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Nephroprotective Effect of *Hertiacheirifolia* Polar Fraction with Selenium against Carbon Tetrachloride in Rats

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ABSTRACT

Various types of research on the biological activities and chemical components of *Asteraceae* were performed, but few kinds of studies on *in vivo* bioactivity of*Hertiacheirifolia*were focused. The objective of the current investigation was to evaluate the potency of *H. cheirifolia*butanol extract (BEHC) alone and in combination withselenium (Se) to protect rats' kidneys from carbon tetrachloride (CCl_4)-induced damage. The plant extract was given orally at doses of 100 and 400 mg/kg, then biochemical and oxidative stress parameters were investigated after two weeks experimental period. Results showed that BEHC contained the highest amounts of polyphenols and administration of CCl_4 induced kidney damage by increasing significantly the levels of urea and malondialdehyde and decreasing GSH, CAT, and SOD levels in renal tissues. Moreover, CCl_4 provokes histopathological kidney lesions. However, pretreatment with BEHC and Se has protected rats against CCl_4 toxicity, significantly reduced oxidative stress, improved renal histological aspects, and ameliorated the biochemical parameters. Due to its potent natural antioxidant activity, the n-butanol extract of *H. cheirifolia* can reduce nephrotoxicity when combined with selenium.

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Keywords: carbon tetrachloride,*Hertiacheirifolia*, nephrotoxicity, oxidative stress, rats, selenium.

Introduction

The kidney has the main role in filtering metabolic waste products and toxic compounds in the blood. This function involves tubular structure and counter-current on the medulla, which is responsible for 25% of the body's circulating blood reabsorption. (Weiner *et al*. 2015). This organ is susceptible to injury from toxic substances in the blood because of the high percentage of filtered blood. (Priyo*et al.* 2018**)**. The main cause of acute kidney injury is chemical and drug-induced nephrotoxicity, which can result from tubular cell toxicity, microangiopathy, rhabdomyolysis, altered intraglomerular hemodynamics, and inflammation. It is acknowledged as the primary contributor to mortality and morbidity. (Shahbazi*et al.* 2012). One of these chemicals is carbon tetrachloride (CCl_4). It is a haloalkane that is frequently used in both industrial and home use as a solvent, cleaner, and degreaser. (Weber *et al*.2003). This substance acts as a very useful experimental model for the investigation of various hepato-nephrotoxic effects and estimates the effectiveness of hepato-protectants (Desai *et al*. 2012). Through the cytochrome P450 complex, CCl_4 is metabolized and converted into trichloromethyl and trichloromethylperoxy radicals, which start a chain reaction that causes lipid peroxidation and damages several organs, including the liver, kidney, brain, lung, and testis (Zhou *et al*. 2010). The bioactive phytochemical components of plants have therapeutic utility for treating oxidative stress-related diseases and tissue damage (Dzoyem and Eloff 2015). A variety of natural sources are being researched to develop new functional component formulations (Priyo*et al*. 2018**)**.

Hertiacheirifolia, which belongs to the *Asteraceae*family, is found throughout South and North Africa as well as South-West Asia(Akhgar et al. 2012). There are twelve species of the genus *Hertia* but in Algeria, it was found only the species *H. cheirifolia*. This endemic plant, also known as *Othonnopsischeirifolia*, is used to cure stomach pain, diarrhea, inflammation, and hyperglycemia (Kada*et al*. 2016).

Numerous research has investigated the biological activities of *H. cheirifolia*'s extracts, including their antioxidant activity, acaricidal effects, and α-glucosidase inhibitory properties(Attia*et al*. 2012; Majouli*et al*. 2016). Butanol extract of *H. cheirifolia*was assessed for its antioxidant

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activity and protective effect against liver and heart mitochondrial oxidative stress in rats and it was tested also for its hepatoprotective effect with Se against CCl_4-induced liver damage in rats (Menakh*et al*. 2020; Menakh*et al*. 2021).

The current study aims to evaluate the potential protective effects of *H. cheirifolia*butanol extract (BEHC) alone and combined with selenium (Se) against carbon tetrachloride (CCl_4) induced nephrotoxicity in rats.

