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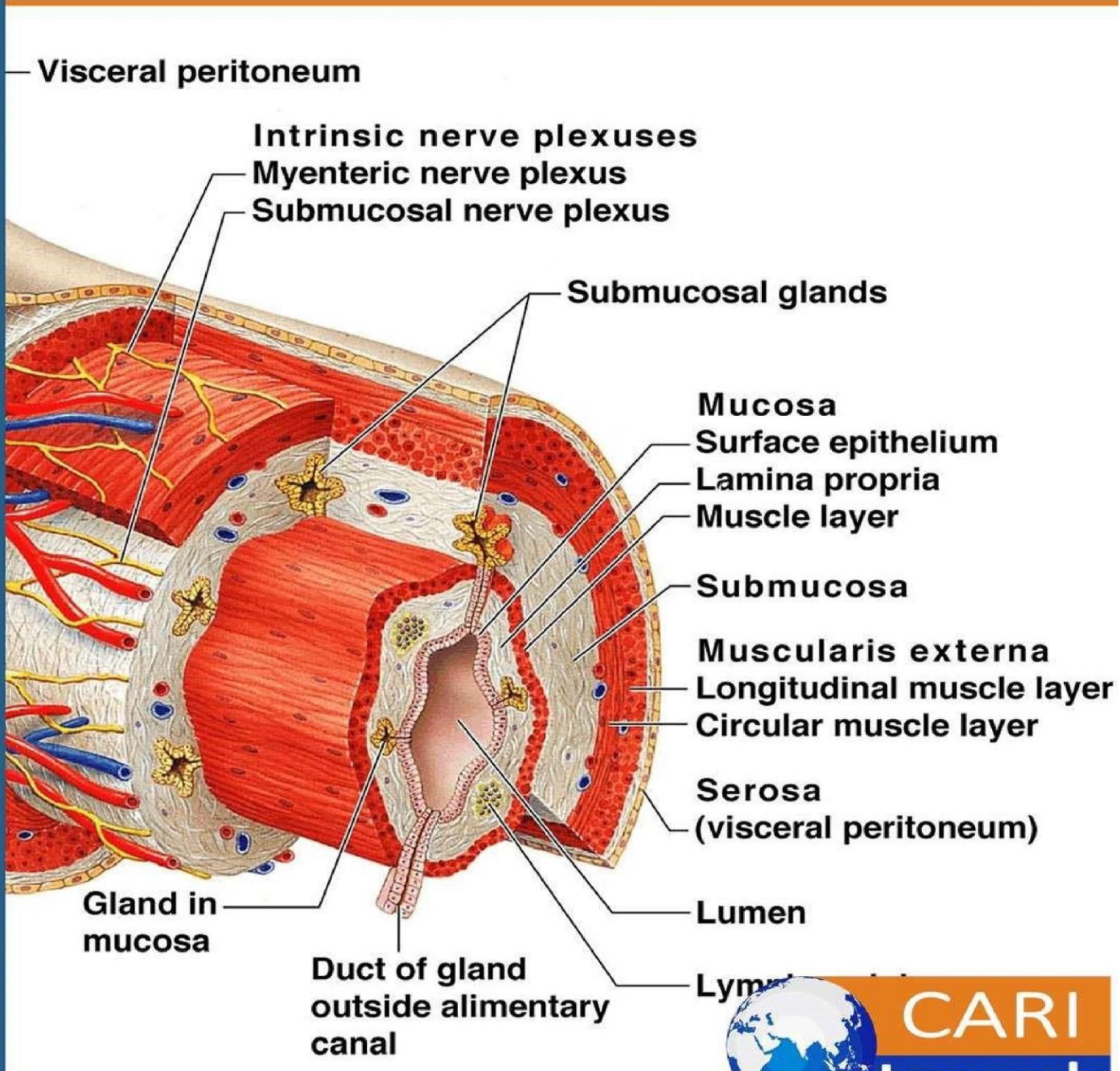

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The Use of Ethanolic Extract of *Sonchus Oleraceus* in the Treatment of Hepatotoxicity Induced by Carbon Tetrachloride Injection in Rats

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Abstract

Purpose: To study the hepatoprotective activity of the ethanolic extract of *Sonchus Oleraceus*. The plant is of the family Asteraceas and was used in the traditional medicine for the treatment of gastrointestinal tract disorders, tumors and inflammatory diseases.

