Original Article

Hepatoprotective Effects of *Hertia cheirifolia* Butanolic Extract and Selenium against CCl_4-induced Toxicity in Rats

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Abstract

Background: *Hertia cheirifolia*, a traditional plant endemic to both Tunisia and Algeria, is used for the treatment of various disorders. This study investigates the antioxidant and protective effects of *H. cheirifolia* butanolic extract (BEHC) alone and combined with selenium (Se) against carbon tetrachloride (CCl_4)-induced liver damage in rats. **Experimental Procedure:** Thirty male Wistar rats were randomly divided into six groups: (1) normal control, (2) hepatotoxic control, (3) positive control received silymarin 100 mg/kg body weight (bw), (4) BEHC (100 mg/kg bw), (5) BEHC (400 mg/kg bw), and (6) BEHC (400 mg/kg bw) + Se (0,3 mg/kg bw) once daily for 14 consecutive days, followed by hepatotoxicity induction with CCl_4 in olive oil 0.6 mL/kg bw intraperitoneally. Some biochemical and oxidative stress parameters were investigated. Quantity and quality of phenolics in BEHC were determined by spectrophotometer and high-performance liquid chromatography with diode-array detection (HPLC-DAD) analysis, respectively. **Results and Conclusion:** BEHC contained high amounts of total phenolics and flavonoids where seven compounds were identified. The pretreatment with BEHC or with BEHC and Se significantly reduced the levels of plasma aminotransferases (alanine aminotransferase [AST] and aspartate aminotransferase [ALT]), alkaline phosphatase, malondialdehyde (MDA), and increasing glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) levels in hepatic tissues. In conclusion, BEHC has a potent natural antioxidant activity that can be used with Se to reduce hepatotoxicity.

Keywords: *Carbon tetrachloride, hepatotoxicity in rat, Hertia cheirifolia, oxidative stress, selenium*

Introduction

Carbon tetrachloride (CCl_4) is a haloalkane hepatotoxin generally employed as a solvent, cleaner, and degreaser both for industrial and home use.[1] This agent proves extremely useful as an experimental model for the study of certain hepatotoxic effects and serves to estimate the efficiency of hepatoprotectants.[2] CCl_4 is metabolized and transformed to trichloromethyl and trichloromethyl peroxy radicals through cytochrome P_{450} complex; these free radicals initiate the chain reaction of lipid peroxidation, which destroys polyunsaturated fatty acids inducing damages in different organs as well as liver, kidney, testis lung, and brain.[3]

Medicinal plants are known as an important source of new drugs. These natural resources appear interesting to develop alternative treatments. Moreover, plants are rich in antioxidants and commonly used against oxidative stress-related disorders and tissue injuries.[4] Some traditional plants have a substantial hepatoprotective effect against various experimental animal models.[5] One of these important traditional hepatoprotective drugs is Silymarin (Silybum marianum); this flavonolignan displays important hepatoprotective effects as it prevents the penetration of hepatotoxic substances, such as CCl_4 by altering cytoplasmic membrane architecture.[6] Selenium (Se) is an essential dietary trace component, which plays an antioxidant role because it is an integral part of various proteins with catalytic and structural functions. The nutritional deficiency of Se in humans leads to chronic degenerative disorders that could be prevented by the supplementation of Se when used alone or in combination.[7]

In this study, we chose the species *Hertia cheirifolia* to estimate its hepatoprotective effects against CCl_4-induced injury in the liver of rat. The genus *Hertia* which belongs to the *Asteraceae* family is distributed with its 12 species in the South and North Africa and South-West Asia.[8] In Algeria, it was found only the species *H. cheirifolia* (L).[9,10]

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This plant, which is also known as *Othonnopsis cheirifolia*, is endemic to both Tunisia and Algeria; it is traditionally used to treat inflammatory disorders, pain of stomach, diarrhea, and to reduce hyperglycemia.[9]

Previous studies showed that *H. cheirifolia* has considerable chemicals and biological activities.[10] Moreover, the methanolic, ethyl acetate, and chloroformic extracts of *H. cheirifolia* were tested for their spasmolytic and anti-inflammatory activities.[11] The antioxidant and the protective activities of *H. cheirifolia* methanol and aqueous extracts against biomolecule oxidative damages were also evaluated.[9] Lots of studies were reported about the biological properties of essential oils from *H. cheirifolia* such as the acaricidal effects,[12] antioxidant activity, and inhibitory properties against α-glucosidase.[13]

This study aimed to evaluate the potential protective effects of *H. cheirifolia* butanolic extract (BEHC) alone and combined with Se against CCl_4-induced liver oxidative stress in rats. In addition, the plant extract was analyzed by high-performance liquid chromatography with diode-array detection (HPLC-DAD) and examined for its antioxidant potential.

