Egypt. J. Chem. **Vol. 68,** No. 07 pp. 145 - 154 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Heprotective Effect and Antioxidant of *Sonchus Oleraceus* Leaves Extract Against Carbon Tetrachloride (CCl_4) Induced hepatotoxicity in Albino Rats

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Abstract

This study examined the effects of *Sonchus Oleraceus* extract from leaves (SO-LE) on carbon tetra chloride induced hepatotoxicity amongst albino rats. The SO-LE exhibited a high content of total phenolic and flavonoid compounds accounting for 127.37± 0.56 mg GAE/g (Gallic Acid Equivalent./gram)extract and 74.23 ± 0.55 mg QE/g(quercetin equivalent) extract, respectively. SO-LE exhibited strong DPPH(1,1diphenyl,2 picrylhydrazyl) scavenging activity with an inhibition ratio of 43.07%. For the *in vivo* assay, 24 adult albino rats were separated into four groups (each of six rats). First group served as the negative group, while the second group received carbon tetrachloride (CCl4) as the positive control. Third group received CCl_4 with 100 mg kg^{-1} SO-LE, while the fourth group received CCl_4 with 200 mg kg^{-1} SO-LE for eight weeks (CCl_4 concentration was 1 mL kg^{-1}). Rat lipid profiles, peroxidation, and indicators of liver and kidney function were evaluated. Treatment with 200 mg kg^{-1} SO-LE reduced creatinine, urea, and uric acid levels to 0.87, 38, and 3.8 mg dL^{-1}, respectively. After eight weeks of treatment, levels of TC, TAG, and LDL were decreased to 129.7, 146.3, and 62.7 mg L^{-1}, respectively. SO-LE reduced AST and ALT activity and decreased malondialdehyde levels, whereas CAT, GSH, and SOD levels increased in SO-LE-treated albino rats. The liver's histopathological analysis revealed that the SO-LE treatment decreased necrosis, cytoplasmic vacuolization, and fatty acid degradation. In conclusion, SO-LE protected albino rats against CCl4-induced hepatotoxicity, a protective effect that may have been enhanced by the antioxidant properties of SO-LE extract. The objective of this study was to investigate the preventive effects of SO-LE on rats' fatty livers caused by carbon tetra chloride.

Keywords: *Sonchus oleraceus* leaf extract, Hepatoprotective, liver, metabolic enzyme, Carbon Tetrachloride (CCl_4), DPPH

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1. Introduction

The liver is a vital organ that plays a significant role in the metabolism of endogenous and exogenous substances. Hepatic injury can lead to disruption of these functions, which makes the liver susceptible to various diseases, such as hepatitis, cirrhosis, alcohol-related disorders, and cancer **[1]**. Exposure to xenobiotics and pollutants in the environmental, such as alcohol, thioacetamide, carbon tetrachloride, paracetamol, and paracetamol, can cause damage to the liver by generating reactive oxygen species (ROS), which is one of the main reasons for these diseases **[2]**. ROS had been proven to enhance fibrosis by increased collagen synthesis, and are known to cause tissue harm by covalent binding and lipid oxidation. **[3, 4]**. Tissue fibrosis can be decreased by defence systems scavenging free radicals. **[5, 6]**. The body's cooperative defence mechanisms, which defend against damage from free radicals, depend on antioxidants and enzymes like glutathione peroxidase (GPH), catalase (CAT), and superoxide dismutase (SOD). The roles of protective enzymes have been extensively studied using in vivo models **[7, 8]**. Industrial solvent CCl4 can be used as a xenobiotic agent to cause chemical liver damage.
Liver disease, which is induced by factors such as alcohol consumption, chemicals, and infections, is a major global health concern. Recent studies have focused on the potential of naturally occurring compounds in the treatment of liver diseases. Certain natural bioactive compounds, including antioxidants, have shown protective effects against liver disease **[9]**. Even in the presence of CCl4, these substances can protect the liver from lipid oxidation and preserve its antioxidant a activity **[10]**. Oxidative stress (ROS), characterized by an imbalance in the production of oxidants and antioxidant defenses, plays a crucial role in liver diseases. Antioxidants can prevent hepatic damage by scavenging free radicals and increasing the activity of intracellular antioxidant enzymes such as SOD, GSH, and CAT **[11]**. Polyphenolic compounds, which possess significant antioxidant properties, can prevent liver damage caused by free radicals **[1]**. Studies have demonstrated the effectiveness due to distinct interactions and synergistic effects, natural antioxidants and plant extracts can reduce oxidative stress-induced liver diseases **[12]**.
In Egypt, sowthistle (SO) is more prevalent than in Saudi Arabia, occurrence depends on precipitation. Sonchus, which consists of about 60 different species and many microspecies, consists of eight parts. This genus, classified as species *Sonchus oleraceus* Sowthistle is the common name for the family Asteraceae, kingdom Plantae, subkingdom Tracheobionta, division Magnoliophyta, class Magnoliopsida, and tribe Lactuceae.

