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مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2021.97377.1472

المجلد الثامن العدد 40 . مايو 2022

الترقيم الدولي

P-ISSN: 1687-3424 E- ISSN: 2735-3346

موقع المجلة عبر بنك المعرفة المصري https://jedu.journals.ekb.eg/

موقع المجلة http://jrfse.minia.edu.eg/Hom

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

Effect of *Sonchus Oleraceus* and *Malva Parviflora* Leaves on Acute Liver Diseases in Rats Fed on Normal Diet.

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ABSTRACT

The present work was carried out to investigate the effect of *Sonchus oleraceus (SO)* and *Malva parviflora (MP)* leaves on acute liver diseases in rats fed on a normal diet. Forty adult male Albino rats of Sprague-Dawley strain were used and divided into two main groups as follows: The first main group (5 rats) was fed on a basal diet "BD", as a control negative group (-ve). The second main group (35 rats) was injected with a single dose of CCL_4 in paraffin oil (50% v/v 4ml/kg) subcutaneous injection to induce acute damage in the liver. Then the rats were divided into 7 groups as a following: group (1): was fed on a basal diet as a control positive group. Groups (2 and 3): were fed on a basal diet containing 2% and 4% SO, respectively. Groups (4 and 5): were fed on a basal diet containing 2% and 4% MP, respectively. Groups (6 and 7): were fed on a basal diet containing (1% SO and 1% MP) and (2% SO and 2% MP), respectively. The results revealed that total lipids and liver enzymes are increased except high-density lipoprotein cholesterol (HDL-c) and protein in acute liver disease rats fed on a basal diet compared to the negative control group.

Key words: *Sonchus oleraceus*, *Malva parviflora*, acute liver disease, lipid profile, liver enzymes.

INTRODUCTION:

The liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1million due to viral hepatitis and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. **(Asrani *et al*., 2018)**. The same authors reported that, approximately 2 billion adults are obese or overweight and over 400 million have diabetes; both of which are risk factors for non-alcoholic fatty liver disease and hepatocellular carcinoma.

The progression of hepatic disorders such as alcoholic liver disease (ALD) or viral hepatitis B/C (VHB/ VHC) usually depends on the nutritional status of an individual **(Serafim *et al*., 2016)**. Egypt ranks 5th amongst all countries for the burden of disease from viral hepatitis **(Stanaway *et al*., 2016)**.

Herbal products are complex mixtures of organic chemicals that can come from any raw or processed part of a plant, including leaves, stems, flowers, roots, and seeds. They usually contain a number of pharmacologically active compounds **(Bent, 2008)**. The plant *Sonchus oleraceus* belongs to the daisy family (Asteraceae) **(Walter *et al*., 2001)** is an upright, annual herb with simple branches. *S.oleraceus* is native to Europe, North Africa, and West Asia. It has spread to North and South America, India, China, southern Australia **(Chauhan *et al*., 2006)**. The genus *Sonchus* comprises about 60 species, and three of them have become common weeds around the world. These are *S. arvensis*, perennial Saw thistle, and the two annual species S. *oleraceus*, common Saw thistle, and *S. asper*, prickly Saw thistle. The main constituents of Sonchus L. were terpenes, steroids, flavones,coumarins , etc. It has hepato- protective activity, antitumor effect,cardiovascular therapy, etc**. (Jiang *et al*., 2007)**.

Malva parviflora L. (family Malvaceae) (cheese weed) is an herb native to Africa, Asia, and Europe. Its common name is cheese weed and is locally known as Sonchal. Pharmacologically, it has been reported to be antibacterial **(Ododo *et al.*, 2016)**, anti-diabetic **(Gutierrez, 2012)**, antifungal **(Wang *et al.*, 2001)**. Traditionally *M. parviflora* is used for the treatment of inflammation, pain, and liver injuries **(Afolayan *et al.*, 2010)**. The plant contains phenolic and flavonoid compounds **(Farhan *et al.*, 2012)**. Therefore, the study aimed to investigate the effects of *Sonchus oleraceus* and *Malva parviflora* leaves on acute liver diseases in rats fed on a normal diet.

Materials and Methods:

Materials:

Plants:

Sonchus oleraceus and *Malva parviflora* leaves were obtained from, Governorate of Beheira, Egypt. A sample of these plants was sent to the Agricultural Research Center to be identified.

Animals:

Forty (40) male albino rats Sprague Dawley strain weighing 150 ± 10g were purchased from Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

Chemicals:

- Casein, Vitamins, Minerals, Cellulose, choline chloride were purchased from EL-Gomhoria company, Cairo, Egypt.
- Starch and soybean oil were obtained from the local market, Cairo, Egypt.
- Kits for biochemical analyses, carbon tetrachloride (CCL_4), and diethyl ether were obtained from Alkan for pharmaceutical and chemical Dokki, Egypt.

Methods:

Samples preparation:

Drying of *Sonchus oleraceus* and *Malva parviflora* leaves:

Sonchus oleraceus and *Malva parviflora* leaves were washed thoroughly with tap water, dehydrated into an air circulated oven at 40-50˚C for 24 hrs. The dried samples were finely powdered by using a coffee grinder and stored in polyethylene bags at - 20˚C until used.

Chemical analysis:

Moisture, protein, fat, fiber, carbohydrate, and active components (flavonoids, total phenols) were determined in *Sonchus oleraceus* and *Malva parviflora* leaves in Agricultural Research Center, Giza, Egypt, according to **(A.O.A.C. 1990).**

Experimental Design:

Forty adult male Albino rats of Sprague-Dawley strain weighing approximately (150 ± 10 g) were housed in well-aerated cages under hygienic conditions and fed on basal diet in the animal house, Faculty of Home Economics, Helwan University. The basal diet was formulated according to **(Reeves *et al.*, 1993)** for one week for adaptation. Then the rats were divided into two main groups as follows: The first main group (5 rats) was fed on a basal diet "BD", as a control negative group (-ve). The second main group (35 rats) was injected with a single dose of CCL4 in paraffin oil (50% v/v 4ml/kg) subcutaneous injection to induce acute damage in the liver **(Jayasekhar *et al.*, 1997)**. After injection, AST, ALT, and ALP were determined to ensure the induction. Then the rats were divided into 7 groups (5 rats per group) as a following:

Group (1): was fed on basal diet as a control positive group. ***Groups (2 and 3):*** were fed on a basal diet containing 2% and 4% *Sonchus Oleraceus*, respectively. ***Groups (4 and 5):*** were fed on a basal diet containing 2% and 4% *Malva parviflora*, respectively.

