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Hepatoprotective Effect of *Corrigiola telephiifolia* Pourr. Root Methanolic Extracts against CCl_4-Induced Hepatic Damage in Mice

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Abstract: Liver disease is a dysfunction that affects all or part of the liver and can lead to death. While the use of medicinal and aromatic plants has been a source of bioactive substances with hepatoprotective properties. This study aims to evaluate the hepatoprotective effect of the methanolic extract of *Corrigiola telephiifolia* roots on hepatic damage induced by carbon tetrachloride (CCl_4) in mice. Animals were treated daily with *C. telephiifolia* methanolic extract (CTME) at doses of 5 and 10 mg/kg b.w for 60 days. In addition, CCl_4 was injected (1 mL/kg, i.p.) for its hepatotoxic effects. At the end of the experiment, the blood of all animals was collected to evaluate biochemical parameters and the liver for histopathological analysis. The administration of CTME showed significant hepatoprotective activity by improving the biochemical parameters and the histological appearance of hepatic cells induced by CCl_4. Consequently, *C. telephiifolia* could be used in the prevention and/or treatment of liver intoxication.

Keywords: *Corrigiola telephiifolia*; carbon tetrachloride; hepatoprotective activity.

1. Introduction

The liver, one of the main organs involved in the metabolism of drugs and toxic chemicals [1,2], plays a central role in the kinetics of absorption, distribution, and elimination of most drugs and several active or inactive metabolites. Liver exposure to toxic chemicals and pollutants results in the formation of reactive oxygen species (ROS) associated with cellular

necrosis and many other disorders increasing in tissue lipid peroxidation and depletion in the tissue GSH levels [3].

CCl_4 is one of the most important and commonly used hepatotoxic agents in liver-related disorders' experimental study. In the liver, CCl_4 is converted into two free radicals: trichloromethyl radical (CCl_3) and proxy trichloromethyl radical ($OOCCl_3$) by cytochrome P450. It can cause severe damages to the liver, such as fatty changes, centrilobular steatosis, inflammation, apoptosis, and cell necrosis [4, 5]. Therefore, the main intercellular structures which are affected by CCl_4 are plasma membrane, endoplasmic reticulum, mitochondria, and Golgi apparatus [6]. As a result of damaging the cell membrane of hepatocytes, enzymes release in circulation [7], inducing an increase in tissue lipid peroxidation, oxidative stress, and serum levels of many biochemical markers such as transaminases, alkaline phosphatase, bilirubin, triglycerides, and cholesterol [8].

Bioactive compounds of medicinal plants showed an important role in managing oxidative stress-induced liver diseases currently and could therefore be a promising strategy to develop drugs with efficacy against these diseases. Numerous studies have shown that plant extracts having antioxidant activities protect against CCl_4-induced hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity [9- 19].

Plants of the genus Corrigiola have been shown to exhibit interesting antioxidant, anti-inflammatory and anti-diabetic activities, herbal remedies containing plants of the Corrigiola genus are part of a traditional remedy given to parturient women [20].

Corrigiola telephiifolia Pourr., (Caryophyllaceae) is a glabrous perennial (height: 80 cm) with a developed root (very fragrant in this case) and a woody, thick, pivoting stump from sandy places in the Mediterranean region, it only develops stems of 10 to 50 cm thin without rigidity, its roots are perpendicular, flowering is all year round. The plant thrives in stony and sandy pastures in the plains, low and medium mountains. It grows in sandy places, on the edges of streams, in France, Allemagne, Switzerland and in North Africa and widely spread in the Atlas Rif mountains in Morocco.

Corrigiola telephiifolia Pourr, has been used extensively in North African traditional medicine in delicate health conditions (e.g., postpartum period) and to treat various ailments such as flu, dermatological diseases, inflammation, ulcer, cough, and jaundice; it is also used as an anesthetic and a diuretic, the ingestion of a little amount of the root powder with either honey is also used by several traditional healers for the treatment of abdominal pain (stomach aches) and, chills, lung diseases and rheumatic diseases, it is considered as fortifying and appetizer [21-23]. Alternatively, they are simply used on food. Traditional Moroccan medicine still often Corrigiola species under the vernacular name of “Sarghina“.

