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Effect of *Sonchus Oleraceus* and *Malva Parviflora* Leaves on Acute Liver Diseases in Rats fed on A High-Fat 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 high-fat diet. Forty-five adult male Albino rats of Sprague-Dawley strain were used and divided into three main groups as follows: The first main group was fed on a basal diet "BD", as a control negative group (-ve). The second main group was fed on a high-fat diet "HFD", as a control positive group (+ve[1]). The third main group 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 the high-fat diet, as a control positive group (+ve[2]) Groups (2 and 3): were fed on a high-fat diet containing 2% and 4% SO, respectively. Groups (4 and 5): were fed on a high-fat diet containing 2% and 4% MP, respectively. Groups (6 and 7): were fed on a high-fat 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 rats fed on HFD (control +ve [1]) and (control +ve[2]) compared to the negative control group.

Key words: *Sonchus oleraceus*, *Malva parviflora*, acute liver disease, total lipids, liver enzymes

INTRODUCTION:

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)**. Dietary fat may be an important modifiable factor in the development of the fatty liver. Multiple animal models have demonstrated that high fat/saturated fat diets (HFD) increase liver fat and promote hepatocyte injury **(Wang *et al*., 2006 and Lieber *et al*., 2004)**. Both studies found that two to three weeks on a low fat/low saturated fat diet (LFD) decreased liver fat compared to an HFD **(Van Herpen *et al*., 2011 and Westerbacka *et al*., 2005)**.

Obesity is a known risk factor for the development of non-alcoholic fatty liver disease (NAFLD); however, it has been suggested that dietary fat, both amount and composition, may play a pivotal role in its development, independent of body fatness. Studies that have investigated the role of dietary fat on liver fat accumulation are reasonably sparse. Overall, it would seem that; total calorie consumption, rather than dietary fat composition, is an important factor in the development of fatty liver disease in humans **(Charlotte and Leanne, 2014)**.

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 high-fat 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-five (45) 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, soybean, oil and saturated fat 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.

Experimental Design

Forty-five 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 three 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 (5 rats) was fed on high fat diet "HFD", as a control positive group (+ve[1]). The third 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 **(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 high fat diet, as a control positive group (+ve^2) ***Groups (2 and 3):*** were fed on high fat diet containing 2% and 4% *Sonchus Oleraceus*, respectively. ***Groups (4 and 5):*** were fed on high fat diet containing 2% and 4% *Malva parviflora*, respectively. ***Groups (6 and 7):*** were fed on high fat diet containing (1% *Sonchus Oleraceus* and 1% *Malva parviflora*) 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:

Total lipids were determined 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)**, Total serum protein **(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:

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 high-fat diet presented in table (1).

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 of rats which were fed on a high-fat diet (control +ve ^{1}) was (16.540 g/day/each rat) (Table 1). Feed intake of acute liver disease group which fed on HFD decreased than that of the control (-ve group). On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve^{2}) led to a decrease in feed intake, as compared to non-injected rats which fed on HFD (control +ve ^{1}). The mean value of feed intake of acute liver diseases group which were treated with high-fat 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 groups (control +ve)$^{1\&2}$.

The highest increase in the mean value of feed intake was recorded for the acute liver disease group which was treated with a high-fat diet containing (4% combination between SO & MP) Followed by the group treated with HFD containing (4% *Malva parviflora* MP), respectively. On the other hand, the mean value of feed intake increased with increasing the levels of *Sonchus Oleraceus* SO, *Malva parviflora* MP and SO & MP together in the diet.

Table (1): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on some nutritional parameters and percentage of liver weight of acute liver diseases rats fed on high fat diet.

Parameters / Groups		Feed Intake g/day/each rat	BWG%	liver weight / body weight%
Control (-ve)		17.00	33.972 ^{b} ± 1.131	2.889 ^{f} ± 0.077
Control (+ve)[1] fed on high fat diet (HFD)		16.540	53.240 ^{a} ± 1.847	4.174 ^{e} ± 0.096
Acute liver disease rats fed on	HFD only (control +ve)[2]	16.043	26.151 ^{c} ± 1.400	5.777 ^{a} ± 0.203
	HFD containing 2% SO	16.654	33.139 ^{b} ± 2.693	5.335 ^{b} ± 0.238
	HFD containing 4% SO	17.163	33.520 ^{b} ± 3.167	4.812 ^{c} ± 0.187
	HFD containg 2% MP	16.900	35.757 ^{b} ± 1.431	5.126 ^{b} ± 0.184
	HFD containing 4% MP	17.342	34.540 ^{b} ± 2.240	4.426 ^{d} ± 0.215
	HFD containing 2% (*SO* + *MP*)	17.225	35.574 ^{b} ± 0.654	4.873 ^{c} ± 0.160
	HFD containing 4% (*SO* + *MP*)	17.453	35.072 ^{b} ± 2.128	4.352 $^{d\,e}$ ± 0.188