Groups (6 and 7): were fed on a basal diet containing (1% *Sonchus Oleraceus* and 1% *Malva parvi*flora) and (2% *Sonchus Oleraceus* and 2% *Malva parviflora*), respectively.

Feed intake, body weight gains, and liver weight to body weight % were estimated at the end of the experiment.

Biochemical analyses:

At the end of the experimental period (4 weeks) rats were fasted overnight, and then sacrificed. Blood samples were collected from the hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3000 rpm (round per minute) for 15 minutes at room temperature, transferred into dry clean Eppendorf tubes, then kept frozen at -20˚C for later determinations. Livers and hearts were removed from rats by careful dissection, washed in saline solution (0.9%), dried using filter paper, and independently weighed. Serum was analyzed in Agricultural Research Center, Giza, Egypt, and analytical lab, Faculty of Home Economics, Helwan University to determine the following parameters:

Determination of total lipids according to **Christopher and Ralph, (1970).** Triglycerides according to **Fossati and prencipe (1982).** Total cholesterol **(Allain *et al*., 1974)**. High density lipoprotein cholesterol (HDL-C) **(Burstein *et al*., 1970).** Low and very low density lipoprotein cholesterol (LDL-c and VLDL-c) **(Friedewald *et al*., 1972)**. Liver enzymes activities, Aspartate transaminase (AST) and Alanine transaminase (ALT) enzymes (Schmidt and Schmidt, 1963). Alkaline phosphatase (ALP) activity **(Belfield and Goldberg, 1971),** and total protein determined according to **(Henry *et al*., 1974)**.

Histopathological Examination:

Specimens from liver tissue were taken immediately after sacrificing animals and fixed in a 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed, and dehydrated embedded in paraffin, cut in sections of 46 microns' thickness and stained with haematoxylin and eosin stain, according to **(Sheehan and Hrapchak, 1980).**

Statistical Analysis:

Results of biochemical analysis and biological evaluation of each group were statistically analyzed. Mean values and standard Error were determined by using a one-way ANOVA test using SAS package, with the level of significance of $P \leq 0.05$ (**SAS, 2004)**.

Results:

Data presented in Table (1) showed the chemical analysis of *Malva parviflora* and *Sonchus oleraceus* on dry weight and wet weight basis. Results in this Table revealed that the moisture content of *Sonchus oleraceus* increased than that of *Malva parviflora* (85.4% vs. 78.6%), respectively. The highest concentration of the two types of leaves was recorded for carbohydrates, followed by protein, ash, fiber, and fat, respectively. The amounts of total flavonoids and total phenols (mg/g) of *Sonchus oleraceus* (SO) increased than that of *Malva parviflora* (MP) (0.158 and 1.020 mg/g) vs. (0.105 and 0.778 mg/g), in wet weight respectively. While their recorded (3.638 and 6.988 mg/g) vs. (0.491 and 1.089 mg/g) in dry weight respectively.

Table (1): Chemical analysis of *Malva parviflora* (MP) and *Sonchus oleraceus* (SO) leaves.

Types of leaves / Parameters	*Malva parviflora*		*Sonchus oleraceus*	
	Wet weight basis (%)	Dry weight basis (%)	Wet weight basis (%)	Dry weight basis (%)
Moisture	78.6	3.694	85.4	3.005
Protein	6.937	32.419	3.996	27.373
Ash	3.095	14.464	2.439	16.709
Fat	0.527	2.463	1.186	8.126
Crude Fibers	1.311	6.13	1.781	12.2
Total Carbohydrates	9.53	40.83	5.198	32.587
Total flavonoids (mg/g)	0.105	0.491	0.158	1.089
Total phenols (mg/g)	0.778	3.638	1.020	6.988

The effect of *Sonchus oleraceus* and *Malva parviflora* leaves on feed intake, body weight gains% & liver weight/body weight% of acute liver diseases rats fed on normal diet presented in table (2).

The mean value of feed intake of healthy rats fed on basal diet was (17 g/day/each rat), while the mean value of feed intake decreased in acute liver diseases rats which were fed on the same diet (14.987 g/day/each rat) (Table 2).

Table (2): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on some nutritional parameters and percentage of liver weight of acute liver diseases rats fed on normal diet.

Parameters / Groups	Feed Intake g/day/ each rat	BWG%	liver weight / body weight%
Control (-ve)	17.00	33.972 [a] ± 1.131	2.889 [e] ± 0.077
Control (+ve)	14.987	8.057 [d] ± 1.217	5.295 [a] ± 0.185
2% *Sonchus Oleraceus (SO)*	15.554	12.963 [c] ± 0.905	4.866 [b] ± 0.266
4% *Sonchus Oleraceus (SO)*	15.872	15.853 [b] ± 0.967	4.274 [c] ± 0.184
2% *Malva parviflora (MP)*	15.421	15.200 [b] ± 0.911	4.228 [c] ± 0.085
4% *Malva parviflora (MP)*	15.633	15.157 [b] ± 0.652	3.401 [d] ± 0.188
2% (*SO* + *MP*)	15.905	15.949 [b] ± 0.814	3.039 [e] ± 0.101
4% (*SO* + *MP*)	16.231	13.676 [c] ± 0.254	2.962 [e] ± 0.109

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

The mean value of feed intake of acute liver diseases group which were treated with basal diet containing (2 & 4% *Sonchus Oleraceus* SO), (2 & 4% *Malva parviflora* MP) & (2 & 4% combination between SO & MP) increased than that of the positive control group. The highest increase in the mean value of feed intake was recorded for the acute liver disease group which was treated with a diet containing (4% combination between SO & MP), followed by the group treated with (2% combination between SO & MP), respectively. Data in this Table revealed that the mean value of feed intake increased with increasing the levels of *Sonchus Oleraceus* SO, *Malva parviflora* MP and SO & MP together.