Materials and methods

Extract preparation

Hertiacheirifolia aerial parts were harvested in Oum El Bouaghi (East of Algeria) during the flowering stage. The identification of the plant was confirmed by Professor Zellagui Amar, and a voucher specimen was deposited at the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben MhidiOumElbouaghi (Algeria), with the reference number ZA 122. Two kilograms of the powdered plant were then macerated three times for 72 hours in an ethanol/water mixture (70:30 v/v) after being cleaned, dried in the shade, and powdered using an electric mill. The resulting solution was concentrated, filtered, and allowed to stand overnight for decantation. We carried out sequential liquid-liquid extractions using four organic solvents of increasing polarity using a separating funnel: petroleum ether, chloroform, ethyl acetate, and *n*-butanol(Devgun et al. 2010; Melakhessou et al.2018).

Total phenolics and flavonoids contents

A volume of 0.5 mL (in triplicate) for each extract was added to 2.5 mL of 10% FC reagent to calculate the total phenolic content in BEHC. After 10 min at room temperature, 2 mL of sodium carbonate (7.5%) was added to the reaction media to alkalinize it. All tubes were shaken and allowed to sit for an hour in the dark before a UV spectrophotometer was used to measure the absorbance at 760 nm. The results were given as milligrams of gallic acid equivalent per gram of extract (mg GAE/g Ext) (Wong et al. 2006).

The flavonoid content of the BEHC was also determined by spectrometric analysis using quercetin (5–20 g/mL) as a reference. AlCl3 (2%), 1mL of extract, and 1mL of the mixture were incubated for 10 minutes at room temperature. The results were represented as milligrams of quercetin equivalent per gram of extract (QE/g extract) after the absorbance was measured at 430 nm. (Miliauskas et al. 2004).

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Experimental Animals

In the current investigation, 30 male Wistar rats weighing 130±13 g were employed. They were purchased from the Pasteur Institute's animal breeding division in Algiers (Algeria), where they were confined in plastic cages with five animals each. The rats were kept in a typical laboratory setting with 12 hours of light and 12 hours of darkness, constant temperature (24±2°C), relative humidity (60%) and free access to water, and a standard pellet rat diet provided by the National livestock food board. International guidelines were followed in the experiment. The Biotechnology Research Centre's Consultative Ethics Committee in Constantine, Algeria, designed and approved the study protocol.

Experimental design

The thirty experimental rats were randomly distributed into six groups of five rats each, and they were treated for 14 days, according to Mahmoodzadeh*et al*. (2017). Group I represented normal control, received daily normal saline solution, and then administered 0.6 ml/kg b.w. of olive oil which served as a vehicle intraperitoneally on the last day of the treatment. Group II served as the hepatotoxic control group, receiving daily saline solution followed by an intraperitoneal injection of 0.6 mL/kg b.w. of CCl_4 (dissolved in olive oil v/v) before 24 h of the sacrifice, which is known to cause toxicity in rats. (Douhri*et al*. 2014). Group III and IV received 100mg/kg b.w. of silymarin and BEHC respectively followed by 0.6 mL/kg b.w. of CCl_4 by intraperitoneal injection before 24 h of the sacrifice. Group V received 400 mg/kg b.w. of BEHC and Group VI received the same dose of BEHC associated with 0.3mg/kg b.w. of Se (as Na2SeO3), at the last of the treatment these groups were injected intraperitoneally by 0.6 mL/kg b.w. of CCl_4

Sample collection

After sedation with Chloroform,rats were euthanized and blood was withdrawn carefully from the heart via injections, and the kidneys were quickly removed, dissected, and washed to eliminate extra blood before being cut into pieces.

Preparation of kidney cytosolic fraction

To determine the levels of CAT, SOD, GSH, and MDA, one g of a kidney sample was homogenized in three volumes of buffer solution of phosphate-buffered saline (0.1M; pH 7.4) and Kcl 1, 17% then homogenates were centrifuged at 2000 rpm for 15 min at 4°C, and the resulting supernatant was centrifuged at 9600 rpm for 45 min at 4°C, and the obtained cytosolic fraction was then used (Iqbal*et al*. 2003).

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Measurement of serum biochemical markers

After a blood samples centrifugation at 2000 rpm for 10 min, plasma was immediately collected and kept at 20°C. Utilizing commercially available standard kits(Spinreact, Espagne), the levels of plasma glucose (GLU), urea (UR), and creatinine (CRE) activities were determined.

Measurement of oxidative stress indicators

To determine the amount of Malondialdehyde (MDA) present in the kidney, tetramethoxypropane was used as a standard and the absorbance was measured at 535 nm MDA levels were represented as nmol MDA/mg protein (Ohkawa et al. 1979).