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Receive Date: 30 September 2024, **Revise Date:** 30 November 2024, **Accept Date:** 05 December 2024
DOI: https://doi.org/10.21608/ejchem.2024.325097.10547

. The plant is used in herbal medicine to treat liver disorders, jaundice, gallbladder issues, and other ailments **[13, 14 and 15]**. Sonchus roots and herbs have also been conventionally used to treat various health issues, including gallbladder and liver disorders **[16, 17].**

Sonchus oleraceus extracts (Petroleum ether, Ethyl acetate, and Methanol) were used for the hepatoprotective study at dosage 200 mg/kg body weight for 8 weeks . Hepatoprotective activity was tested in rats with experimentally-induced hepatotoxicity by CCl4. The tested extract proved significant hepatoprotective capacity under in-vitro conditions and in-vivo experiment showed that prominent hepatoprotective activity was shown by ethyl acetate and petroleum ether extracts (44).

Sonchus oleraceus L. extracts (ethanolic and aqueous) at a concentration of 150 mg/kg b.w were evaluated for hepatoprotective activity against liver toxicity induced by paracetamol in rats . showed a significant protection against-induced alteration in (AST),(ALT), (ALP), (SOD) and glutathione peroxidase activities compared with paracetamol group (45).

Hepatoprotective activity of the ethanolic extract of Sonchus Oleraceus at dose rates of 200 and 400 mg /kg BW induced by administration of carbon tetrachloride (CCL4) at dose rate of 0.2 mg /kg BW was evaluated according to (46). They found that high dose (400 mg), hence the level of (ALT), while (AST), Total protein, Albumin, Bilirubin and (ALP) were significantly decreased. The Histological appearance of the given Sonchus Oleraceus. ethanolic extract (400mg/kg BW) showed few hepatocytes necrosis that has amorphous eosinophilic cytoplasm and areas of necrosis characterized by disintegration and disappearance of cells in many areas .

The objective of this study was to investigate the preventive effects of SO-LE on rats' fatty livers caused by carbon tetra chloride.

2. Materials and Methods:

Leaves of *Sonchus oleraceus*, which was collected in January 2023 in the stage of plant maturity, were naturally cultivated on a farm at the Faculty of Agriculture's research station at Moshtohor, Benha University, Egypt. The experiments' chemicals and standards were all acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). The animals used for the study were male albino rats (Wister strain) that were one month old and in good health. A total of 24 were housed in a stainless-steel bottom cage with the temperature regulated at 25 ± 2 ºC. Rats were housed in a healthy environment for 14 days and provided a basal diet, 50% relative humidity, 12 h light-dark cycle, and free access to water. Starch comprised 65% of the diet, followed by casein, 15%, maize oil, 10%, cellulose, 4% minerals, and 1% vitamins .They were obtained from the Egyptian Drug Authority in EL-Manial, Cairo, Egypt, and were almost the same weight . Research Ethics Committee approved the experimental animal design numbered (REC-FOABU.5/0006).

2.1. Extract Preparation for Biological Investigations and experiment design

Clean and dried *S. oleraceus* leaves were used. After being mechanically ground and soaked in (1:10) ethanol 80% for one week at room temperature (in the range of 25 and 30 ◦C), the dried leaves were gently mixed every day. The solution had been evaporated using a rotary evaporator to dryness after being filtered using a Büncher funnel. After that, the extract was diluted with distilled water and orally administered to experimental animals **(18).**

To investigate the protective function of SO-LE against CCl_4-induced hepatotoxicity, twenty-four albino rats have been used. To prevent CCl_4-induced liver damage, olive oil and CCl_4 were combined in a 1:1 (w/w) ratio and applied as a hepatoprotective agent. The four equal groups of six rats each were assigned at random, and the animals received treatment as follows: For eight weeks, rats in Group 1 (negative control group) were fed a normal synthetic diet. The second group of rats, known as the positive control group, were given an ordinary meal in addition to CCl_4 and olive oil orally twice a week via gastric gavage, at a dose of 1 mL/kg (b.w.) for a duration of eight weeks.

Group 3: Rats were given a normal diet and given 100 mg kg-1 of SO-LE orally twice a week via gastric gavage , also they were given an equal amount of CCl4 1 mL kg-1. Group 4: Rats were given a normal meal and given 200 mg kg-1 of SO-LE orally twice a week in addition rats were given 1 mL kg-1 of CCl4 .

2.1.1. Biochemical evaluating of blood Sample

Using tiny capillary heparinised tubes, blood samples were taken from each rat's retro-orbital plexus vein at the final stage of the experiment. After collecting the blood samples and letting it coagulate, the serum was separated using centrifugation for 15 minutes at 3000 rpm. Serum was utilised for investigation the biochemical markers, such as the lipid profile and tests evaluating liver and kidney function. The aspartate transaminase (AST) and alanine transaminase (ALT) activity of the liver, as well as serum total bilirubin (TB), total protein, and serum albumin, were measured using methods of **(22 , 23, 24 and 25**). The serum total protein level was subtracted from albumin to determine the globulin level. Urea, uric acid, and creatinine indicators of kidney function were assessed using the method described by (**26).** Triglycerides (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured using the methods outlined by **(27).**

2.1.2. Histopathological examination

The liver tissue samples were analysed using a histology examination. Rats in each group provided tissue samples, which were quickly preserved by fixing them in buffered formalin neutral 10% .Thin paraffin sections were then made in accordance with conventional protocols and examined under a microscope. Haematoxylin and eosin (H&E) stain was used on

the sections for histological abnormalities in the tissues of the live and renal tissues, following the guidelines provided by (33).

