Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Evaluation of Hepatoprotective Activity of *Sonchus oleraceus* L. Extract against CCl_4-induced Hepatic Damage in Wistar Rats

Kusum S. Akki*[1], Rashmi H C[1], Pratiksha Akki[2]

[1]*Department of Pharmacognosy, K.L.E. College of Pharmacy, Vidyanagar, Hubli, Karnataka, India*
[2]*Department of Pharmaceutics, K.L.E. College of Pharmacy, Vidyanagar, Hubli, Karnataka, India*

ARTICLE INFO

Article history:
Received: 23 November, 2021
Revised: 16 June, 2022
Accepted: 20 June, 2022
Published: 30 July, 2022

Keywords:
CCl_4-induced Hepatotoxicity, Hepatoprotective activity, *Sonchus oleraceus* L.

DOI:
10.25004/IJPSDR.2022.140401

ABSTRACT

Sonchus oleraceus L. (Asteraceae) has a widespread world distribution and is also common in Brazil. It is a dietary and traditional medicinal plant in Chinese folk culture that can be cooked and eaten to treat inflammatory diseases. The aim of the present study was to offer a novel perspective on the medicinal product originating from this species and to test its hepatoprotective activity. Petroleum ether, Ethyl acetate, and Methanol successive extracts obtained from the aerial parts were used for the hepatoprotective study. Compounds evaluated for this activity were Flavonoids and Sesquiterpene glycosides, that are identified and quantified by Column chromatography and Proton nuclear magnetic resonance (^{1}H- NMR) and Mass Spectra, respectively. Hepatoprotective activity was assessed *in-vitro*, using various biochemical parameters *i.e.,* serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), Systematic Assessment of Licensee Performance (SALP), bilirubin (total and direct), and isolated liver. Hepatoprotective activity was tested in rats with experimentally-induced hepatotoxicity by CCl_4. The tested extract proved significant hepatoprotective capacity under *in-vitro* conditions. Results of the *in-vivo* experiment showed that prominent hepatoprotective activity was shown by successive ethyl acetate and petroleum ether extracts. In this way, the present study offered a novel perspective on the medicinal uses of the species, proving significant amounts of Flavonoids and Sesquiterpene glycosides in the composition of the aerial parts, that has proved hepatoprotective activity.

Introduction

Sonchus oleraceus L. (Asteraceae) has a widespread world distribution and is also common in Brazil. It is considered native to Europe and North Africa and is commonly problematic as an invader of many crops.[1] *S. oleraceus* is a plant belongs to the family Asteraceae which is in use in folklore medicine in treatment of gastrointestinal tract disorder in addition to its used as food in some parts of Asia and Africa.[2] *S. oleraceus* is edible to humans as a leaf vegetable and is frequently consumed in Mediterranean countries.[3-7] Australia [8] and New Zealand, particularly by the native Maori.[9] In Brazilian traditional medicine, the aerial parts of *S. oleraceus* are used mostly in salad, infusions or decoctions and are administered orally to treat stomach pain, hepatitis, infections, inflammation, headaches, general pain, rheumatism, and even as a general tonic.[10-12] In addition, the antioxidant, antinociceptive and anxiolytic properties of *S. oleraceus* extract have previously been reported.[13-16] A previous phytochemical investigation of *S. oleraceus* resulted in the isolation of flavonoids and terpenes.[17-18] The plant contains taraxasterol, apigenin 7-glucuronide, and luteolin 7-glucoside.[19] Alkaloids, coumarins, flavonoids, and saponins have also been detected.[19-21] In addition, antioxidant properties of the *S. oleraceus* extract have previously been reported.[22-23] However, the said plant is not evaluated previously for its hepatoprotective activity. Hence, in the present study, the researchers have investigated the hepatoprotective activity of *S. oleraceus* L. against CCl_4-induced hepatotoxicity.

***Corresponding Author:** Kusum S. Akki

Address: Department of Pharmacognosy, K.L.E. College of Pharmacy, Vidyanagar, Hubli, Karnataka, India

Email: kusum_akki@yahoo.com.

Tel.: 9448822195

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

MATERIAL AND METHODS

Plant Material

The aerial parts of *S. oleraceus* L. were collected from the local surrounding areas of GADAG and Hubli, Dharwad district of Karnataka, and authenticated by Dr. B. D. Huddar, Head, Department of Botany, Shri Kadasiddheshwar Arts College and H.S. Kotambari Science institute, Vidyanagar, Hubli.

Study of Morphological and Microscopical characters

Morphological features and organoleptic features viz. color, odor, taste, shape, and size, were observed and evaluated botanically. Transverse sections of stem and leaf were observed to study the microscopical characters. The shade-dried aerial parts of *S. oleraceus* L. were coarsely powdered and then stained with phloroglucinol and HCl in a 1:1 ratio, observed under high power (40 x), for different diagnostic characters such as trichomes, stomata, lignified fibers, xylem, cork cells, starch grains, and calcium oxalate crystals, etc. The cytomorphological study viz., Stomatal index, vein islet, and vein termination number were also conducted. The proximate values *viz.,* extractive value, moisture content, and ash value were also determined.

Preparation of Plant Extract

The plant extract from *S. oleraceus* L**.** was obtained by using the method given by Vilela *et al.,* 2009 with some modifications.[24] The *S. oleraceus* L**.** aerial parts were shade dried at room temperature, pulverized and 100 g of coarse powder was extracted exhaustively with 95% ethanol at temperature 40–60°C in a soxhlet extractor. The extract was concentrated in a rotary flash evaporator and the residue was dried in a desiccator over sodium sulfite. 70–80 g per batch of shade-dried *S. oleraceus* L**.** aerial parts powder was successively extracted with petroleum ether (40–60), ethyl acetate and methanol in increasing order of polarity. The extracts were concentrated under reduced pressure using a rotary flash evaporator and the residues were dried in desiccator over sodium sulfite. After drying, the respective extracts were weighed and the percentage yield was determined. The extracts were subjected for phytochemical investigations by qualitative chemical tests.