The effect of *Sonchus oleraceus* and *Malva parviflora* leaves on BWG% of acute liver diseases rats fed on normal diet presented in

the same table. The mean value of BWG% of healthy rats fed on basal diet increased significantly p≤0.05, as compared to acute liver diseases rats fed on the same diet (basal diet). The results in this table revealed that all treated acute liver diseases groups with normal diet containing 2% and 4% (SO, MP, and the combination between SO& MP) showed a significant increase in the mean values of BWG%, as compared to the positive control group. From these data, it could be observed that a non-significant change in BWG% was appeared between all treated groups, except the groups which were treated with (2% SO) and (4% SO + MP) which showed a significant decrease, as compared to other treated groups.

The mean value of liver weight/body weight % of acute liver disease rats fed on a basal diet (Control +ve group) increased significantly (p≤ 0.05), as compared to healthy rats (Control –ve group) fed on a basal diet (Table 2). All treated acute liver diseases groups with normal diet containing 2% and 4% (SO, MP, and the combination between SO& MP) showed a significant decrease (p≤ 0.05) in the mean values of liver weight /body weight%, as compared to the positive control group. The best results in liver weight/body weight % between all treated groups recorded for the groups which were fed on a normal diet containing 4% combination between (SO & MP), followed by the group fed on 2% combination between (SO & MP), respectively compared to the other treated groups.

The effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum cholesterol and triglycerides of acute liver diseases rat fed on normal diet presented in Table (3). The mean value of serum cholesterol and triglycerides in the acute liver disease group fed on the basal diet increased significantly (P≤ 0.05), as compared to the healthy group fed on the basal diet. Injected rats with CCl4 (the positive control group) increased the mean value of serum cholesterol and triglycerides by about 79.097% and 61.332% than that of non-injected rats (the negative control group).

All treated groups with the two levels from SO, MP, and "SO & MP together" recorded a significant decrease ($P \leq 0.05$) in serum cholesterol and triglycerides, as compared to the positive control group. On the other hand, the mean value of serum cholesterol and triglycerides decreased gradually with increasing the levels of (SO, MP, and "SO & MP together) in the diet.

The best results in serum cholesterol recorded for acute liver disease group which treated with the combination between SO and MP with the level of (4%), followed by acute liver disease groups treated with (the combination between SO and MP with the level of 2%) and 4% SO, respectively. Treating the acute liver disease group with the combination of SO and MP with the level (4%) decreased the mean value of serum cholesterol by about 26.589% than that of the positive control group. The highest decrease in serum triglycerides was recorded for the group which treated with a 4% combination between (SO & MP), followed by the groups which were treated with a 2% combination between (SO & MP), (4% SO), and (4% MP), these treatments showed a significant decrease ($P \leq 0.05$), as compared to the other treated groups.

Table (3): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum cholesterol and triglycerides of acute liver diseases rats fed on normal diet.

Parameters / Groups	Cholesterol	Triglycerides
	mg/dl	
Control (-ve)	81.418 [f] ± 3.135	51.395 [e] ± 2.526
Control (+ve)	145.818 [a] ± 4.941	82.917 [a] ± 3.788
2% *Sonchus Oleraceus (SO)*	129.180 [b] ± 4.405	72.071 [b] ± 3.698
4% *Sonchus Oleraceus (SO)*	113.211 [d] ± 4.316	63.377 [c] ± 3.887
2% *Malva parviflora (MP)*	133.714 [b] ± 4.031	75.721 [b] ± 3.212
4% *Malva parviflora (MP)*	121.952 [c] ± 4.273	65.309 [c] ± 1.509
2% (*SO* + *MP*)	116.611 [d] ± 2.446	64.309 [c] ± 4.026
4% (*SO* + *MP*)	107.046 [e] ± 3.169	56.803 [d] ± 2.167

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

The results in Table (4) illustrated the effect of two levels (2% & 4%) SO, MP, and SO &MP together on HDL-c, LDL-c, and VLDL-c of acute liver disease rats. The mean value of serum HDL-c in the acute liver disease group fed on a basal diet (control positive) decreased significantly ($P \leq 0.05$), as compared to the control negative group (24.948 ± 2.227 mg/dl vs. 49.977 ± 1.819 mg/dl), respectively. The mean value of serum HDL-c in the positive control group decreased by about 50.081% than that of the negative control group. On the other hand, the mean values of LDL-c and VLDL-c increased significantly in the positive group, ($P \leq 0.05$), as compared to the negative group.

Treating acute liver disease groups with a normal diet containing 2% and 4% (SO, MP, and "SO+MP' leaves) led to a significant increase ($P \leq 0.05$) in HDL-c, while these treatments induced significant decrease ($P \leq 0.05$) in LDL-c & VLDL-c, as compared to the control positive group. The results in this table

revealed that the mean value of HDL-c increased gradually with increasing the level of (SO) and (MP) leaves in the diet, while LDL-c and VLDL-c decreased gradually.

The best results of HDL-c, LDL-c, and VLDL-c were recorded for the groups treated with a 4% combination from SO & MP leaves, followed by the group fed on a normal diet containing 4% SO, and the group which treated with 2% combination between (SO&MP leaves), respectively. Mathematically, the treated acute liver disease group with a 4% combination from SO & MP leaves increased the mean value of serum HDL-c by about 62.169%, while LDL-c and VLDL-c decreased by about 47.04% and 31.496% respectively than that of the positive control group.

Table (4): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum lipoproteins of acute liver diseases rats fed on normal diet.

Parameters / Groups	HDL-c	LDL-c	VLDL-c
	mg/dl		
Control (-ve)	49.977 ^{a} ± 1.819	21.162 ^{h} ± 1.363	10.279 ^{e} ± 0.505
Control (+ve)	24.948 ^{f} ± 2.227	104.286 ^{a} ± 2.297	16.583 ^{a} ± 0.575
2% *Sonchus Oleraceus (SO)*	32.030 $^{d e}$ ± 3.206	82.736 ^{c} ± 1.524	14.414 ^{b} ± 0.739
4% *Sonchus Oleraceus (SO)*	37.085 $^{b c}$ ± 2.526	63.450 ^{f} ± 1.968	12.674 ^{c} ± 0.777
2% *Malva parviflora (MP)*	30.161 ^{e} ± 2.817	88.409 ^{b} ± 1.348	15.144 ^{b} ± 0.642
4% *Malva parviflora (MP)*	34.703 $^{c d}$ ± 1.949	74.187 ^{d} ± 4.579	13.061 ^{c} ± 0.302
2% (*SO + MP*)	36.130 ^{c} ± 3.682	67.609 ^{e} ± 2.584	12.872 ^{c} ± 0.806
4% (*SO + MP*)	40.458 ^{b} ± 3.271	55.226 ^{g} ± 2.041	11.360 ^{d} ± 0.433

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

The effect of two levels from *Sonchus oleraceus* SO and *Malva parviflora* MP leaves on serum protein of acute liver diseases rats fed on normal diet presented in Table (5). Injected rats with CCl4 to induce acute liver disease caused a decrease in serum protein

level significantly ($p \leq 0.05$), as compared to the negative control group. This decrease was estimated mathematically by about 17.561%, compared to the negative control group.