2.2. Estimating the Total Phenolic Content (TPC)

Using a colorimetric oxidation/reduction technique and a UV spectrophotometer designed by Azzota, Claymont, DE, USA (SM1600UV-vis Spectrophotometers), and total phenol content in ethanol extract was identified, as described by **[19]**. As the oxidising agent, Folin-Ciocalteu reagent was used. Briefly, 100 µL of the diluted extract 10% (10 mg in 10 mL solvent) was added to 2.5 ml of Folin-Ciocalteu reagent and 2 mL of Sodium Carbonate (Na2CO3) 7.5%. Water was added to the mixture in a 10 mL volumetric flask and shaken. The solution was then incubated at ambient temperature for 30 min. Absorbance was measured at 760 nm. Gallic acid equivalent (GAE) is an expression of the total phenolic content.

2.3. Determination of Total Flavonoids Content (TFC)

The method is given by **[20]** was utilised to determine total flavonoid content.

In brief, 0.5 mL of the extract solution (10 mg in 10 mL of solvent) was mixed with 1.5 mL of the 20 g L^{-1} AlCl3 ethanolic solutions. When the absorbance at 420 nm was measured at room temperature one hour after addition, flavonoids were detected by the yellow colour. TFC is expressed as quercetin equivalent (QE).

2.4. The activity of DPPH (2, 2-Diphenylpicryhydrazyl) Radical-Scavenging

By bleaching the purple DPPH solution, the resulting extracts' ability to donate electrons was assessed using the methodology described in reference **[21**]. Extract (0.1 mg /10 mL solvent) was added in 100 µL increments to 3 mL of 0.1 mM DPPH dissolved in ethanol. The absorbance at 517 nm was measured after 30 minutes at room temperature and compared to a standard. The free radical DPPH's % antioxidant activity was calculated using the following formula:

Antioxidant activity (inhibition) % =

$$\left[\frac{(A(control) - A(sample)}{A(control)}\right] \times 100$$

Where A (sample) is the absorbance of the plant extract and A (control) is the absorbance of the control reaction (100 µL methanol with 3 mL DPPH).

2.5. Antioxidant markers

Antioxidant markers , including CAT , SOD,GST,GSH and MAD were determined.

The serum CAT activity was determined using the method described by (28). First, 2.8 ml 30 mM H2O2 was placed in a blind tube, and 0.2 ml phosphate buffer was added to it. Then 2.8 ml 30 mM H2O2 was added to the sample tube. To both of these tubes, 0.2 ml enzyme was added, and the tubes were mixed . The absorbances at 240 nm were read twice at 30 s intervals to determine the activity.

The serum SOD activity was determined using the method recommended by (29). This method is based on reading the optical density (at 560 nm) of blue formazan dye formed in the reaction of nitro blue tetrazolium with the superoxide radicals generated using xanthine and xanthine oxidase. The SOD found in the sample eliminates the superoxide radicals from the medium, inhibiting the formazan reaction. One unit of SOD inhibits the nitro blue tetrazolium reduction rate by 50% under assay conditions.

The assay to determined the glutathione- S-transferase(GST) was performed by measuring the formation of a conjugate of glutathione and 1-chloro-2, 4, dinitro benzene (CDNB) according to the method described by (30). According to this method, reaction mixture containing 20 mM CDNB, 100 mM reduced glutathione (GSH), GST assay buffer (100mM Potassium phosphate buffer adjusted to pH 6.5, 0.1% TritonX-100) and 20 µl blood lysate was prepared and mixed thoroughly. CDNB was incubated at 37ºC for 10 minutes before use and absorbance was taken at 340nm. The molar extinction coefficient of CDNB is 0.0096 μM^{-1} u $^{-1}$.

The GSH level was determined using the method described by (31). First, 200 µl serum was diluted in 800 µl phosphate buffer, and the first absorbance (OD1) was measured at 412 nm. Then 100 µl Ellman's reagent was added to the same tube, and the second absorbance value (OD2) was recorded. The GSH concentration was calculated using the formula C/1000 = (OD2 − OD1)/13,600 × E1 × 5/2 × [(C: mmol/glutathione (mg/dl).

MDA, one of the peroxidation products formed by the reaction of fatty acids with free radicals, was measured as the formation of its colored form with thio barbituric acid (32). For each patient, 200 µl serum was transferred to a tube, and 800 µl phosphate buffer, 25 µl butylhydroxytoluene solution and 500 µl 30% trichloroacetic acid were added. The tubes were mixed and incubated on ice for 2 h. They were then centrifuged at 2000 rpm for 15 min, and 1 ml of each supernatant was transferred to a new tube, after which 75 µl ethylenediamine tetra acetic acid and 25 µl thio barbituric acid were added. The tubes were mixed and incubated in a hot water bath for 15 min. They were then brought to room temperature, and the absorbance at 532 nm was read on a UV/Vis spectrophotometer.