In an earlier study, we demonstrated that *C. telephiifolia* was among the most widely used plants in traditional cancer medicine. This led us first to study anticancer activity in vitro. We demonstrated that *C. telephiifolia* extracts exert an important cytotoxic action against cancer cells CT-26 and WM-266 [24]. Indeed, the methanolic extract showed strong inhibition of cell proliferation compared to the cyclohexane and dichloromethane extracts. These results prompted us to investigate the anticancer properties of this plant extract in animal models. Scientifically, there are no valid reports available on the hepatoprotective and curative activity of this plant. Hence, the present study aimed to investigate this plant's hepatoprotective properties against CCl_4-induced hepatotoxicity in mice.

2. Materials and methods

2.1. Plant collection and extract preparation.

The collection of *C. telephiifolia* roots was carried out 4 km south of Ben Slimane city (Morocco), with the authorities' agreement and in compliance with the United Nations Convention of Biodiversity, and with the assistance of a traditional medical practitioner. A botanist authenticated the plant material from the Scientific Institute (Pr. M. Ibn Tatou) and a voucher specimen "RAB77666" was deposited at the Herbarium of Botany Department of the Scientific Institute of Rabat, Morocco. The extracts were prepared from dried roots (970 g) of *C. telephiifolia* extracted successively with cyclohexane, dichloromethane, and methanol by maceration at room temperature (22°C) over a period of 24 hours. This process was repeated three times. The extracts were then filtered through Whatman paper and the solvents were vacuum distilled in a rotary evaporator (Büchi brand). The extracts were finally dried in an oven at 30°C for 2 hours to ensure the removal of any residual solvent. All extracts were stored (−20°C) until use.

2.2. Phytochemical screening of plant extract.

The extracts were subjected to tests for secondary metabolites such as tannins, flavonoids, steroids, glycosides, cardiac glycosides, and saponins. The tests were carried out using standard methods of analysis [25].

2.3. Experimental animals.

Male Swiss mice (20–25 g) (Iffa-credo, France) aged about 8 to 10 weeks were used in pharmacological tests. Females of the same strain in the LD50 calculation were supplied from the animal experimentation center of the Faculty of Medicine and Pharmacy in Rabat. During the experiment, mice were housed in cages under standard light/dark cycle (12/12h) and temperature (22–24°C) culture conditions with free access to water and food. They were used in accordance with the directives of the Official Journal of the European Community concerning the care and use of laboratory animals.

2.4. Acute toxicity.

Median lethal dose (LD50) values were determined as described by Litchfield and Wilcoxon [26]. Seven groups of mice of both sexes (n = 10, 5 males and 5 females) received or no single oral doses at different concentrations (500, 1000, 1500, 2000, 3000, and 5000 mg/kg, body weight). Also, for the group of control, only water solution was administrated as a single dose and the mice were placed in individual clear plastic boxes to observe side effects at 6 h and at 24 h time interval (symptoms observed include the occurrence of excitement, breathing difficulty, hemorrhage, diarrhea, convulsions, sedation, and death). In the first period, the tested products were administrated to an animal at doses of 500 and 1000 mg/kg. Moreover, if no mortality was observed, the doses were increased to 1500, 2000, 3000, and 5000 mg/kg. Then, the possible mortalities and weights of mice were registered during 14 days. Finally, all used mice were sacrificed for macroscopic tissue examination [27], and then the LD_{50} was determined.

2.5. Pharmacological Evaluations

2.5.1. Antioxidant activity.

The free radical scavenging activity of the extracts of *C. telephiifolia* root parts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the method of Sánchez-Moreno [28]. 0.2 mmol l-1 solution of DPPH in methanol was prepared and 0.5 mL of this solution was added to 2.5 mL of plant extract and were allowed to stand at room temperature for 30 min, and then absorbance was read using a UV-VIS spectrophotometer at 517 nm against blank samples. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation:

$$RSA\ (\%) = \frac{A_D - A_E}{A_D} \times 100$$

where A_D is the absorbance value of the DPPH blank sample, and A_E is the absorbance value of the test solution. A_E was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

2.6. Preparation of doses and treatments.

The activity of methanolic extract from *C. telephiifolia* as a hepatoprotective plant using CCl_4-induced hepatic damage in mice. Mice have fasted for 16 hours before treatment with CCl_4. Subsequently, CCl_4 was administered intraperitoneally at a dose of 1 ml/kg with maize oil as a vehicle. The methanolic extract of *C. telephiifolia* roots was administered at doses of 5 and 10 mg/kg, which were selected based on a previous sub-acute toxicity study [24]. Cyclophosphamide (5 mg/kg) was administered to animals by gavage.