Feeding acute liver disease groups on a normal diet containing (2% and 4%) SO leaves, MP leaves & "SO + MP" leaves together increased the mean value of serum protein significantly ($p \leq 0.05$), as compared to the positive control group. On the other hand, the data in this table indicated that serum protein increased in acute liver disease groups with increasing the level of these leaves in the diet. The highest improvement in serum protein recorded for acute liver disease group fed on a normal diet containing 4% combination between (SO leaves & MP leaves), followed by the group fed on the same diet containing 2% combination between (SO leaves & MP leaves) and the group which treated with 4% *Sonchus Oleraceus* (SO), respectively.

Table (5): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum protein of acute liver diseases rats fed on normal diet.

Parameters / Groups	Protein g/dl
Control (-ve)	6.725 ± 0.203 [a]
Control (+ve)	5.544 ± 0.075 [g]
2% *Sonchus Oleraceus (SO)*	5.718 ± 0.071 [e f]
4% *Sonchus Oleraceus (SO)*	5.870 ± 0.056 [c d]
2% *Malva parviflora (MP)*	5.665 ± 0.043 [f]
4% *Malva parviflora (MP)*	5.820 ± 0.032 [d e]
2% (*SO + MP*)	5.963 ± 0.034 [c]
4% (*SO + MP*)	6.175 ± 0.065 [b]

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

Treating acute liver disease rats with a normal diet containing 4% combination between (*Sonchus Oleraceus* leaves SO & *Malva parviflora* leaves MP) increased the mean value of serum protein by about 11.381% than that of the positive control group.

The effect of *Sonchus oleraceus* "SO" and *Malva parviflora* "MP" leaves and their combination on liver enzymes including (Aspartate Amino Transferase AST, Alanine Amino Transferase

ALT and Alkaline phosphatase ALP) of acute liver diseases rats fed on normal diet presented in Table (6).

Table (6): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on liver enzymes of acute liver diseases rats fed on normal diet.

Parameters / Groups	AST	ALT	ALP
	U/l		
Control (-ve)	59.266 ^{e} ± 4.337	17.678 ^{f} ± 1.219	80.671 ^{e} ± 3.733
Control (+ve)	111.420 ^{a} ± 5.246	59.605 ^{a} ± 3.052	147.382 ^{a} ± 4.102
2% *Sonchus Oleraceus (SO)*	99.798 ^{b} ± 3.627	52.368 ^{b} ± 1.724	138.865 ^{b} ± 1.912
4% *Sonchus Oleraceus (SO)*	90.496 ^{c} ± 2.875	45.948 $^{c\ d}$ ± 2.661	128.680 ^{c} ± 2.832
2% *Malva parviflora (MP)*	103.884 ^{b} ± 2.324	53.091 ^{b} ± 1.618	141.563 ^{b} ± 1.519
4% *Malva parviflora (MP)*	93.498 ^{c} ± 2.324	48.376 ^{c} ± 1.606	131.336 ^{c} ± 2.886
2% (*SO* + *MP*)	91.981 ^{c} ± 2.064	44.673 ^{d} ± 2.040	129.219 ^{c} ± 3.209
4% (*SO* + *MP*)	85.334 ^{d} ± 1.869	40.339 ^{e} ± 1.236	120.927 ^{d} ± 4.026

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

The means value ± SD of serum AST, ALT, and ALP in the control negative group which fed on basal diet were (59.266 ± 4.337 U/l), (17.678 ± 1.219 U/l) and (80.671 ± 3.733 U/l), while injected rats with CCl4 and fed on a basal diet "control positive group" induced a significant increase in the mean value of these parameters (111.420 ± 5.246 U/l), (59.605 ± 3.052 U/l) and (147.382 ± 4.102 U/l), respectively (Table 7). The mean value of serum AST, ALT, and ALP in the positive control group increased significantly, as compared to the negative control group. Treating acute liver diseases rats with normal diet containing (2% & 4%) from (*Sonchus Oleraceus* SO, *Malva parviflora* MP and the combination between them "SO & MP") improved the mean

values of all parameters, as compared to the positive control group.

The mean value of serum AST, ALT and ALP of the acute liver disease group which was treated with 2% SO recorded a non-significant change, as compared to the group treated with 2% MP, the same trend was observed when used the level (4%). The highest improvement in serum liver enzymes recorded for the group which treated with 4% combination from (SO & MP leaves), followed by the groups which were treated with (2% combination from SO & MP leaves), (4% *Malva parviflora* MP) and (4% *Sonchus Oleraceus* SO), respectively.

The high level from the combination 4% (SO & MP) decreased the mean value of serum AST, ALT and ALP by about (23.412%, 32.322% and 17.949%) respectively, than that of the control positive group.

Histopathological examination of liver:

Microscopically, the liver of rats from group 1 "Control (-ve)" revealed the normal histological structure of the hepatic lobule (Photo. 1). Moreover, severe histopathological alterations were observed in the liver from group 2 "Control (+ve)". Examined sections showed steatosis of hepatocytes, apoptosis of hepatocytes and hemorrhage (Photo. 2), necrosis as well as inflammatory cells infiltration. Liver from group 3 which was treated with "2% *Sonchus Oleraceus* (SO)" showed no changes except cytoplasmic vacuolization of some hepatocytes (Photo. 3) or steatosis of focal hepatocytes (Photo. 4). Some sections from group 4 which was treated with "4% *Sonchus Oleraceus* (SO)" revealed no histopathological changes (Photo. 5), whereas, other sections showed slight dilatation of hepatic sinusoids (Photo. 6) and small vacuoles in the cytoplasm of some hepatocytes (Photo. 7). Some examined sections from group 5 which was treated with "2% *Malva parviflora* (MP)" showed macrovesicular steatosis of hepatocytes (Photo. 8) and mononuclear cells infiltration (Photo. 9), whereas, other sections revealed cytoplasmic vacuolization of hepatocytes and congestion of hepatic sinusoids (Photo. 10).