2.6. Statistical analysis

ANOVA, one-way analysis of variance, was used to analyse all the data. Duncan's recently developed multiple-range test was used to ascertain variations in the treatment means. The software as SPSS was used for all statistical analyses (version 11.0, Surrey, UK: SPSS Ltd. A p-value of 0.05 was determined to be significant. The ratio values were not prior to the statistical analysis arcsine-transformed **[34].**

3. Results

3.1. The yield of the SO extract :

Based on the results, SO-LE produced a yield of 33.4 %. Also we found that the extract contained phenolic compounds in total flavonoid and activity of DPPH was (127.37 mg GAE/g), (74.23 mgQE/g), **(**43.07**%)** respectively**.**

3.2. Effect of treatment with (SO-LE), on liver function, and kidney function, comparison to the negative and positive (CCl4) control groups in rats

The effects of Sowthistle (SO-LE) on liver enzymes in the rats are shown in **Table 1**.. According to the results, the ALT and AST values of the positive control group, which received an injection of CCl4 at a rate of 1 mL kg^{-1} body weight (b.w.), were significantly higher ($P<0.05$) than those of the negative control group. This is because of the destructive effect of the drug on liver cells **[36, 47]**.

Table (1): Effect of treatment with (SO-LE), on liver function comparison to the negative and positive (CCl4) control groups in rats

G.	Treatment	Liver function					
		AST U L^{-1}	ALT U L^{-1}	T. Protein mg dL^{-1}	Albumin mg dL^{-1}	Globulin mg dL^{-1}	Bilirubin mg dL^{-1}
1	**Negative control**	61.2^{b}± 1.61	68.3^{b}± 1.5	1.4^{c} ±0.42	1.6^{b} ± 1	0.63^{b}± 0.21	0.13^{b} ± 0.01
2	**Positive control (CCl_4)**	195^{a}± 4	163.7^{a}± 54.4	6.2^{a} ±0.75	4.2^{a}± 0.31	2.8^{a}± 0.33	1.1^{a} ± 0.08
3	**CCl_4 + 100 mg kg^{-1} SO-LE**	59^{b}± 8.7	63.3^{b}± 3.2	2^{c} ±0.55	1.2^{b}± 0.35	0.80^{b}± 0.20	0.11^{b} ± 0.03
4	**CCl_4 + 200 mg kg^{-1} SO-LE**	59.3^{b}± 4.5	67.3^{b}± 2.1	4^{b} ±0.16	2.4^{b}± 0.42	1.6^{b}± 0.58	0.13^{b} ± 0.01

SD: Standard deviation for every group, n = 6. SO-LE: *Sonchus oleraceus* Extract; carbon tetrachloride (CCl_4). Note: Within each column's values, individual letters (a, b, c, and d) show significant differences ($p < 0.05$).

SO-LE effects on kidney function indicators in CCl_4-treated rats are shown in **Table 2**. Urea, uric acid, and creatinine levels in the negative group (group 1) after 8 weeks of experimentation were 36, 2.9, and 0.90 mg dL^{-1}, respectively. It could be seen that treatment with CCl_4 (group 2) elevated the urea, creatinine, and uric acid levels to 63.7, 1.6, and 5.2 mg dL^{-1}, respectively.

Table (2): Effect of treatment with (SO-LE), on kidney function comparison to the negative control and positive control (CCl4) groups in rats

G.	Treatment	urea mg dL^{-1}	uric Acid mg dL^{-1}	creatinine mg dL^{-1}
1	**Negative control**	36^{b} ± 4.4	2.9^{b} ± 0.15	0.90^{a} ± 0.36
2	**Positive control (CCl_4)**	63.7^{a} ± 5.5	5.2^{a} ± 0.70	1.6^{a} ± 0.38
3	**CCl_4 + 100 mg kg^{-1} SO-LE**	42.7^{b} ± 4	3.8^{ab} ± 0.87	1.1^{a} ± 0.33
4	**CCl_4 + 200 mg kg^{-1} SO-LE**	38^{b} ± 4.6	3.8^{ab} ± 0.31	0.87^{a} ± 0.15

SD: Standard deviation for every group, n = 6. SO-LE: *Sonchus oleraceus* Extract; carbon tetrachloride (CCl_4). Note: Within each column's values, individual letters (a, b, c, and d) show significant differences ($p < 0.05$).

3.3. Effect of treatment with SO-LE, on lipid profile and antioxidant markers comparison to the negative control and positive control (CCl4) groups in rats

Results in **Table 3** show that the positive control group had significantly elevated levels of low-density lipoprotein and decreased serum levels of high-density lipoprotein compared to the negative group. Rats treated with 100 and 200 mg kg^{-1} SO-LE had lower mean LDL values than the positive group. When compared to the positive control group, the mean values of HDL were greater in all groups treated with SO-LE at different dosages.

Table (3): Effects of (SO-LE) treatment on the lipid profile comparison to the negative and positive (CCl4) control groups in rats

G.	Treatments	Total Cholesterol mg dL^{-1}	Triglyceride mg dL^{-1}	HDL-Cho mg dL^{-1}	LDL-Cho mg dL^{-1}
1	**Negative control**	$133^{b} \pm 3.6$	$124.3^{b} \pm 5$	$54^{a} \pm 3.6$	$57^{c} \pm 2$
2	**Positive control (CCl_4)**	$210.3^{a} \pm 27.8$	$234.3^{a} \pm 55.2$	$35^{c} \pm 4.3$	$92.7^{a} \pm 3.2$
3	**CCl_4 + 100 mg kg^{-1} SO-LE**	$151^{b} \pm 1$	$164^{ab} \pm 26.9$	$41.3^{bc} \pm 3.2$	$85^{b} \pm 1$
4	**CCl_4 + 200 mg kg^{-1} SO-LE**	$129.7^{b} \pm 1.5$	$146.3^{b} \pm 12.5$	$45.7^{ab} \pm 3.1$	$62.7^{c} \pm 2.5$

SD: Standard deviation for every group, n = 6. SO-LE: *Sonchus oleraceus* Extract; carbon tetrachloride (CCl4). Note: Within each column's values, individual letters (a, b, c, and d) show significant differences ($p < 0.05$).