A colorimetric method based on the yellow color change that occurs when substances with sulfhydryl groups are introduced to DTNB (5.5 dithiobis-2-nitrobenzoic acid) was used to measure kidney glutathione (GSH)levels. At 412 nm, the absorbance was measured, and the results were reported as nmol GSH/mg protein (Ellman 1959).

According to Claiborne (1985), Catalase (CAT) activity was evaluated by the UV colorimetric method using H_2O_2 as substrate, and the enzymatic activity of samples was measured in International Units (IU) / mg of proteins.

Measurement of the Superoxide dismutase (SOD) levels was performed utilizing the oxidizing reaction of nitrobluetetrazolium (NBT). At 560 nm, the absorbance was recorded, and each sample's specific activity was determined in U/min/mg of protein. (Beauchamp and Fridovich 1971).

Histopathological studies

Renal tissues were excised, washed with normal saline, fixed in 10% neutral formalin, dehydrated in graded alcohol, and then implanted in paraffin. Thin sections (4 - 5 mm) were cut and stained with routine hematoxylin-eosin(Danish et al. 2015)**.** The severity grades were assessed based on the extent of the morphologic change in the examined tissue sections to be clearly defined in the pathology report for critical lesions impacting study interpretation(Gibson-Corley et al 2013).

Statistical analysis and calculation

Using Statistica software (Version 5.1, StatSoft France, 1997), a one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to determine whether the results were statistically

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significant. Mean ± standard error of the mean (S.E.M) are used to express all data. Accordingto the following equations, change and improvement percentages were calculated (Ibrahim et al. 2018):

% 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 flavonoids contents

Data presented in **(Table 1)** showed that the highest total phenolic content (TPC) was detected in the *n*-but extract (203.52 ± 1.81 mg GAE/g) followed by EtAcO extract (202.06±4.34 mg GAE/g) and $CHCl_3$ extract (43.88±1.11 mg GAE/g). A similar trend was observed in the flavonoid content (FC), with the highest levels detected in *n*-butanol extract (104.86 ±0.57 mg QE/g) followed by EtAcO extract (35.61±0.58 mg QE/g) and $CHCl_3$ extract (13.69±0.76 mg QE/g).

Table 1. Total phenolics and flavonoids contents of *H. cheirifolia* extracts

Extract	Total phenolics (a)	Flavonoids (b)
Chloroform extract	43.88±1.11	13.69±0.76
Ethyl acetate extract	202.06±4.34	35.61±0.58
***n*-butanolic extract**	203.52±1.81	104.86±0.57

Results are expressed as means ± standard deviation of triplicate.

(a) Microgram of Gallic Acid Equivalent per milligram of extract.

(b) Microgram of Quercetin Equivalent per milligram of extract.

Effect of BEHC on plasma biochemical parameters

The effect of *n*-butanol extract (BEHC) onplasma biochemical parametersis shown in **(Table 2)**. Urea (UR) values increased significantly in the intoxicated group as compared to the control ones ($p < 0.01$). Moreover, groups treated with BEHC 400mg/kg with and without Selenium (Se) and Silymarin showed a marked amelioration with a percentage of improvement recorded at 95.42%, 72.72%, and 122.72% respectively. Whereas, no significant variations were observed in glucose (GLU) and creatinine (CRE) values among the different groups ($p > 0.05$) as shown in Table 2. However, these parameters exhibited percentages of changes that amounted to 39.02% and

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29.85% respectively. But, the pretreatment with BEHC 100mg/kg, BEHC (400mg/kg), BEHC (400mg/kg) + Se and silymarin recorded percentages of amelioration 20.73%, 36.58%, 35.36% and 31.70% for GLU and 14.82%, 19.23%, 19.03% and 15.63% for CRE respectively.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
GLU (g/L)	0.82 ± 0.04	1.14 ± 0.13	0.88 ± 0.17	0.97 ± 0.13	0.84 ± 0.14	0.85 ± 0.09
Improvement %	_	_	31.7	20.73	36.58	35.36
CRE (mg/dL)	4.99 ± 0.38	6.48 ± 0.20	5.70 ± 0.53	5.74 ± 0.66	5.52 ± 0.68	5.53 ± 0.32
Improvement %	_	_	15.63	14.82	19.23	19.03
UR (g/L)	0.44 ± 0.08	0.98± 0.02**	0.45 ± 0.09##	0.73 ± 0.10	0.66 ± 0.12#	0.56 ± 0.08#
Improvement %	_	_	122.72	56.81	72.72	95.45

Table 2. Effect of BEHC on plasma biochemical parameters.
Values are mean ± S.E.M., n = 5 animals in each group.* p ≤.05; ** p ≤.01; *** p ≤.001 as compared to normal control group, # p ≤.05; ## p ≤.01; ### p ≤.001 as compared to intoxicated control group.