Materials and Methods

Extract preparation

The aerial parts of *Hertia cheirifolia* (HC) were collected from Oum El Bouaghi (East of Algeria) during the flowering period (April 2015). The plant was identified by Professor Zellagui Amar and a voucher specimen was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum Elbouaghi (Algeria) under number ZA 122. Samples were cleaned, dried in shade, and powdered using an electric milling, and then 2 kg of powdered plant was macerated three times in an ethanol or water mixture (70:30 v/v) from 48 to 72 h by renewing the solvent each time. The obtained solution was filtered, concentrated, and kept standing overnight for decantation. Using a 1-L separating funnel, we proceeded to successive liquid–liquid extractions, by four organic solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, and *n*-butanol.[14]

Total phenolics and flavonoids contents

To determine total phenolic content in BEHC in each tube, a volume of 0.5 mL (in triplicate) for each extract was added to 2.5 mL of 10% FC reagent. After 10 min of incubation at ambient temperature, the reaction medium was alkalinized with 2 mL of sodium carbonate (7.5%). All tubes were shaken and incubated for 1 h in the dark before measuring the absorbance at 760 nm by a ultraviolet (UV) spectrophotometer. The results were expressed as milligram of gallic acid equivalent per gram of extract (mg GAE/g extract).[15]

The BEHC was also analyzed spectrometrically to determine flavonoid content using quercetin (5–20 µg/mL) as standard. 1 mL of extract (1 mg/mL) was added to 1 mL of $AlCl_3$ (2%), incubated for 10 min at room temperature. Then, the absorbance was measured at 430 nm, and the results were expressed as milligram quercetin equivalent per gram of extract (QE/g extract).[16]

High-performance liquid chromatography analyses

The analysis of phenolic constituents present in the BEHC was performed using a Shimadzu reverse phase HPLC-DAD (Shimadzu Cooperation, Japan). The column temperature was set at 35°C. The separation was carried out on a Inertsil ODS-3 (4 µm, 4.0 mm × 150 mm) column and Inertsil ODS-3 guard column; mobile phases were aqueous acetic acid 0.1% (A) and methanol (B). The injected volume was 20 µL. The separation was carried out using a diode-array detector (DAD) at 254-nm wavelength. All the samples and standards were filtered with an Agilent 0.45 µm filter.[17]

Free radical scavenging activity

The antiradical activity of the BEHC was measured using the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Briefly, 39.4 mg of DPPH was dissolved in methanol to prepare 0.1 mM of solution and 4 mL of this solution was added to 1 mL of BEHC in methanol at different concentrations. The resulting solution was shaken vigorously, and after 30 min of incubation in the dark at room temperature, its absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) and butylatedhydroxylanisole (BHA) were used as standards antioxidants. The ability to scavenge the DPPH was calculated using the following formula:

Scavenging activity % = [Abs (control) – Abs (sample)]/Abs (control)× 100.

The results were expressed as half-maximal inhibitory concentration (IC_{50}) (µg/mL), which represented the concentration of extract required to cause a 50% DPPH inhibition.[18]

Experimental animals

Thirty male Wistar rats, weighing 130 ± 13 g, were used in this study. They were purchased from the breeding division of animals at Pasteur Institute located in Algiers (Algeria), and housed in plastic cages (5 animals/cage). The animals were maintained under standard laboratory conditions of constant temperature (24 ± 2°C), relative humidity (60%), 12 h light: 12 h dark cycle, and allowed free access water and standard pellet rat diet provided by National Livestock Food Board (Bejaia, Algeria). All experiments were performed according to the international guidelines. The study protocol was designed and approved by the Consultative Ethics Committee of the Biotechnology Research Centre in Constantine, Algeria (CCE-8-10-2014).

Experimental design

The 30 experimental rats were divided randomly into six groups, five rats in each group, and treated for 14 days[19] as follows:

- Group 01: Normal control—rats of this group received daily normal saline solution for 14 days, and then administered

0.6 mL/kg bw of olive oil, which served as vehicle intraperitoneally on the last day of the treatment.

- Group 02: Hepatotoxic control—rats of this group received daily normal saline solution for 14 days, followed by 0.6 mL/kg bw of CCl_4 (dissolved in olive oil v/v) by intraperitoneal injection before 24 h of the sacrifice, which is clearly documented to induce hepatotoxicity in rats.[20]
- Group 03: Standard (positive control) group—rats of this group received daily a single dose (100 mg/kg bw) of silymarin for 14 days, followed by 0.6 mL/kg bw of CCl_4 by intraperitoneal injection before 24 h of the sacrifice.
- Group 04: Based on previous studies which showed that polar extract from *H. cheirifolia* was safe and has no toxicity effects even at 2000 mg/kg.[21] Rats of this group received daily 100 mg/kg bw BEHC for 14 days, followed by 0.6 mL/kg bw of CCl_4 by intraperitoneal injection before 24 h of the sacrifice.
- Group 05: Rats of this group received daily 400 mg/kg bw BEHC for 14 day, followed by 0.6 mL/kg bw of CCl_4 by intraperitoneal injection before 24 h of the sacrifice
- Group 06: Rats of this group received daily 400 mg/kg bw BEHC associated with 0.3 mg/kg bw of Se (as Na_2SeO_3) for 14 days followed by 0.6 mL/kg bw of CCl_4 by intraperitoneal injection before 24 h of the sacrifice.