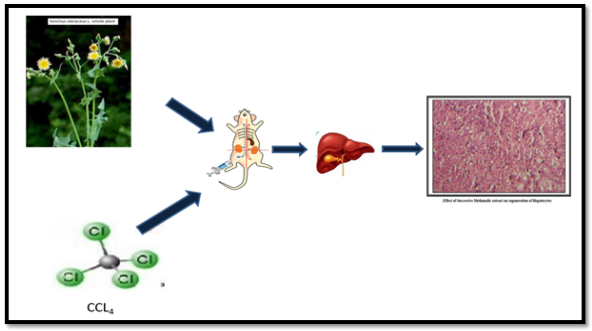

Fig. 1: CCl_4-induced Hepatotoxicity and Hepatoprotective activity of *S. oleraceus* L.

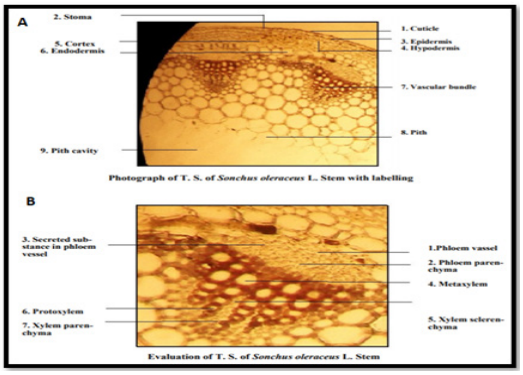

Fig. 2: (A) T.S. of *S. oleraceus* L stem and (B) Vascular bundles

Qualitative Chemical identification

All the extracts of *S. oleraceus* L**.** aerial parts were subjected to qualitative chemical tests to detect the presence of various phytoconstituents *viz*., carbohydrates, proteins and amino acids, sterols and triterpenoids, glycosides, alkaloids, phenolic compounds, flavonoids, tannins and steroidal glycosides.

Chromatographic study and isolation of Phytoconstituents

Successive methanolic extract, ethyl acetate extract and petroleum ether extracts were evaluated by TLC for the presence of different phytoconstituents. Column chromatographic technique has been adopted for isolation purpose and two phytoconstituents (A and B) were isolated from successive petroleum ether extract. Isolated compounds (A and B) were dissolved in pure methanol separately and evaporated on the hot water bath. After 90% evaporation, filtered to separate the powder appeared. Similarly, one phytoconstituent (C) was isolated from the methanolic extract and purified by adopting the same method as for Compound A and B. Similarly, sesquiterpene glycosides were isolated by Miyase *et al.,* (1987). Flavonoids and glucosides were isolated and antioxidant activity was estimated by Yin j. *et al.,* (2008).[21, 25]

Characterization of isolated Phytoconstituents (A, B and C)

The isolated compounds were subjected to physical, chemical spectral characterization.

Pharmacological screening of S. oleraceus L. for Hepatoprotective activity

Experimental Animals

Hepatoprotective activity of *S. oleraceus* L. was assessed as per the method given by Ouassou H *et al.,* (2021).[26] The experiment was carried out using wistar albino mice of either sex weighing between 25–50 gm for acute toxicity study and wistar albino rats of either sex weighing around 150–200 gms were used for the Hepatoprotective activity. The animals are grouped into six of six animals each and maintained at normal laboratory conditions and were given a standard diet and water ad libitum.

The organisation for economic co-operation and development (OECD) guideline no-420 fixed dose method was adopted and accordingly, doses of successive petroleum ether, ethyl acetate and methanolic extracts were calculated.

Preparation of Doses and Treatments

Successive petroleum ether, ethyl acetate and methanolic extracts of aerial parts of *S. oleraceus* L. were screened for its Hepatoprotective property. The dried extracts were suspended in distilled water using 1% Tween 80 as an emulsifying agent and employed for the study. The safest dose of successive petroleum ether, ethyl acetate and methanolic extracts is 2000 mg/kg body weight. The safe dose was found to be 2000 mg/kg body weight; hence $1/10^{th}$ of the dose was taken as effective dose, which is found to be 200 mg/kg body weight. Liv-52 syrup was used as positive control and carbon tetrachloride (CCl_4) was used as hepatotoxins to induce hepatotoxicity. The liv-52 dose taken was 1-mL/kg body weight by oral route and carbon tetrachloride [CCl_4] 0.7 mL/kg body weight by intraperitoneal route.

CCl_4-Induced Hepatotoxicity Model in Rats

After the adaptation, the animals were divided into 6 experimental groups of 6 animals each, and treated as follows: Tween-80 (1%) is given to I, II, III, IV, V and VI groups as a vehicle for 7 days by oral route. Liv-52 is administered to III group at the dose of 1-mL/kg body weight by oral route for 10 days. The extracts are administered to IV, V and VI groups respectively at a dose of 200 mg/kg body weight by oral route for 10 days. CCl_4 at a dose of 0.7 mL/kg of body weight is injected to II, III, IV, V and VI groups on 2, and 3 days by Intraperitonial route, over a period of 8 weeks. At the end of the experiment (8 weeks), the animals are anesthetized and sacrificed by mild ether anaesthesia and blood is collected from the Carotid artery, serum is separated and used for the estimation of various biochemical parameters like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), Serum Alkaline Phosphatase (SALP) and bilirubin (total and direct). Livers were excised and fixed in formalin for assessment of histopathological studies and liver weight.