2.7. CCl_4-induced hepatotoxicity in mice.

Seven days after adaptation, the animals were randomly divided into five groups of 10 mice each and treated for 60 days as follows: the normal group received maize oil (1 mg/kg). The control group was treated with CCl_4 only at a dose of 1 mL/kg. The CCl_4-treated positive group received cyclophosphamide (5 mg/kg) four times per week. Two CCl_4-treated curative groups received *C. telephiifolia* extract at doses of 5 and 10 mg/kg/day four times a week (Figure 1). All animals were observed daily during the period of the experiment [29].

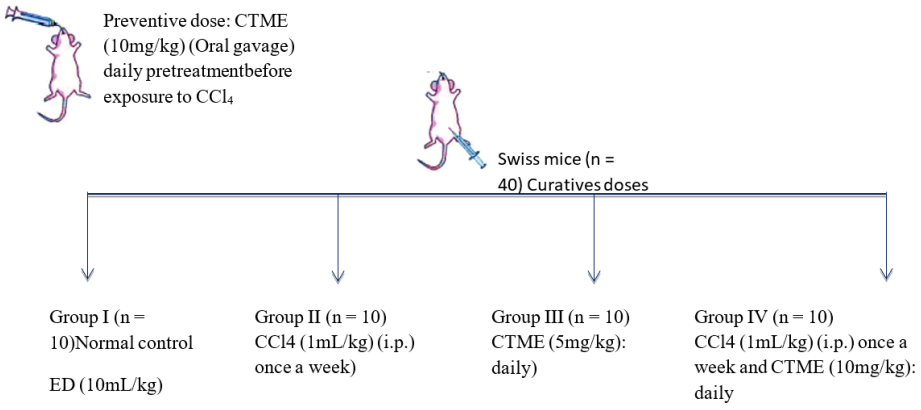

Figure 1. Experimental design for CTME treatment in the experimental mice injected with CCl_4, n: number of mice.

2.8. Biochemical parameters.

At the end of the experiment, the animals were anesthetized and sacrificed by decapitation, then the blood was collected and serum was separated. Aspartate aminotransferase (AST), serum liver enzymes such as alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were estimated in all groups and expressed as international unit per liter (UI/L) [30].

2.9. Histopathology.

After weighing, the mice livers in each group were fixed for 24 hours in 10% buffered formalin, dehydrated in increasing alcohol concentrations, clarified in xylene, and incorporated in liquid paraffin. Sections of 5 microns were performed and stained with hematoxylin-eosin. The sections were observed using a light microscope. In order to quantify the morphological changes, sections of hepatic necrosis were graded using an arbitrary scale [31]; 0 negative results (0% necrosis), 1 slight (about 20 to 30% necrosis), 2 moderate (about 50% necrosis), 3 marked (about 75% necrosis), and 4 very intense (about 90 to 100% necrosis).

2.10. Statistical analysis.

Data are expressed as means ± SD. Statistical significances between control and treated groups were determined by one-way analysis of variance (ANOVA). The differences were considered statistically significant at $p< 0.05$.

3. Results

3.1. Phytochemical screening.

The phytochemical analysis of *C. telephiifolia* Pourr. have revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenes and, quinones, the concentration of saponins were high, Steroids, terpenes and quinones were of moderate concentration, with flavonoids and tannins of low concentrations. The preliminary screening tests may be useful in the detection of bioactive principles and may lead to drug discovery and development.

Plants with alkaloids and phenolic compounds are potent antioxidants and free radical scavengers that prevent oxidative cell damage, have strong anticancer activity [32- 34], and anti-arthritic activity. Saponins are glycosides occurring widely in plants. They are abundant in many foods. Saponins are used as mild detergent and in intracellular histochemistry, staining allows antibody access to intracellular proteins. In medicine, it is used in antioxidant, anticancer, anti-inflammatory and central nervous system activities, etc. Tannins were reported to inhibit HIV replication selectively besides uses as diuretics and are recognized for their pharmacological properties.

3.2. Acute Toxicity of Corrigiola telephiifolia methanolic extract.

Following oral administration of *C. telephiifolia* extract at the doses of 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, bodyweight no toxicity and no significant changes in the body weight between the control and treated group was demonstrated at these doses (table 1). This result indicates that the LD_{50} was higher than 5000 mg/kg. The histopathological examination showed normal morphology of the animals' vital organs, no significant or pathological changes

were detected in the kidney, lung, liver and heart. Given that the LD_{50} value for this extract was beyond 5000 mg/kg for oral administration, as determined by Litchfield and Wilcoxon, our results suggest a remote risk of acute toxicity and good tolerance of this extract in traditional medicine.