Sample collection

At the end of the experiment, rats were anesthetized with chloroform, blood samples were collected immediately from the heart into heparinized tubes, and the livers were removed rapidly, dissected, and washed to remove excess blood and cut into pieces.

Measurement of serum biochemical markers

Blood samples were centrifuged at 2000 rpm for 10 min, and then the plasma was removed immediately and stored at −20°C. The levels of plasma total protein (TP), total bilirubin (TB), cholesterol (CHOL), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured using commercially standard kits (Spinreact, Espagne).

Preparation of liver cytosolic fraction

Approximately 1 g of liver was homogenized in 3 volume of buffer solution of phosphate-buffered saline (0.1M, pH 7.4) and Kcl 1, 17%. Homogenates were centrifuged at 2000 rpm for 15 min at 4°C, and the obtained supernatant was centrifuged at 9600 rpm for 45 min at 4°C, and the resultant cytosolic fraction was used for the determination of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) levels.[22]

Measurements of oxidative stress indicators

CAT activity was estimated by the UV colorimetric method using H_2O_2 as substrate and enzymatic activity of each sample was measured in international units (IU)/mg of proteins.[23] SOD enzyme activity measurement was conducted using the oxidizing reaction of nitroblue tetrazolium (NBT). The absorbance was determined at 560 nm, and the specific activity of each sample was estimated in U/min/mg of protein.[24]

Measurement of liver GSH was performed using a colorimetric technique based on the change of a yellow color when DTNB [5,5 dithiobis-(2-nitrobenzoic acid)] is added to compounds containing sulfhydryl groups. The absorbance was recorded at 412 nm and the results were expressed as nmol GSH/mg protein.[25]

MDA content in liver was estimated using tetramethoxypropane as a standard. The absorbance was recorded at 535 nm and MDA levels were expressed as nmol MDA/mg protein.[26]

Statistical analysis and calculation

All data are expressed as mean ± standard error of the mean (SEM). Testing for statistical significance was assessed by a one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test using Statistica software (version 5.1, StatSoft, France). Values of $P < 0.05$ were considered significant. Percentages of change and improvement were calculated according to the following equations:[27]

% Change = [(mean of control − mean of treated)/mean of control] ×100%, Improvement = [(mean of disease (CCl_4) − mean of treated)/mean of control] ×100.

Results

Total phenolics and flavonoid contents

Total phenolic and flavonoid contents of *n*-butanolic extract of *H. cheirifolia* are presented in Table 1. The results showed that BEHC had a high amount of total phenolics (203.52 ± 1.81 mg GAE/g) and flavonoids (104.86 ± 0.57 mg QE/g).

High-performance liquid chromatography profiling

The results of HPLC analysis are given in Table 2. Seven compounds were detected in *n*-butanolic extract of *H. cheirifolia.* Rutin was the major compound (36.32 µg/g), followed by trans-2-hydroxycinnamic acid (5.41 µg/g), ferulic acid (3.04 µg/g), 6, 7 dihydroxycoumarin (2.28 µg/g), chlorogenic acid (2.21 µg/g), 4-hydroxybenzoic acid (0.49 µg/g), and a trace of 2, 4,-dihydroxybenzoic acid.

Free radical scavenging activity

The BEHC was found to have a higher scavenging activity of DPPH [Figure 1] with an IC_{50} of 91.60 µg/mL as compared with that of both used standard antioxidants BHT (IC_{50}= 22.32 µg/mL) and BHA (IC_{50}= 5.73 µg/mL).

Effects of *Hertia cheirifolia* butanolic extract on plasma biochemical parameters and liver enzymes

It was found that TB, ALP, AST, ALT, TG, and CHOL values increased significantly in the hepatotoxic group as compared to control ones ($P < 0.01$ for TB, and $P < 0.001$ for ALP, AST, ALT, TG, and CHOL) [Figure 2] with percentages increase of 183.33%, 449.36%, 182.77%, 283.79%, 112%, and 46.66%,

Table 1: Total phenolics and flavonoids contents in *n*-butanolic extract of *Hertia cheirifolia*

Plant extract	Total phenolics (mg GAE/g)	Total flavonoid (mg QE/g)
n-butanolic extract	203.52 ± 1.81	104.86 ± 0.57

mg GAE/g = mg of gallic acid equivalent per g of dry plant extract, mg QE/g = mg of quercetin equivalent per g of dry plant extract, SEM = standard error of the mean
Values are mean ± SEM of triplicate determinations

Table 2: Total phenolics and flavonoids compounds quantification and qualification of *Hertia cheirifolia* butanolic extract (BEHC)