Effects of BEHC on renal MDA, GSH, CAT, and SOD

Table 3 showed the effects of BEHC on renal Malondialdehyde (MDA), glutathione (GSH), Superoxide dismutase (SOD), and catalase (CAT) levels after induction of nephrotoxicity using CCl_4. Results revealed a significant increase in the level of MDA in the intoxicated control group compared with the control ones ($p<0.001$). Conversely, pretreatment with silymarin, BEHC (400 mg/kg) with and without Se showed a significant decrease in MDA levels ($p<0.01$ for BEHC (400mg/kg) with and without Se; $p<0.001$ for silymarin). However, pretreatment with BEHC (100 mg/kg) showed an insignificant decrease in MDA levels. Furthermore, a highly marked improvement was observed in the MDA level with improvement percentages of 442.33%, 292.83%, 301.94%, and 307.60% for groups treated with silymarin, BEHC (100mg/kg), BEHC

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(400mg/kg) alone and (400mg/kg) with Se, respectively.Our results showed a significant decrease in the levels of GSH, CAT, and SOD in the intoxicated group compared with those of the control ones ($p<0.001$).However, GSH and SOD levels exhibited a significant increase in groups treated with silymarin and BEHC (400mg/kg) with and without Se compared to the intoxicated group ($p<0.05$). But pretreatment with BEHC (100 mg/kg) showed an insignificant increase in GSH and levels. We observed that the percentage improvement in SOD of BEHC 400mg/kg+ Se (23.52%) was higher than that shown in the silymarin pretreatment group (19.36%). In addition, CAT level was significantly elevated in groups treated with silymarin, BEHC with and without Se ($p<0.01$ for BEHC (100mg/kg); $p<0.001$ for silymarin and (400mg/kg) with and without Se) when compared with the intoxicated group. We observed also that the percentage improvement in CAT of BEHC 400mg/kg+ Se (48.94%) was higher than that recorded in the silymarin pretreatment group (42.34%).

Treatment groups	Parameters/ Improvement %			
	MDA (nmol/mg prot)	GSH (nmol/mg prot)	CAT (UI/mg prot)	SOD (UI/mg prot)
Group I	0.45±0.03	7.62±0.30	3.38±0.11	4.98±0.30
	-	-	-	-
Group II	2.96±0.20***	4.57±0.15***	0.46±0.10***	3.28±0.18***
	-	-	-	-
Group III	0.97±0.15$^{###}$	5.33±0.32***$^{\#}$	1.89±0.25***$^{###}$	4.24±0.34*$^{\#}$
	442.32%	10.16%	42.33%	19.35%
Group IV	1.64±0.45**	4.88±0.15***	1.23±0.17***$^{\#\#}$	3.80±0.25**
	292.82%	4.16%	22.72%	10.36%
Group V	1.60±0.24*$^{\#\#}$	5.21±0.41***$^{\#}$	1.64±0.14***$^{###}$	4.13±0.13*$^{\#}$
	301.94%	8.57%	34.83%	16.97%
Group VI	1.58±0.04*$^{\#\#}$	5.38±0.40***$^{\#}$	2.11±0.10***$^{###}$	4.45±0.70*$^{\#}$
	307.59%	10.75%	48.93%	23.51%

Table 3. Levels of renal MDA, GSH, CAT and SOD in all groups.

Values are mean ± S.E.M., n = 5 animals in each group.* $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$ as compared to normal control group, # $p \leq .05$; ## $p \leq .01$; ### $p \leq .001$ as compared to intoxicated control group.