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

The effect of *Sonchus oleraceus* and *Malva parviflora* leaves on BWG% of acute liver diseases rats fed on high-fat diet presented in table (1). The mean value of BWG% of healthy rats fed on a basal diet decreased significantly p≤0.05, as compared to healthy rats fed on HFD (control +ve [1]) (33.972 ± 1.131 vs. 53.240 ± 1.847), respectively. On the other hand, feeding the acute liver disease group on HFD (control +ve[2]) induced a significant decrease in BWG%, as compared to (control +ve [1]) (26.151 ± 1.400) vs. (53.240 ± 1.847), respectively. Injected group with CCl_4 and fed on HFD decreased BWG% by about 50.88% than that of the non-injected group which fed on the same diet. The results in this Table revealed that, all treated acute liver diseases groups which were fed on a high-fat diet containing 2% and 4% (SO, MP and the combination between SO& MP) showed a significant decrease in the mean values of BWG%, as compared to the positive control group[1] (control +ve[1]) while BWG% of all treated groups recorded a significant increase, as compared to the positive control group[2]. On the other hand, a non-significant change in BWG% was observed between all treated groups.

The mean value of liver weight/body weight % increase in the positive control group[1], as compared to the negative control group. All treated groups recorded a significant increase in liver weight / body weight%, as compared to the positive control[1], while the results in liver weight / body weight% in all treated groups recorded a significant decrease, as compared to the positive control[2].

The best results in liver weight / body weight % between all treated groups recorded for the group which was fed on a high-fat diet containing 4% combination between (SO & MP). This group showed a significant decrease in the mean value of liver weight / body weight %, as 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 high-fat diet presented in Table (2). The mean value of serum cholesterol in the acute liver disease group fed on a high-fat diet increased significantly ($P \leq 0.05$), as compared to the healthy group fed on a basal diet. On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant increase $P \leq 0.05$ in serum cholesterol, as compared to non-injected rats which fed on HFD (control +ve [1]). Treated groups with 4% SO and the combination between SO and MP with the level of (4%) recorded a significant decrease ($P \leq 0.05$) in serum cholesterol, as compared to (the positive control group)[1]. All treated groups recorded a significant decrease in the mean value of serum cholesterol, as compared to the positive control group[2]. Data in this table showed that, feeding normal rats with HFD increased the mean value of serum triglycerides significantly ($P \leq 0.05$) as compared to the healthy group fed on a basal diet.

Table (2): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum cholesterol and triglycerides of acute liver diseases rats fed on high fat diet.

Groups	Parameters	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control (-ve)		81.418 ^{h} ± 3.135	51.395 ^{g} ± 2.526
Control (+ve)[1] fed on high fat diet (HFD)		134.709 ^{e} ± 5.083	86.129 ^{d} ± 3.804
Acute liver disease rats fed on	HFD only (control +ve)[2]	166.775 ^{a} ± 6.642	111.598 ^{a} ± 5.095
	HFD containing 2% SO	144.634 ^{c} ± 2.789	94.512 $^{b\,c}$ ± 3.350
	HFD containing 4% SO	126.604 ^{f} ± 2.943	78.798 ^{e} ± 3.752
	HFD containing 2% MP	151.444 ^{b} ± 4.371	98.752 ^{b} ± 3.879
	HFD containing 4% MP	137.243 $^{d\,e}$ ± 2.389	86.849 ^{d} ± 3.110
	HFD containing 2% (*SO* + *MP*)	140.062 $^{c\,d}$ ± 2.317	90.398 $^{c\,d}$ ± 3.096
	HFD containing 4% (*SO* + *MP*)	120.377 ^{g} ± 3.759	72.807 ^{f} ± 3.963

Least significant differences at $P \leq 0.05$.
Means with the same letter are insignificantly difference.

The mean value of serum triglycerides increased by about 67.58% in the positive control group one than that of the negative control group. On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant increase $P \leq 0.05$ in serum triglycerides, as compared to non-injected rats which fed on HFD (control +ve [1]). Two treated groups showed a significant decrease ($P \leq 0.05$), as compared to the positive control group one (acute liver disease group which fed on HFD containing 4% SO and the group fed on HFD containing 4% combination between SO and MP). On the other hand, the mean value of serum triglycerides decreased gradually with increasing the level of SO, MP, and (SO&MP together) in the diets

The results in Table (3) 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 high-fat diet decreased significantly ($P \leq 0.05$), as compared to the control negative group (17.079 ± 2.149 mg/dl vs. 49.977 ± 1.819 mg/dl), respectively. The mean value of serum HDL-c in the positive control group one decreased by about 36.81% than that of the negative control group. On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant decrease $P \leq 0.05$ in serum HDL-c, as compared to non-injected rats which fed on HFD (control +ve [1]).