Table 1. Changes in the body weights in mice treated with *C. telephiifolia* root extract in the acute toxicity study. Each point represents the mean ± SD (n = 10). No significant difference.

Days	Control	500 mg/kg	1000 mg/kg	1500 mg/kg	2000 mg/kg	3000 mg/kg	5000 mg/kg
D1	26.22 ±0.31	26.35±0.34	26.1±0.43	26.17±0.28	27.12 ±0.35	27.04 ±0.42	27.22 ±0.15
D2	26.20 ±0.25	27.42±0.32	26.13±0.44	26.21±0.21	27.15±0.41	27.13±0.44	27.25±0.21
D3	26.31 ±0.25	27.43±0.30	26.24±0.44	26.22±0.25	27.22±0.32	27.16±0.35	27.27±0.23
D4	26.41 ±0.25	27.46±0.33	26.25±0.45	26.25±0.24	27.27±0.35	27.2±0.35	27.31±0.15
D5	26.49 ±0.26	27.51±0.29	26.33±0.47	26.3±0.23	27.32±0.28	27.22±0.33	27.32±0.22
D6	26.61±0.29	27.54±0.29	26.35±0.49	26.32±0.18	27.36±0.29	27.23±0.32	27.35±0.24
D7	26.73±0.28	27.57±0.31	26.39±0.50	26.35±0.13	27.38±0.31	27.23±0.32	27.37±0.26
D8	26.79±0.27	27.60±0.28	26.42±0.21	26.37±0.25	27.41±0.26	27.24±0.34	27.4±0.25
D9	26.89±0.28	27.62±0.27	26.43±0.29	26.39±0.28	27.44±0.28	27.27±0.37	27.42±0.28
D10	26.98±0.31	28.65±0.31	26.47±0.46	27.39±0.32	28.47±0.44	28.02±0.31	28.44±0.22
D11	27.11±0.32	28.67±0.27	26.48±0.43	27.4±0.25	28.491±0.27	28.1±0.29	28.44±0.21
D12	27.27±0.35	28.68±0.32	27.13±0.46	27.42±0.22	28.55±0.23	28.12±0.27	28.46±0.25
D13	27.35±0.40	28.69±0.35	27.15±0.47	27.43±0.18	28.57±0.42	28.13±0.43	28.47±0.27
D14	27.58±0.44	28.69±0.34	27.24±0.42	27.45±0.12	28.59±0.32	28.18±0.41	28.48±0.26

3.3. Antioxidant of Corrigiola telephiifolia.

Antioxidant activity measured in plant extract obtained using DPPH assay was measured three times to test the reproducibility of the assays. The DPPH radical scavenging is a commonly used method to evaluate the ability of plant extracts to scavenge free radicals generated. From DPPH reagent Mrabtib *et al*. [35]. The DPPH scavenging activity in the extract was concentration-dependent (increasing from 31.25 µg/mL to 1000 µg/mL). The methanolic extract was able to inhibit the formation of DPPH radicals with a percentage inhibition of 82.07 ± 0.31% at the highest concentration. At this concentration, the DPPH radical scavenging capacity of the plant extract of *C. telephiifolia* was almost similar to the Trolox (96.80 %, IC_{50}=4.71 ± 0.83 µg/mL), ($P<0.001$).

3.4. Macroscopic changes.

As already mentioned, the toxicity study carried out previously facilitated the selection of the therapeutic doses of *C. telephiifolia*, which were used in the antitumor activity *in vivo* [24]. Usually, injection of CCl_4 causes necrosis, steatosis, and peripheral hepatobiliary damage. This compound is metabolized in the liver to trichloromethyl free radical ($^{\bullet}CCl_3$), which reacts with oxygen to produce the trichloromethyl peroxyl radical ($CCl_3OO^{\bullet}$), subsequently leading to the destruction of the cell membrane, centrilobular necrosis, and lipid degeneration [36]. Additionally, a study showed that intraperitoneal administration of CCl_4 inhibits the expression of several cytochromes and induces that of inducible NO synthase and TNFα [37]. In fact, in this study, the injection of CCl_4 into mice experimentally induced a solid tumor in the form of a solitary encapsulated nodule of whitish or yellowish color, which developed until the death of certain animals. This nodule was either located below the ventral surface of the liver lobes or originated from one lobe extremity. The tumor appeared as a cyst filled with necrotic material, and the tumor capsule was thick with a firm consistency.