Compounds (µg/g)	Retention time (min)	BEHC
4-hydroxybenzoic acid	8.01	0.49
6,7 dihydroxycoumarin	11.62	2.28
2,4,-dihydroxybenzoic acid	15.54	tr
Chlorogenic acid	17.05	2.21
Ferulic acid	19.76	3.04
trans-2-hydroxycinnamic acid	21.98	5.41
Rutin	22.01	36.32

BEHC = *Hertia cheirifolia* butanolic extract, (tr) = trace, SEM = standard error of the mean
Values are mean ± SEM of triplicate determinations

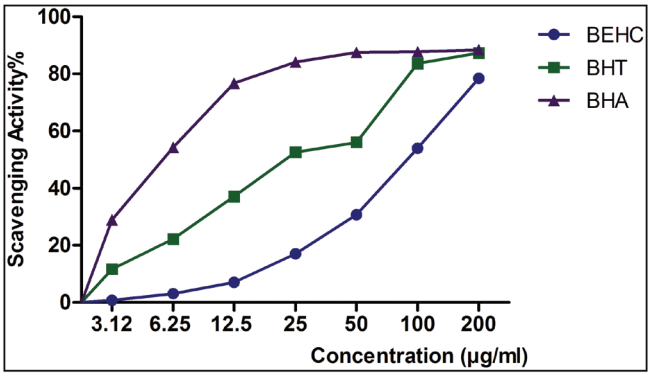

Figure 1: Free radical scavenging activity of *n*-butanolic extract of *Hertia cheirifolia* (BEHC), BHA, and BHT. Values are mean ± SEM of triplicate determinations

respectively. However, a highly significant decrease in liver function biomarkers (ALP, AST, ALT, and TB) was observed in groups treated with silymarin and BEHC with and without Se when compared with hepatotoxic control group ($P < 0.001$ for ALP, AST, and ALT; $P < 0.01$ for TB) showing a remarkable improvement. In addition, we noticed also that the pretreatment with BEHC (400 mg/kg) +Se showed a higher ameliorative effect for ALP, AST, ALT, and TB (421%, 165.96%, 217.57%, and 166.67, respectively) which was better than that shown by silymarin (288.55%, 36.22%, 127.45%, and 147.22%, respectively) [Figure 3].

In addition, TG and CHOL values decreased significantly in groups treated with silymarin and BEHC with and without Se compared to hepatotoxic group ($P < 0.01$) [Figure 2]. Moreover, marked improvement noticed in the lipid parameters (TG and CHOL) in groups treated with BEHC (100 and 400 mg/kg) with percentage of improvement recorded 30% and 88%, respectively, for CHOL and 33 and 88%for TG, respectively. However amelioration was recorded for CHOL and TG in BEHC (400 mg/kg) +Se-treated group (33.33% and 92%, respectively, compared to silymarin treated group ones (35% and 96%) [Figure 3].

Moreover, TP values decreased significantly in the hepatotoxic control group as compared to control ones ($P < 0.01$) with percentages of changes amounted 26%. However, the pretreatment with BEHC 100 mg/kg, BEHC (400 mg/kg), BEHC (400 mg/kg)+ Se and silymarin increased this parameter significantly when compared to hepatotoxic group and recorded percentages of amelioration 18.36%, 16.47%, 24.56%, and 17.5%, respectively [Figure 3].

Effects of *Hertia cheirifolia* butanolic extract on oxidative stress markers

Our results showed in hepatotoxic control group a significant increase in MDA levels compared with control ones ($P < 0.001$) with percentages of changes amounted to 347.87%. In contrast, pretreatment with BEHC (400 mg/kg) and silymarin showed a significant decrease in MDA levels ($P < 0.05$ for BEHC (400 mg/kg); $P < 0.01$ for silymarin. MDA production was reduced significantly ($P < 0.01$) when Se was combined with the plant extract as silymarin did. However, pretreatment with BEHC (100 mg/kg) showed an insignificant decrease in MDA levels [Figure 4]. Moreover, a highly marked improvement was observed in MDA level with improvement percentages of 248.94%, 178.72%, 227.66%, and 201.06% for groups treated with silymarin, BEHC (100 mg/kg), and BEHC (400 mg/kg) with and without Se, respectively [Figure 5].

Results in Figure 4 showed a significant decrease in the levels of CAT, GSH, and SOD in hepatotoxic group as compared with those of control ones ($P < 0.001$), and showed percentage of changes 69.9%, 72.25%, and 49.62%, respectively. These marked changes were accompanied with a high significant amelioration by increasing in the levels of CAT and GSH