Treating acute liver disease groups with a high-fat diet containing 4% (SO), 4% MP and 4% "SO+MP' leaves together) led to a significant increase ($P \leq 0.05$), as compared to acute liver disease groups with a high-fat diet containing 2% (SO), 2% MP and 2% "SO+MP' leaves together), respectively. All treatments increased the mean values of serum HDL-c significantly, as compared to the control positive group two. From these results, we can be observed that the mean value of HDL-c increased gradually with increasing the level of these leaves in the diet. The mean value of serum LDL-c in the high-fat diet group and acute liver disease group fed on high fat diet Control +ve group [1&2]) increased significantly ($p \leq 0.05$), as compared to healthy rats (control –ve group) fed on basal diet.

On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant increase $P \leq 0.05$ in serum LDL-c, as compared to non-injected rats which fed on HFD (control +ve [1])

All treated groups recorded a significant decrease in the mean value of serum LDL-c, as compared to the acute liver disease group fed on HFD (control +ve)[2], while these treatments showed a significant increase in this parameter, as compared to the HFD group (control +ve)[1], except a group of rats which was treated with HFD containing a combination of 4% (SO + MP) together. The mean value of serum VLDL-c in the high-fat diet group and acute liver disease group fed on a high-fat diet (Control +ve group [1&2]) increased significantly ($p \leq 0.05$), as compared to healthy rats (Control –ve group) fed on basal diet.

On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant increase $P \leq 0.05$ in serum VLDL-c, as compared to non-injected rats which fed on HFD (control +ve [1]). The mean value of serum VLDL-c increased by about 68.62 % in the positive control group one than that of the negative control group. On the other hand, the mean value of serum VLDL-c decreased gradually with increasing the levels of SO, MP and (SO&MP together) leaves in the diet. The highest decrease in serum VLDL-c recorded for the group which treated with 4% combination between (SO & MP) leaves and 4% (SO) leaves, 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 lipoproteins of acute liver diseases rats fed on high fat diet.

Groups \ Parameters		HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control (-ve)		49.977^{a} ± 1.819	21.162^{h} ± 1.363	10.279^{g} ± 0.505
Control (+ve)[1] fed on high fat diet (HFD)		31.577^{cd} ± 1.979	85.906^{e} ± 3.480	17.225^{d} ± 0.760
Acute liver disease rats fed on	HFD only (control +ve)[2]	17.079^{g} ± 2.149	127.376^{a} ± 5.126	22.319^{a} ± 1.018
	HFD containing 2% SO	27.423^{e} ± 1.112	98.308^{c} ± 2.179	18.903^{bc} ± 0.668
	HFD containing 4% SO	33.316^{c} ± 2.381	77.528^{f} ± 2.661	15.759^{e} ± 0.750
	HFD containing 2% MP	24.226^{f} ± 2.423	107.468^{b} ± 2.623	19.750^{b} ± 0.776
	HFD containing 4% MP	29.876^{de} ± 2.303	89.997^{d} ± 1.705	17.369^{d} ±0.622
	HFD containing 2% (*SO* + *MP*)	31.227^{cd} ± 0.942	90.410^{d} ± 1.337	18.079^{cd} ± 0.619
	HFD containing 4% (*SO* + *MP*)	36.057^{b} ± 2.156	69.759^{g} ± 3.492	14.561^{f} ± 0.792

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 a high-fat diet is presented in Table (4). The mean value of serum protein in the high-fat diet group and acute liver disease group fed on a high-fat diet (control +ve group [1&2]) decreased significantly (p≤ 0.05), as compared to healthy rats (control –ve group) fed on basal diet. On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant decrease P≤ 0.05 in serum protein level, as compared to non-injected rats which fed on HFD (control +ve [1]). This decrease was estimated mathematically by about 16.99% in (control +ve[2]), compared to the (control +ve[1]).

Feeding acute liver disease groups on a high-fat diet containing 2% (SO or MP), 4% (SO or MP) and 2 &4% (SO and MP together) increased the mean values of serum protein, as compared to acute liver disease group fed on HFD, on the other hand, these treatments proved to be decreasing when compared to the (control +ve)[1].

The highest improvement in serum protein was recorded for the acute liver disease group fed on a high-fat diet containing 4% combination between (SO leaves & MP leaves), because this treatment increased the mean value of serum protein by about 15.801% than that of the positive control group two.