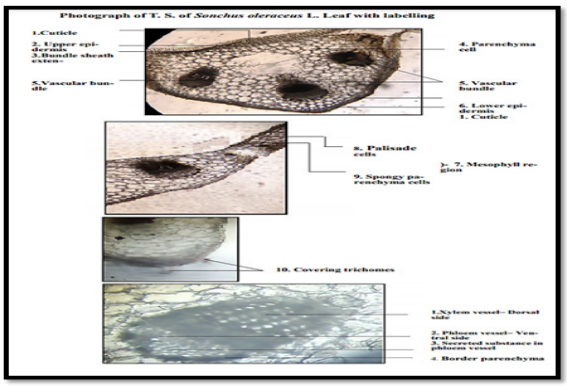

Fig. 3: Photograph of T. S. of *S. oleraceus* L. Leaf with labelling

Biochemical Parameters Determination

Biochemical parameters such as SGPT, SGOT, SALP and bilirubin (total and direct) were determined according to the standard procedure prescribed by the manufacturer (Transasia Biomedicals Ltd., Daman, India).

Estimation of SALT, SAST, AST, SAP and Total Bilirubin

After collecting the blood for the estimation of biochemical parameters, the liver was isolated, washed with alcohol and weight was determined by recording liver weight with respect to body weight per 100 grams. The isolated livers were preserved in 10% neutral formalin solution for histopathological studies. SALT is also called as SGPT, which is located in the cytosol of the liver cell. During liver cell inflammation and breakdown of liver cells, they are released into circulation due to increased permeability of cell membrane. Hence determination of SALT is an index of the extent of liver damage. Its normal serum is 0-40 IU/L. SAST is located on the cytosol of liver. The hepatic cell damage leads to an increased level of SGOT in blood serum. Its normal serum is 5-34 IU/L. Serum aspartate amino transferase (AST) also known as SGOT is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha amino acids. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma and viral hepatitis, will also increase serum AST levels. Many

Table 1: Morphological features of *S. oleraceus* L.

S. No.	*Features*	*Observations*	
		Stem	***Fresh leaf***
1	Color	Glabrous-green to purple	Glabrous-green
2	Odor	Characteristic	Characteristic
3	Taste	Peculiar	Peculiar
4	Shape	Cylindrical	Lyrate
5	Angle	Five angled hollow stems	-
6	Size	1–6 ft. in length, 0.5–1 cm in width	25 cm in length, 2.5–3 inches in width
7	Fracture	Fibrous and exude a milky latex	-
8	Surface	Smooth	-
9	Internode	10–12 cm long	-

Table 2: Botanical evaluation of *Sonchus oleraceus* L. Leaf.

S. no.	*Leaf portion*	*Observation*
1	Leaves	Sessile
2	Phyllotaxy	Alternate, Spiral
3	Apex	Acute auricles
4	Margin	Dentate with soft spines
5	Lamina	
	Shape	Oblong, Runcinate, Lanceolate
	Composition	Simple
	Texture	Soft, thin
	Venation	Reticulate, Divergent
	Midrib	Distinct, On ventral surface more distinct
	Surface	Glabrous
	Dorsal Surface	Dark green, Smooth, Glabrous
	Ventral Surface	Light green, Smooth, Glabrous
	Leaf Base	Asymmetrical

tissues produce serum alkaline phosphatase, especially bone, liver, intestine and placenta and excreted in the bile. Most of the normal serum alkaline Phosphatase (range 15–112 IU/L) is derived from bone. Elevation in activity of this can be found in the disease of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase level generally reflects hepatobiliary disease.

Histopathological Study

The isolated organ was cut into small pieces and preserved in formalin (10% solution) for at least 2 days. The liver pieces were washed in running water for about 12 hours. This was followed by dehydration with alcohol of increasing strength (70, 80 and 90%) for 12 hours each. The tissue was again cleaned by using xylene 2 times for 15 to 20 minutes each and the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit. Hard paraffin was melted and was poured into square-shaped blocks. The liver pieces were then quickly dropped into the liquid paraffin and allowed to cool. The blocks were cut using microtome to get section of thickness 5 microns. The section was then taken on a microscope slide on which egg albumin (sticky substance) was applied. The sections were allowed to remain on the sticky substance for three days till it sticks firmly on the slide. The section should be dried completely before staining. Eosin is an acidic stain and haematoxylin is a basic stain used for staining. All the slides were observed for change in histopathological characteristics and photographs were taken.

RESULTS

Initially, the plant parts were investigated for morphological and microscopic characteristics. The successive ethyl acetate, petroleum ether and methanolic extracts were obtained and phytochemical investigation was carried out. The active phytoconstituents were isolated and characterized. Finally, the extracts were evaluated for hepatoprotective activity in Wistar rats.

Pharmacognostical Investigations

Morphological Evaluation of S. oleraceus L.

The morphological features of *S. oleraceus* L. stem and fresh leaf are depicted in Table 1 and the botanical evaluation is presented in Table 2.

Microscopic Observations

Examination of diagnostic characters of the powdered leaves and stem indicated the presence of spiral xylem vessels, uniseriate multicellular covering trichomes, wavy walled epidermal cells with anomocytic type of stomata, collenchyma cells, lignified phloem fibres, simple and polyhedral starch grains, prism and acicular raphides type calcium oxalate crystals (Fig 2 (A) and (B)).