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Histopathological examination of kidney

As shown in **(Figure 1)**, a normal histological pattern in the kidney was observed in control animals, whereas histological alterations were observed in CCl_4 intoxicated rats especially in the renal cortex as evidenced by the focal glomerular changes including mild dilation of Bowman's space with adhesion between the visceral and parietal layers of Bowman's capsule and glomerular atrophy (necrosis). In addition, proximal and distal tubules were severely dilated, and their epithelial cells tended to be flattened, detached, and finally degenerated indicating tubular necrosis. Moreover, inflammatory cell infiltration was also detected in the renal interstitium. However, the histopathological kidney lesions induced by the administration of CCl_4 were remarkably improved by silymarin and BEHC at the dose of 400mg/kg .bw with and without Se pretreatment. By examining the histological score given to the kidney tissues as shown in Table 4, it was feasible to conclude that the highest dose of BEHC (400 mg/kg) with Selenium pretreatment only provided good protection against CCl4-induced kidney damage.

Table 4. Grades of renal histopathological changes in different groups.

Groups/ Parameters	Congestion	Tubular necrosis	Glomerular atrophy	Dilation of Bowman's space	Infiltration of lymphocytes
Group I	-	-	-	-	-
Group II	+++	+++	+++	+++	+++
Group III	++	+	+	-	++
Group IV	+	++	++	+++	+++
Group V	-	+	-	+	+
Group VI	-	+	-	-	+

Scoring was done as follows: none (-), mild (+), moderate (++) and severe (+++).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3032 - 3049
Received 08 November 2021; Accepted 15 December 2021.

