hepatocytes and thus generate an inflammatory reaction whose duration and scale explain the level of lesions observed in the liver parenchyma.[4] It is important to notice that patients are not aware of the development of non-infectious hepatitis for several reasons. First, exposure to the toxic substances that cause this form of hepatitis is unconscious, and these substances, once in the body, gradually create cellular damage. Second, the liver damage is not painful and, therefore, goes unnoticed until the signs of liver failure appear and early diagnosis techniques are not available. The damage to the liver is then irreversible. Statistics on non-infectious hepatitis are almost non-existent, although this disease is just as dangerous, and we believe that it would be more dangerous if it was associated with infectious hepatitis, which prevalence increases gradually.[5]

Most toxic substances such as CCl_4 cause tissue damage through the excessive production of free radicals. CCl_4 is metabolized by hepatocytes into pro-oxidant compounds, including the highly reactive trichloromethyl peroxyl radical (CCL_3O_2) which reacts with cell components and leads to liver injury.[6] Liver diseases require long and expensive hospital stays, which justifies the importance of early and effective treatment. Since the liver performs many vital functions for the body, a drug that would help maintain its proper functioning would go a long way toward solving liver disorder problems. We also believe that drugs which are effective at a lower dose and can be administered preventively have the best therapeutic profile.

Liver injury due to toxic substances is clinically managed with drugs such as betaine citrate, silymarin, and heparin.[4,6] Even though that these drugs bring relief to patients, their use is limited by individual patient intolerance and physiological status (pregnancy and breastfeeding), but also by the shortage and relatively high cost of care in poor or developing countries.[7]

Nearly 80% of the population in poor countries in the world are dependent on traditional medicine for their health needs.[8] This rush to use natural resources for protection against disease is justified not only by the sustainability of this approach but also by studies which show that these resources have health-benefiting biological activities. Thus, it has been reported that about 160 phytoconstituents of 101 medicinal plants have hepatoprotective activity.[9] Among these plants, we find *Coclospermum tinctorium* (Cochlospermaceae) which is a well-known plant in phytotherapy.[10-13]

The rhizome of *C. tinctorium* is used against fever, hepatitis, inflammation, abdominal pain, helminth infections, and bilharziasis.[11] In Senegal and Niger, *C. tinctorium* is used to treat liver disease, but also rickets, colic, helminthiasis, and beriberi.[14] The root of this plant is used for the treatment of hepatobiliary disorders, such as jaundice and hematuric bile fever. Jaundice is relieved by powdering the root in water or in millet beer. In case of stomach aches, a drink based on root infusion is recommended. The decoction of the root is administered against orchitis, bilharzia, and fever. Root decoction is used as a bath, rubbing, or drink against epilepsy, pneumonia, intercostal pain, bronchial affections, and generalized edemas. The root is given in instillations against conjunctivitis and as a sitz bath against hemorrhoids. It is advised to suck a root stick against indigestion and to apply locally the powder to cure snake bites. *Cochlospermum* is used in combination with other plants such as *Melanthera gambica* and *Combretum glutinosum* for the treatment of yellow fever and jaundice.[15]

Toxicological studies carried out on the cold aqueous extract of roots and leaves of *C. tinctorium* showed no signs of intoxication in animals. These studies showed that the 50% lethal dose was >5 g regardless of the type of extract.[16-18]

Many studies have highlighted various properties of this plant. Ndouyang *et al*.[10] have shown that the whole meal of *C. tinctorium* roots stimulates lipid accumulation in rats, has hypoglycemia and hypercholesterolemia effects, and also boosts the activity of the rat liver. Another study has demonstrated a strong *in vitro* radical scavenging activity of *C. tinctorium* root powder.[11]

An investigation by Diallo *et al*.[19] showed the hepatoprotective effect of the aqueous, ethanolic, and hydroethanolic extracts of *C. tinctorium* rhizome on CCl_4 and tert-butyl hydroperoxide model of liver injury. Etuk *et al*.[13,16] have shown that the cold aqueous extract of the root of *C. tinctorium* has a preventive and curative protective activity against CCl_4 intoxication in rats. Oluseyi *et al*.[12] also showed that the methanol extract

of the leaves of *C. tinctorium* has hepatoprotective and curative activity against CCl_4 intoxication in rats. Diaw[20] demonstrated that the decoction or maceration of the root of *C. tinctorium* has anabolic and choleretic effects, which would justify its exploitation for the management of jaundice due to obstruction of the bile ducts. The administration of a mixture of *C. tinctorium* root powders and *Combretum micranthum* (Combretaceae) leaves to patients suffering from hepatitis B improves their clinical condition and contributes to the reduction of transaminase levels.[21] Studies conducted by Adam *et al*.[17,18] have demonstrated the hepatoprotective effects of the cold aqueous extract of leaves and roots of *C. tinctorium* against acute exposure to CCl_4-induced hepatotoxicity. Despite these numerous studies on the various extracts from *C. tinctorium*, little is known about the effect of low doses of aqueous leafy stem extract of the plant against subacute exposure to CCl_4-induced hepatotoxicity. Therefore, this study was planned to evaluate the hepatoprotective activity of aqueous leafy stem extract of *C. tinctorium* on liver injury caused by subacute exposure of rats to CCl_4.

MATERIAL AND METHODS

Drugs and chemicals

Silymarin (Silybon-140) was purchased from Micro Labs Limited (Bengaluru, India). Chemicals such as 1,1-Diphenyl-2-picryl-hydrazyl, Folin–Ciocalteu reagent, and gallic acid were procured from Sigma-Aldrich (St. Louis, Missouri, USA). All kits for biochemical analysis were obtained from Chronolab Systems S.L. (Barcelona, Spain).

Plant material

The plant material consisted of the leafy stem (aerial part of the plant) of *C. tinctorium*, harvested in September in Moundou, the southwestern part of the province of Logone Occidental-Tchad, between 6 and 10 am. The plant was identified by Dr. Fawa Guidawa, a botanist at the University of Ngaoundere, and identification was confirmed by botanist at the National Herbarium of Cameroon, by comparison with the Letouzey R. 6680 material of the specimen of the herbarium collection n°7890 SRF/Cam. The leafy stem was cleaned with clean water, cut into small pieces, and dried in the shade for a week. Dried small pieces of leafy stem were then pulverized using a grinder and the obtained powders were kept away from light and moisture in hermetically sealed bottles.

Experimental animals

The study was carried out on 2.5-month-old male albino rats of Wistar strains, weighing between 150 and 180 g, reared at the Animal House of the University of Ngaoundere. These rats had free access to drinking water and food and were reared under natural lighting conditions (photoperiod 12/24 h), at average temperature within the animal house of 25.6°C, and an average hygrometry of 79%. They were given a standard commercial diet purchased from LANAVET (Garoua, Cameroon) and water *ad libitum*. The experiment was conducted in accordance with an institutional protocol approved by the National Ethical Committee (Reg. No FWAIRD 0001954).