Methodology: Sixty rats of both sexes were used, divided into 5 groups. The hepatotoxicity was induced by administration of carbon tetrachloride **(CCL_4)** at dose rate of ***0.2 mg /kg BW***. The plant extract was administered orally at dose rates of ***200*** *and* ***400 mg /kg BW***.

Findings: were compared to the standard drug known ***Silymarin*** at a dose rate of ***100 mg/kg BW***. The protective effect of ethanolic extract of *Sonchus. Oleraceus* was found to be better with the high dose ***(400 mg),*** hence the level of serum alanine amino transferase **(ALT),** serum Aspartate amino transferase **(AST),** were significantly decreased. Total protein, Albumin, Bilirubin and Alkaline Phosphatase **(ALP)** were also 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.

Unique Contribution to Theory, Practice and Policy: According to the obtained results, it would be important to isolate the active ingredient of the plant used that responsible for the treatment of hepatotoxicity.

Keywords: *Sonchus. Oleraceus, Hepatotoxicity, Carbon tetrachloride, ALT, AST*

1- Introduction

Plant-based, traditional medicine system continues to play an essential role in health care, with about **80%** of the world's inhabitants relying mainly on traditional medicines for their primary health care **(1),** Though, synthetic and semi synthetic drugs are available in today's market, there is need for ones from natural origin to cope up with the increased evolution of multiple resistant strains. Liver is one of the largest organs in human body and it is involved with almost all the biochemical pathways, such as carbohydrates, protein and fat metabolism. Besides its central role of detoxification, secretion of bile and storage of vitamin, fight against disease, nutrient supply, energy provision and reproduction **(2).** Therefore, it has a surprising role in the maintenance, performance and regulating homeostasis of the body. Since liver plays a fundamental role in drugs metabolism, it is the most vulnerable tissue for drugs toxicity. More than 900 drugs, toxins and herbs have been reported to cause liver injury, and drugs account for 20%- 40% of all instances of hepatic failure **(3).** Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders **(4); (5).**Thus, to maintain a healthy liver is a crucial factor for overall health and wellbeing. However, it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, and alcohol and prescribed & over-the-counter drug, which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease **(6); (7).** The common causative agents of liver injuries are toxic chemicals (e.g., **CCL_4**, aflatoxin etc.). Therapeutic drugs (e.g. antibiotics anti- tubercular drugs etc). Alcohol, and microbial agents (e.g. hepatitis virus, leptospira, malarial parasites). **(7). CCL_4** is well known hepatotoxin, which is widely used to induce toxic liver injury in laboratory animals. Hepato cellular injury due to **CCL4** occurs due to the toxic metabolite trichloromethyl radical **(CCL3),** and the drug metabolizing enzymes activate **CCL4** to produce electrophilic metabolites. These potent agents bind covalently to cell proteins and induce necrosis. In addition, metabolites with unpaired electrons are produced by oxidative reaction of cytochrome P450. These free radicals also damage proteins and unsaturated fatty acids causing lipid peroxidation. The administration of **CCL4** results in elevated activities of liver enzymes in serum, which is an indicator of cellular leakage and loss of activities of cell membranes in liver **(8).** The elevation of liver enzymes, especially **ALT** has more importance as a specific marker of liver injury due to toxic drugs. Numerous naturally occurring phytochemicals are present in plant tissues and many phytochemical plants (extracted plants) are used in treatment of many liver disorders like jaundice, liver cirrhosis and fatty liver.