Moreover, an improved picture was noticed in the liver from group 6 which was treated with "4% *Malva parviflora* (MP)", examined sections revealed no changes except macrovesicular steatosis of sporadic hepatocytes with congestion of hepatic sinusoids (Photo. 11). Meanwhile, most sections from group 7 which was treated with "2% combination between (SO + MP)" revealed no histopathological alterations except slight vacuolation of sporadic hepatocytes with slight congestion of hepatic sinusoids (Photo. 12), whereas, few sections showed macrovesicular steatosis of some hepatocytes (Photo. 13). The liver of rats from group 8 which was treated with "4 % combination between (SO + MP)" revealed mild histopathological changes, examined sections showed macrovesicular steatosis of sporadic hepatocytes and slight Kupffer cells activation (Photo. 14).

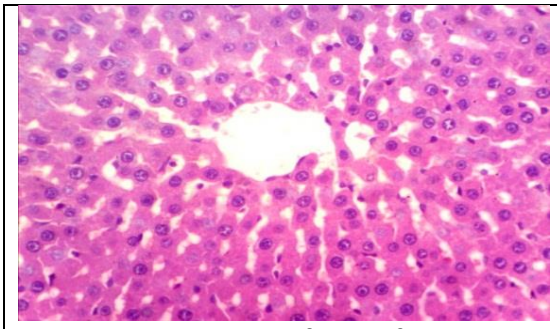

Photo (1): Liver of rat from group 1"Control (-ve)" which was fed on basal diet showing the normal histological structure of hepatic lobule (H & E X 400).

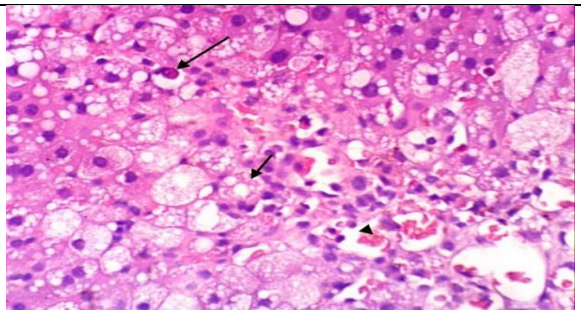

Photo (2): Liver of rat from group 2 "Control (+ve)" which was fed on basal diet showing steatosis of hepatocytes, apoptosis of hepatocytes and haemorrhage (H & E X 400).

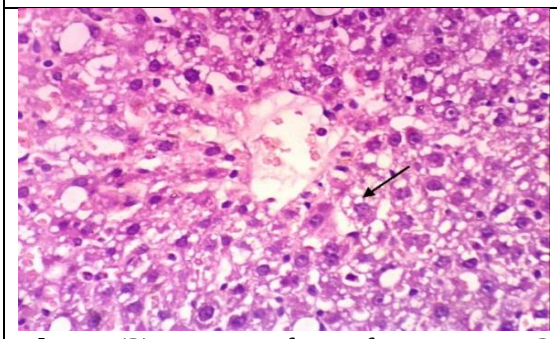

Photo (3): Liver of rat from group 3 which was treated with "2% *Sonchus Oleraceus (SO)*" showing cytoplasmic vacuolization of some hepatocytes (H & E X 400).

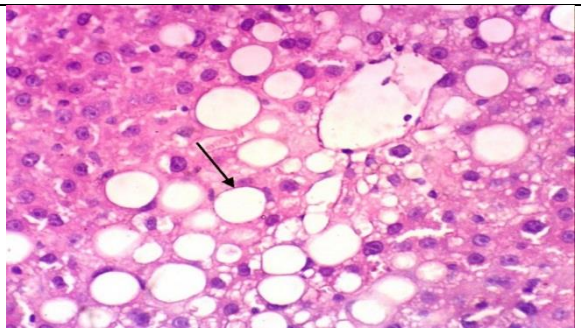

Photo (4): Liver of rat from group 3 which was treated with "2% *Sonchus Oleraceus (SO)*" showing steatosis of focal hepatocytes (H & E X 400).

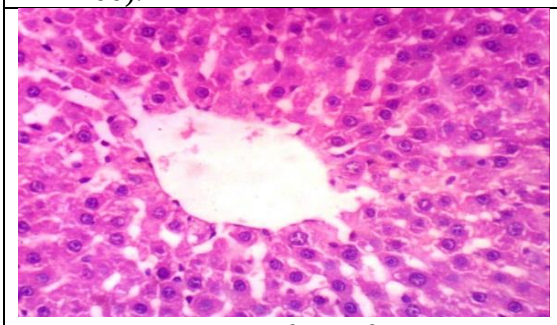

Photo (5): Liver of rat from group 4 which was treated with "4% *Sonchus Oleraceus (SO)*" showing no histopathological changes (H & E X 400).

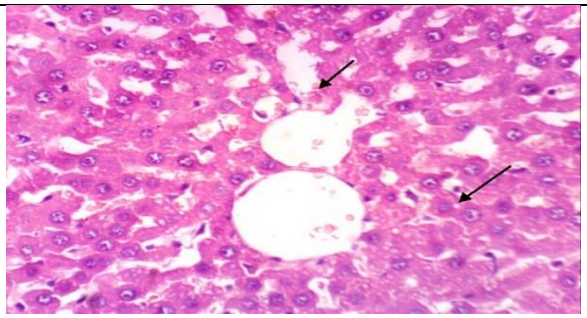

Photo (6): Liver of rat from group 4 which was treated with "4% *Sonchus Oleraceus (SO)*" showing slight dilatation of hepatic sinusoids and small vacuoles in the cytoplasm of some hepatocytes (H & E X 400).

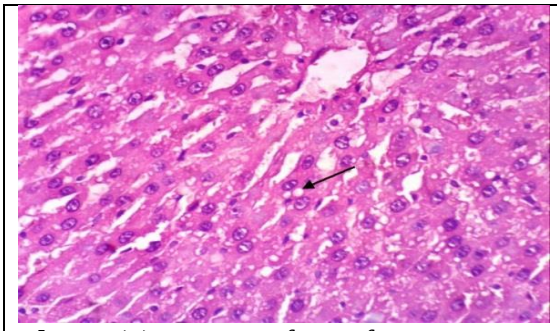

Photo (7): Liver of rat from group 4 which was treated with "4% *Sonchus Oleraceus (SO)*" showing small vacuoles in the cytoplasm of some hepatocytes (H & E X 400).