Methods

Preparation of plant aqueous leafy stem extract

The leafy stem powder of *C. tinctorium* (200 g) was infused in distilled water maintained at 55 ± 5°C for 24 h in a water bath. The aqueous extract was collected by filtration of the mixture following the protocol previously described by Temdie *et al*. with some modifications.[22] Aliquots were prepared from the filtrate and were frozen at −20°C for further usage, and a small quantity of filtrate (3 mL) was evaporated in an oven (37°C) to determine its concentration (56.7 mg/mL). The yield of the leafy stem aqueous extraction was 6.26%.

Phytochemical analysis of aqueous leafy stem extract

Qualitative phytochemical investigations of aqueous leafy stem extract were performed to screen some principal families of compounds such as alkaloids, polyphenols, flavonoids, saponins, steroids, and triterpenes using standard methods previously described.[23] The total polyphenol and flavonoid contents of aqueous leafy stem extract were determined following the UV spectrophotometer standard methods previously described by Momeni *et al*.[24] and Guergouri and Zohra,[25] respectively.

Evaluation of the effects of the aqueous leafy stem extract of C. tinctorium on subacute liver injury due to CCl_4

The activity of the aqueous leafy stem extract of *C. tinctorium* was evaluated on subacute hepatotoxicity according to the protocol described by Sangare *et al*.[8] with some modifications on the frequency of injection of CCl_4 and the parameters evaluated. Briefly 30 rats were selected among healthy animals in the Animal House to form a population that was randomly divided into six homogeneous groups of five rats each. The random draw is founded on simple random sampling. This sampling technique (randomization technique) is based on the principle that all the rats in the previously constituted population have an equal probability of being part of the sample which is experimental groups in this study. Thus, five rats were randomly chosen from the population of selected rats so that each had the same probability of being selected.[26] They were fasted for 12 h and weighed. Substances were administered orally at a daily dose for 14 days:

- Group 1 and Group 2 (control and healthy control) were given distilled water (10 mL/kg);
- Group 3 (positive control) received silymarin (25 mg/kg);
- Group 4 (extract control) and Group 5 (test 50) received the aqueous leafy stem extract at 50 mg/kg;
- Group 6 (test 25) received the aqueous leafy stem extract at 25 mg/kg.

On the 4th and 11th day of treatment, hepatotoxicity was induced by intraperitoneal injection of 0.5 mL/kg CCl_4 solution mixed with olive oil (1/1) to all groups of animals except Group 2 and Group 4 (healthy control and extract control rats). Rats of these Groups 2 and 4 received an equal volume of olive oil. The animals were weighed regularly after induction of hepatotoxicity. On the 15th day of the study, all the rats were sacrificed under anesthesia (ethyl ether). Blood was collected in dry tubes and left to rest at room temperature for 5 min, then centrifuged at 5000 rpm for 10 min to obtain serum, which was stored at −20°C.[26] Livers and kidneys were carefully collected, rinsed in 0.9% NaCl, and weighed for the assessment of organs' relative weight (organ weight/body weight*100). Liver and kidney samples (0.4 g) were collected and cold grounded in a 2 mL phosphate buffer solution (1.15 M, pH; 7.4). The 20% homogenate was then centrifuged at 5000 rpm for 15 min and the supernatant was collected and stored at −20°C.[26] Further, liver and kidney samples were introduced into buffered formalin (10%) for histological studies.[27] Serum and supernatant were preserved for biochemical studies.

Analysis of biochemical parameters

The analysis of liver and kidney biomarkers such as transaminases, gamma-glutamyl-transferase, triglycerides, total protein, creatinine, total cholesterol, and total bilirubin was carried out according to the protocol of the kits Chronolab Systems (Barcelona, Spain), revised in May 2017. Oxidative stress parameters such as superoxide dismutase, malondialdehyde, catalase, and reduced glutathione were also evaluated.[28]

Histopathological analysis

Histological tests were conducted according to the protocol previously described by Hould.[29] Briefly, liver and kidney samples were removed from 10% formaldehyde, and a section of each organ was dehydrated with ethylic alcohol. After clarification with xylene, the tissue samples were soaked in paraffin (60°C) and then placed oriented in molds which were filled with paraffin and cooled to form blocks, before a 5 µm section was made. These sections were dewaxed and rehydrated before staining with hematoxylin-eosin. The stained tissues were then observed under a microscope.

Statistical analysis

The results obtained were expressed as mean ± SEM or photomicrographs. Analysis of variance was performed by the ANOVA one-way test followed by Dunnett's *post hoc* test for multiple comparisons, using GraphPad Prism software V5.03. The difference compared to control or healthy control was considered statistically significant at 5% level ($P < 0.05$) and highly significant at 1% level ($P < 0.01$).

RESULTS

Phytochemical analysis

The phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, and polyphenols in the aqueous leafy stem extract of *C. tinctorium*; steroids and triterpenes were not present in this aqueous extract [Table 1]. The aqueous leafy stem extract contained polyphenols, 40 mg EAG/g of dry extract (the equivalent of gallic acid per gram of dry extract) and flavonoids, 2.89 mg EQ/g of dry extract (the equivalent of quercetin per gram of dry extract).

Effects of the aqueous leafy stem extract of *C. tinctorium* on subacute hepatotoxicity due to CCl_4

Effects of aqueous leafy stem extract on body weight gain and relative liver and kidney weights

The results showed normal growth of the animals (healthy control) with a body weight gain of 6.92 ± 0.63% body weight on day 6 and 15.33 ± 2.10% body weight on day 14. Administration of CCl_4 (0.5 mg/kg) resulted in a significant ($P < 0.01$) loss of body mass of the animals compared to the healthy control, with negative weight gain on the 6th day (−6.19 ± 1.84% body weight) and 14th day (−2.43 ± 1.16% body weight). The administration of silymarin at 25 mg/kg did not prevent a loss of animal body mass compared to the healthy control. Treatment of the animals with the aqueous leafy stem extract of *C. tinctorium* at a dose of 50 mg/kg resulted in a slight increase in the body mass of the animals

Table 1: Phytochemical composition of the aqueous leafy stem extract of *Cochlospermum tinctorium*.

Searched compound	Results	
	Qualitative	Quantitative
Steroids	Absent	-
Triterpenes	Absent	-
Alkaloids	Present	-
Saponins	Present	-
Flavonoids	Present	2.89 mg EQ/g dry extract
Polyphenols	Present	40 mg EAG/g dry extract

EAG/g of dry extract: Equivalent of gallic acid per gram of dry extract, EQ/g of dry extract: Equivalent of quercetin per gram of dry extract

with a significant weight gain of 5.57 ± 1.54% body weight on the 9th day and 5.56 ± 0.21% body weight on the 14th day. At a dose of 25 mg/kg, the aqueous leafy stem extract caused an improvement in the body mass of the animals with a significant weight gain of 11.01 ± 1.56% body weight on the 9th and 10.85 ± 2.39% on the 14th day, compared to the control [Table 2]. When given alone to healthy animals, the aqueous leafy stem extract did not alter the growth of rats compared to healthy control within the period of treatment.