Histological studies of the leaf showed the arrangement of tissues in the lamina and midrib region as follows: upper and lower epidermis consists of single layered rectangular cells covered with a cuticle, some of lower epidermal cells are interrupted with anomocytic type of stomata and unisereate multicellular covering trichomes. Mesophyll region is differentiated into palisade cells and spongy parenchyma which contain starch grains and square type calcium oxalate crystals. The midrib region consists of collateral type of vascular bundles and on either side

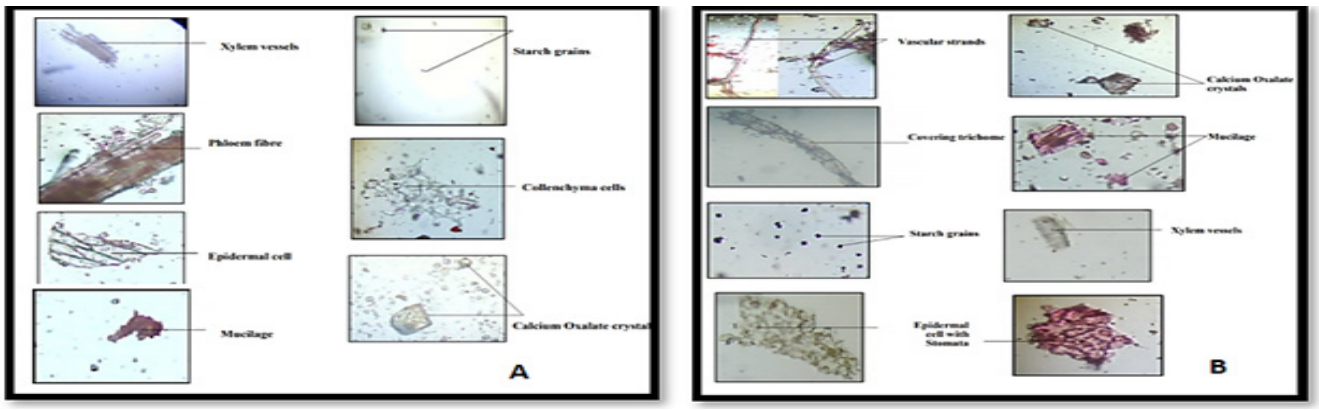

Fig. 4: (A) Powdered Microscopy of *Sonchus oleraceus* L. dried stem and (B) Powdered characteristics of *Sonchus oleraceus* L. dried leaves.

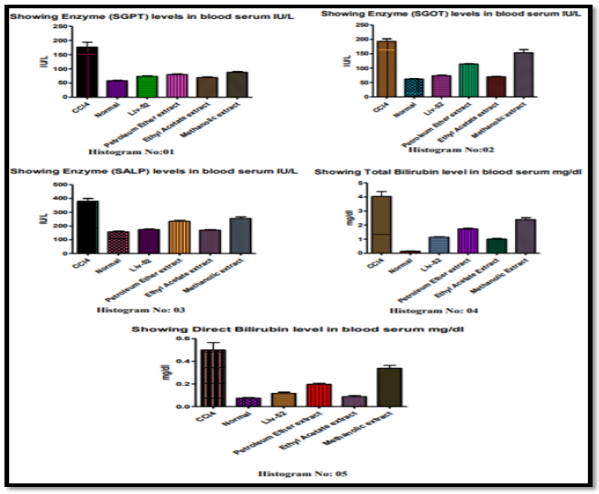

Fig. 5: Level of SGPT, SGOT, SALP and total bilirubin levels in blood serum

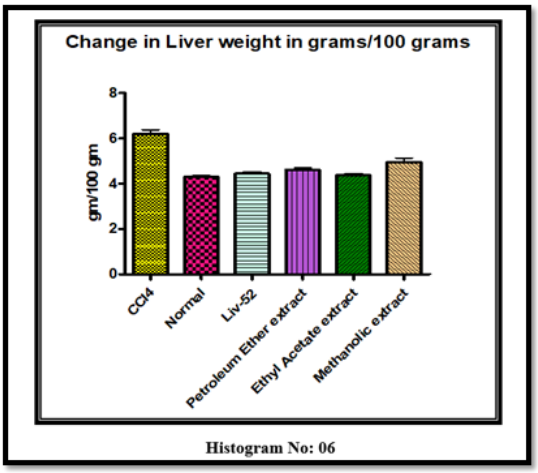

Fig.6: Effect of different successive extracts of *S. oleraceus* L. on liver weight

Table 3: Percentage yield and physical characteristics of various extracts of *S. oleraceus* L. aerial parts

Extract	*% Dry wt. in gm*	*Colour*	*Odour*	*Consistency*
Total Alcoholic	11.85	Blackish green	Characteristic	Sticky
Aqueous	24.32	Brownish green	Characteristic	Sticky
Successive extraction				
Petroleum Ether (40-60° C)	3.5	Dark green	Characteristic	Waxy
Ethyl acetate	3.81	Brownish green	Characteristic	Sticky
Methanol	20.22	Reddish Brown	Characteristic	Sticky
Aqueous	3.2	Brownish green	Characteristic	Sticky

of vascular bundle below the upper and lower epidermis are collenchyma cells. Histological studies of the stem showed the arrangement of tissues as follows: epidermis consisting of single-layered cubical cells covered with a cuticle, hypodermis, cortex of parenchyma cells which contain starch grains and square type calcium oxalate crystals, endodermis followed by open collateral type of vascular bundle (Fig. 3). Leaf surface data like stomatal index, vein islet and veinlet termination number were found to be 33-34, 6-7 and 16-17, respectively.

Proximate values

Various proximate values for the aerial parts of *S. oleraceus* L. were performed and were found to be, alcohol soluble extractive value (10.2%), ether soluble extractive value (2%), water-soluble extractive value (11.6%), moisture content (12.80%), total ash (15%), insoluble acid ash (13.33%), water-soluble ash (9%), sulfated ash (31%). These values are criterion to judge the identity and purity of crude drug.