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

Groups \ Parameters		Protein g/dl
Control (-ve)		6.725 [a] ± 0.203
Control (+ve)[1] fed on high fat diet (HFD)		6.313 [b] ± 0.287
Acute liver disease rats fed on	HFD only (control +ve)[2]	5.240 [g] ± 0.134
	HFD containing 2% SO	5.716 [e f] ± 0.079
	HFD containing 4% SO	5.925 [c d] ± 0.058
	HFD containing 2% MP	5.534 [f] ± 0.137
	HFD containing 4% MP	5.783 [d e] ± 0.135
	HFD containing 2% (*SO* + *MP*)	5.876 [c d e] ± 0.070
	HFD containing 4% (*SO* + *MP*)	6.068 [c] ± 0.069

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

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 a high-fat diet presented in Table (5).

The mean value ± SD of serum AST enzyme in the control negative group which fed on basal diet was (59.266 ± 4.337u/l), while injected group with CCl_4 and fed this group on a high-fat diet "control positive group two" induced a significant increase in the mean value of serum AST enzyme (143.148 ± 4.665 u/l) (Table 5). The mean value of serum AST enzyme in the positive control group one increased by about 54.145% than that of the negative control group. On the other hand, injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve^2) led to significant increase $P \leq 0.05$ in serum AST enzyme, as compared to non-injected rats which fed on HFD (control +ve ^{1}). All Treated acute liver diseases rats with a high-fat diet containing (SO) and (MP) leaves don't improve the mean values of serum AST enzyme, as compared to the positive control group one.

The mean value of serum ALT enzyme in the high-fat diet group and acute liver disease group fed on a high-fat diet (Control +ve group $^{1\&2}$) increased significantly ($p \leq 0.05$), as compared to healthy rats (Control –ve group) fed on basal diet. On the other hand injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve^2) led to a significant increase $P \leq 0.05$ in serum ALT enzyme, as compared to non-injected rats which fed on HFD (control +ve ^{1}).

Table (5): Effect of *Sonchus oleraceus* and *Malva parviflora* leaves on liver enzymes of acute liver diseases rats fed on high fat diet.

Groups \ Parameters		AST	ALT	ALP
		U/l		
Control (-ve)		59.266 ^{g} ± 4.337	17.678 ^{h} ± 1.219	80.671 ^{g} ± 3.733
Control (+ve)[1] fed on high fat diet (HFD)		91.356 ^{f} ± 4.398	40.255 ^{g} ± 2.006	135.293 $^{c\,d}$ ± 4.088
Acute liver disease rats fed on	HFD only (control +ve)[2]	143.148 ^{a} ± 4.665	76.641 ^{a} ± 4.661	167.357 ^{a} ± 4.624
	HFD containing 2% SO	125.968 ^{b} ± 2.925	62.621 ^{c} ± 3.067	138.166 $^{b\,c}$ ± 5.376
	HFD containing 4% SO	114.811 ^{d} ± 4.398	53.609 ^{e} ± 1.298	127.474 ^{e} ± 3.740
	HFD containing 2% MP	130.568 ^{b} ± 4.177	66.482 ^{b} ± 4.377	143.516 ^{b} ± 4.711
	HFD containing 4% MP	120.493 ^{c} ± 4.475	57.882 ^{d} ± 1.573	131.295 $^{d\,e}$ ± 3.991
	HFD containing 2% (*SO* + *MP*)	119.941 ^{c} ± 1.660	57.372 ^{d} ± 3.334	132.425 $^{cd\,e}$ ± 3.369
	HFD containing 4% (*SO* + *MP*)	102.566 ^{e} ± 3.628	47.470 ^{f} ± 1.927	120.253 ^{f} ± 4.732

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

All treated groups with the two levels (2% and 4%) from (SO, MP and the combination between them) showed a significant decrease in serum ALT, as compared to the positive control group two. Feeding normal rats on a high-fat diet (control +ve [1]) and the rats injected with CCl_4 to induced acute liver diseases and feeding on HFD (control+ve[2]) showed a significant increase ($P\leq 0.05$) in serum ALP, as compared to the negative control group fed on basal diet. The induction of the acute liver disease group by CCl_4 and fed on HFD (control +ve[2]) increased the mean value of serum ALP by about 107.456% than the negative control group. On the other hand injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve[2]) led to a significant increase $P\leq 0.05$ in serum ALP, as compared to non-injected rats which fed on HFD (control +ve [1]). Feeding acute liver disease groups on a high-fat diet containing (4%) SO leaves, MP leaves and their combination (2% & 4%) showed a significant decrease (P≤ 0.05) in this parameter, as compared to the positive control group one.