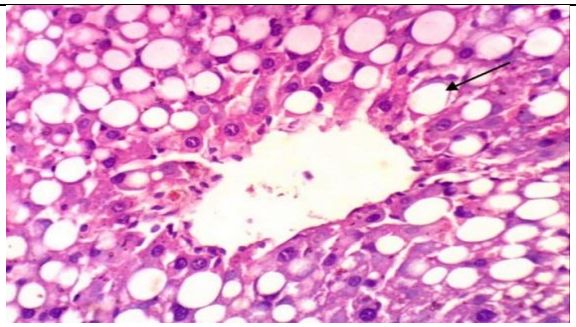

Photo (8): Liver of rat from group 5 which was treated with "2% *Malva parviflora (MP)*"showing macrovesicular steatosis of hepatocytes (H & E X 400).

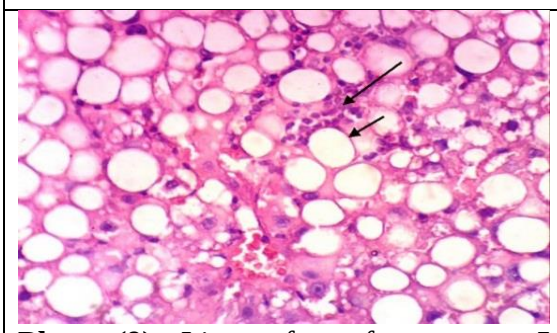

Photo (9): Liver of rat from group 5 which was treated with "2% *Malva parviflora(MP)*"showing macrovesicular steatosis of hepatocytes and mononuclear cells infiltration (H & E X 400).

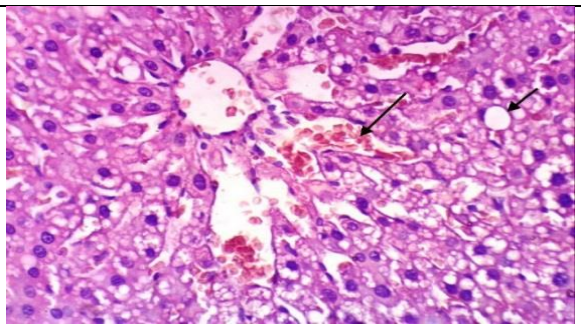

Photo (10): Liver of rat from group 5 which was treated with "2% *Malva parviflora (MP)*"showing cytoplasmic vacuolization of hepatocytes and congestion of hepatic sinusoids (H & E X 400).

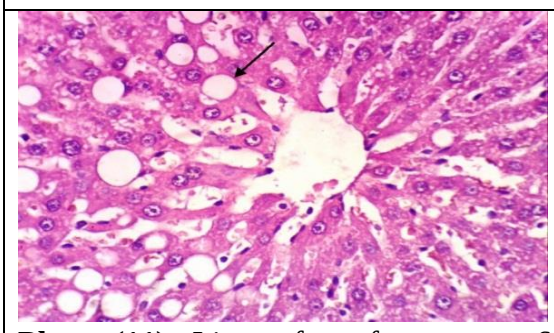

Photo (11): Liver of rat from group 6 which was treated with "4% *Malva parviflora (MP)*"showing macrovesicular steatosis of sporadic hepatocytes (H & E X 400).

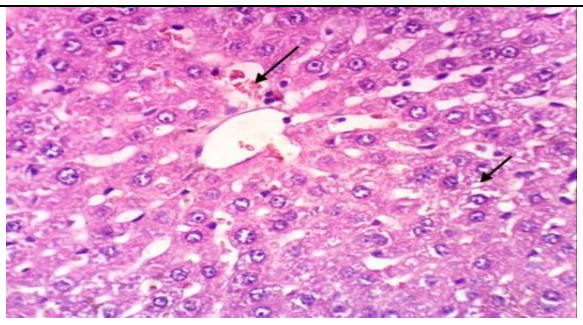

Photo (12): Liver of rat from group 7 which was treated with "2% combination between (*SO + MP*)" showing slight vacuolation of sporadic hepatocytes with slight congestion of hepatic sinusoids (H & E X 400).

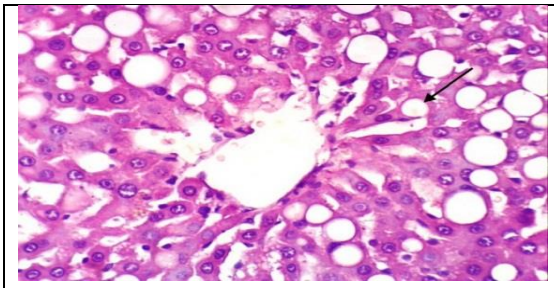

Photo (13): Liver of rat from group 7 which was treated with "2% combination between (*SO* + *MP*)" showing macrovesicular steatosis of some hepatocytes (H & E X 400).

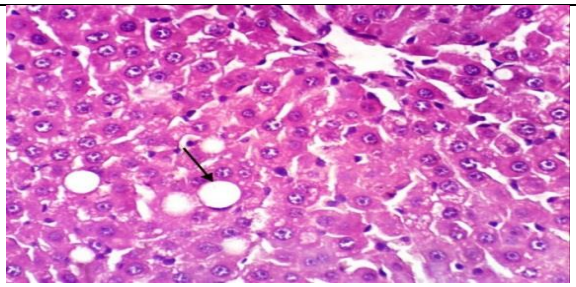

Photo (14): Liver of rat from group 8 " which was treated with 4% combination between (*SO* + *MP*)" showing macrovesicular steatosis of sporadic hepatocytes (H & E X 400).