2- Materials and Methods

2.1. Plant

The leaves of ***Sonchus. Oleraceus,*** family of ***Asteraceae*** were collected from Hag yousif in Khartoum Bahary at Desember- 2021; and dried at room temperature(10 to 15 days). The plant was used in this area for relieve hepatitis and malaria and also used in nutrition. The plant was authenticated by the botanists in medicinal and aromatic plants research institute.

2. 2. Animals

Sixty Wister albinos rats weighting 100-150 gm; were obtained from the Veterinary Research Institute. They were housed in laboratory cages , maintained in a room under standard environmental condition, controlled temperature **(22±2°C),** relative humidity **(60%)** with free access to water and formula rat feed (2.5 M cal and **20%** crude protein). Animals were apparently healthy and they were identified by color tail marks. One week was allowed as a preliminary adaptive period.

2.3. Preparation of extract:

Extraction was carried out according to method described by **(9).** 300 g of each plant sample was successively extracted by 80% ethanol for about seventy two hours; with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus. The yield percentage was calculated as followed:

Weight of extract / weight of sample * 100

Table (1) the yield of Sonchus Oleraceus leaves ethanolic extract

Sample	Tested material	Weight (g)	Extract	Yield %
Sonchus. Oleraceus	**Leaves**	**36.9**	**Ethanol**	**12.3**

Figure (1): *Sonchus. Oleraceus*

2.4. Phytochemical screening

Phytochemical screening for the active constituents of *Sonchus oleraceus* was carried out using the methods described by **(10), (11), (12)** with many few modifications**.**

Table: (2): Result of phytochemical screening of *Sonchus. Oleraceus* leaves ethanolic extract

Test	***Sonchus. Oleraceus***
Alkaloids	+
Sterols	+++
Triterpenes	++
Flavonoids	+++
Saponins	++
Cumarins	+
Tannins	+
Anthraquenones	-
Cyanogenic	-

2.5 Biochemical tests:

Samples were collected before treatment started at 0, and after 5 days and 10 days, and analyzed for liver function test. Rats were observed for signs of toxicity. The specimens of liver were collected immediately after slaughtering and were fixed in 10 % neutral formal saline for histopathology.

2.6 Histological methods:

The specimens were collected immediately after slaughter and fixed in 10% formal saline, embedded in paraffin wax, sectioned at 5 um and stained with haemotoxylin and eosin (H&E) using Mayer's haemalum

2.7 Biochemical analysis

Blood samples obtained, from the ocular vein of rats were used to prepare sera for the chemical methods. Venous blood samples were allowed to clot. Serum was separated by centrifugation at 3000 r.p.m. for 5 minutes and stored at -20C until analyzed. Spectrophotometer, (Merck mega, version 0.6 (1995); E. Merck, Darmstadt, Germany) was used to recorded serum activities of enzymes AST, ALT and ALP and serum metabolites, albumin, total protein, bilirubin.

2.8. Antihepatotoxic activity

The hepatoprotective activity of the tested plant carried out by studying the effect of the two doses (***200- 400 mg/Kg***) of ethanolic extract of ***Sonchus. Oleraceus*** leaves; against **CCL_4** induced hepatotoxcity in rats. The effect of hepatoprotective activity of the tested plants was compared with the known hepatoprotective drug or natural product, ***Silymarin***.