Histopathological examination of liver:

Microscopically, the liver of rats from group **1 ''Control (-ve)**'' which was fed on basal diet revealed the normal histological structure of hepatic lobule (Photo. 1). Meanwhile, the liver of rats from group **2 ''Control (+ve)[1]** which was fed on a high-fat diet **(HFD)''** revealed macrovesicular steatosis of hepatocytes (Photo. 2) and mononuclear cells infiltration (Photo. 3). Also, liver of rats from group **3 ''Control (+ve)[2]** acute liver disease rat fed on high fat diet (HFD)'' revealed macrovesicular steatosis of hepatocytes (Photo. 4) and mononuclear cells infiltration (Photo. 5). However, the liver of rats from group **4** which was treated with HFD containing **''2% *Sonchus Oleraceus (SO*)''** showed the improved picture, as vacuolar degeneration of hepatocytes (Photo. 6) and slight dilatation of sinusoids (Photo. 7). Meanwhile, liver from group **5** which was treated with HFD containing **''4% *Sonchus Oleraceus (SO)* ''** revealed macrovesicular steatosis of hepatocytes (Photo. 8). The improved picture was noticed in the liver from **group 6** which was treated with HFD containing **''2% *Malva parviflora (MP)*''**, sections revealed microvesicular steatosis of hepatocytes (Photo. 9) and small focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo. 10). Macrovesicular steatosis of hepatocytes was observed in the liver from group **7** which was treated with HFD containing **''4% *Malva parviflora (MP)*''** (Photo. 11). However, liver from group **8** which was treated with HFD containing "2% combination between **(*SO* + *MP*)''** revealed regressed changes as macrovesicular steatosis of some hepatocytes (Photo. 12). Meanwhile, some liver sections from group **9** which was treated with HFD containing a combination between' **'4% (*SO* + *MP*)''** showed macrovesicular steatosis of hepatocytes (Photo. 13), whereas, other sections revealed slight vacuolization of hepatocytes (Photo. 14).

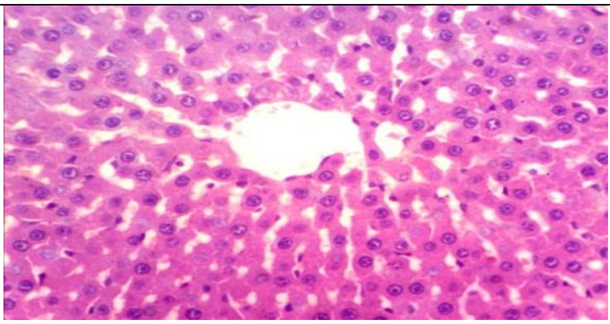

Photo (1): Liver of rat from group 1"Control (-ve)" which was fed on basal diet showing the normal histological structure of hepatic lobule (H & E X 400).

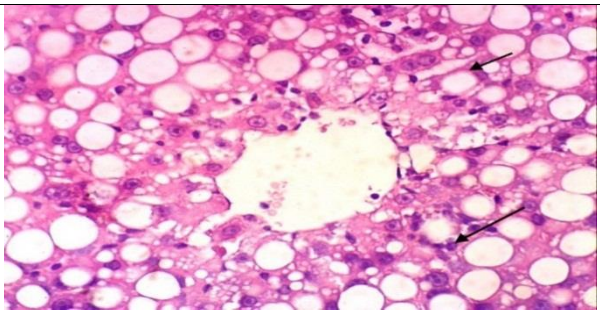

Photo (2): Liver of rat from group 2 "Control (+ve)[1] fed on high fat diet (HFD)" showing macrovesicular steatosis of hepatocytes and mononuclear cells infiltration (H & E X 400).

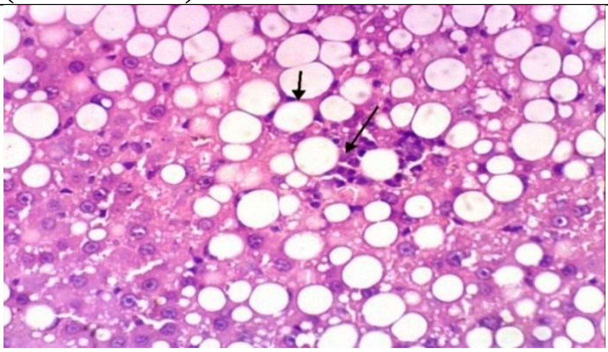

Photo (3): Liver of rat from group 2"Control (+ve)[1] fed on high fat diet (HFD)" showing macrovesicular steatosis of hepatocytes and mononuclear cells infiltration (H & E X 400).