Powder Microscopy

Powdered microscopy of *S. oleraceus* L dried stem revealed that the xylem vessels were spiral and lignified phloem with rectangular shape single layer of epithelial cells. Rethenium red stained mucilage, simple and rounded starch grains, polygonal-shaped collenchyma cells and prism and square shaped calcium oxalate crystals were also found (Fig.4(A)). Similarly, spiral and lignified xylem vessels with wavy walled epidermal cells comprising of anomocytic type of stomata were present in powdered sample of dried leaves of *S. oleraceus*. Uniseriate, multicellular covering trichomes, square & prism shaped calcium oxalate crystals, simple polyhedral starch grains and rethenium red stained mucilage were also seen in the powder microscopy (Fig. 4(B)).

Phytochemical Investigations

Qualitative chemical examinations of various extracts revealed the presence of terpenoids, steroids, carbohydrates, phenolic compounds, glycosides and alkaloids. Further, TLC studies confirmed the presence of terpenoids in successive petroleum ether extract and flavonoids in successive ethyl acetate and successive methanolic extracts (Table 3 and 4).

Chromatographic Studies

It has been found that the plant *S. oleraceus* L. consists of flavonoids and sesquiterpene glycosides as major phytoconstituents. So, an attempt was made to isolate these phytoconstituents by column chromatography and characterization of the isolated compounds was performed by spectral studies. The results of the study were summarized in Table 5.

Based on the characterization of isolated compounds, it was concluded that compound A and B were sesquiterpene

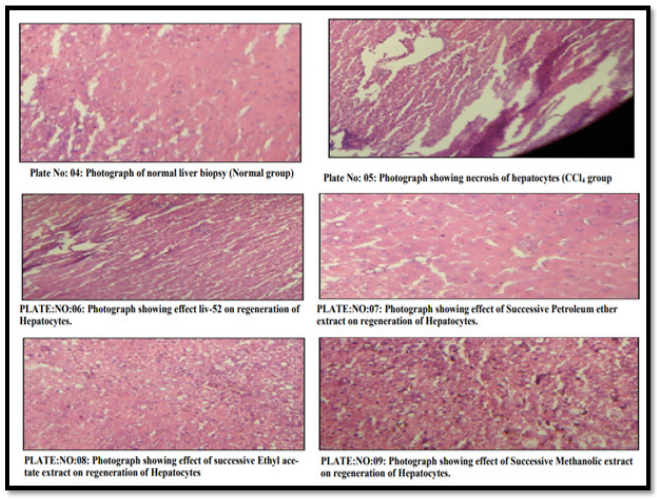

Fig. 7: Histopathology of Liver and hepatoprotective effect of *S. oleraceus* L. extracts

glycosides while the compound C was confirmed as a flavonoid.

Pharmacological investigation

Enzymes (SGPT, SGOT, SALP and total Bilirubin) levels in blood serum IU/L

An increased levels of SGOT, SGPT, SALP and Bilirubin (Total and Direct) in blood serum was observed due to hepatotoxicity induced by CCl_4 (Table 6, Fig 5).

Effect of Different Extracts of *S. oleraceus* L. on Liver Weight

Liver weights of all animals were recorded and the difference among all the groups was noted. In the CCl_4 treated group there was an increase in the liver weight. The extracts treated groups reduce the increased liver weight to the normal level in the following range: successive ethyl acetate extract > successive petroleum ether extract > successive methanolic extract. The results were depicted in Table 7 and Fig. 6.

Hepatoprotective Activity

The successive petroleum ether, ethyl acetate and methanolic extracts of aerial parts of *S. oleraceus* L. were evaluated for hepatoprotective activity and the results indicated that the elevated levels of SGOT, SGPT, SALP and Bilirubin (Total & Direct) in blood serum were significantly reduced in successive petroleum ether, successive ethyl acetate and successive methanolic extracts. The reduction was significant in the following range: successive ethyl acetate extract > successive petroleum ether extract > successive methanolic extract. The results of the study shown in Table 8.

Table 4: Qualitative chemical analysis of various extracts of *S. oleraceus* L aerial parts

Nature of phytoconstituents	*Total Alc.*	*Aqueous*	*Successive Extraction*			
			P.E	*E.A*	*Met*	*Aq*
Flavonoids	+	--	--	+	+	--
Triterpenoids	+	--	+	--	--	--
Alkaloids	+	+	--	+	--	--
Steroids	+	--	+		--	--
Carbohydrates	+	+	--	+	+	+
Tannins	+	+	--	+	+	+
Protein & Amino acid	--	--	--	--	--	--
Glycoside	+	+	+	--	+	--

Histopathological Study

The Histopathological studies of liver were carried out to support the above activity. Hepatocytes from central vein and portal traids were shown in the liver section of normal group. A fatty change with lobular necrosis was observed in CCl_4 control group. Liv-52 treated group showed the foci of necrosis and binucleate hepatocytes were seen with regenerative activity. In successive petroleum ether extract treated group, hepatocytes with moderate necrosis were seen while, regenerating hepatocytes were also seen. Minimal hepatocyte necrosis with a good number of binucleate regenerating hepatocytes were observed in successive ethyl acetate extract treated group. Dense foci of necrosis were seen in successive methanolic extract treated groups. Some hepatocytes showing fatty change and few regenerating hepatocytes were also observed in this group (Fig. 7).