Administration of CCl_4 (0.5 mL/kg) resulted in a non-significant increase in the relative liver mass of the control (3.24 ± 0.09% body weight) compared to the healthy control (2.90 ± 0.09% body weight). Treatment of the animals with silymarin 25 mg/kg and the aqueous leafy stem extract of *C. tinctorium* (50 mg/kg or 25 mg/kg) did not influence the relative liver mass of these animals compared to both control and healthy control. CCl_4 (0.5 mL/kg) caused a significant ($p < 0.05$) increase in relative kidney mass in the control group (0.63 ± 0.01% body weight) compared to the healthy control (0.56 ± 0.01% body weight). Treatment of the animals with silymarin 25 mg/kg and aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) prevented a significant increase in the relative kidney mass of the different animals [Table 2].

Effects of the aqueous leafy stem extract of *C. tinctorium* on biomarkers of liver and kidney functions

Induction of subacute hepatotoxicity by CCl_4 (0.5 mL/kg) resulted in a significant increase ($p < 0.01$) in serum alanine aminotransferase (ALT) activity in control animals (773.21 ± 28.46 U/L) compared to the healthy control (348.10 ± 92.76 U/L). Two-week treatment of the animals with silymarin (25 mg/kg) resulted in a significant decrease ($P < 0.01$) in the activity of ALT (339.45 ± 72.11 U/L), compared to control. Administration of the aqueous leafy stem extract (50 or 25 mg/kg) resulted in a significant drop ($P < 0.01$) in ALT activity (94.79 ± 14.99 U/L or 228.20 ± 28.86 U/L, respectively) compared to the control.

Administration of CCl_4 (0.5 mL/kg) resulted in a significant increase ($P < 0.01$) in aspartate aminotransferase activity (AST) after 14 days (463.63 ± 58.89 U/L) compared to the healthy control (265.32 ± 35.92 U/L). Animals treated with silymarin (25 mg/kg) showed a significant reduction ($P < 0.01$) in AST activity (295.28 ± 21.00 U/L) compared to the hepatitis control. The results show that at the end of treatment with aqueous leafy stem extract (50 or 25 mg/kg), there was a significant reduction ($P < 0.01$) in the activity of AST (169.40 ± 10.22 or 178.62 ± 2.25 U/L, respectively). Even in healthy rats, the aqueous leafy stem extract reduced ($P < 0.01$) the activity of AST, but this effect was non-significant, in reference to the healthy control.

Hepatotoxicity induced by a preparation containing (0.5 mL/kg CCl_4 and 50% of olive oil) caused a significant increase ($P < 0.01$) in the gamma-glutamyl transferase activity (γGT) in the control group (42.44 ± 6.41 U/L) compared to the healthy control (11.79 ± 2.06 U/L). The results show a significant reduction ($P < 0.05$) in the activity of γGT (22.74 ± 4.42 U/L) after treatment with silymarin (25 mg/kg). There was also a significant decrease ($P < 0.01$) in the activity of γGT (10.08 ± 5.40 or 13.6 ± 5.92 U/L) following treatment with aqueous leafy stem extract at 50 or 25 mg/kg, respectively, compared to the control.

Induction of subacute hepatotoxicity by CCl_4 (0.5 mL/kg) resulted in a significant ($P < 0.01$) increase in total bilirubin (0.47 ± 0.01 mg/dL) compared to the healthy control (0.27 ± 0.05 mg/dL). There was a significant decrease ($P < 0.01$) in total bilirubin level (0.19 ± 0.10 mg/dL) in the silymarin-treated animals compared to the control. Results also showed a significant decrease ($P < 0.01$) in serum total bilirubin (0.26 ± 0.01 or 0.15 ± 0.08 mg/dL) in animals treated with aqueous leafy stem extract at 50 or 25 mg/kg, respectively, compared to the control.

Treatment of the animals with CCl_4 did not significantly ($P > 0.05$) alter plasma total protein level in the control (6.20 ± 0.57 mg/dL) compared to the healthy control (6.34 ± 0.52 mg/dL). Silymarin administration resulted in a

Table 2: Effects of aqueous leafy stem extract of *Cochlospermum tinctorium* on body weight gain and relative mass of liver and kidney of rats submitted to carbon tetrachloride-induced subacute liver injury.

Groups	Treatment	Change in body weight (%)			Relative Mass	
		Day 6	Day 9	Day 14	Liver	Kidney
Control	H_2O (10 mL/kg)	−6.19±1.84$^{\#\#}$	1.92±0.84$^{\#}$	−2.43±1.16$^{\#\#}$	3.24±0.09	0.63±0.01$^{\#}$
Healthy control	H_2O (10 mL/kg)	6.92±0.63**	6.44±1.22*	15.33±2.10**	2.90±0.09	0.56±0.01*
Positive control	Sily (25 mg/kg)	−4.95±0.59$^{\#\#}$	1.11±0.52$^{\#\#}$	−5.57±1.84$^{\#\#}$	3.16±0.15	0.59±0.03
Extract control	ALSE (50 mg/kg)	2.07±0.62**	11.96±1.10$^{**\#\#}$	8.90±0.75$^{**\#}$	2.86±0.09	0.61±0.02
Test 50	ALSE (50 mg/kg)	−3.27±1.44$^{\#\#}$	5.57±1.54	5.56±0.21$^{**\#\#}$	3.02±0.02	0.60±0.01
Test 25	ALSE (25 mg/kg)	0.22±2.33$^{**\#\#}$	11.01±1.56$^{**\#}$	10.85±2.39**	3.17±0.18	0.61±0.01

The values represent the means±ESM, n=05. $^{*}P<0.05$ and $^{**}P<0.01$ compared to the control. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to the healthy control.
ALSE: Aqueous leafy stem extract of *Cochlospermum tinctorium*. Sily: Silymarin

significant ($P < 0.05$) drop ($P < 0.05$) in plasma total protein level (4.42 ± 0.54 mg/dL) compared to the healthy control. Administration of the aqueous leafy stem extract alone (extract control) did not influence on protein levels. However, a significant increase ($P < 0.01$) in plasma total protein levels (8.88 ± 0.64 or 8.07 ± 0.40 mg/dL) was observed in animals treated with the aqueous leafy stem extract at 50 or 25 mg/kg, respectively, compared to both control and healthy control.

The results show that the induction of hepatotoxicity by CCl_4 (0.5 mL/kg) did not cause, after 2 weeks of treatment, any significant increase ($P > 0.05$) in plasma creatinine level (0.64 ± 0.06 mg/dL) in rats compared to the healthy control (0.55 ± 0.03 mg/dL). Treatment of the animals with silymarin (25 mg/kg) resulted in an important ($P < 0.05$) lessening in plasma creatinine level (0.34 ± 0.07 mg/dL) compared to the control. It is also noted that even as the aqueous leafy stem extract of *C. tinctorium* had no effect in healthy animals (extract control), it caused a significant decrease ($P < 0.05$) in plasma creatinine levels (0.37 ± 0.10 or 0.50 ± 0.09 mg/dL) in sick animals at 50 or 25 mg/kg, respectively, compared to the control [Table 3].