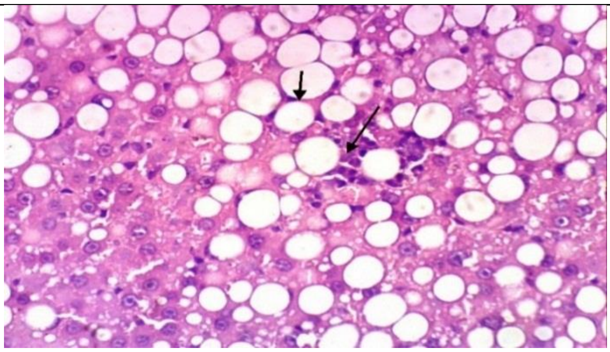

Photo (4): Liver of rat from group 3"Control (+ve)[2] acute liver disease rat fed on high fat diet (HFD)" showing macrovesicular steatosis of hepatocytes and mononuclear cells infiltration (H & E X 400).

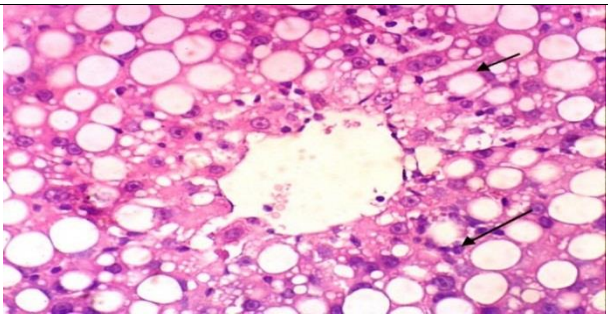

Photo (5): Liver of rat from group 3 "Control (+ve)[2] acute liver disease rat fed on high fat diet (HFD)" showing macrovesicular steatosis of hepatocytes and mononuclear cells infiltration (H & E X 400).

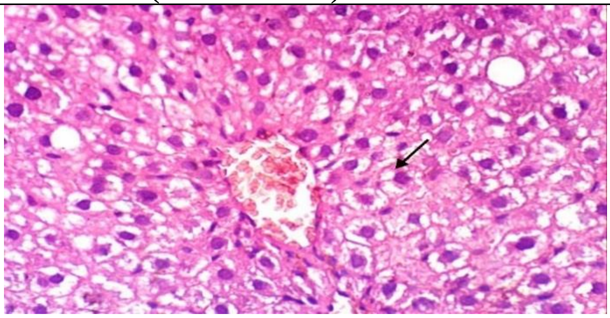

Photo (6): Liver of rat from group 4 which was fed on HFD containing "2% *Sonchus Oleraceus* (*SO*)"showing vacuolar degeneration of hepatocytes (H & E X 400).

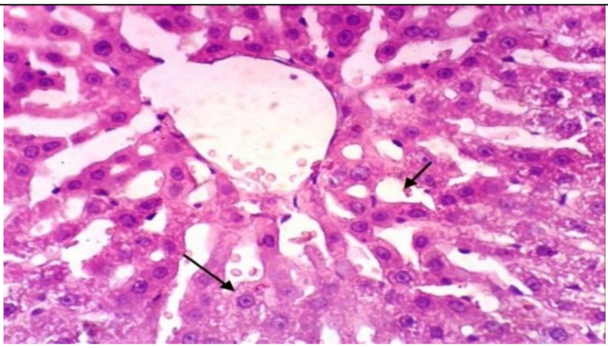

Photo (7): Liver of rat from group 4 which was fed on HFD containing "2% *Sonchus Oleraceus (SO)*"showing slight dilatation of hepatic sinusoids and slight vacuolar degeneration of hepatocytes (H & E X 400).

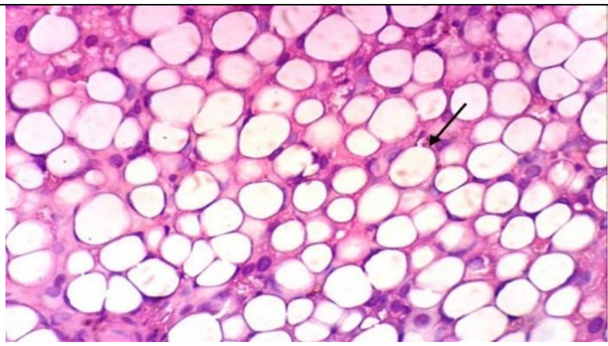

Photo (8): Liver of rat from group 5 which was fed on HFD containing "4% *Sonchus Oleraceus (SO)* " showing macrovesicular steatosis of hepatocytes (H & E X 400).