3.4. Effect of treatment with SO-LE, on antioxidant markers comparison to the negative control and positive control (CCl4) groups in rats

As shown in **Fig. 1**, after 8 weeks, the MDA (mM^{-1}) level in CCl_4-treated rats (group 2) were significantly higher than the negative group (24.3 and 10), respectively. Lower MDA levels were seen in groups 3 and 4, which received SO-LE treatment (100 and 200 mg kg^{-1} body weight, respectively). We can conclude that after CCl4 was administered, SO reduced the elevation of MDA levels. CCl_4 treated rats' levels of GST, SOD, CAT, and GSH were significantly reduced (group 2). In contrast to the positive control group, SO-LE treatment (groups 3 and 4) significantly elevated levels.

3.4 Effects of SO-LE, on body weight in albino rats as compared to negative and positive control (CCl_4) groups

At time zero, no group's body weight showed any significant variations between treatments. Table 4 shows the significant drop in the final body weight of the CCl4 rats as compared to the negative group. Rats treated with CCl4 and rats in negative group showed enhanced final body weights due to SO-LE (100 mg $kg^{-1}$and 200 mg kg^{-1}) after 56 days of treatment. Phenolic and flavonoid compound of the extract might have been responsible for this phenomenon.

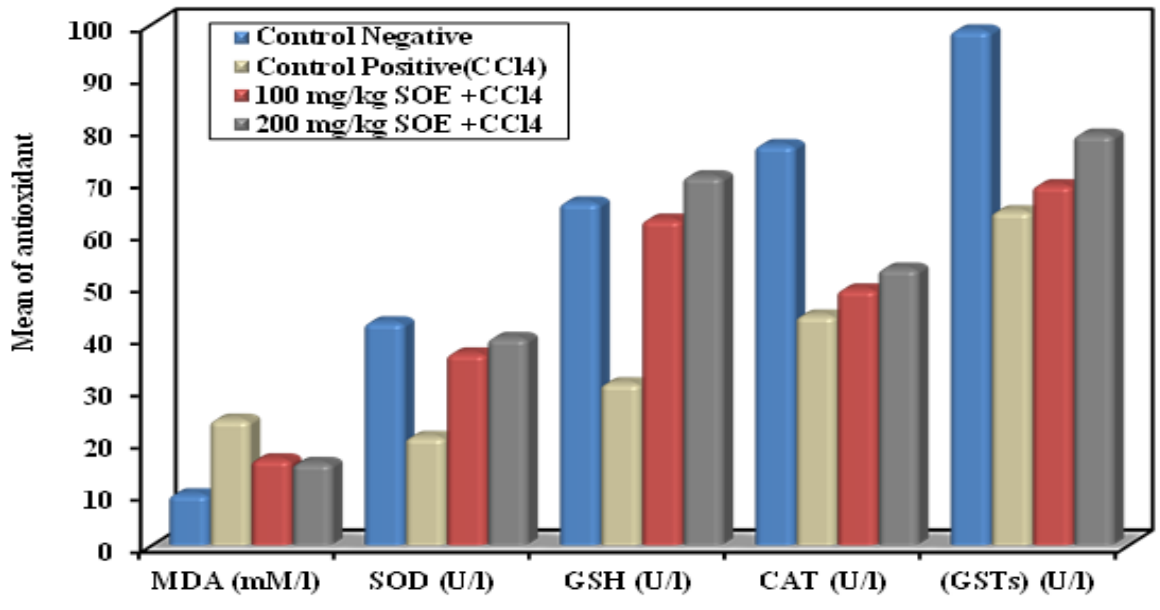

Fig 1. Effects of (SO-LE) treatment on antioxidant marker

Table (4) Effects on body weight gain, comparing between the various groups.

G.	Treatment	Initial body weight (g)	Final body weight (g)	Change body weight gain	
				(gram)	(%)
1	**Negative control**	$151^{a} \pm 8.1$	$280^{a} \pm 12.3$	$129^{a} \pm 29.6$	$85.43^{a} \pm 16.2$
2	**Positive control (CCl_4)**	$155.16^{a} \pm 15$	$252^{b} \pm 2.3$	$96.84^{b} \pm 17$	$62.41^{b} \pm 11.1$
3	**CCl_4 + 100 mg kg^{-1} SO-LE**	$155..83^{a} \pm 15.4$	$293.\ 3^{a} \pm 9.1$	$137.47^{a} \pm 19.2$	$88.22^{a} \pm 10.9$
4	**CCl_4 + 200 mg kg^{-1} SO-LE**	$148.4^{a} \pm 9.7$	$264.5^{ab} \pm 6.8$	$116.1^{a} \pm 15.3$	$78.23^{a} \pm 13.1$

SD: Standard deviation for every group, n = 6. SO-LE: *Sonchus oleraceus* Extract; carbon tetrachloride (CCl_4). Note: Within each column's values, individual letters (a, b, c, and d) show significant differences ($p < 0.05$).