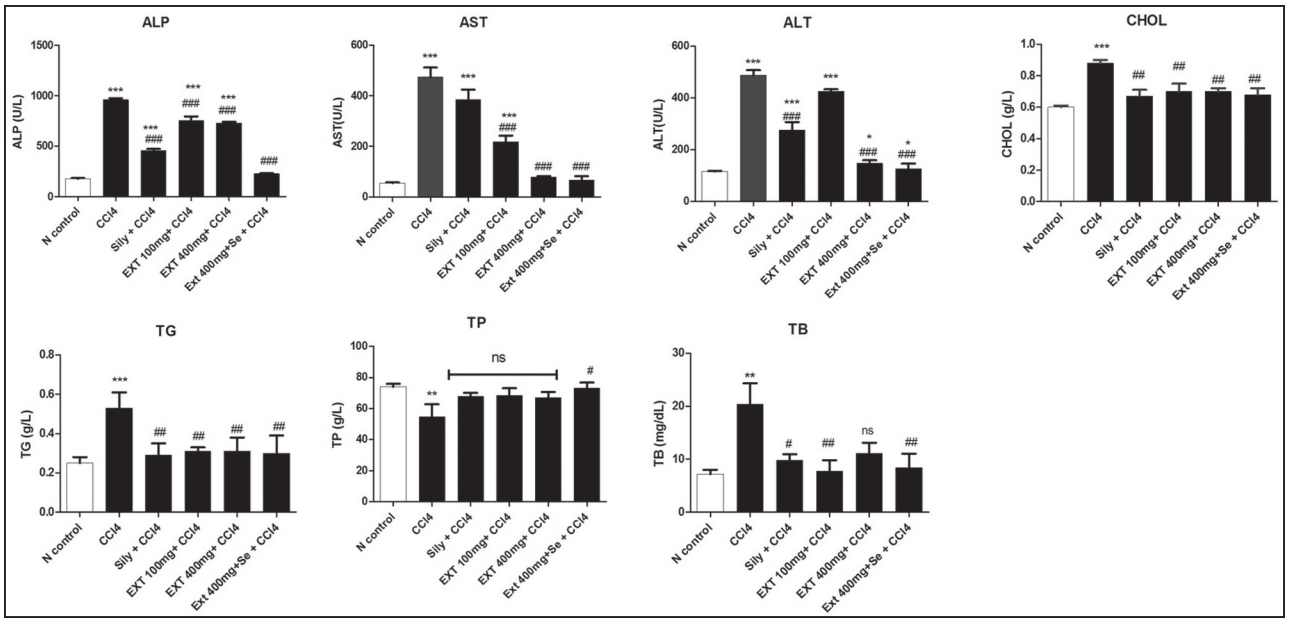

Figure 2: Effects of BEHC on plasma biochemical parameters and liver enzymes. N control = normal control, CCl4 = intoxicated control, sily+CCl4 = standard group (silymarin 100 mg/kg bw) +CCl4; EXT100 mg+CCl4; EXT400 mg+CCl4 and EXT400 mg+Se+CCl4: groups treated with *n*-butanolic extract (100 mg/kg bw+CCl4; 400 mg/kg bw+CCl4; 400 mg/kg bw+selenium 0.3 mg/kg bw+CCl4), respectively. Values are mean ± SEM, $n = 5$ animals in each group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ as compared to normal control group. #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ as compared to hepatotoxic control group