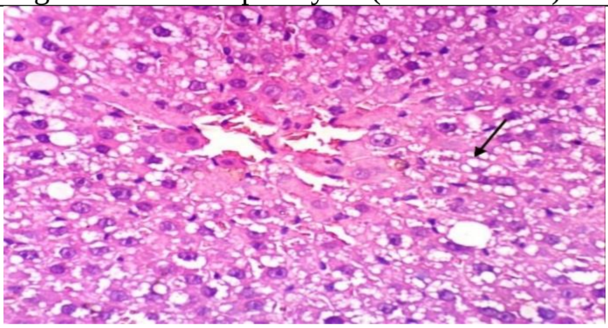

Photo (9): Liver of rat from group 6 which was fed on HFD containing "2% *Malva parviflora (MP)*"showing microvesicular steatosis of hepatocytes (H & E X 400).

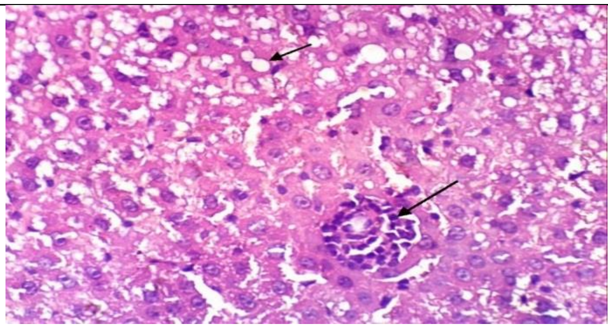

Photo (10): Liver of rat from group 6 which was fed on HFD containing "2% *Malva parviflora (MP)*"showing microvesicular steatosis of hepatocytes and small focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400).

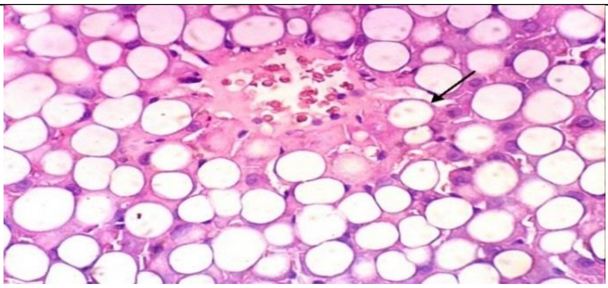

Photo (11): Liver of rat from group 7 which was fed on HFD containing "4% *Malva parviflora (MP)*" showing macrovesicular steatosis of hepatocytes (H & E X 400).

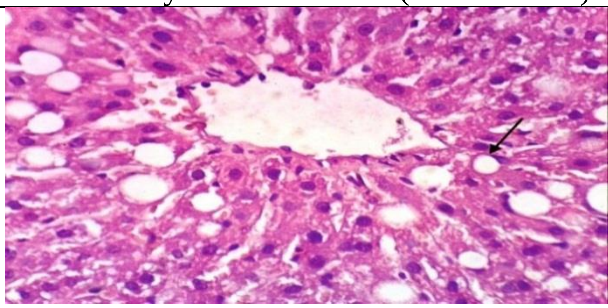

Photo (12): Liver of rat from group 8 which was fed on HFD containing "2% combination between (*SO* + *MP*)" showing macrovesicular steatosis of some hepatocytes (H & E X 400).

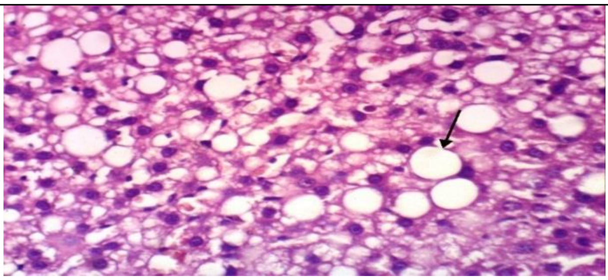	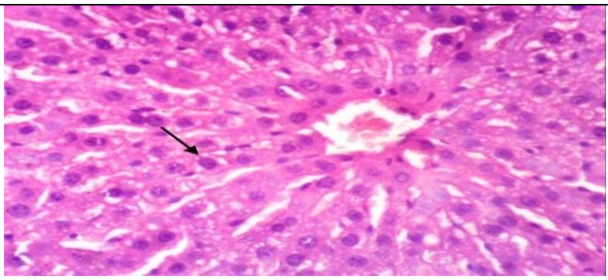
Photo (13): Liver of rat from group 9 which was fed on HFD containing "4% combination between (*SO* + *MP*)" showing macrovesicular steatosis of hepatocytes (H & E X 400).	**Photo (14):** Liver of rat from group 9 which was fed on HFD containing "4% combination between (*SO* + *MP*)" showing slight vacuolization of hepatocytes (H & E X 400).