During all of the laparotomies, we noticed heavy bleeding, a sign of portal hypertension. In addition, histological examination showed that the death of the mice was due either to pneumonia, cirrhosis with ascites, or hepatocarcinoma. Neoplastic liver nodules were

also observed in mice of both sexes with sclerosis, enlarged spleen and liver (a sign of portal hypertension), and collateral circulation. The liver became cirrhotic, the internal part of which was filled with necrotic material formed from cellular debris. The hepatic parenchyma surrounding the solitary nodule was cirrhotic. Cellular hyperplasia and extensive per lobular and dissecting fibrosis were noted.

Furthermore, CTME acted as a cytoprotective agent against induced hepatic toxicity in mice. Indeed, chronic administration (60 days) of an oral dose of 5 mg/kg/day showed moderate improvement in the general appearance of the tumor mice's liver, but the tumor persisted. While at a dose of 10 mg/kg/day for the same duration, there was a reduction in tumor induction and a significant decrease in transaminase (AST and ALT) levels compared to the control group.

3.5. Effect on body and liver weight.

As shown in table 2, the normal mice treated with plant extract (10 mg/kg) did not affect the liver weight and liver index compared with those of the normal control mice, indicating that the dose of the extract may have no liver toxicity in rats. After CCl4 administration, the liver and body weight significantly increased in mice ($p < 0.001$), indicating serious hepatomegaly that was markedly suppressed by a dose of CTME (5 mg/kg) and (10 mg/kg) ($p < 0.01$ and $p < 0.001$; respectively). Moreover, CCl_4 administration resulted in a gain of liver weight which CTME successfully countered

Table 2. Effect of *C. telephiifolia* extracts on body and liver weight of mice against CCl4 induced toxicity.

Group Treatment	Treatment	Body weight (g) (BW)	Liver weight (g) (LW)
Group I	Control	29.07±1.71	1.752±0.178
Group II	CCl4 1ml/kg (i.p.)	26.3975±1.25	1.87±0.153
Group III	*C. telephiifolia* 5mg/kg + CCl4	27.925±1.45	1.73±0.18
Group IV	*C. telephiifolia* 10mg/kg + CCl4	28.66±1.18	1.74±0.52

Values are means ± S.E.M, n = 10.

3.6. Biochemical parameters.

After the experimental induction of hepatotoxicity by CCl_4, the hepatoprotective effect of CTME in mice was first evaluated by analyzing hepatic transaminases (AST and ALT), which are enzymatic markers of hepatocyte lesions [38]. After injection of CCl_4, the levels of transaminases increased significantly compared to the control groups, indicating an enzymatic leak induced by the alteration of the hepatic cells' cellular and intracellular membranes, a sign of hepatocyte necrosis.

Compared to the control group, the ALT and AST activities in serum of mice treated with CCl4 increased significantly ($p < 0.001$) after eight weeks, reaching 60 and 190 UI/L, respectively, indicating acute hepatocellular damage. Nevertheless, the oral administration of plant extract (dose of 10 mg/kg b.w.) during intoxication showed a significant decrease in AST and ALT compared to CCl_4 only-treated mice.

The administration of the extract in different doses protected the liver and inhibited the increase of these enzymes (Figure 2). It can then be assumed that one of the bioactive compounds' sites would be located at a cell and/or intracellular membranes of hepatocytes while protecting them from the lipoperoxidation induced toxicant, subsequently limiting the secretion of ALT.

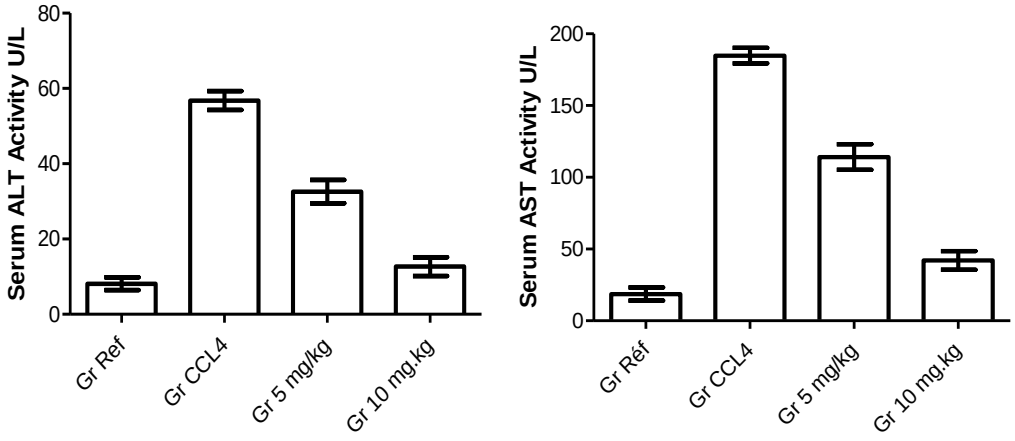

Figure 2. Transaminase values in mice before and after oral administration of CTME at different doses.