Effects of the aqueous leafy stem extract of *C. tinctorium* on total cholesterol and triglyceride levels in rats

Induction of hepatotoxicity by intraperitoneal injection of CCl_4 (0.5 mL/kg) to the animals resulted in a significant increase ($P < 0.01$) in total cholesterol concentration (85.83 ± 4.95 mg/dL) compared to the healthy control (47.22 ± 6.22 mg/dL). Treating the animals with silymarin (25 mg/kg) prevented a significant increase in total cholesterol (55.65±5.80 mg/dL) compared to the control. There was a significant decrease ($P < 0.01$) in total cholesterol levels (53.35 ± 0.40 or 52.24 ± 0.92 mg/dL) in animals treated with the aqueous leafy stem extract of *C. tinctorium* at 50 or 25 mg/kg, respectively, compared to the control [Table 4].

Induction of liver injury with CCl_4 (0.5 mL/kg) resulted in a significant increase ($P < 0.01$) in triglyceride level (80.20 ± 2.62 mg/dL) compared to the healthy control (63.13 ± 0.75 mg/dL). Animals treated with silymarin (25 mg/kg) showed a significant decrease ($P < 0.01$) in triglyceride level (65.16 ± 1.29 mg/dL) after 14 days of treatment compared to the control. Daily administration of the aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) caused a significant decrease ($P < 0.01$) in triglycerides (37.03 ± 1.38 or 32.36 ± 1.68 mg/dL, respectively), compared to both control and healthy control groups [Table 4].

Effects of the aqueous leafy stem extract of *C. tinctorium* on oxidative stress in rats

Effects on hepatic oxidative stress

The results indicate that intraperitoneal injection of CCl_4 (0.5 mL/kg) in the animals resulted in a significant increase ($P < 0.01$) in the hepatic concentration of malondialdehyde (3.14 ± 0.02 µmol/mg protein) compared to the healthy control (2.49 ± 0.01 µmol/mg protein). Daily administration of silymarin (25 mg/kg) to the animals resulted in a significant reduction ($P < 0.01$) in the hepatic concentration of malondialdehyde (2.37 ± 0.07 µmol/mg protein) compared to the control. Treatment with the aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) prevented a significant increase ($P < 0.01$) in malondialdehyde concentrations (2.67 ± 0.05 or 2.72 ± 0.05 µmol/mg protein, respectively), compared to the control [Figure 1].

Induction of hepatotoxicity by CCl_4 leads to a significant increase ($P < 0.05$) in catalase activity (103.31 ± 4.30 µmol/min/mg protein) compared to the healthy control (75.68 ± 2.38 µmol/min/mg protein). Administration of silymarin (25 mg/kg) for 14 days stimulated a significant increase ($P < 0.05$) in hepatic

Table 3: Effects of aqueous leafy stem extract of *Cochlospermum tinctorium* on biomarkers of liver and kidney function of rats submitted carbon tetrachloride-induced subacute liver injury.

Groups	Treatment	ALT (U/L)	AST (U/L)	Gamma-Gt (U/L)	Total bilirubin (mg/dL)	Total proteins (mg/dL)	Creatinine (mg/dL)
Control	H_2O (10 mL/kg)	773.21 ± 28.46$^{\#\#}$	463.63 ± 58.89$^{\#\#}$	42.44 ± 6.41$^{\#\#}$	0.47 ± 0.01$^{\#\#}$	6.20 ± 0.57	0.64 ± 0.06
Healthy control	H_2O (10 mL/kg)	348.10 ± 92.76**	265.32 ± 35.92**	11.79 ± 2.06**	0.27 ± 0.05**	6.34 ± 0.52	0.55 ± 0.03
Positive control	Sily (25 mg/kg)	339.45 ± 72.11**	295.28 ± 21.00**	22.74 ± 4.42*	0.19 ± 0.10**	4.42 ± 0.54$^{\#}$	0.34 ± 0.07*
Extract control	ALSE (50 mg/kg)	346.63 ± 27.78**	145.69 ± 15.00**	12.89 ± 7.57**	0.21 ± 0.03**	6.12 ± 0.48	0.58 ± 0.02
Test 50	ALSE (50 mg/kg)	94.79 ± 14.99**	169.40 ± 10.22**	10.08 ± 5.40**	0.26 ± 0.01**	8.88 ± 0.64**$^{\#\#}$	0.37 ± 0.10*
Test 25	ALSE (25 mg/kg)	228.20 ± 28.86**	178.62 ± 2.25**	13.60 ± 5.92**	0.15 ± 0.08**	8.07 ± 0.40*	0.50 ± 0.09

The values represent the means ± ESM, n = 05.*$P < 0.05$ and **$P < 0.01$ *compared* to the control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ in comparison with the healthy control. ALSE: Aqueous leafy stem extract of *Cochlospermum tinctorium*. Sily: Silymarin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 4: Effects of aqueous leafy stem extract of *Cochlospermum tinctorium* on total cholesterol and triglyceride levels of rats submitted to carbon tetrachloride-induced subacute liver injury.

Groups	Treatment	Total cholesterol (mg/dL)	Triglycerides (mg/dL)
Control	H_2O (10 mL/kg)	85.83±4.95$^{\#\#}$	80.20±2.62$^{\#\#}$
Healthy control	H_2O (10 mL/kg)	47.22±6.22**	63.13±0.75**
Positive control	Sily (25 mg/kg)	55.65±5.80**	65.16±1.29**
Extract control	ALSE (50 mg/kg)	42.20±1.46**	32.36±1.68**$^{\#\#}$
Test 50	ALSE (50 mg/kg)	53.35±0.40**	37.03±1.38**$^{\#\#}$
Test 25	ALSE (25 mg/kg)	52.24±0.92**	43.29±1.55**$^{\#\#}$

The values represent the means±ESM, n=05. *P<0.05 and **P<0.01 compared to the control. $^{\#}P$<0.05 and $^{\#\#}P$<0.01 compared to the healthy control. ALSE: Aqueous leafy stem extract of *Cochlospermum tinctorium*. Sily: Silymarin