DISCUSSION:

From data presented in Table (1), it could be concluded that the mean value of feed intake in the acute liver disease group which was fed on HFD decreased than that of the normal group. The highest increase in the mean value of feed intake was recorded for the acute liver disease group which was treated with a high-fat diet containing 4% (combination between SO & MP), followed by the group treated with (4% *Malva parviflora* MP), respectively. The mean value of BWG% of the positive control group one fed on a high-fat diet increased significantly (P≤0.05), as compared to the negative control group which was fed on normal diet. Non-significant changes in BWG% was observed between all treated groups, these treatments showed a significant increase in BWG%, as compared to the positive control [2] (acute liver disease group fed on HFD), on the other hand all treated groups recorded a significant decrease in BWG%, as compared to the positive control [1].The mean value of liver weight/body weight % increase in the positive control group[1], as compared to the negative control group. All treated groups recorded a significant increase in liver weight / body weight%, as compared to the positive control[1], while the results in liver weight / body weight% in all treated groups recorded a significant decrease, as compared to the positive control[2]. The best results in liver weight / body weight % between all treated groups recorded for the group which was fed on a high-fat diet containing 4% combination between (SO &

MP). This group showed significant decrease in the mean value of liver weight / body weight %, as compared to the other treated groups.

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 (2 and 3) showed the effect of *Sonchus oleraceus* and *Malva parviflora* leaves on the lipid profile of acute liver diseases rats fed on a high-fat 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 the HFD group and acute liver disease group which was fed on HFD, while HDL-c recorded a significant decrease in the "control +ve group " [1 & 2], as compared to healthy rats which were fed on "basal diet". On the other hand, feeding acute liver disease groups on a high-fat diet containing (4%) SO leaves and 4% combination between "SO & MP together" improved the mean value of all lipid profile parameters, as compared to the positive control group one.

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 (4) showed the effect of *Sonchus oleraceus* and *Malva parviflora* leaves on serum protein of acute liver diseases rats fed on a high-fat diet. From these data, we could be concluded that: Feeding normal rats on HFD decreased the mean value of serum protein, on the other hand injected rats with CCl_4 to induce acute liver disease for the HFD 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 HFD 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, CCL_4 significantly increases ALT, AST, ALP activities and total bilirubin levels while decreasing total protein, albumin and total cholesterol. So **(Maher *et al.*, 2015)** reported that, CCl_4-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* contain 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 (5) 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 high-fat diet. Feeding rats on HFD (control +ve ^{1}) and injected rats with CCl_4 to induce acute liver disease and fed on HFD (control +ve^{2}) led to a significant increase in serum AST, ALT and ALP enzymes, as compared to rats fed on basal diet. Treating acute liver disease rats with the level (4%) from (*Sonchus Oleraceus* SO, *Malva parviflora* MP and the combination between "SO & MP" (2% & 4%) led to significant decrease in liver enzymes, as compared to the positive control group one in ALP enzyme only. The high level from the combination of (SO leaves & MP leaves) achieved the best results in decreasing the mean value of serum (ALP) enzyme and non – significant changes in AST and ALT 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 CCl_4-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 a 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 عالية الدهن

تم إجراء هذه الدراسة لفحص تأثير أوراق الخبيزة والجعضيض على أمراض الكبد الحادة في الفئران التي تتغذى على وجبات غذائية عالية الدهن.تم استخدام خمسة وأربعون من ذكور الفئران البالغة من سلالة سبراجو– داولى وتم تقسيمها إلى ثلاث مجموعات رئيسية على النحو التالي: المجموعة الأولى الرئيسية تم تغذيتها على نظام غذائى أساسى كمجموعة ضابطة سلبية. المجموعة الرئيسية الثانية تم تغذيتها على نظام غذائى عالى الدهن كمجموعة ضابطة إيجابية أولى. المجموعة الرئيسية الثالثة 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 عالى الدهن كمجموعة ضابطة إيجابية ثانية .المجموعات (الثانية والثالثة): تم تغذيتها نظام غذائى عالى الدهن يحتوى على 2٪ و 4٪ جعضيض على التوالي.المجموعات (الرابعة والخامسة): تم تغذيتها على نظام غذائى عالى الدهن يحتوى على 2٪ و 4٪ خبيزة على التوالي.المجموعات (السادسة والسابعة): تم تغذيتها على نظام غذائي عالى الدهن يحتوي على (1٪ جعضيض و 1٪ خبيزة) و (2٪ جعضيض و 2٪ خبيزة) على التوالي. اشارت النتائج الي حدوث ارتفا ع فى الدهون الكلية وإنزيمات الكبد فيما عدا كوليسترول البروتين الدهني عالي الكثافة و البروتين في الفئران التي تم تغذيتها على نظام غذائى عالى الدهن (مجموعة ضابطة إيجابية أولى وثانية) مقارنة بالمجموعة الضابطة السلبية.

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