3.5. Histopathology of liver

Hepatic cords, portal triad structures, biliary system, vascular tributaries, sinusoids, Von Kupffer's cells, supporting stroma, and central veins were all maintained in liver sections from negative group (rats (G 1) **(Fig. 2, 3 A**). No inflammatory, degenerative, or apoptotic alterations were observed in any of the tissues under investigation. Sections from CCl_4-intoxicated rats (G2) revealed features identical to those of CCl_4 toxicity, as moderate periportal hepatocytes signify. Strong portal biliary proliferation and inflammatory responses accompany degenerative and necrotic alterations, which are then followed by fibroblastic hyperplasia and the development of fine fibrous strands encircling the hepatic lobules. Some hepatocytes showed compensatory regenerative changes, as they were large in size with large hyperchromatic or double nuclei, there was a noticeable necrosis with eccentrically positioned pyknotic nuclei with hepatocellular pathognomonic ballooning degeneration. The other hepatocytes showed either micro/macrosteatosis. There was perivascular and interstitial bleeding sporadically along with dilated portal blood vessels. The von Kuffer cells appeared hypertrophied with hyperchromatic nuclei (**Fig 2 B)**. liver sections of carbon tetra chloride-intoxicated 100 mg kg^{-1} SO-LE treated rats **(G3)** demonstrated a significantly higher protective effect of the substance, as seen by the fully regenerating hepatocytes with cytoplasmic basophilia and large hyperchromatic nuclei that had previously been injured. The hepatic parenchyma assumed a normal architectural morphology comparable to control group, occasionally with the existence of double-nucleated hepatocytes. A few sections demonstrated mild portal vascular dilatation, early apoptotic cells, and Von-Kupffer cell hypertrophic reactive changes (**Figs. 2 C)**. Carbon-tetrachloride-intoxicated 200 mg kg^{-1} SO-LE-treated rats **(G4)** revealed a significantly increased protective effect of the substance, as evidenced by the presence of hepatocytes that seemed normal but had large hyperchromatic nuclei and cytoplasmic basophilia. Remnants of CCL_4 toxic effects were seen in about (30-35% of examined cases) and represented by hepatocellular hydropic degeneration, scattered macro-steatosis, and sinusoidal dilatation; necrotic, hemorrhagic or fibroblastic changes were not recorded (**Figs. 2 D).**

CCl_4-intoxicated rats (G2, B) exhibited increasing hepatocytes periportally. Necrotic and degenerative alterations (black arrows) associated by inflammatory responses and portal biliary growth (yellow circles, green, and red arrows). Hepatocytes show micro/macrosteatosis (yellow arrowheads and green stars). Liver sections of carbon tetrachloride-intoxicated plant extract 1 treated rats **(G3, C)** demonstrate hepatic parenchyma, assuming a normal architectural morphology comparable to control free group, occasionally with the existence of some double-nucleated hepatocytes (black arrows). A few sections show mild portal vascular dilatation, early apoptotic cells (orange arrowhead), and von Kupffer cell hypertrophic reactive changes (green star). Carbon tetra chloride is an intoxicated-plant extract 2 treated rats **(G4, D)** show remnants of CCL4 toxic effects represented by hepatocellular hydropic degeneration (black arrows) scattered micro-steatosis (yellow arrowhead) and sinusoidal dilatation (green star). H&E X 100, 200, 400.