DISCUSSION:

From these results in Table (1) it could be observed that the moisture content of *Sonchus oleraceus* increased than that of *Malva parviflora*. The highest concentration of the two types of leaves was recorded for carbohydrates, followed by protein, ash, fiber, and fat, respectively. Also, the results indicated that the amounts of total flavonoids and total phenols (mg/g) of *Sonchus oleraceus* (SO) increased than that of *malva parviflora* (MP). in this respect, **Jimoh *et al*., (2011)** showed that the percentage of moisture content, ash content, crude protein, crude lipid, crude fibre, and carbohydrate in the leaves of S. *oleraceus*, are 85.4, 14.3, 17.5,7.0, 46.0, and 15.3%, respectively while its calorific value is 317.3 Kcal/100 g. Total Polyphenols of the acetone, methanol and water extracts of the leaves of *Sonchus oleraceus* are 10.71 ± 0.32, 9.72 ±1.06 and 6.07 ± 0.07, respectively (Total polyphenol is expressed as mg tannic acid/g of dry plant material) while flavonoids are 1.09±0.01, 1.21±0.01 and 0.66±0 respectively, (Flavonoid is expressed as mg quercetin/g of dry plant material).

Abdalla *et al*., (2016) indicated that the percentage of moisture, crude protein, crude fat, crude fiber, total ash, and total carbohydrates are 78.64, 44.77, 3.39, 9.81, 10,47 and 41,37 % respectively while total energy is 285.29 kcal/100g in *malva*

parviflora (mallow) on a dry weight basis. While the amount of total phenolic content (TPC) and total flavonoid content (TFC) in the leaves of *Malva parviflora* (mg CAE/g dry weight) in aqueous and ethanol are 1.99 ± 0.070, 0.83 ± 0.063 and 2.24 ± 0.031, 1.07 ± 0.031, respectively **(Farhan *et al*., 2012),** This research significantly supported our results.

From data presented in Table (2), it could be concluded that the mean value of feed intake and BWG% in the acute liver disease group (+ve group) decreased, while liver weight/body weight % increased significantly than that of the normal group. Treating acute liver disease groups with (2% & 4%) SO, MP, and SO&MP together increased the mean value of feed intake and BWG%, while these treatments decreased the percent weight of liver, as compared to the positive control group. The best results in these parameters were recorded for the group treated with 4% SO&MP together.

In this respect, **(Muna *et al*., 2016)** reported that the raw and cooked leaves in *malva parviflora* had a high content of total dietary fiber (41.45%), including [insoluble dietary fiber (32.51%) and soluble dietary fiber (8.94%)]. Neutral detergent fiber (28.98%), acid detergent fiber (25.61%), and acid detergent lignin (8.93%).

In adipose tissue, luteolin (flavonoids) in (*sonchus oleraceus*) increased Peroxisome proliferator-activated receptor gamma (PPARg) protein expression to attenuate hepatic lipotoxicity, which may be linked to the improvement in circulating fatty acid (FA) levels by enhancing FA uptake genes and lipogenic genes and proteins in adipose tissue. Interestingly, luteolin also upregulated the expression of genes controlling lipolysis and the tricarboxylic acid (TCA) cycle prior to lipid droplet formation, thereby reducing adiposity **(Eun *et al*., 2015).** Polyunsaturated fatty acids present in *malva parviflora* exhibit antiobesity, anti steatosic and anti-inflammatory effects. Bioactive fatty acids provide health benefits through modification of fatty

acid composition and modulating the activity of liver cells during liver fibrosis **(Eva *et al*., 2016).**

Bitter vegetable (*Sonchus Oleraceus*) lipid extracts (BVL) can effectively inhibit adipogenesis through, at least in part, stimulating the AMP-activated protein kinase (AMPK) pathway and attenuate HFD-induced obesity. These findings suggest that BVL can be a promising dietary supplement for protection against obesity, and the affective component of BVL can be potentially developed as anti-obesity drugs **(Chen *et al*., 2021)**.

Tables (3and 4) showed the effect of *Sonchus oleraceus* and *Malva parviflora* leaves on the lipid profile of acute liver diseases rats fed on a normal diet. From these data in these Tables, we could be concluded that the mean value of serum cholesterol, triglycerides, and lipoprotein fractions including "LDL-c and VLDL-c" increased significantly in acute liver disease rats fed on a basal diet, while HDL-c decreased significantly, as compared to healthy rats which were fed on a "basal diet". On the other hand, feeding acute liver disease groups on a normal diet containing (2% & 4%) SO leaves, MP leaves, and "SO & MP together" decreased the mean value of all lipid profile parameters, except HDL-c which showed a significant increase, as compared to the positive control group. In addition to, serum cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c improved gradually with increasing the levels of SO leaves, MP leaves and "SO & MP together.In this respect,

Boll et al., (2001) reported that CCl_4 increased the synthesis of fatty acids and triglycerides and the rate of lipid esterification. Cholesterol and phospholipid synthesis from acetate was also increased. This could be due to the transport of acetate into the liver cell, resulting in increased substrate (acetate) availability. ***Kamalakkannan et al., (2005)*** cleared that carbon tetrachloride (3 ml/ kg/1 wk) administration to albino Wistar rats increased the levels of lipids, cholesterol, triglycerides, and free fatty acids in plasma and tissues (liver, kidney, heart, and brain). Phospholipid

levels increased in plasma, heart, and brain but decreased in liver and kidney.

In *Sonchus oleraceus*, it is suggested that there is a possibility of synergistic effects of quercetin and kaempferol (flavonoids) that enhance the LDL uptake more effectively together than its single compounds alone. The decrease in cell viability was higher in mixture combinations of quercetin and kaempferol (1:1, 2:1, and 1:2) than in individually treated quercetin and kaempferol (1:0 and 0:1) **(Yusof *et al*., 2016).** Apigenin lowered plasma levels of free fatty acid, total cholesterol, apolipoprotein B, and hepatic dysfunction markers and ameliorated hepatic steatosis and hepatomegaly, without altering food intake and adiposity **(Un Ju *et al*., 2016).**

In mice treated orally with (α, β-amyrin) (present in *Malva parviflora*) (10, 30, and 100 mg/kg), the HFD-associated rise in serum TC and TGs were significantly less. The hypocholesterolemic effect of α, β-amyrin appeared more prominent at 100 mg/kg with significant decreases in VLDL and LDL cholesterol and an elevation of HDL cholesterol. Besides, the atherogenic index was significantly reduced by α, β-amyrin **(Flávia *et al*., 2012).**

The results of this experiment found clear support for **(Khan *et al*., 2012)** which showed that the administration of *Sonchus asper (SAME)* and silymarin significantly lowered cholesterol, low-density lipoprotein, and triglycerides while elevating high-density lipoprotein levels. Also, **Flávia *et al*., (2012)** showed that the hypocholesterolemic effect of α, β-amyrin (among the components of *Malva parviflora*) appeared more prominent at 100 mg/kg with significant decreases in VLDL and LDL cholesterol and an elevation of HDL cholesterol. Besides, the atherogenic index was significantly reduced by α, β-amyrin.