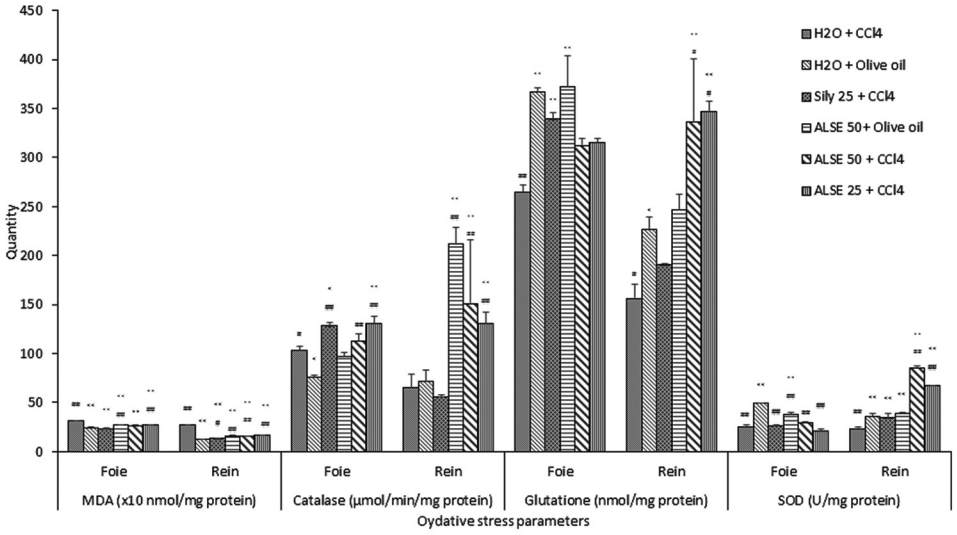

Figure 1: Effects of the aqueous leafy stem extract of *Cochlospermum tinctorium* on hepatic (a) and kidney (b) oxidative stress caused by carbon tetrachloride-induced subacute hepatitis. The bars represent mean ± ESM, n = 05. *p<0.05; **p<0.01, compared to the control. #p<0.05; ##p<0.01, in comparison with the healthy control. MDA: Malondialdehyde, SOD: Superoxide dismutase. ALSE 50 or 25 = Aqueous leafy stem extract of Cochlospermum tinctorium 50 or 25 mg/kg. Sily 25 = Silymarin.25 mg/kg.

catalase activity (128.51 ± 2.69 µmol/min/mg protein) compared to healthy and sick animals. Treatment of the animals with the aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) resulted in a significant increase (P < 0.01) in the hepatic activity of catalase (112.52 ± 7.19 or 131.03 ± 6.99 µmol/min/mg protein, respectively) compared to healthy and hepatitis controls [Figure 1]. It should also be noted that the aqueous leafy stem extract of *C. tinctorium* induced in healthy animals a slight increase in catalase activity (96.66 ± 4.71 µmol/min/mg protein).

Hepatotoxicity induced by intraperitoneal injection of CCl_4 (0.5 mL/kg) caused a significant decrease (P < 0.01) in hepatic glutathione concentration (264.71 ± 7.13 nmol/mg protein) compared to the healthy control (366.72 ± 4.40 nmol/mg protein). Animals that were given silymarin (25 mg/kg) had a significantly higher liver glutathione level (339.85 ± 6.31 nmol/mg protein) compared to the control. The aqueous leafy stem extract (50 or 25 mg/kg) administered to the animals did not significantly (P > 0.05) influence the hepatic glutathione concentration (312.65 ± 6.84 or 315.00 ± 4.32 nmol/mg protein, respectively) compared to the control, although this concentration remained relatively high [Figure 1].

The results presented in [Figure 1] show that intraperitoneal injection of CCl_4 (0.5 mL/kg) leads to a significant decrease (P < 0.01) in hepatic superoxide dismutase activity (25.67 ± 1.25 U/mg) compared to the healthy control (49.17 ± 0.26 U/mg). A non-significant increase (P > 0.05) in the hepatic activity of superoxide dismutase (26.92 ± 0.23 U/mg) was noted in animals provided with silymarin (25 mg/kg) compared to the control. Administration of the aqueous leafy stem extract (50 or 25 mg/kg) did not significantly (P > 0.05) alter the hepatic activity of superoxide dismutase (29.17 ± 1.73 or 20.96 ± 1.67 U/mg), respectively,

compared to the control. Healthy rats receiving only the plant aqueous leafy stem extract showed a significant reduction in superoxide dismutase activity (37.67 ± 1.92 U/mg) compared to the healthy control [Figure 1].

Effects on renal oxidative stress

Intraperitoneal injection of CCl_4 (0.5 mL/kg) caused inflammation that induced a significant increase ($P < 0.01$) in the renal concentration of malondialdehyde (2.75 ± 0.02 nmol/mg protein) compared to the healthy control (1.23 ± 0.01 nmol/mg protein). Treatment of the animals with silymarin (25 mg/kg) resulted in a significant decrease ($p < 0.01$) in the renal concentration of malondialdehyde (1.34 ± 0.01 nmol/mg protein) compared to the control. There were also significant decreases ($P < 0.01$) in the renal concentration of malondialdehyde (1.59 ± 0.03 or 1.68 ± 0.02 nmol/mg protein) in sick animals which received the aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg, respectively), compared to the control [Figure 1].

The results indicate that the induction of hepatotoxicity by CCl_4 resulted in a non-significant ($P > 0.05$) decrease in renal catalase activity (65.47 ± 4.15 µmol/min/mg protein) compared to the healthy control (71.42 ± 6.47 µmol/min/mg protein). Treatment of the animals with silymarin (25 mg/kg) did not significantly influence the renal catalase activity (56.26 ± 2.36 µmol/min/mg protein) compared to the control. Administration of the aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) resulted in a significant increase ($P < 0.01$) in the renal activity of catalase (151.38 ± 2.15 or 131.03 ± 6.99 µmol/min/mg protein), respectively, compared to the control and healthy control groups [Figure 1]. Treatment of healthy rats with the aqueous leafy stem extract induced a significant increase in renal catalase activity compared to the control and healthy control groups (212.63 ± 4.84 µmol/min/mg protein).

The introduction of CCl_4 (0.5 mL/kg) into the intraperitoneal cavity of the animals caused a great ($P > 0.05$) decrease in renal glutathione concentration (156.76 ± 13.68 nmol/mg protein) compared to the healthy control (226.76 ± 12.21 nmol/mg protein). Aqueous leafy stem extract of *C. tinctorium* (50 or 25 mg/kg) induced a significant increase ($P < 0.01$) in renal glutathione concentrations (336.18 ± 64.88 or 347.08 ± 10.81 nmol/mg protein, respectively), compared to control and healthy control [Figure 1].

The results show that intraperitoneal administration of CCl_4 to the animals caused a significant ($P < 0.01$) decrease in the renal activity of superoxide dismutase (23.66 ± 1.25 U/mg) compared to the healthy control (35.95 ± 2.50 U/mg) whereas administration of silymarin (25 mg/kg) for 14 days resulted in a significant increase ($P < 0.01$) in the renal activity of superoxide dismutase (35.00 ± 3.63 U/mg) compared to the control. There was also a significant increase ($P < 0.01$) in the renal activity of superoxide dismutase (86 ± 1.50 or 67.2 ±0.2 U/mg) in the animals that received the aqueous leafy stem extract (50 or 25 mg/kg, respectively), compared to the control and healthy control groups [Figure 1].