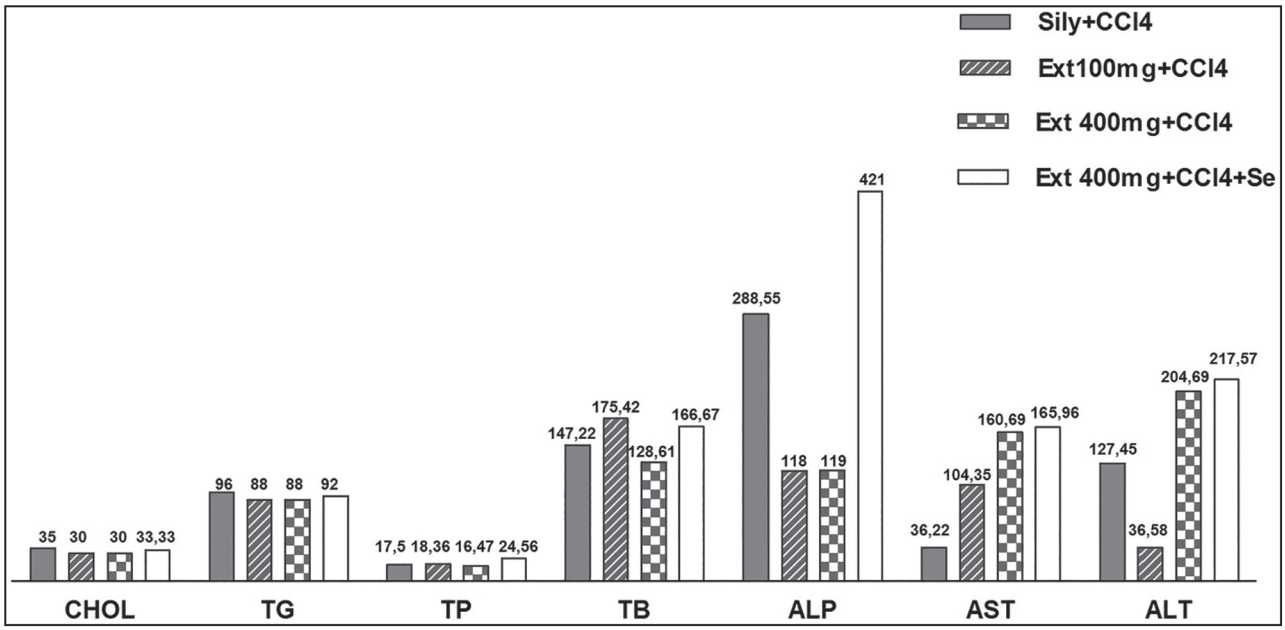

Figure 3: Percentages of improvement in different biomarkers of CCl4-intoxicated rats treated with silymarin, BEHC 100 mg/kg, 400 mg/kg, and BEHC 400 mg/kg+selenium

in groups treated with BEHC and silymarin compared to hepatotoxic group ($P < 0.05$ for BEHC (100 mg/kg); $P < 0.001$ for silymarin, BEHC (400 mg/kg) with and without Se). SOD level was significantly elevated in silymarin, BEHC (400 mg /kg) alone and BEHC (400 mg/kg) with Se groups ($P < 0.05$) as shown in Figure 4.

We observed also that percentages improvement in CAT, GSH, and SOD of BEHC 400 mg/kg+ Se (40.28%, 32.72%, and 28.84% for CAT, GSH, and SOD, respectively) were higher than those recorded in silymarin pretreatment group (37.62%, 25.87%, and 28.53%, respectively) [Figure 5].

Discussion

Several researches were carried out about chemical composition and biological properties of *Asteraceae*, but few studies on biological activities of *H. cheirifolia* were focused. This study investigated the protective effect of BEHC and Se against oxidative damages induced by CCl_4 in rats.

The free radical scavenging activity of BEHC was tested by measurement of the capacity of this extract to scavenge the stable free radical formed in solution, by donating of a hydrogen atom or an electron.[28] As indicated from the results, BEHC showed concentration-dependent free radical scavenging activity. Comparing our results with those in previous studies, BEHC showed higher scavenging activity of DPPH than the butanolic extract of Tunisian *H. cheirifolia* flowers and roots (210 ± 0.01 µg/mL) and (98 ± 0.006 µg/mL), respectively.[29,30] This capacity is probably highly related to the higher polyphenols and flavonoids contents in BEHC as shown in the results. In our previous study, a variety of *in vitro* antioxidant assays were performed to determine the antioxidant status of BEHC and the results indicated that the BEHC showed an interesting antioxidant activity when compared with those of standard antioxidants in all tests, and the highest activity was recorded in the ß-carotene-linoleic acid assay (IC_{50} = 9.99 ± 0.53 µg/mL).[31]

In fact, the HPLC analysis of BEHC showed the presence of some important flavonoids such as Rutin (quercetin-3-rhamnosyl glucoside) as the major compound, which had been reported to possess a high antioxidant and anti-inflammatory activity.[32] In another study, Rutin represented also the major constituent in methanol extract of *H. cheirifolia*.[21]

Liver is the main organ of detoxification, which plays a major role in diverse metabolisms and transformation xenobiotics into compounds with low toxicity and excretes them from the body. It has been reported that various toxic chemicals such as antibiotics, chemotherapeutic agents, and CCl_4 damage liver cells.[33]

Our results showed that the administration of CCl_4 caused liver injuries in rats, as shown by the significant increase in plasma levels of AST, ALT, and PAL. It is well known that the activity of AST, ALT, and PAL enzymes in plasma reflect the dysfunctional activity of the liver, and they are mainly