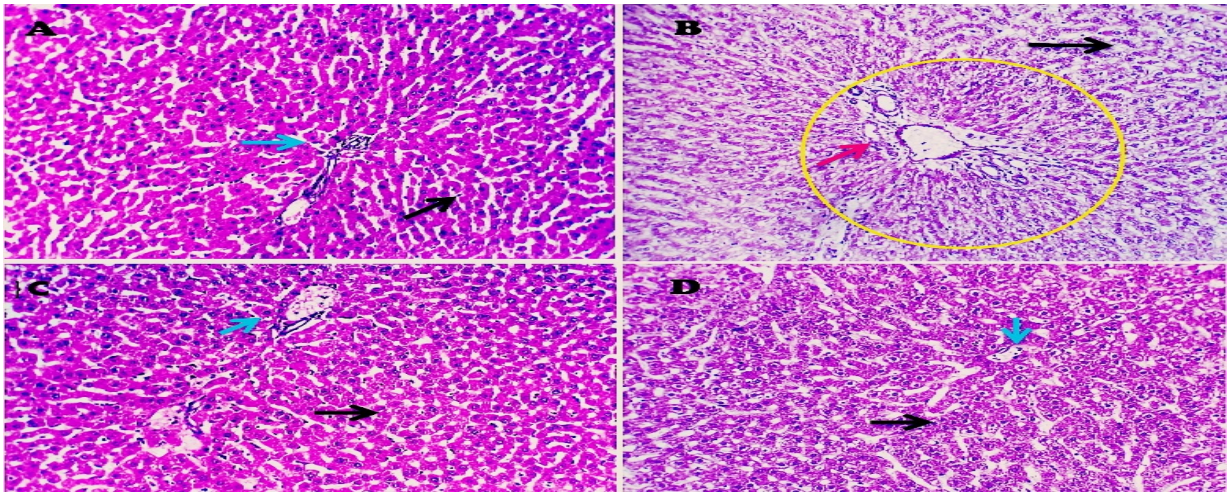

Fig 2: Photomicrographs from the liver of control free rats (G 1, A), carbon tetrachloride treated rats (G2, B), carbon tetrachloride-intoxicated -plant extracts treated rats(G 3 C, G4, D) showing (black arrows) maintained hepatic cords, (blue arrows) portal triad structures, vascular tributaries, biliary system, central veins, sinusoids(green stars), Von Kupffer's cells, and supporting stroma in control rats (G1). H&E X 100, 200, 400.

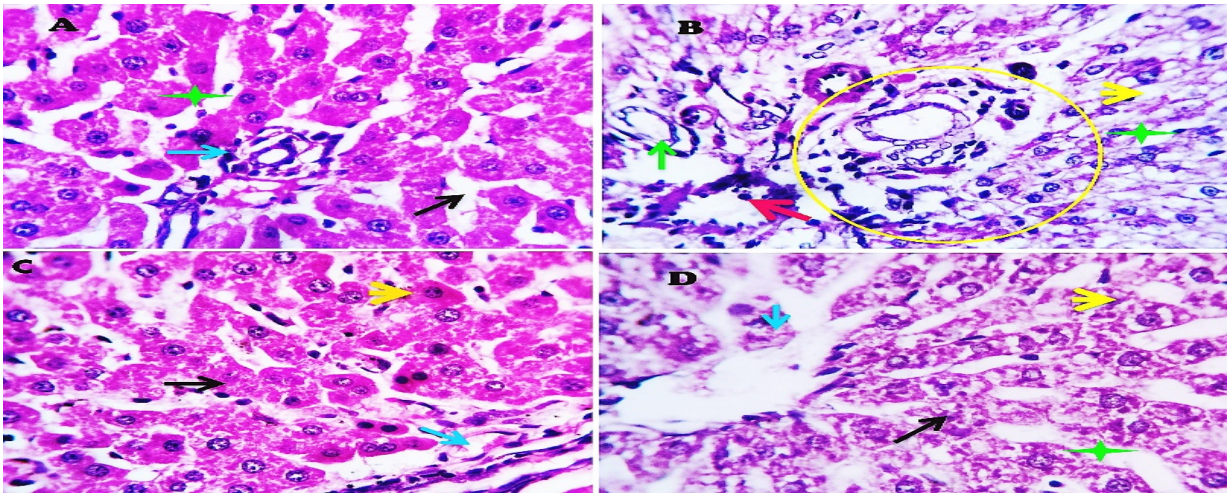

Fig 3 CCl_4-intoxicated rats (G2, B) exhibited increasing hepatocytes periportally. Necrotic and degenerative alterations (black arrows) associated by inflammatory responses and portal biliary growth (yellow circles, green, and red arrows). Hepatocytes show micro/macrosteatosis (yellow arrowheads and green stars). Liver sections of carbon tetrachloride-intoxicated plant extract 1 treated rats (G3, C) demonstrate hepatic parenchyma, assuming a normal architectural morphology comparable to control free group, occasionally with the existence of some double-nucleated hepatocytes(black arrows). A few sections show mild portal vascular dilatation, early apoptotic cells(orange arrowhead), and von Kupffer cell hypertrophic reactive changes (green star). Carbon tetra chloride is an intoxicated-plant extract 2 treated rats (G4, D) show remnants of CCL4 toxic effects represented by hepatocellular hydropic degeneration(black arrows) scattered micro-steatosis (yellow arrowhead) and sinusoidal dilatation(green star). H&E X 100, 200, 400.

4. Discussion

Antioxidant compounds, like phenolic compounds, are highly regarded for their capacity to neutralise free radicals, which are associated with metabolic disorders. These radicals include hydrogen peroxide, hydroxyl radicals, and superoxide anions. [**39 and 40]**. This study revealed that *SO-LE* could be a potential source of active chemicals powerful in antioxidants. There was a higher phenolic content observed in *the SO-LE, which had a higher* flavonoid content (74.23 mgQE/g) The data were consistent with those of **(35**).