Treating acute liver disease with SO or MP or their combination improved the structure of the liver. **(Simin *et al*., 2018)** reported that, Stigmasterol and β-sitosterol (active compounds in

malvaparviflora) significantly ameliorated high-fat Western-style diet (HFWD) induced fatty liver and metabolic abnormalities, including elevated levels of hepatic total lipids, triacylglycerols, cholesterol, and liver histopathology.

Table (5) showed the effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum protein of acute liver diseases rats fed on a normal diet. From these data, we could be concluded that: injected rats with CCl4 to induce acute liver disease for the normal group led to a significant decrease in the mean value of serum protein, as compared to the negative control group. While feeding acute liver disease rats which were fed on a normal diet containing two levels from (*Sonchus Oleraceus* leaves SO, *Malva parviflora* leaves MP and their combination) increased the mean value of serum protein, as compared to the positive control group.

In this respect, **Essam *et al*., (2012)** showed that there was a decrease in total protein concentrations observed in cirrhosis and liver cancer (hepato-cellular Carcinoma) patients compared to the control group. On the other hand, **(Bigoniya *et al*., 2009)** reported that, CCL4 significantly increases ALT, AST, ALP activities and total bilirubin level while decreasing total protein, albumin and total cholesterol. So **(Maher *et al*., 2015)** reported that, CCl4-significantly altered serum and hepatic enzymes, total protein, albumin, globulin, oxidative stress markers and lipid profiles.

On the other side, *Malva parviflora* L and , *S. oleraceus* contains high amounts of protein (on a dry weight basis) which may be increased serum protein in acute liver disease rats. (*Malva parviflora* L contain 44.77% crude protein **Muna *et al*., 2016**) and *S. oleraceus*, contain 17.5 % protein **(Jimoh *et al*., 2011)**. Treated rats with KBrO3 led to a significant decrease in serum protein, globulin, and albumin, as compared to the control group, on the other hand, administration of various concentrations of *Sonchus* erased the toxication of KBrO3 thereby increased the level of serum total protein, globulin, and albumin in a dose-dependent way (**Khan *et al*., 2012)**.

Table (6) showed the effect of *Sonchus oleraceus* and *Malva parviflora* leaves on liver enzymes, including (AST, ALT, and ALP) of acute liver diseases rats fed on a normal diet. Injected rats with CCl4 to induce acute liver disease led to a significant increase in serum AST, ALT, and ALP enzymes, as compared to non-injected rats.

Treating acute liver disease rats with two levels (2% and 4%) from (*Sonchus Oleraceus* SO, *Malva parviflora* MP, and the combination between "SO & MP" led to a significant decrease in liver enzymes, as compared to the positive control group. The high level from a combination of (SO leaves & MP leaves) achieved the best results in decreasing the mean value of serum (AST, ALT, and ALP enzymes).The administration of CCl_4 resulted in marked alteration in serum hepatic enzymes (like AST, ALT and ALP), oxidant parameters (like GSH and MDA) and pro-inflammatory cytokine TNF-α release from blood leukocytes indicative of hepatic injury **(AL-Harbi *et al*., 2014).**

Flavonoids (present in SO and MP) prevent hepatosteatosis by increasing fatty acid oxidation in the liver **(Akhlaghi, 2016)**. As an important category of phytochemicals, natural polyphenols (present in SO and MP) have attracted increasing attention as potential agents for the prevention and treatment of liver diseases. The striking capacities in remitting oxidative stress, lipid metabolism, insulin resistance, and inflammation put polyphenols in the spotlight for the therapies of liver diseases **(Sha Li *et al*., 2018)**. Our results strongly agree with **(Khan *et al*., 2012)** who said that the administration of *Sonchus asper (SAME)* and silymarin significantly lowered the CCl4-induced serum levels of hepatic marker enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase). On the other hand, **(Mallhi *et al*., 2014)** found that the extract of *M. parviflora* produced significant ($p<0.001$) reduction in liver enzymes and total bilirubin.

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المستخلص العربى

تأثير أوراق الخبيزة والجعضيض على أمراض الكبد الحادة فى الفئران التى تتغذى على وجبات غذائية قياسية

تم إجراء هذه الدراسة لفحص تأثير أوراق الخبيزة والجعضيض على أمراض الكبد الحادة في الفئران التي تتغذى على وجبات غذائية طبيعية. تم استخدام أربعون من ذكور الفئران البالغة من سلالة سبراجو– داولى وتم تقسيمها إلى مجموعتان رئيسيتان على النحو التالي: المجموعة الأولى الرئيسية (5 فئران) تم تغذيتها على نظام غذائى أساسى كمجموعة ضابطة سلبية. المجموعة الرئيسية الثانية (35 فئران) تم حقنهم بجرعة واحدة من رابع كلوريد الكربون في زيت البارافين (50٪ حجم / حجم 4 مللي / كجم) تحت الجلد لإحداث تلف حاد في الكبد. ثم قسمت الفئران إلى 7 مجموعات كالتالي: المجموعة (الأولى) تم تغذيتها على نظام غذائى أساسى كمجموعة ضابطة إيجابية . المجموعات (الثانية والثالثة) تم تغذيتها نظام غذائى أساسى يحتوى على 2٪ و 4٪ جعضيض على التوالي. المجموعات (الرابعة والخامسة) تم تغذيتها على نظام غذائى أساسى يحتوى على 2٪ و 4٪ خبيزة على التوالي. المجموعات (السادسة والسابعة) تم تغذيتها على نظام غذائي أساسى يحتوي على (1٪ جعضيض و 1٪ خبيزة) و (2٪ جعضيض و 2٪ خبيزة) على التوالي. اشارت النتائج الي حدوث ارتفاع فى الدهون الكلية وإنزيمات الكبد فيما عدا كوليسترول البروتين الدهني عالي الكثافة و البروتين في مجموعة الفئران المصابة بأمراض الكبد الحادة التي تم تغذيتها على الغذاء الأساسي مقارنة بالمجموعة الضابطة السالبة.

الكلمات الدليلية:– الجعضيض ، الخبيزة ، أمراض الكبد الحادة ، معدل الدهون ، إنزيمات الكبد.