Effects of the aqueous leafy stem extract of *C. tinctorium* on inflammatory lesions due to CCl_4

Microphotography of the liver of rats treated with CCl4 shows a characteristic invasion of the portal space and the whole hepatic parenchyma in particular, by leukocyte cells. The hepatocyte parenchyma shows dilated sinusoidal capillaries, and the hepatocytes are less well organized in trabeculae, with darker nuclei, indicating pycnotic probably due to chromatin condensation, which could be due to reduced cell activity. This alteration of the cell nucleus would lead to hepatocyte necrosis [Figure 2a]. Microphotography of the liver shows normal and uniformly stained hepatocytes with one or two round nuclei.

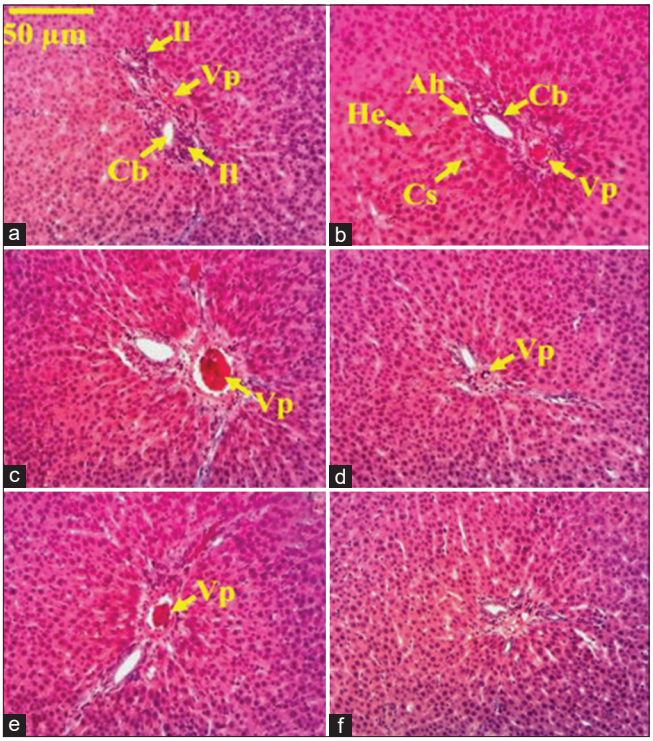

Figure 2: Microphotography of the liver showing the effects of the aqueous leafy stem extract of *Cochlospermum tinctorium* on the histological inflammatory lesions due to carbon tetrachloride. Histological sections stained with hematoxylin eosin (×100), (a) hepatitis control, (b) healthy control, (c) silymarin 25, (d) ALSE 50, (e) ALSE 25 , and (f) extract control. Vp: Hepatic portal vein, He: Hepatocyte, Cs: Sinusoid capillary, Ah: Hepatic artery, Cb: Biliary duct, Il: Leukocyte infiltration. ALSE 50 or 25: Aqueous leafy stem extract of *Cochlospermum tinctorium* 50 or 25 mg/kg. Sily 25: Silymarin 25 mg/kg.

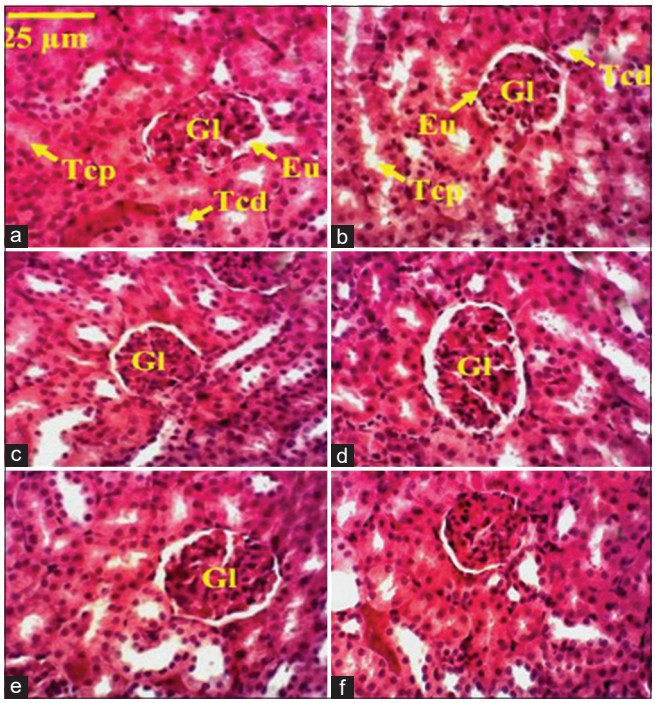

Figure 3: Microphotograph of the kidney showing the effects of the aqueous leafy stem extract of *Cochlospermum tinctorium* on the histological inflammatory lesions due to subacute hepatitis by carbon tetrachloride. Histological sections stained with hematoxylin eosin (×200), (a) hepatitis control, (b) healthy control, (c) silymarin 25, (d) ALSE 50, and (e) ALSE 25 , and (f) extract control. Gl: Glomerulus, Eu: Urinary tract, Tcd: Distal contoured tubule, Tcp: Proximal contoured tubule. ALSE 50 or 25: Aqueous leafy stem extract of *Cochlospermum tinctorium* 50 or 25 mg/kg. Sily 25: Silymarin 25 mg/kg.

These cells are well-organized in trabeculae which start from the portal space where the different vessels that make up the liver can be seen clearly. These trabeculae are separated by sinusoids [Figure 2b]. Treatment of the animals with silymarin (25 mg/kg) revealed a reduction in necrosis and leukocyte infiltration [Figure 2c]. On microphotography of the animals that received the aqueous leafy stem extract (50 or 25 mg/kg), an improvement in the histopathological parameters such as reduction of leukocytes infiltration and cells damage was observed compared to the control [Figure 2d-f].

The control group [Figure 3a] shows the onset of urinary tract damage and an important reduction in the lumen of the proximal and distal contoured tubes compared to the healthy control [Figure 3b]. No alterations were observed in animals treated with silymarin and aqueous leafy stem extract of *C. tinctorium* [Figure 3c-f].

DISCUSSION

C. tinctorium is a medicinal plant widely used in Africa, particularly in South Chad, for the treatment of parasitosis, malnutrition, and inflammatory disorders. This work aimed to evaluate the activity of aqueous leafy stem extract of this plant on CCl_4-induced subacute liver injury in rats. Plant aqueous leafy stem extract protected the animals against CCl_4-induced subacute hepatotoxicity.

Induction of hepatotoxicity with CCl_4 causes a considerable reduction in body weight gain.[30] Furthermore, the previous studies have shown that the loss of body mass can be explained by increased catabolism of lipids and proteins in the body under the toxic effect and prolonged fasting during the experiments.[31] However, pre-treatment of animals with the aqueous leafy stem extract of *C. tinctorium* showed a considerable improvement in body weight. This could be due to the ability of the plant aqueous leafy stem extract to either reduce harmful effects of CCl_4 or maintain the normal hepatic physiological process, which may have been altered by the accumulation of free radicals. These are in agreement with the results obtained by Tikare *et al*.[32]

An increase in the relative weights of the liver and kidney following treatment of animals with hepatotoxic substances can be explained by tissue hypertrophy or swelling due to the intense accumulation of substances in these target organs in response to the inflammatory process.[33] We observed a significant increase in relative mass of kidneys, and animals treated with silymarin and aqueous leafy stem extract of *C. tinctorium* showed normalization of relative liver and kidney weights. These results suggest that treatment with the aqueous leafy stem extract significantly neutralized the toxic effect of CCl_4 and, therefore, improved the general health of the treated animals, thereby supporting the anti-inflammatory effect of this extract.