The liver is a vital organ for excretion and metabolism is constantly detoxified. Hepatotoxic agents include minerals, bacterial metabolites, fungi, viruses, toxins from the environment, and chemotherapeutic drugs can induce various organ disorders **[41].** Liver diseases, like cirrhosis,fibrosis, hepatitis, and hepatocellular carcinoma, seem to be the most serious of the numerous health issues. Hepatotoxins, which include acetaminophen, ethanol, and CCl4, cause liver injury that shows in various degrees of hepatocyte degeneration and cell death **[42]**. This study investigated how defence enzymes, which are crucial for protecting biological components from oxidative damage, are controlled by the SO-LE extract. Cardiovascular lesions may develop as a result of oxidative stress **[43]**. Superoxide dismutase (SOD) expression is lower in CCL_4-induced participants in multiple studies. After this, SOD activity was improved by treatments with SO-LE at both doses. Additionally, the study demonstrated a significant decrease in the total concentration of GSH (glutathione) in livers of CCL4 rats. In CCL_4 rats, administration of the SO-LE extract increased GSH levels in liver. Thus, it is probable that the decrease in SOD and GSH levels played a role in the reduction of oxidative stress. As seen in Fig. 1, the results of the investigation demonstrated that the positive group (injection of CCL_4) had significantly higher levels of malondialdehyde (MDA), a biomarker of oxidative stress, than the normal group, MDA levels reduced after an 8-week SO-LE extract dosing duration compared to the positive group. Our results show that SO-LE might be effective for the hepatoprotective treatment, without having a negative impact on kidney or liver function, or lipid profile. The current study contrasted rats given CCl4 to produce degenerative changes in their livers with a negative control group which liver histological structure was normal. The mean values of ALT and AST were significantly lower in the treated groups receiving SO-LE 100 and 200 mg kg^{-1} for eight weeks than in the positive control group. This result is in agreement with those reported by **(37).** With a similar duration of treatment with 200 mg kg^{-1} SO-LE , levels of Total Protein, Albumin, Globulin, and Bilirubin were (4, 2.4, 1.6, and 0.13 mg dL^{-1}) respectively. Treatment with SO-LE decreased the creatinine, urea, and uric acid levels in the treatment of 100 mg/kg were (1.1, 42.7 , 3.8, mg dL^{-1}) respectively and in the fourth group treated with 200 mg/kg levels were(0.87 , 38 , 3.8 mg dL^{-1}) respectively if it compared with positive group .These results were in agreement with those reported by **(38).**

Table 4 shows the significant drop in the final body weight of the CCl4 rats as compared to the normal group. Rats treated with CCl4 and rats in negative group showed enhanced final body weights 293.3 , 264.5 g respectively due to SO-LE (100 mg kg-1and 200 mg kg-1) after 56 days of treatment. And there is increase in the percentage of change body weight gain by 88.22 and 78.23 % respectively in the treated groups if it compared with positive group. The results presented agree with the results published by (18)

Rats treated with 100 and 200 mg kg^{-1} SO-LE had lower mean LDL values than the positive group. When compared to the positive control group, the mean values of HDL were greater in all groups treated with SO-LE at different dosages. All lipid indicators (TC, TAG, and LDL) significantly increased with CCl4 treatment, with the exception of HDL. For an eight-week investigation, the positive control group's (group 2) levels of TC, TAG, and LDL were 210.3, 234.3, and 92.7 mg dL^{-1}, respectively., there were a considerable reduced in the levels of TC, TAG, and LDL, with respective values of 129.7, 146.3, and 62.7 mg dL^{-1} with 200 mg kg^{-1} SO-LE treatment. Also, treatment with both doses of SO-LE enhanced HDL levels, whereas treatment with CCl4 caused HDL levels to decrease. This result is in agreement with those reported by (38).

It's interesting to observe that SO-LE treatment at both dosages significantly improved liver histological features, indicating the drug's potential as a therapeutic strategy to support liver health. Based on the comprehensive findings, it was deduced that SO-LE demonstrated antiradical and antioxidant properties, potentially offering protection against oxidative liver damage caused by CCl4.

5. Conclusion

This study evaluated the pharmacological use of Sonchus Oleraceus extract against CCl4 induced liver injuries. SO-*LE* rich source of health promoting antioxidants , improving blood lipid levels , AST and ALT in albino rats intoxicated with carbon tetrachloride

Abbreviations:

SO-LE: *Sonchus oleraceus* extract; CCl4: carbon tetrachloride; MDA: malondialdehyde; DPPH: 1,1dipheny l, 2 picrylhydrazyl; ALT: Alanine Transaminase; LDL: low-density lipoprotein; HDL: highdensity lipoprotein; AST: Aspartate amino ferase; SOD: Superoxide Dismutase; GSH: Glutathione Reductase; GST: GlutathioneS-Transferases; CAT: catalase enzyme; TPC: Total Phenolic; TFC: Total Flavonoid; QE: quercetin equivalent and GAE: Gallic Acid Equivalent.

Ethics approval: Accordance with the Declaration of Helsinki, World Health Organization the study was performed and approved by the Ethics Committee of, Benha University, Faculty of Agriculture protocol code REC-FOABU.5/0006

Consent to participate: Not applicable

Consent to Publish: We confirm that all participants have provided their consent for the publication of data and findings derived from this study. Participants were informed about the nature of the publication, including the potential for public and scientific community access. Written consent for publication was obtained from each participant, ensuring they understand and agree to the dissemination of their data in this manuscript.

Data Availability statement: All of the data gathered or analyzed during this investigation are included in this article.

Conflicts of Interest: No conflicts of interest are disclosed by the authors.

Funding: There was no external funding for this study.

Author Contributions: Conceptualization, Nesrein Salim; methodology, Mohamed Abdel-Alim and Nesrein Salim; validation Nesrein Salim and Mohamed Abdel-Alim; formal analysis, Mohamed Abdel-Alim; investigation, Nesrein Salim, and Mohamed Abdel-Alim; data curation, Mohamed Abdel-Alim; original draft preparation, Nesrein Salim; review and editing, visualization, Nesrein Salim and Mohamed Abdel-Alim.

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