2.9 Standard drug (Silymarin):

Silymarin, a flavonolignan from 'milk thistle' (Silybum marianum) plant is used almost exclusively for hepatoprotection. The use of silymarin may replace the polyherbal formulations and will avoid the major problems of standardization, quality control and contamination with heavy metals or bacterial toxins. Silymarin consists of fourflavonolignan isomers namely-silybin, isosilybin, silydianin and silychristin. Among them, silybinbeing the most active and commonly used. Silymarin is orally absorbed and is excreted mainly through bile as sulphates and conjugates. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts by antioxidative, anti-lipid peroxidative, antifibrotic, and anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms. Silymarin may prove to be a useful drug for hepatoprotection in hepatobiliary diseases and in hepatotoxicity due to drugs

2.10 Treatments

- Carbon tetrachloride **(CCL4)** Analytical Rear 12117 England; dissolved in liquid paraffin (1:9), and administrated I/p **(13).**
- ***Silymarin*** (Silymarin Simepar – mepha) dissolved in 5% w/v acacia mucilage and administered orally by nasogastric tube, **(14).**

2.11 Experimental design

Rats were divided into five groups each group of twelve rats.

- Group **A** (Control negative): received only food and water.
- Group **B** (Control positive): was given a single intraperitoneal dose of (0.2mg/kg BWCCL4) for 10 days.
- Group **C** (Reference drug): received ***Silymarin*** at dose rate ***100 mg/kg BW*** together with ***(0.2 mg/kg BW CCL4)*** for 10 days.
- Group **D:** received, ***CCL4*** together with ***200 mg/kg BW*** of the plant extract for 10 days

- Group **E**: received, **CCL4** together with ***400 mg/kg BW*** of the plant extract for 10 days.

Clinical signs were recorded, and blood samples were taken from the ocular vein prior to experimental dosing, after 5 days, and at the end of the trial; for hematological investigation and serum analysis. Sera were analyzed for the activities of **ALT**, **AST**, **ALP,** and for the concentration of metabolic indicators, Total protein, Urea, Albumin, parameters were determined Calorimetrically, using Commercial Kit (Randox laboratories Ltd, U.K). After 10 days the rats were dissected and the liver tissues were fixed in 10% neutral buffered formalin and processed for histopathology.

2.12 Statistical analysis: The values were expressed as mean ± SD. Statistical analysis and comparison between groups was performed by one-way analysis of variance (ANOVA) using SPSS version 10.0.

Table (3): Experimental plan of rats given ethanolic extract of *Sonchus oleraceus* leaves simultaneously with CCL4

Groups	***Animals number***	***Doses***	***Time of slaughtering***
Group A (Control)	12 rats	—	10 days
Group B (CCL_4 control)	12 rats	0.2 ml/Kg CCL_4	10 days
Group C (Standard drug)	12 rats	0.2 ml/Kg CCL_4 + 100 mg/Kg of Silymarin in 5% acacia mucilage orally	10 days
Group D (200mg/kg *Sonchus. Oleraceus leave extract)*	12 rats	0.2ml/kg CCL_4 +200mg/kg Sonchus. Oleraceus leave extract)	10 days
Group E (400mg/kg *Sonchus. Oleraceus leave extract)*	12 rats	0.2ml/kg CCL_4 +400mg/kg Sonchus. Oleraceus leave extract)	10 ays

3- Results

3.1. *Biochemical Profile Results*

The hepatoprotective effect of *Sonchus. Oleraceus* leaves ethanolic extract is shown in tables **(3,4,5,6),** where the plant produced significant decrease in the concentration of enzymes alanine aminotransferase **(ALT)**, aspartate aminotransferase **(AST),** alkaline phosphatase **(ALP)**,

bilirubin, albumin and total protein concentration in treated groups compared to both control groups **(A and B),** and standard drug group **(C).** The results were found to be better with the dose ***400 mg/kg BW,*** (***P≤0.05- P≤ 0.01***).

3.2. *Histopathology Result*

Post mortem findings in liver sections of rats injected with CCL4 group (C); revealed fibrosis and severe fatty change. The liver of the rats treated with ***400 mg/kg BW*** of the plant extract; appeared to be normal sections **(E).** The Histological appearance; of groups received **(E and D);** ***Sonchus. Oleraceus*** leaves ethanolic extract; was quite similar to that of the control group; tissue damage and necrosis were of less extent in group (**D)** and (**E)** than **CCl4** treated group.