Biotransformation of CCl_4 into the trichloromethyl free radical is done by cytochrome P450 isozymes of the endoplasmic reticulum or microsomes. As soon as the trichloromethyl radical is formed, it reacts instantaneously with molecular oxygen to form another highly toxic trichloromethyl peroxyl radical. Both free radicals then react covalently with proteins and lipids or remove a hydrogen atom from polyunsaturated fatty acids in the lipids of biological membranes, thereby, initiating lipids peroxidation. The propagation of these chains of events results in the breakdown of membrane structure and disruption of cell energy processes and protein synthesis which, thereafter, causes cell injury.[34,35] The hepatoprotective effect of a preparation should be based on its capability to reduce the harmful effects of hepatotoxic by preserving the physiological functions of the liver and its architecture. Excess of these radicals due to bioactivation of CCl_4 leads to the release of intracellular enzymes such as transaminases (ALT and AST) into the bloodstream.[36,37] An increase in transaminase activity is an indicator of hepatocyte destruction.[16] The results of the study show a significant increase in serum transaminase (ALT and AST) activity in

animals treated with CCl_4. This is thought to be due to the presence of hepatocellular injury. Treatment of the animals with the aqueous leafy stem extract resulted in a significant decrease in ALT and AST activities in rats intoxicated by intraperitoneal injection of CCl_4. This indicates that aqueous leafy stem extract protected the hepatocytes either by preserving the integrity and permeability of the plasma membrane, by neutralizing free radical attacks, or by inhibiting the cytochrome P450 2E1 (CYP2E1) which is implicated in the bioactivation CCl_4. It was demonstrated that the activity of CYP2E1 could be influenced by exposure to some substances. As pyrazole, acetone, or other aliphatic alcohols (methanol and ethanol) enhance CCl_4 bioactivation when compared to untreated animals, the possibility that *C. tinctorium* aqueous leafy stem extract inhibits the CCl_4-dependent hepatotoxicity may be suggested.[38] These results agree with those obtained by Oluseyi *et al*. who showed that the methanol leaf extract of *C. tinctorium* induced a decrease in transaminases.[12] The ALT and AST values lower than normal as shown by treatment with the plant aqueous leafy stem extract at the dose of 50 mg/kg are not usually seen as a concern clinically, although it can be somehow indicative of a Vitamin B6 deficiency or a kidney disease. Deep investigations are also needed to understand the mechanisms underlying these observations.

The results of the study show a significant increase in the serum gamma-glutamyltransferase activity and total bilirubin level in CCl_4-treated animals compared to healthy control. The higher serum level of bilirubin and/or the activity of gamma-glutamyltransferase are signs of obstructive jaundice and hepatic cholestasis. Both parameters are useful tools to evaluate the blockage in the biliary system.[3,39] When bile cannot flow through the normal route to be excreted out of the liver, it may leak into the blood system, especially in the presence of hepatocellular injury. Treatment with the aqueous leafy stem extract causes an appreciable reduction of serum bilirubin level and gamma-glutamyltransferase activity suggesting the efficacy of the aqueous leafy stem extract to protect liver function. In agreement with this study, Oluseyi *et al*. showed that methanol leaves extract of *C. tinctorium* decreased the level of bilirubin.[12]

The liver is the main source of protein synthesis, an abnormally low level of protein in the blood is generally associated with impaired anabolic function of the liver.[40] A non-significant reduction in total protein was observed in animals after induction of hepatotoxicity by CCl_4. This could be attributed to the decrease in protein synthesis by hepatocytes after cellular injury.[3] Similarly, creatinine levels increased under CCl_4 treatment, indicating increased protein catabolism.[41] Saba *et al*. demonstrated that an increase in blood creatinine level is due to the activation of protein catabolism during hepatotoxicity.[42] A significant increase in total protein was also observed in animals treated with the aqueous leafy extract of *C. tinctorium*. These results support those obtained by Oluseyi *et al*. who showed that the methanol leaves extract of *C. tinctorium* increases the total protein level.[12] Etuk *et al*. also reported an increase in the serum total protein and albumin levels following administration of aqueous root extract of *C. tinctorium*.[13,16] All these works strengthen the argument that this plant could be involved in the synthesis of plasma proteins. This implies the capacity of the leafy stem aqueous extract to stimulate a normal functional liver despite the toxic effects of CCl_4 on hepatocytes. There was also a decrease in creatinine levels in CCl_4-treated animals treated with the aqueous leafy stem extract of *C. tinctorium*, suggesting a decrease in protein catabolism. It would be advisable to specifically assess the quality of the secreted proteins to determine the type of protein as well as its role in the body. An increase in the quantity of proteins after induction of hepatotoxicity with CCl_4 could be attributed to a possible increase in globulins due to the heavy secretion of antibodies in response to inflammation.

The decreased creatinine level under the effect of the aqueous leafy stem extract observed in this study corroborates the findings of Saba *et al*.[42] and Messaoudi[3] who showed that the aqueous extract of the leaves of *Cnidoscolus aconitifolius* and the ethanolic extract of the aerial part of *Santolina chamaecyparissus* decreased the level of creatinine. It is known that the kidney and the central nervous system are among the target organs of the toxic effects of CCl_4.[39] The most sensitive biochemical biomarkers used to assess renal function are urea and creatinine. Elevated level of creatinine in the blood may be due to renal dysfunction.[43] Our results revealed a non-significant increase in serum creatinine level of CCl_4-treated rats compared to the healthy group. This may be due to the short duration of the study. However, the aqueous leafy stem extract prevented the elevation of creatinine level in CCl_4-treated rats, moreover, treatment of rats only with the aqueous leafy stem extract did not alter the level of this parameter, indicating that *C. tinctorium* aqueous leafy stem extract was not only protective against alteration due to CCl_4 but is also not nephrotoxic in rats during the treatment period.