3.7. Histopathological changes.

The results of liver sections are presented in Figures 3-5. In fact, the histological observations of the mice liver treated with CCl_4 alone marked certain lesions, namely cytolytic hepatitis and cholestasis especially intralobular and more rarely periportal with hepatocyte suffering manifested in the form of ballooning, clarification, anisokaryosis, and anisocytosis. Intracytoplasmic bile deposits were also observed. In addition, CCl_4 produces liver damage identical to that caused by viral hepatitis [39].

In mice with tumors treated with different doses of CTME, it was noted that there were less biliary deposits, no necrosis foci and signs of hepatocyte suffering were less visible with rare bile deposits. These results clearly showed that CTME inhibited the onset of hepatic fibrosis. Interestingly, administration of the extract at a dose of 10 mg/kg/day resulted in a reduction in chemo-induced cirrhosis and an improvement in the general condition of the mice. The extract of this plant can prevent liver damage or lead to the reconstruction of damaged liver parenchyma. CCl_4 also alters calcium homeostasis through the degradation of transmembrane proteins carrying calcium and increases lipid peroxidation.

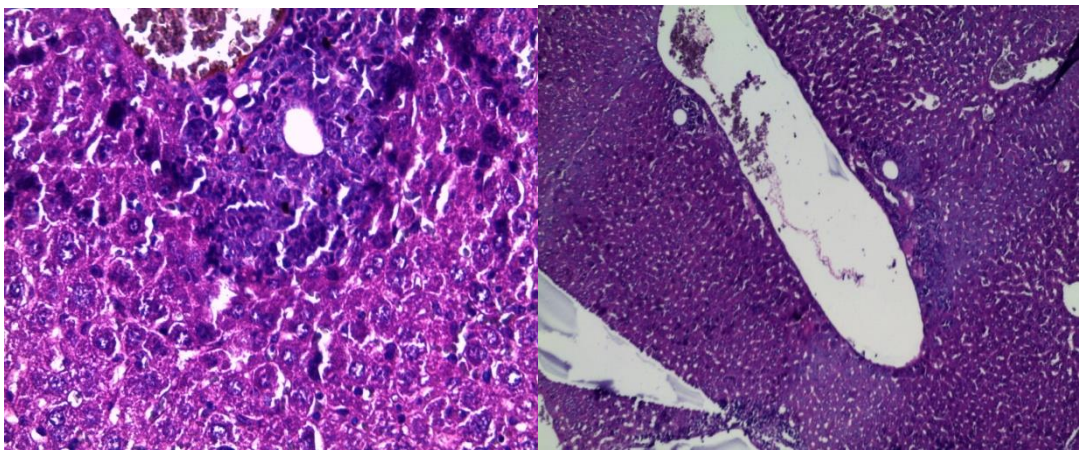

Figure 3. Histological section of mice liver treated with CCl_4 showing lesions of chronic hepatitis with centrilobular necrosis (around the hepatic veins in the center of the hepatic lobules).

Importantly, less hepatotoxicity was observed in pre-treated mice. However, the pathological hepatic lesions induced by the administration of CCl_4 were remarkably ameliorated by *C. telephiifolia* extract in a dose-dependent manner and this was in good agreement with the results of serum biochemical parameters and hepatic oxidative stress level.

The maximum protection was observed at the dose of 10 mg/kg B.W. of *C. telephiifolia* extract and the liver sections of the rats from these groups showed minor pathomorphological changes that were more similar to the control group.