Table 5: Characterization of Isolated phytoconstituents A, B and C

Isolated Compounds	*U V Spectra (λ max)*	*IR spectra (wave no.)*	*NMR spectra (d Values)*	*MS Spectra*
Compound A	410 nm	1737.23 cm^{-1} for OH Stretching and 3452.66 cm-1 for -C=0 Stretching.	0.97 (CH_3 Proton), 1.13 (CH_3 Proton), 1.15 (CH_3 Proton), 2.25 (CH_2 Protons), 3.5 (O-CH_2-)	m/z (relative intensity) at 413.4, Base peak at 353.3
Compound B	350 nm	1735 cm^{-1} for OH Stretching and 3369 cm^{-1} for -C=0 Stretching	0.95 (CH_3 Proton), 1.15 (CH_3 Proton), 1.16 (CH_3 Proton), 2.7 (CH_2 Protons), 3.87 (O-CH_2-)	m/z (relative intensity) at 542, Base peak at 353.3
Compound C	269 nm	1687.66 cm^{-1} for OH Stretching and 3491.95 cm^{-1} for -C=0 Stretching	7.7 (resorcinol protons), 7.6 (phenolic-aromatic) 6.9 and 6.8 (OH Protons)	m/z molecular ion peak at 331.3

Table 6: Enzymes (SGPT, SGOT, SALP and total Bilirubin) levels in blood serum IU/L

Enzyme	*Values*	*Normal*	*CCl_4*	*Liv-52*	*Successive extracts*		
					Petroleum ether	*Ethyl acetate*	*Methanol*
SGPT	Mean	58.15	176.53	73.38	79.83	69.48	87.88
	S. D *p-value*	3.628 <0.0001	42.035	7.093 <0.0001	5.950 <0.0001	5.932 <0.0001	5.840 <0.0001
SGOT	Mean	62.15	193.23	73.63	114.2	69.91	153.68
	S. D *p-value*	2.966 < 0.0001	21.325	4.880 < 0.0001	5.399 < 0.0001	4.944 < 0.0001	26.032 < 0.0001
SALP	Mean	157.76	380.20	174.23	234.56	169.93	254.85
	S. D	16.187	48.283	9.557	19.815	11.586	29.200
	p-value	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001
Total bilirubin	Mean	0.138	4.035	1.138	1.725	1.008	2.39
	S. D *p-value*	0.0365 <0.0001	0.858	0.1017 < 0.0001	0.1179 <0.0001	0.106 <0.0001	0.328 <0.0001
Direct bilirubin	Mean	0.075	0.5	0.116	0.196	0.088	0.338
	S. D *p-value*	0.0151 <0.0001	0.1589	0.0258 <0.0001	0.0250 <0.0001	0.0231 < 0.0001	0.0621 < 0.0001

Table 7: Effect of different successive extracts of *S. oleraceus* L. on liver weight in CCl_4 induced hepatotoxicity

Animal No.	*Normal*	*CCl_4*	*Liv-52*	*Successive extracts*		
				Petroleum ether	*Ethyl acetate*	*Methanol*
1	4.12	6.2	4.21	4.35	4.12	5.23
2	4.52	6.3	4.62	4.81	4.55	4.91
3	4.15	6.8	4.33	4.53	4.21	5.41
4	4.22	5.6	4.41	4.62	4.30	4.32
5	4.28	5.9	4.37	4.45	4.35	4.53
6	4.50	6.5	4.72	4.92	4.68	5.32
Mean	4.29	6.21	4.44	4.61	4.36	4.95
S. D	0.1733	0.4262	0.1905	0.2171	0.210	0.447
S.E.M	0.070	0.174	0.077	0.088	0.086	0.182
p-value	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001

Table 8: Effect of different successive extracts of *S. oleraceus* L. on different biochemical parameters in CCl_4 induced hepatotoxicity

Group	*SGPT*	*SGOT*	*SALP*	*Total bilirubin*	*Direct bilirubin*	*Liver weight*
Normal	58.15 ± 1.481	62.15 ± 1.211	157.76 ± 6.608	0.138 ± 0.019	0.075 ± 0.0061	4.29 ± 0.070
CCl_4	176.53 ± 17.161	193.23 ± 8.706	380.20 ± 19.711	4.035 ± 0.3505	0.5 ± 0.0648	6.21 ± 0.174
Liv-52	73.38 ± 2.896***	73.63 ± 1.992***	174.23 ± 3.902***	1.138 ± 0.0415***	0.116 ± 0.0105***	4.44 ± 0.077***
Successive petroleum ether extract	79.83 ± 2.429***	114.2 ± 2.204***	234.56 ± 8.090***	1.725 ± 0.048***	0.196 ± 0.0102***	4.61 ± 0.088***
Successive ethyl acetate extract	69.48 ± 2.422***	69.91 ± 2.018***	169.93 ± 4.730***	1.008 ± 0.043***	0.088 ± 0.0094***	4.38 ± 0.086***
Successive methanolic extract	87.88 ± 2.384***	153.68 ± 10.628***	254.85 ± 11.921***	2.39 ± 0.134***	0.338 ± 0.0253***	4.95 ± 0.182***

*Results are expressed as mean ±SEM, n=4, ***p < 0.01 vs CCl_4-intoxicated group using one way ANOVA followed by Dunnet's post test

From the above studies, it is evident that successive ethyl acetate and successive petroleum ether extracts of aerial parts of *S. oleraceus* L. plays a promising role in the treatment of liver disease and worth for further investigations for isolation of more bioactive phytoconstituents for the above treatment.