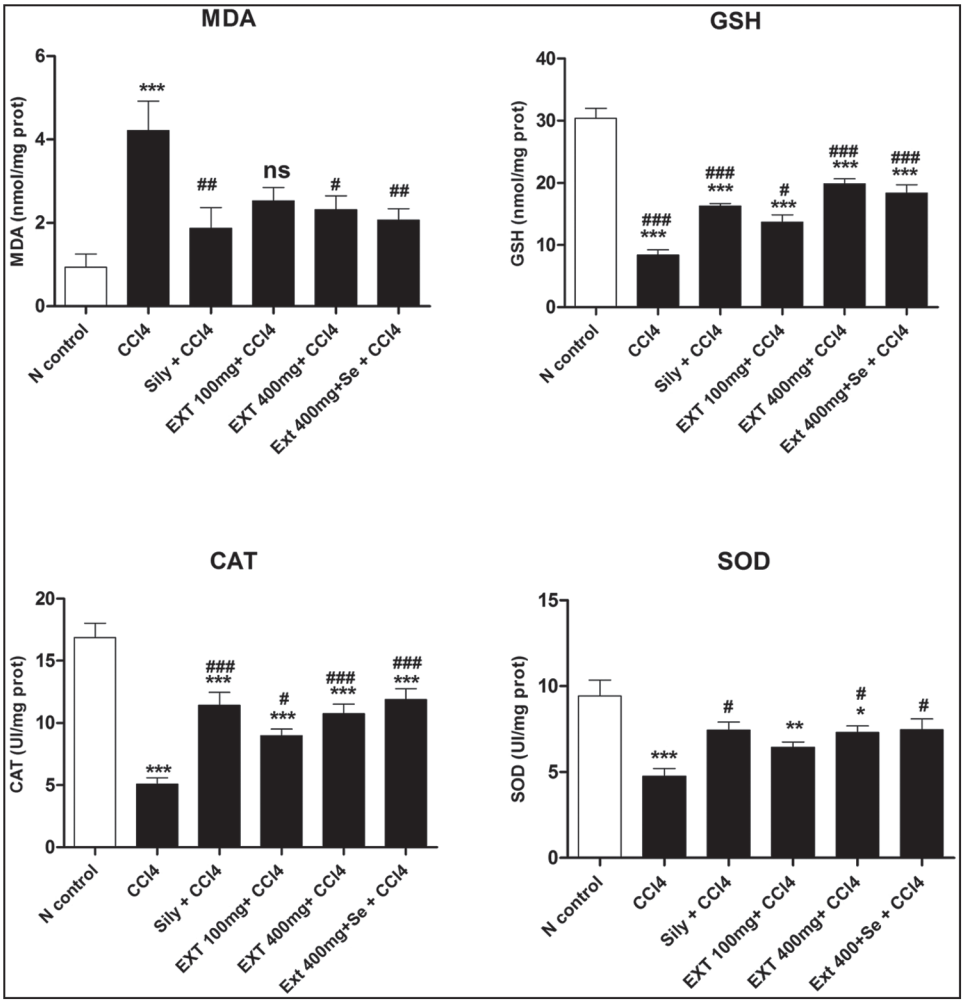

Figure 4: Effects of BEHC on plasma biochemical parameters and liver enzymes. N control: normal control; CCl4: intoxicated control; sily+CCl4: standard group (silymarin 100 mg/kg bw) +CCl4; EXT100 mg+CCl4; EXT400 mg+CCl4 and EXT400 mg+Se+ CCl4: groups treated with *n*-butanolic extract (100 mg/kg bw+CCl4; 400 mg/kg bw+CCl4; 400 mg/kg bw+selenium 0.3 mg/kg bw+CCl4), respectively. Values are mean ± SEM, *n* = 5 animals in each group. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 as compared to normal control group. #*P* < 0.05, ##*P* < 0.01, and ###*P* < 0.001 as compared to hepatotoxic control group

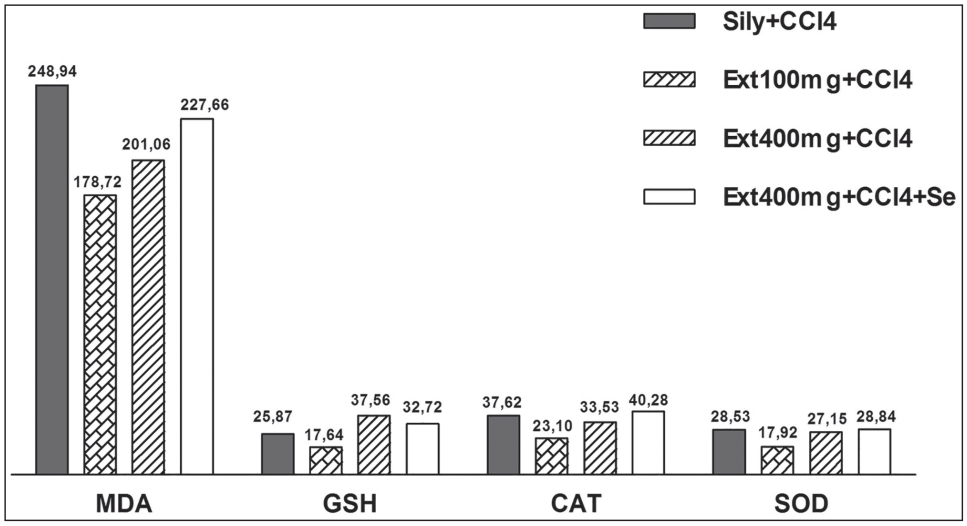

Figure 5: Percentages of improvement in MDA, GSH, CAT, and SOD of CCl4-intoxicated rats treated with silymarin, BEHC 100 mg/kg, 400 mg/kg, and BEHC 400 mg/kg + selenium

used in the assessment of liver damage and dysfunction.[34] This increase in the enzymatic activity was confirmed also by previous reports on CCl_4-induced liver damage.[2,5,6] This result might be due to the change of the plasma membrane permeability and consequently the leakage of enzymes from the tissue to the plasma or due to the onset of liver necrosis.[35] According to previous investigation, extracts of *H. cheirifolia* were regarded as being safe or practically nontoxic by oral route at doses greater than 400 mg/kg b w.[36] In our study, treatment with BEHC at the dose of 100 mg/kg, and 400 mg/kg with and without Se improved the deleterious effects of CCl_4 in decreasing significantly the levels of enzymes compared with hepatotoxic control group especially. However, the pretreatment with BEHC (400 mg/kg) accompanied with Se showed higher ameliorative effect which was better than that showed by silymarin.