3.3. *Phytochemical Screening Result*

Phytochemical screening of ***Sonchus. Oleraceus*** leaves ethanolic extract resulted in moderate concentration of ***sterol***, ***flavonoids*** (+++), and trace level of both ***cumarin*** and ***tannins***.

Table (4): Effect of *Sonchus. Oleraceus* leaves ethanolic extract administered simultaneously with CCL4 on ALT and AST in rats.

Groups	*ALT (Mean ± S.E.)*			*AST (Mean ± S.E.)*		
	Day 0	***Day 5***	***Day 10***	***Day 0***	***Day 5***	***Day 10***
A	11.20±0.00	11.50±0.00	12.00±0.00	12.00±0.00	12.10±0.00	12.20±0.00
B	11.10±0.00	14.00±0.00	17.00±0.00*	11.50±0.00	13.20±0.00	15.20±0.00*
C	9.70±0.00	14.00±0.00	10.10±0.00*	10.20±0.00	13.20±0.00	11.30±0.00*
D	10.50±0.28	16.00±1.41	12.80±1.13*	11.40±0.57	17.30±1.41°	12.00±.057
E	10.50±0.28	16.00±1.41	10.80±1.13**	11.40±0.57	17.30±1.41	11.00±.057**

A *(Control Negative),* ***B*** *(Control Positive),* ***C*** *(Standard Drug),* ***D*** *(200mg/kg BW Sonchus. Oleraceus,* ***E*** *(400 mg/kg BW* ***Sonchus. Oleraceus****)* **** = P≤0.05, **= P≤0.04, ***= P≤0.01.***

Table (5): Effect of *Sonchus. Oleraceus* leaves ethanolic extract administered simultaneously with CCL4 on serum concentration Albumin and Total Protein in rats

Groups	*Total Protein (Mean ± S.E.)*			*Alb (Mean ± S.E.)*		
	Day 0	***Day 5***	***Day 10***	***Day 0***	***Day 5***	***Day 10***

A	7.40±0.00	7.50±0.00	7.30±0.00	5.00±0.00	4.60±0.00	4.90±0.00
B	7.30±0.00	9.70±0.00	10.15±0.00*	4.80±0.00	9.10±0.00	10.20±0.00*
C	7.20±0.00	10.20±0.00*	6.90±0.00	4.10±0.00	7.00±0.00**	4.50±0.00
D	7.75±0.35	10.50±.028	8.40±0.57**	4.55±.021	8.75±0.78	6.65±.078*
E	7.75±0.35	10.50±.028	8.40±0.57*	4.55±.021	8.75±0.78	5.65±.078

A** (Control Negative), **B** (Control Positive), **C** (Standard Drug), **D** (200mg/kg BW Sonchus. Oleraceus, **E** (400 mg/kg BW **Sonchus. Oleraceus**)* * = **P≤0.05,** ** = **P≤0.04,** *** = **P≤0.01.

Table (6): Effect of *Sonchus. Oleraceus* leaves ethanolic extract administered simultaneously with CCL4 on total and direct Bilirubin in rats.

Groups	***Total BiL (Mean ± S.E.)***			***Direct Bil (Mean ± S.E.)***		
	Day 0	***Day 5***	***Day 10***	***Day 0***	***Day 5***	***Day 10***
A	1.20±0.00	1.10±0.00	0.96±0.00	0.25±0.00	0.24±0.00	0.25±0.00
B	1.20±0.00	2.80±0.00	3.90±0.00**	0.26±0.00	1.80±0.00	10.20±0.00*
C	1.30±0.00	2.90±0.00	1.40±0.00*	0.35±0.00	1.16±0.00**	0.21±0.00
D	1.04±0.08	3.00±0.28*	1.01±0.13**	0.25±0.07	1.90±0.28	0.55±.035*
E	1.04±0.08	3.00±0.28	1.01±0.13*	0.25±0.07	1.90±0.28	0.55±.035**

A** (Control Negative), **B** (Control Positive), **C** (Standard Drug), **D** (200mg/kg BW Sonchus. Oleraceus, **E** (400 mg/kg BW **Sonchus. Oleraceus**)* * = **P≤0.05,** ** = **P≤0.04,** *** = **P≤0.01.