DISCUSSION

Liver disease is a metabolic disorder which is the most common cause of mortality and morbidity worldwide. Hence, medicinal herbs with hepatoprotective properties have received considerable attention from researchers. Recently, researchers have utilized medicinal herb in experiments to investigate their hepatoprotective properties on animals.[27] In this study, we aimed to investigate the hepatoprotective effect of *S. oleraceus* extract on liver damage by measuring serum levels of various biochemical parameters as enzyme markers of hepatocellular damage. Liver injuries are induced by carbon tetrachloride in rat models. CCl_4 is a commonly used model for the investigation of hepatoprotective activity on various experimental animals.[28] The liver damage caused by CCl_4 is similar to that produced by viral hepatitis.[29] The elevated serum enzyme levels of enzymes *viz.*, SGPT, SGOT and SALP and bilirubin have been attributed to the damaged structural integrity of the liver because they are cytoplasmic in origin and are released into the blood after hepatic damage.[30] Our findings showed that SGPT, SGOT and SALP activities were increased in rats with the CCl_4 treatment alone in comparison with the normal control group. This elevation in hepatic markers has been attributed to the cells damaged or cell membranes became leaky and they are released into the circulation.[31-32] In contrast, a significant reduction in plasma activities of SGPT, SGOT, and SALP was found in rats with *S. oleraceus* extract+ CCl_4 in comparison with the CCl_4-treated group. This finding suggests that *S. oleraceus* extracts protected the liver tissue from CCl_4-induced injury. Besides, S. oleraceus extracts ameliorated the excretory function of the liver, and this effect was shown by suppressing the elevation of the bilirubin (total and direct) serum level. This effect indicated that the extract improved metabolic function by restoring serum triglycerides (TG) and VLDL levels to normal values compared to the CCl_4-treated group. The other lesion of hepatic injury was hepatomegaly. While the liver index was an objective indicator to reflect hepatomegaly, eliminating individual variation led to the difference of liver weights. In the present study, the liver index significantly enlarged in the CCl_4-treated group, which indicated that CCl_4 caused hepatic damage and hepatomegaly. However, the treatment with *S. oleraceus* extract (200 mg/kg) restored the liver weight and the liver index to the condition, almost like in the normal group. Free-radical production plays a key role in the mechanism pathway of CCl_4-induced acute liver injury. Hence, the scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the hepatotoxicity of CCl_4 and reduce liver damage.[33] The antioxidant properties of the *S. oleraceus* extract have previously been reported.[22-23] The phytoconstituents such as flavonoids, glycosides, triterpenoids, alkaloids, and saponins are known to possess hepatoprotective activity. Flavonoids have been known for their antioxidant and antiperoxidative properties leading to hepatoprotective activities.[34] However, further studies are required before we can conclude on the exact mechanism(s) involved in the hepatoprotective activity of the *S. oleraceus*.

CONCLUSIONS

Experimental evidence obtained in the present study showed that the administration of *S. oleraceus* extracts exerted favorable hepatoprotective activity against carbon tetrachloride-induced liver damage. This activity may be due to the presence of flavonoids and other components present in the plant. Significant results were obtained from the successive ethyl acetate and petroleum ether extracts. However, complementary *in-vitro* and *in-vivo* studies will be necessary to confirm these findings and explore the mechanism responsible for this hepatoprotective effect.

REFERENCES

1. Vieira BS, Barreto RW. First Record of Bremia lactucae Infecting Sonchus oleraceus and Sonchus asper in Brazil and its Infectivity to Lettuce. Journal of Phytopathology. 2006;154 (2):84-87. doi:10.1111/J.1439-0434.2006.01064.X
2. Hussain J, Muhammad Z, Ullah R, et al. Evaluation of the Chemical Composition of Sonchus eruca and Sonchus asper. Journal of American Science. 2010; 6(9): 231-235.
3. Jose Luis Guil-Guerrero,1* Antonio Gimenez-Gimenez,1 Iganacio Rodrguez-Garca3 and Mara Esperanza Torija-Isasa2. Nutritional composition of Sonchus species (S asperL, S oleraceusL and S tenerrimusL). J Sci Food Agric 1998; 76: 628È632.
4. PM G. Food medicine and minor nourishment in the folk traditions of Central Italy (Marche, Abruzzo and Latium). Fitoterapia. 2003;74(6):515-544. doi:10.1016/S0367-326X(03)00122-9.
5. Michael Heinrich MLSNWP. "Local food - Nutraceuticals": An example of a multidisciplinary research project on local knowledge, J Physiol Pharmacol. 2005; 56 Suppl 1:5-22.
6. Guarrera PM, Salerno G, Caneva G. Food, flavouring and feed plant traditions in the Tyrrhenian sector of Basilicata, Italy. Journal of Ethnobiology and Ethnomedicine 2006 2:1. 2006;2(1):1-6. doi:10.1186/1746-4269-2-37
7. Lentini F, Venza F. Wild food plants of popular use in Sicily. Journal of Ethnobiology and Ethnomedicine 2007 3:1. 2007;3(1):1-12. doi:10.1186/1746-4269-3-15
8. Liu L, Howe P, Zhou Y-F, Hocart C, Zhang R. Fatty acid profiles of leaves of nine edible wild plants: an Australian study. Journal of Food Lipids. 2002; 9(1): 65-71. doi:10.1111/J.1745-4522.2002.TB00209.X
9. RC C, LR F. Potential functional foods in the traditional Maori diet. Mutation research. 2003;523-524:109-117. doi:10.1016/S0027-5107(02)00344-5
10. FC V, M de MP, L DS-E-S, G A-S, A G-P. Evaluation of the antinociceptive activity of extracts of Sonchus oleraceus L. in mice. Journal of ethnopharmacology. 2009;124(2):306-310. doi:10.1016/J.JEP.2009.04.037
11. MF A, GS B, K N, IJ B, VP C. Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. Journal of Ethnopharmacology. 2007;111(2): 383-395. doi:10.1016/J.JEP.2006.12.007.
12. G.S. VendruscoloL.A. Mentz. Ethnobotanical survey of the medicinal plants used by the community of Ponta Grossa neighborhood, Porto Alegre, Rio Grande do Sul, Brazil. Iheringia Serie Botanica. 2006; 61(1):83-103.
13. SN E, S K. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. International journal of food sciences and nutrition. 2004; 55(1):67-74. doi:10.1080/09637480310001642501