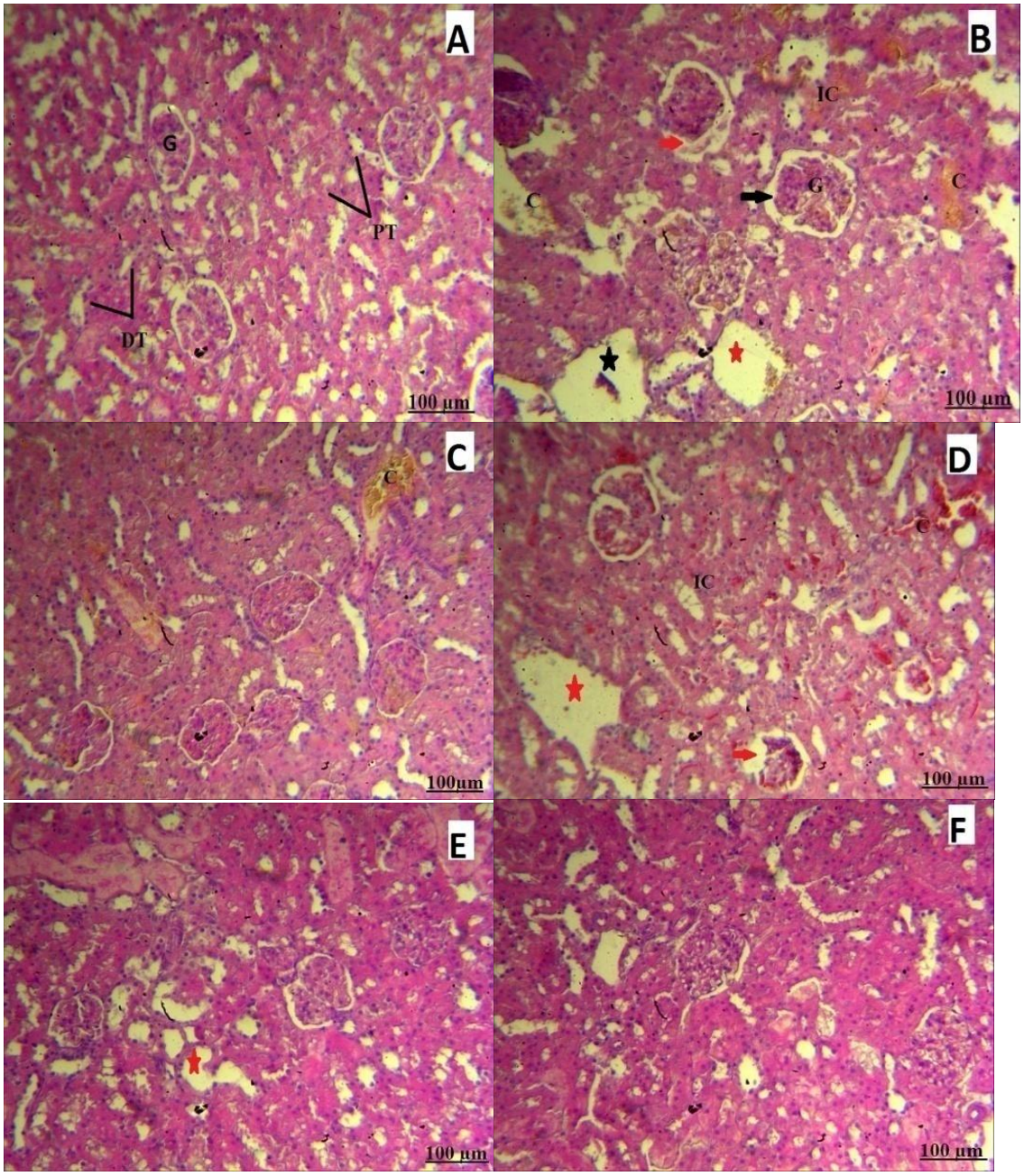

Figure 1: Photomicrographs of kidney sections from A: Group I; B: Group II; C: Group III; D: Group IV; E: Group V; F: Group VI. (H&E; 100 X). G: glomerulus; PT: proximal tubule; DT: distal tubule; C: congestion; IC: inflammatory cells; Black arrow: dilatation of Bowman's space, Red arrow: glomerular atrophy; Red star: tubular necrosis; Black star: glomerular necrosis.

Discussions (Times New Roman, bold, 12)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3032 - 3049
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DISCUSSION

The utilization of natural plant products derived from traditional medicinal plants in the creation of innovative therapies for the treatment of various diseases is becoming more and more popular in the modern era, in which extracts, derived fractions, and isolated active constituents exhibited potent protective action mainly related to their antioxidant properties (Ali et al. 2018). The use of traditional medicinal plants as sources of secondary metabolites, antioxidants, and antimicrobials for treatment has shown promise, and they can also guard against tissue damage caused by free radicals (Hasan, 2022).

In the current study, we investigate the potential protective effects of *H. cheirifolia*butanol extract (BEHC) alone and combined with selenium (Se) against carbon tetrachloride (CCl_4) induced nephrotoxicity in rats for the first time according to our knowledge. Taking into account the polarity of the extracts, it appears that the content of total phenolic and flavonoids in *H. cheirifolia* L. increased with the polarity of extract where results indicate that BEHC contains the highest concentrations of total phenolic compounds and flavonoïds. In another study, it was shown that ethyl acetate extract of Tunisian *H. cheirifolia* roots contains the highest content of total phenolics and flavonoids followed by butanol extracts (Majouli et al. 2017). Moreover, BEHC had higher polyphenols content than the other extracts of *Hertiacheirifolia* shown in previous studies (Kada et al. 2016; Majouli et al. 2017). Our results were related to the high polyphenols content of the plant aerial part, furthermore, the extraction of polar compounds with *n*-butanol was more efficient than ethyl acetate. Additionally, the phenolic content of a plant was influenced by certain numbers of intrinsic (genetic) and extrinsic factors (climatic conditions, maturity at harvest, and storage conditions) (Falleh et al. 2008). The BEHC can have the strongest antioxidant effect since it was rich in polyphenols that have shown multiple biological activities including adsorbing and neutralizing free radicals. In a range of *in vitro* antioxidant assays, the BEHC demonstrated an intriguing antioxidant activity, according to a prior study (Menakh et al. 2020).

Carbon tetrachloride (CCl_4) is a known hepatonephrotoxin, frequently used to induce liver and kidney damage in animal models. The administration of CCl_4 initiated the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes in the liver, kidney, testis, lung, pancreas, nervous system, and blood tissues by producing free radicals (Weber et al.2003)**.** It has been reported that excess cellular ROS can cause perturbations

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in mitochondrial function and play a role in the pathogenesis of diabetes complications and trigger b-cells apoptosis (Mihailovic et al. 2013). In this study, plasma GLU levels in the CCl_4 intoxicated group did not show any significant variation ($p > 0.05$) when compared to the control group. This result was similar to a previous study (Mihailovic et al. 2013). However, this parameter exhibited percentages of changes that amounted to 39.02%. This elevation of glucoselevel could be caused by decreasing the pancreatic secretion of insulin from β-cells since the levels of fatty changes in the cells of islet result from the toxicity of CCl_4.While, the pretreatment with and without Selenium ameliorates plasma GLU levels may be by enhancing insulin secretion from beta cells, and/or increasing pancreatic tissue regeneration.