Table (7): Effect of *Sonchus. Oleraceus* leaves ethanolic extract administered simultaneously with CCL4 on indirect Bilirubin and ALP

Groups	***Indirect BiL (Mean ± S.E.)***			***ALP (Mean ± S.E.)***		
	Day 0	***Day 5***	***Day 10***	***Day 0***	***Day 5***	***Day 10***
A	0.95±0.00	0.86±0.00	0.71±0.00	99.00±0.00	99.10±0.00	96.00±0.00

B	0.94±0.00*	1.00±0.00**	1.00±0.00	95.00±0.00	112.00±0.00	130.00±0.00**
C	0.95±0.00	1.74±0.00**	1.19±0.00*	98.00±0.00	115.00±0.00*	99.10±0.00
D	1.02±0.10	1.10±0.00	0.46±.023	96.50±2.12	121.00±5.66	97.50±0.71*
E	1.02±0.10	1.10±0.00	0.46±.023	96.50±2.12	121.00±5.66	97.50±0.71**

A *(Control Negative),* ***B*** *(Control Positive),* ***C*** *(Standard Drug),* ***D*** *(200mg/kg BW Sonchus. Oleraceus,* ***E*** *(400 mg/kg BW* ***Sonchus. Oleraceus****)* * = ***P≤0.05,*** **= ***P≤0.04,*** ***= ***P≤0.01.***

Figure (2): Control group showed normal histopathological appearance

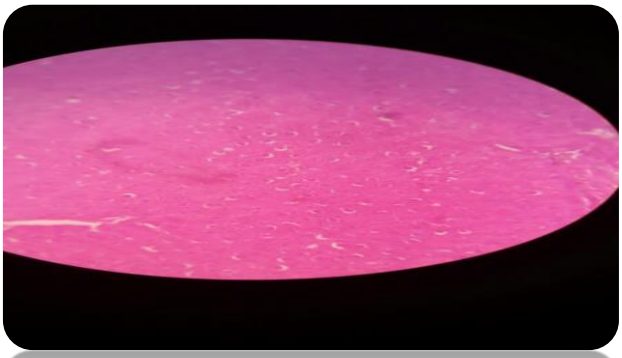

Figure (3): CCL4 group showed hepatocellular degeration with sever fatty changes and sever koliocytosis

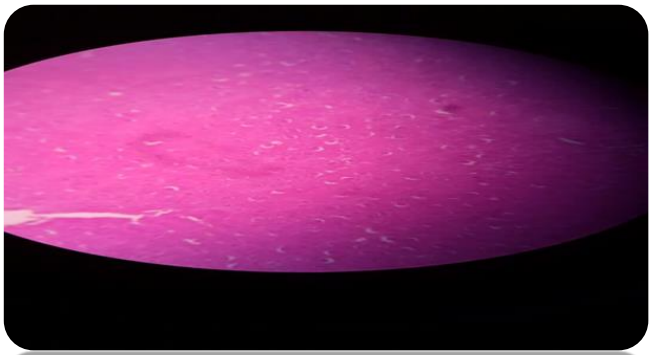

Figure (4): Standard drugs group showed mild fatty changes, focal necrosis and hydrophobic changes.