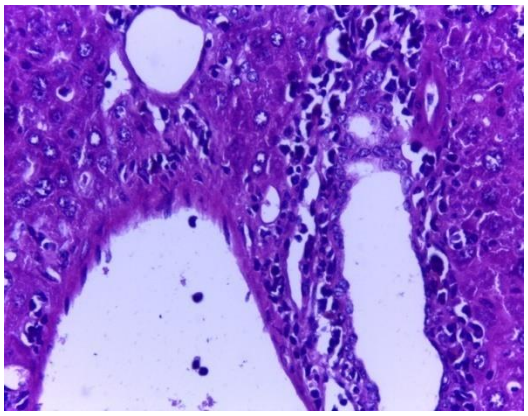

Figure 4. Histological section of mice liver treated with CTME at a dose of 5 mg/kg showing the persistence of necrosis (inflammation) around certain centrilobular veins.

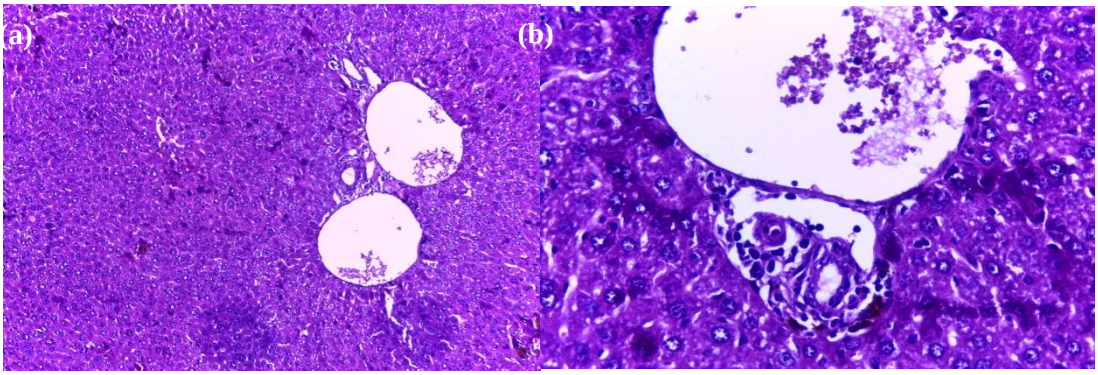

Figure 5. Histological section of mice liver treated with CTME at a dose of 10 mg/kg showing (**a**) rare isolated inflammatory cells and no interface hepatitis or perlobularnecrosis (HE x 400). (**b**) Unclarified hepatocytes with an absence of inflammatory infiltrate and intralobular necrosis (HE x 40).

3.8. Discussion.

The liver is considered the target organ and the main site of metabolism in the body and the first passage for toxins absorbed through the intestines. Hence, viral infiltration through ingestion or infection as well as consumption of toxic chemicals and drugs can cause its damage [40, 41]. The objective of using CCI_4 in our study was to assess the hepatoprotective activity of *C. telephiifolia in vivo*. Obviously, the action of this toxic substance is based on the lipid peroxidation of the cell membrane and the induction of the CCl_3 radical. This suggests that the defense mechanism of CTME involves antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which convert oxygenated molecules into non-toxic compounds [42]. In addition, CCl_4 induced hepatotoxicity characterized by an adipose liver, with cirrhosis and necrosis. This is due to the formation of intermediate reagents such as trichloromethyl free radical metabolized by P450 in the endoplasmic reticulum [43].

This is the first study to investigate the hepatoprotective effect of *C. telephiifolia* methanolic extract to the best of our knowledge. However, in a very recent study conducted by Hebi and Eddouks [44]; the evaluation of a dose (5 mg/kg b.w) administered orally of the aqueous extract of *C. telephiifolia* aerial parts for 15 days, in streptozotocin-induced diabetic rats improved the hepatic architecture of these animals compared to the control group, which corroborates perfectly with our results. Moreover, the methanolic extract of another species

(*Silene villosa*) of the Caryophyllaceae family has been evaluated for its hepatoprotective potential *in vivo*. In fact, the authors injected CCl_4 (1 mL/kg) intraperitoneally into rats to cause liver damage represented by increased levels of liver enzymes (ALT, AST, ALP, and γ-glutamyl transferase) and serum bilirubin (BRN) as well as induction of hepatic oxidative stress. Therefore, treatment of these animals with doses of 250 and 500 mg/kg b.w of the extract significantly decreased liver function biomarkers and BRN levels and increased antioxidant enzymes' activities in liver tissues in a dose-dependent manner.