Triglyceride and total cholesterol levels in blood were significantly increased in CCl_4-treated rats. A previous study has demonstrated an increase in the level of triglycerides and total cholesterol after the induction of hepatotoxicity with de CCl_4.[43] Different mechanisms have been put forward to explain this situation, notably the inhibition of lipases which would lead to a reduction in the hydrolysis of triglycerides; the lysis of hepatocytes following intoxication of rats by CCl_4 which would cause hypercholesterolemia because of a dysfunction of the lipid metabolism in the liver;[43] another mechanism may involve the possibility of disruptions in the mechanisms of association of triglycerides with the

appropriate apoprotein to form the lipoprotein carrier molecule.[44] In fact, CCl_4 reduces the activity of the enzymes such as lipoprotein lipase and triglyceride lipase and decreases the oxidation of fats, thus causing the accumulation of triglycerides in the serum and increasing the bioavailability of free fatty acids.[45] The increase in total cholesterol was thought to be due to an increase in the activity of the enzyme β-hydroxymethylglutaryl CoA, which leads to an increase in cholesterol synthesis and its massive release into the blood.[45] Treatment of animals with *C. tinctorium* aqueous leafy stem extract significantly reduced triglyceride and total cholesterol levels. These results are in agreement with those obtained by Murugesan *et al*. who showed that the ethanol extract of *Ceriops decandra* leaves decreases triglyceride and cholesterol levels in the CCl_4 model of hepatotoxicity.[46] This implies that *C. tinctorium* aqueous leafy stem extract may contain bioactive compounds with lipid-lowering properties. These bioactive compounds would inhibit intestinal absorption of dietary cholesterol or cholesterol synthesis by the liver and stimulate biliary excretion of cholesterol in the feces to reduce total cholesterol levels.[47] The significant lipid-lowering effect observed on triglycerides in the test groups is beneficial to the rats and is related to the direct capacity of *C. tinctorium* aqueous leafy stem extract to interfere with the lipid metabolism in the presence or absence of CCl_4 administration. These results are not consistent with those of Ndouyang *et al*. who found that the root powder of *C. tinctorium* increases the serum lipid levels in rats and thereby leading to a rise in the atherogenic index.[10]

Attacks by free radicals cause acute and chronic liver toxicity, leading to necrosis, fibrosis, steatosis, and cirrhosis.[35] The metabolism of CCl_4 by liver cells leads to the formation of free radicals which reduce the levels of antioxidant enzymes, increase malondialdehyde, and cause oxidative stress.[48] The results of our study demonstrated that the MDA level in the liver and renal tissue increased significantly in response to the administration of CCl_4. These effects exerted by *C. tinctorium* aqueous leafy stem extract are similar to those of silymarin, a compound extracted from the seeds of *Silybum marianum* whose hepatoprotective activity is associated with its ability to act as a free radical scavenger, and reduce membrane permeability and inflammation.[49,50] Our study shows that both substances significantly decreased hepatic and renal MDA levels thus supporting that the aqueous leafy stem extract of *C. tinctorium* can protect the liver against lipid peroxidation and oxidative chain reactions due to CCl_4 treatment.

Catalase, glutathione peroxidase, and superoxide dismutase are the main antioxidant enzymes that play a primary role in the body's defense against oxidative stress.[51] SOD limits the accumulation of superoxide anion (O^{2-}) and generates hydrogen peroxide (H_2O_2) which is involved in the formation of a hydroxyl radical by the Fenton reaction. Catalase is responsible for the elimination of H_2O_2 by converting it into H_2O and O_2. Glutathione peroxidase uses reduced glutathione as a substrate, to degrade H_2O_2 and organic hydroperoxides into harmless compounds. The role of these endogen antioxidant defense enzymes is to maintain reactive oxygen species at non-cytotoxic level.[52] Our findings show that administration of the aqueous leafy stem extract of *C. tinctorium* alone or after the intoxication of animals with CCl_4 increases the levels of glutathione and the activity of catalase and SOD. These results suggest that the hepatoprotective effect of plant aqueous leafy stem extract seems to be due to the activation of the endogenous antioxidant defense mechanisms.

Disruption of cell membranes causes leakage of intracellular substances which interact with the immune system and consequently induces an inflammatory process that increases tissue damage.[33,38] The histopathological study of liver sections from CCl_4-intoxicated rats provides evidence in favor of the previous biochemical analyses. Examination of liver sections showed that intoxication with CCl_4 caused liver lesions, such as steatosis, necrosis, and leukocyte infiltration. Treatment with *C. tinctorium* aqueous leafy stem extract reduced these lesions and significantly preserved the morphology of hepatic parenchyma by reducing cells necrosis and immune cell infiltration. This hepatoprotective activity of *C. tinctorium* aqueous leafy stem extract could be due to its antioxidant and anti-inflammatory properties.

All the hepatoprotective effects against CCl_4-induced hepatotoxicity reported in the present work may be due to the presence of bioactive compounds in the studied aqueous leafy stem extract. In fact, the phytochemical analysis revealed the presence of polyphenols, flavonoids, alkaloids, and saponins which may act individually or in combination. Moreover, some authors show that phenolic compounds mediate the expression of antioxidant enzymes that play an important role in protecting the liver against oxidative stress.[53] Flavonoids could directly protect the mitochondria, by inhibiting cytochrome P450 2E1 which is accountable for the bioactivation of CCl_4.[38] Inhibiting effects of tannins on lipoprotein lipase, triglyceride lipase, and/or β-hydroxymethylglutaryl CoA are responsible for its lipid-lowering properties.[54] At this stage, further investigations are necessary to identify and elucidate the mechanism of bioactive compounds of *C. tinctorium* aqueous leafy stem extract.

Results obtained during this study at the dose of 50 mg/kg, whether significant or not, are substantially comparable to those of the dose of 25 mg/kg except for the ALT activity. These observations could be explained by saturation; if we consider that the aqueous leafy stem extract acts in a dose-dependent way, we can suggest that at a dose of 50 mg/kg, we tend toward or we have reached the maximum effect.

However, it should be noted that plant extracts might not always have dose-dependent effects, but considering the efficacy obtained at a dose of 25 mg/kg, the preservation of the environment while harvesting the plant material, and the potential toxicity of this plant aqueous leafy stem extract, it can be envisaged that subsequent studies should be carried out at doses below 50 mg/kg.

CONCLUSION

We evaluated the hepatoprotective activity of aqueous leafy stem extract of *C. tinctorium* on CCl_4-induced subacute hepatotoxicity in rats and showed that this aqueous extract protects the liver against liver injury induced by the subacute administration of CCl_4. This was evidenced by the restoration of liver enzyme levels and the improvement of the histological structure of the liver of animals treated with CCl_4. This hepatoprotective activity could be attributed to the anti-inflammatory and antioxidant activities of secondary metabolites found in the aqueous leafy stem extract. The present work supports the use of this plant in folk medicine for the treatment of liver diseases. However, further investigations are required to understand the molecular and cellular mechanisms underlining the hepatoprotective effects observed in this work. In addition, characterization of the crude extract will be done to identify the different components as well as their proportion in the extract. Moreover, subacute and chronic toxicity studies would be performed before the clinical study.

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Ethical Approval

All authors hereby declare that experiments were conducted in agreement with the revised principles of laboratory animal care (NIH publication No. 85-23, 1985) as well as in accordance with an institutional protocol approved by the Cameroon National Ethical Committee (Reg. No FWAIRD 0001954).

Authors' contributions

All authors conceived and designed experiments, conducted the experiments, contributed to data analysis, interpretation, manuscript preparation, and approved the final version of the article.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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