14. Schaffer S, Schmitt-Schillig S, Müller WE, Eckert GP. Antioxidant properties of Mediterranean food plant extracts: Geographical differences. Journal of Physiology and Pharmacology. 2005; 56(SUPPL. 1):115-124.
15. F CV, R S, A G-P. Anxiolytic-like effect of *Sonchus oleraceus* L. in mice. Journal of Ethnopharmacology. 2009;124(2): 325-327. doi:10.1016/J.JEP.2009.04.029
16. FC V, AD B, LD C, LS F, R S, A G-P. Anti-inflammatory and antipyretic effects of *Sonchus oleraceus* in rats. Journal of ethnopharmacology. 2010;127(3): 737-741. doi:10.1016/J.JEP.2009.11.030
17. MIYASE T, FUKUSHIMA S. Studies on Sesquiterpene Glycosides from *Sonchus oleraceus* L.(Pharmacognosy, Chemical). Chem Pharm Bull. 1987; 35: 2869-2874.
18. Yin JC-LM-H. Antioxidant Activity of Flavonoids and Their Glucosides from *Sonchus oleraceus* L. Journal of Applied Biological Chemistry. 2008; 51(2):57-60. doi:10.3839/JABC.2008.010
19. Ghazanfar SA. Handbook of Arabian medicinal plants. Published August 24, 1994 by CRC Press, 272 Pages.
20. Guarrera PM, Lucchese F, Medori S. Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). Journal of Ethnobiology and Ethnomedicine 2008; 4(1): 1-11. doi:10.1186/1746-4269-4-7
21. Miyase T, Fukushima S. Studies on Sesquiterpene Glycosides from *Sonchus oleraceus* L.(Pharmacognosy,Chemical). Chem Pharm Bull. 1987; 35: 2869-2874.
22. SN E, S K. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. International journal of food sciences and nutrition. 2004; 55(1): 67-74. doi :10.1080/09637480310001642501
23. Schaffer S, Schmitt-Schillig S, Müller WE, Eckert GP. Antioxidant properties of Mediterranean food plant extracts: Geographical differences. Journal of Physiology and Pharmacology. 2005;56(SUPPL. 1):115-124.
24. FC V, M de MP, L DS-E-S, G A-S, A G-P. Evaluation of the antinociceptive activity of extracts of *Sonchus oleraceus* L. in mice. Journal of Ethnopharmacology. 2009;124 (2): 306-310. doi:10.1016/J.JEP.2009.04.037
25. Yin JC-LM-H. Antioxidant Activity of Flavonoids and Their Glucosides from *Sonchus oleraceus* L. Journal of Applied Biological Chemistry. 2008; 51(2): 57-60. doi:10.3839/JABC.2008.010
26. Ouassou H, Bouhrim M, Daoudi E, et al. Evaluation of Hepatoprotective Activity of Caralluma europaea Stem Extract against CCl 4-Induced Hepatic Damage in Wistar Rats. Published online 2021. doi:10.1155/2021/8883040
27. Okaiyeto K, Nwodo UU, Mabinya L V., Okoh AI. A review on some medicinal plants with hepatoprotective effects. Pharmacognosy Reviews. 2018;12 (24): 186-199. doi:10.4103/PHREV.PHREV_52_17
28. Bouhrim, Ouassou H, Choukri M, et al. Hepatoprotective effect of Opuntia dillenii seed oil on CCl4 induced acute liver damage in rat. Asian Pacific Journal of Tropical Biomedicine. 2018; 8(5): 254. doi:10.4103/2221-1691.233006
29. G P, A A, VK G, PT L, C G. NF-κB Activation and proinflammatory cytokines mediated protective effect of Indigofera caerulea Roxb. on CCl4 induced liver damage in rats. International Immunopharmacology. 2014; 23(2): 672-680. doi:10.1016/J.INTIMP.2014.10.021
30. Ouassou H, Bouhrim M, Daoudi E, et al. Evaluation of Hepatoprotective Activity of Caralluma europaea Stem Extract against CCl 4-Induced Hepatic Damage in Wistar Rats. Published online 2021. doi:10.1155/2021/8883040
31. Kharchoufa L, Bouhrim M, Bencheikh N, et al. Acute and Subacute Toxicity Studies of the Aqueous Extract from Haloxylon scoparium Pomel (Hammada scoparia (Pomel)) by Oral Administration in Rodents. BioMed Research International. BioMed Research International Volume 2020, Article ID 4020647, 11 pages. doi:10.1155/2020/4020647
32. Ko JH, Lim KT. Glycoprotein isolated from Ulmus davidiana NAKAI protects against carbon tetrachloride-induced liver injury in the mouse. Journal of Pharmacological Sciences. 2006;101(3):205-213. doi:10.1254/JPHS.FP0051053
33. Jain S, Dixit VK, Malviya N, Ambawatia V. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Amorphophallus campanulatus Roxb. tubers. Acta Poloniae Pharmaceutica - Drug Research. 2009; 66(4): 423-428.
34. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sciences. 1999; 65(4): 337-353. doi:10.1016/S0024-3205(99)00120-4

HOW TO CITE THIS ARTICLE: Akki KS, C RH, Akki P. Evaluation of Hepatoprotective Activity of *Sonchus oleraceus* L. Extract against CCl_4-induced Hepatic Damage in Wistar Rats. Int. J. Pharm. Sci. Drug Res. 2022;14(4):364-372. **DOI:** 10.25004/IJPSDR.2022.140401