Regarding histological observations, animals exposed to CCl_4 exhibited hydropic degeneration, neutrophil infiltration, and liver tissue necrosis. While treatment with the different doses of the extract reduced the severity of the signs recorded, which was in agreement with the findings of our study. Interestingly, the probable mechanisms involved in the hepatoprotective activity of *C. telephiifolia* may be attributed to its compounds having the capacity to eliminate hepatotoxic metabolites as well as to its antioxidant potential. In the same context, several studies have examined this potential using various methods [24, 45, 46]. Indeed, antioxidant activity and the inhibition of free radical production are important in preventing CCl4-induced hepatopathies. The body has effective defense mechanisms to prevent and neutralize free radicals.

DPPH and hydrogen peroxide radical scavenging assays are important tools for the assessment of the antioxidant potential of extracts [47]. In DPPH assay, the radical scavenging potential of *C. telephiifolia* extracts may be attributed to a direct role in trapping free radicals by donating electrons or hydrogen atoms. Moreover, hydrogen peroxide is involved in the generation of hydroxyl radicals, which cause further damage to the cells [48].

In CCl_4-induced liver damage, the balance between ROS production and the antioxidant defense system is disturbed due to oxidative stress, disrupting cellular functions through some events and causes liver damage and necrosis. The antioxidant activity of *C. telephiifolia* root extract against the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was consequently inhibited by the extract tested with a significative inhibition (IC50=6.03±0.43 µg/mL), which was similar to that of the reference antioxidant.

In another study, the ethanolic extract of *C. telephiifolia* aerial parts showed promising results concerning the prevention of lipid peroxidation (LP) and the scavenging capacity on free radicals such as DPPH (IC_{50}=0.673±0.061 mg/mL), ABTS (IC_{50}=0.737±0.079 mg/mL), and hydroxyl (IC_{50}=0.1792±0.0040 mg/mL) as well as the chelation capacity of metal ions (IC_{50}=0.287±0.013 mg/mL) [45]. This was in line with the findings of Hebi and Eddouks [46], who recorded an *in vitro* antioxidant activity of the aqueous extract of this plant.

On the other hand, the hepatoprotective activity of this plant may be linked to its main compounds. Indeed, triterpene saponin (TS) constitutes the most abundant molecule of *C. telephiifolia*. This, in turn, showed considerable hepatoprotective potential in numerous studies [49- 53]. Indeed, Tran and colleagues isolated and identified dammarane-type TS from *Panax vietnamensis*, which possesses hepatocytoprotective effects [49]. As a result, these molecules exhibited an important protective effect *in vitro*, against experimentally induced cell death, in mouse hepatocytes in primary cultured. In a subsequent study, the same bioactive constituents from *Panax notoginseng*, showed potent hepatoprotective activity [50]. Additionally, an Indian research team tested the possibility of using TS as a protective alternative against liver disease by evaluating *in vivo* the protective activity of these compounds from *Centella asiatica* on chemical-induced hepatotoxicity in rats [51]. Administration of TS improved several parameters indicative of severe hepatic toxicity, including increased LP, ALT, and hepatic

mRNA level of certain cytokines (TNF-α, IL-2, and IFN-γ). TS also reduced liver damage detected in histological sections. In the same year, a Chinese research laboratory extracted TS from the roots of *Glycyrrhiza inflata* in an *in vitro* study using primary rat hepatocytes damaged by a hepatotoxic substance [52]. Accordingly, the isolated molecules lowered the levels of transaminases and inhibited the activity of phospholipase A_2, considered as a target for hepatoprotective agents [54]. A year later, the same method was adopted in a Japanese study. The authors revealed an inhibition of hepatocyte injury by TS identified in *Bupleurum falcatum* roots [53]. Overall, we speculate that the hepatoprotective effect of CTME may be associated with the presence of TS and/or other phytoconstituents and also with the inhibition of cytochrome suppression and anti-inflammatory interleukin levels, and reduction of NO production. However, further investigations are needed to elucidate the exact mechanism(s) responsible for this activity and phytochemical studies to isolate the bioactive compounds involved.

4. Conclusions

Based on the present study's findings, oral administration of CTME had a favorable hepatoprotective effect against liver injury induced by CCI_4. It may be concluded from the current study that hepatoprotective activity of *Corrigiola telephiifolia* is likely due to free radical scavenging. The antioxidant effects can protect the liver against damages induced by free radicals, which are produced as the result of CCl4 metabolism. Further studies regarding the determination of bioactive compounds as well their hepatoprotection activities should be carried out. Other *in vitro* and *in vivo* studies must be carried out to validate these results and discover the mechanisms linked to this protection. The findings of this study are expected to play a vital role in the development of new and effective hepatoprotective remedies.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to declare.

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