Kidney markers like creatinine and urea were also estimated to confirm the renal damage, their elevation is considered a significant marker of renal dysfunction (Shahbazi et al. 2012). According to our data, CCl_4 administration considerably enhanced the level of UR., whereas no significant variations were observed in CRE values among the different groups ($p > 0.05$), which is similar to a previous study (Mihailovic et al. 2013). However, CRE showed percentages of changes amounted to 29.85%, which is consistent with reports confirming that the level of serum creatinine increases only if at least half of the kidney nephrons are already damaged (Bhattacharya and Gomez 2005). After the treatment with BEHC and selenium CRE and UR values are decreased when compared to the intoxicated group, these results suggest that pretreatment with BEHC and Selenium could prevent the CCl_4-induced alterations in kidney function of rats. It has been reported that adding selenium to some plants increases their effectiveness against oxidative stress. Selenoenzymes, which are known to shield many cellular components from oxidative damage, seemed to be primarily responsible for the protective effects of Se (Yu et al. 2009).

Cell membranes are damaged by toxic radicals such as the superoxide radical, trichloromethyl, and trichloromethylperoxy, which also cause lipid peroxidation (Mahmoodzadeh et al. 2017). In the present study administration of CCl_4 showedanincreased level of MDA compared to control rats in kidney tissues. Significant reductions in MDA levels were seen in groups receiving 400mg/kg of BEHC with and without selenium. The presence of Rutin, Ferulic acid, and Chlorogenic acid in plant extract, as shown by HPLC analysis (Menakh et al. 2021), could be the cause of these outcomes. Free radical scavenging and lipid peroxidation-reducing abilities of these flavonoids are well-known.

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To understand the mechanisms of BEHC for its protective effect against CCl_4-induced kidney issues damage, we determined the activities of antioxidant enzymes (CAT and SOD), as well as the levels of GSH in the kidney of rats. SOD, CAT, and GSH have been reported to constitute a mutually supportive defense mechanism against ROS (Ali et al. 2018).The coordinated action of the antioxidant system is essential for the detoxification of free radicals. By diminishing their levels in the rat kidney tissues, these enzymes' antioxidant activity was found to be reduced in the current investigation. This is likely because free radicals are responsible for protein inactivation. Similar results have also been shown previously for antioxidant enzyme systems in the liver during oxidative stress induced by CCl_4 (Mihailovic et al. 2013; Mahmoodzadeh et al. 2017).By significantly improving the levels of oxidative enzymes in comparison to a normal control group, treatment with BEHC at a dose of 400 mg/kg along with selenium alleviated the harmful effects of CCl_4; This antioxidant activity of BEHC was most possibly associated with its capacity to lessen the generation of free radicals. The histological changes seen in the kidney of rats intoxicated with CCl_4 were characterized by some nephrotoxic lesions, as evidenced by the dilation of Bowman's space, glomerular atrophy, tubular necrosis, and inflammatory cell infiltration. Our results confirmed previous findings that had found degenerative changes in kidneys of rats exposed to CCl_4 and detected glomerular necrosis and histologic alterations in proximal and distal tubules (Ben Hsouna et al. 2011). Bowman’s space dilatation was caused by an increase in Glomerular Filtration Rate (GFR) due to the sodium reabsorption process and hyperfiltration on the glomerulus. As result, tubuloglomerular feedback will be reduced and Bowman’s space will be dilated (Priyo et al. 2018). These histologic alterations can be explained by lipid peroxidation and the breakdown of the membranes. The result of histological observation on treatment groups showed that BEHC at the dose of 400 mg/kg was capable to protect the kidney from nephrotoxic lesions after administration of CCl_4. Moreover, observation results also proved that BEHC and selenium exhibited the maximum protection of kidney tissue. The positive effect of BEHC and Se on CCl4-induced nephrotoxicity may be the result of their contribution to cell membrane biosynthesis. On the other hand, the ability of BEHC to protect against oxidative stress and free radicals is related to its antioxidant compounds.

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Conclusion

The present investigation justifies the hypothesis that the pretreatment with *H. cheirifolia*butanol extract with selenium protected rats against CCl_4-induced kidney damage and significantly reduced oxidative stress by decreasing lipid peroxidation, increasing antioxidant enzyme activities, and improved kidney histological lesions.Further investigations are essential to elucidate the precise mechanism of active agents of BEHC protection against CCl_4-induced nephrotoxicity and it has to be tested against other biological parameters.

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