Se is a trace element with a great importance for health and its deficiency can lead to several diseases. It is essential for normal metabolic processes, as well as the metabolism of thyroid hormones, antioxidant defense and immune function.[37] The protective effects of Se appeared to be mainly associated in selenoenzymes, which are known to protect several cellular components against oxidative damage.[38] In agreement with these results; a previous study showed that Se has protective effects against hepatotoxicity induced by CCl_4.[39]

Liver has a fundamental role in the metabolism of lipids, glucides, and proteins. This study has also revealed that the CCl_4 injection-induced liver metabolic disorders including the destruction of lipid synthesis. In fact, data presented a highly significant increase in plasma levels of CHOL and TG. The increased esterification of fatty acids, inhibition of fatty acid β-oxidation, and decreased excretion of cellular lipids could explain the rise in CHOL levels.[40] CCl_4 stimulates the transport of acetate to liver cells that enhance CHOL synthesis. It also increases fatty acids and TG synthesis from acetate and increases lipid esterification.[1] In addition, inhibition lysosomal lipase activity and very low density lipoprotein (VLDL) secretion may cause accumulation of TG in the liver.[41] However, pretreatment with BEHC and Se decrease significantly plasma levels of CHOL and TG lessening the CCl_4-induced liver injure owing to their capacity to inhibit the damaging effects of reactive oxygen species (ROS) and avoid low density lipoprotein (LDL) and cell membrane oxidation. On the contrary, this action causes the decrease of acetate transfer to liver cells and diminishing the synthesis of CHOL, free fatty acids, and TG.[19]

Bilirubin is a metabolic waste product formed from the destruction of aged or abnormal erythrocytes.[42] Significant elevated level of plasma bilirubin in CCl_4-treated group is possibly because of its leakage from hepatocytes to plasma, generally due to the hepatic obstruction to bile outflow and cholestasis.[43] In addition, CCl_4 also significantly reduced plasma TP probably causing endoplasmic reticulum destabilization and destruction of protein synthesis.

But the treatment of BEHC associated with Se well-improved plasma levels of TB and TP through stabilizing biliary obstruction and resynthesizing protein. These findings suggest that the combination of BEHC with Se is effective to stabilize CCl_4-induced liver dysfunction in rats.

Oxidative stress dysfunction results from free radicals and reactive oxygen species; these reactive molecules are involved in many physiological processes and human diseases, such as cancer, aging, arthritis, Parkinson's syndrome, ischemia, and liver injury. MDA level is the primary measure of oxidative damage in liver. Toxic radicals as the superoxide radical and also trichloromethyl and trichloromethyl peroxy destabilize cell membranes induced lipid peroxidation.[44,45] Our data revealed that CCl_4 treatment significantly increased the level of MDA in liver tissues. These findings are in line with a previous

report which revealed that CCl_4 led to an increase in lipid peroxidation.[35] A significant amelioration of MDA levels was observed in groups treated with BEHC at the dose 400 mg/kg and BEHC associated with Se. These results could be explained by the potential activity of rutin, ferulic acid, and chlorogenic acid that were present in plant extract as found by HPLC analysis. These three components are known for their capacity of quenching free radicals and diminishing lipid peroxidation and therefore acting as potential therapeutic agents.[46,47]

Antioxidant enzymes such as GSH, CAT, and SOD are considered as the first line of cellular defense against oxidative damage. In this study, the treatment of rats with CCl_4 decreased the antioxidant activity of these enzymes by decreasing their levels in the hepatic tissues of the rat. Our results are similar to other reports that showed that CCl_4 causes a decrease in antioxidants enzymes and an increase in lipid peroxidation.[19,33] Pretreatment with BEHC at the dose of 400 mg/kg associated with Se ameliorated the deleterious effects of CCl_4 by increasing significantly the levels of oxidative enzymes compared with the normal control group; this antioxidant effect of BEHC was probably related to its capability to reduce the accumulation of free radicals.

The current research is the first one to show the potent antioxidant and protective effect of BEHC in association with Se against oxidative damage *in vivo*.

Conclusion

The present investigation proves that BEHC combined with Se prevented CCl_4-induced liver damage in rats and significantly reduced oxidative stress by increasing antioxidant enzymes activities, decreasing the level of MDA, and preventing the increase in plasma aminotransferases levels. Therefore, due to its higher polyphenol content and potent antioxidant activities, *H. cheirifolia* confirmed its reliability with Se as a hepatoprotective plant to rat against CCl_4-induced liver damage.

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Conflicts of interest

There are no conflicts of interest.

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