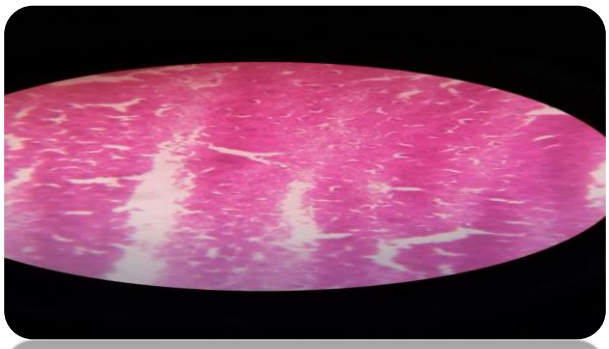

Figure (5): Group D liver section (200mg/kg) showed mild hepatocellular degeneration and fatty changes.

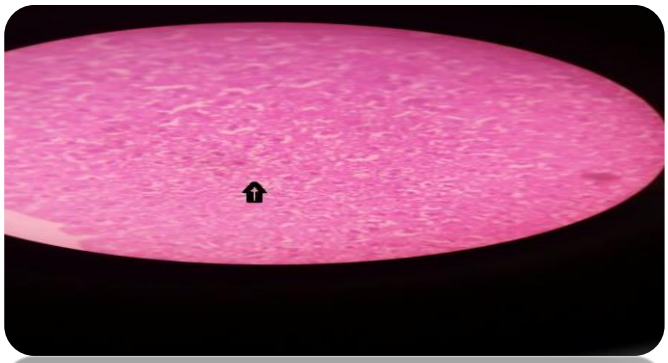

Figure (6): Group E liver section (400mg/kg) showed few hepatocyte immediately adjacent to the central vein in affected areas and mild hepatocyte swelling.

4- Discussion

Hepatotoxicity was defined as impairment of the liver function caused by exposure to xenobiotic such as chemical, drugs, food additives and other. In **CCL4** induced hepatotoxicity model, upon administration of **CCL_4** to animals, it undergoes enzymatic activation, into the trichloromethyl free radicals **(CCL_3)** within the membrane of the endoplasmic reticulum, These free radicals can bind with polyunsaturated fatty acids to produce alkoxy **(R)** and peroxy radicals **(ROO),** that , in turn , generate lipid peroxides, which are highly reactive, causing changes in enzyme activity, and finally induce injury or necrosis with corresponding health problems **(15)** . This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acids of the endoplasmic reticulum membrane phospholipids **(16).** These processes are known as lipid peroxidation, leading to functional and structural disruption of hepatocytes **(17).**Carbon tetrachloride **(CCL4**); is a potent environmental hepatotoxin **(18),** that, in addition to hepatic problems, causes dysfunction of the kidneys, lungs, testis, brain, and blood by generating free radicals **(19)**.

The level of the enzymes ALT, AST and ALP was decreased significantly; when the extract of ***Sonchus. Oleraceus*** leaves, was administered concomitantly with **CCL4**. The decrease was found to be significant ***(P≤ 0.05- 0.01).*** At the end of the experiment, these levels returned gradually to the normal levels. The results agreed with that of ***Shrma et al (2009)***; where he used curcumin as a natural product to produce hepatoprotectivity. Other work that agreed with our results is that of **(20)**; who used ethyl acetate extract of ***Sterculia. Setigera***; against carbon tetrachloride induced hepatotoxicity in rats. The hepatoprotective activity of some Sudanese medicinal plants was revered by **(21),** which support our results.

The present study revealed the presence of flavonoids, saponosides- triterpenies and glucosides, which is in an agreement with findings of previous workers **(22)**.These bioactive compounds may exhibit antioxidant activity **(23)**, that could be the reason behind the protection ability against CCL4 liver damage in rats.

Conclusion

It can be concluded that, there was variety of phytochemicals in the plant ***Sonchus. Oleraceus*** with hepatoprotective activity against carbon tetrachloride induced toxicity. Down regulation of liver enzymes represents evidence of that protection activity. The protective effect is dose dependent where the most efficient result was obtained with higher dose.

There is need to validate the efficacy of some of the medicinal plants with active components which can be candidate for therapeutic purposes.

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