Effect of marjoram leaves on injured liver in experimental rats

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Abstract: This Study aimed to determinate some chemical composition of marjoram and investigates the treatment effect of marjoram leaves, hydro-alcoholic extract and essential oils on injured liver in experimental rats. 30 adult male Sprague Dawely rats classified into normal control (five rats) and liver injured rats groups which were non-treated group and treated groups with silymarin, marjoram leaves powder , oil and extract(each five rats).The chemical composition of marjoram leaves showed higher value of ash, protein, carbohydrate and fiber in dry weight than marjoram weight weight. Phenolic fractionations of marjoram in descending manner were protocathoic, chlorogenic, catechol, coumarone, cinnamic, caffein, vanillic, caffeic, synergic and chrisin. Marjoram leaves oils fractionations in descending manner were terpinene-4-ol, P-cymene, myrcene, γ-terpinene, β-pinene borneol, limonene, α-Pinene ,eugenol and sabinene. In compared to non treated group, the treated group with silymarin showed a significant decrease in final weight, weight gain, weight gain percent and FER and also serum ALT &AST, ALP, creatinine and total bilirubin , MDA ,liver cholesterol and total lipids but a significant increase in serum globulin ,serum CAT, liver TAC and in both serum and liver SOD . The treated group with marjoram leaves showed a significant decrease in gain percent and serum ALT &AST, creatinine, uric acid .total bilirubin , MDA, liver triglycerides, liver cholesterol and liver total lipids but a significant increase in serum globulin , serum CAT , liver TAC and liver SOD. The treated group with marjoram oil showed a significant decrease in final weight, weight gain, weight gain percent and FER and also serum ALT, ALP, creatinine, uric acid, total bilirubin ALP, MDA and liver triglycerides but a significant increase in globulin, liver cholesterol while treated group with marjoram extract showed a significant decrease in final weight, weight gain, weight gain percent and FER and also serum ALT, ALP, creatinine ,uric acid , total bilirubin ,liver triglycerides but a significant inecrease in globulin ,liver cholesterol.The treated groups with silymarin, marjoram leaves, oil and extract showed a significant decrease in serum cholesterol, triglycerides, LDL-c , VLDL-c and CHO/HDLc and a significant increase in serum HDL-c but a significant increase in serum TAC and liver CAT but a significant decrease in liver MDA.

[Abd El-Ghany, M. A & Nanees , Y. El-Metwally. **Effect of marjoram leaves on injured liver in experimental rats**. Report and Opinion 2010;2(12):181-191]. (ISSN: 1553-9873). http://www.sciencepub.net.

Key words: marjoram leaves; injured liver; rats; hydro-alcoholic.

1. Introduction

The liver is one of the most important organs of the human body affects all the systems. Liver injury is caused by chemical substances, medicines, alcohol, liver tumors, viral liver diseases and ischemia occurring after surgical operations .Medicinal treatment is necessary for a rapid recovery of liver injury However, medicines used in the treatment of liver have too many side effects. There is an increasing interest in the use of herbal drugs for therapeutic purposes. The new liver medicine is silymarin which is a plant derived flavonoid extracted from the fruits and seeds of the milk thistle and has been used for liver health (Katiyar, 2005).

Marjoram (Origanum majorana L) is a plant found throughout the world. For food uses, marjoram is employed to flavour sausages, meats, salads and soups. Traditionally, it is used as a folk remedy against cramps, depression, dizziness, gastrointestinal disorders, nervous headaches, paroxysmal coughs, asthma, and rheumatism and as a diuretic (Zargari, 1996 and Novak et al., 2000)). It contains phenolic terpenoids, flavonoids, tannins, phenolic glycosides and sitosterol .The antiviral, bactericidal, antiseptic and antifungal effects of marjoram are attributed to ursolic acid and essential oil and in particular to thymol and carvacrol (Kelly , 2004 and El-Ashmawy et al., 2005).

Commercial Origanum majorana L. oil or sweet marjoram is used as a spice and condiment. The volatile aromatic compounds are employed in the food industry as a flavoring. The oils exist in two forms. One with terpinen-4-ol and sabinene hydrate is major components and the other with thymol and/or carvacrol is predominant compounds. Volatile oils are being used by people for some respiratory, digestive and excretory system disorders. Volatile oils also have antiaging, antibacterial, antifungal and

antiseptic properties (Vera and Chane-Ming 1999 and Baser, 2002).

The study aimed to investigate the chemical composition of marjoram and the treatment effect of marjoram leaves, hydro-alcoholic extract and essential oils of on injured liver on experimental rats.

2. Material and Methods

I-Materials:

A- Marjoram leaves:

Dry plant leaves of Marjoram (Origanum Majorana) were purchased from the local market of Mansoura city, Egypt. All unwanted materials like stems, flowers, roots or stones were removed from the leaves.

B- Chemicals and drugs:

Carbon tetrachloride and pure ethanol were obtained from El-Gomhoria Company for chemicals, Egypt. CCL4 was diluted in olive oil (1:2)and gived to rats in dose 1.5 ml/kg b.w once a week for the first three weeks of the experiment .Pure ethanol dose was 0.4% of the diet daily for the first three weeks of the experiment (Demoria et al., 2006 and Goldania et al., 2007).Silymarin drug is brown color capsules each one contains 140 mg of silymarin and it was obtained from (CID). The rat dose was 7.6 mg/200 g b.w daily for 8 weeks (Katiyar, 2005).

C- Experimental animals:

This work was carried out on 30 adult male rats of Sprague Dawely strain. The mean weight of the male rats ranged from 137 ± 8g. The animals were purchased from the Agricultural Research Center, Giza, Egypt.

D- Standard diet

The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC (1995).

II-Methods:

A-Chemical study:

1-Determination of marjoram chemical composition:

Protein, fat, ash, and moisture of marjoram leaves were determined while total carbohydrates were calculated by difference as following: Carbohydrates % = 100 - (moisture % + protein % + fat % +ash) according to the methods of the A.O.A.C. (1995)

2- Preparation of hydro-alcoholic extract:

The dry leaves of marjoram were ground by domestic model electronic mixer. Hydro-alcoholic extraction procedure was carried out according to Charles et al., (1993).

3- Extraction of oil and determination of marjoram phenolic and oils fractionation:

The extraction of oil and fractionation of leaves oils were carried out according to the method recommended by Radwan, (1978). Fractionation and identification of phenolic compounds were determined in Central Laboratory of Food Tech. Res. Inst., Agric. Res. Center, Giza, according to Singleton and Rossi (1965).

B- Biological study:

1- Grouping of rats and experimental design:

The animals were kept under observation for five days before the start of the experiment for adaptation and fed on standard diet. Food and water was provided ad-libtum. The rats were randomly classified into six groups (5 rats each). The first group kept as normal control fed standard diet only. The other five groups administered CCL4, pure ethanol and also 5% lard fat instead of corn oil in diet for 8 weeks of the experiment to induce liver injury. They classified into non-treated group and treated groups with silymarin, marjoram leaves powder (3% instead of diet cellulose), and marjoram oil (0.5 ml/kg b.w daily) and marjoram extract (250 mg/kg b.w daily) all over all period of the experiment.

2-Calculation of food intake, body weight gain percentage and food efficiency ratio in rats:

The study was assigned for eight weeks. The food intake was calculated daily and the body weight gain was recorded daily. Food efficiency ratio was determined according to the method of Chapman et al., (1959).

3- Collection of blood samples:

At the end of the experimental period all rats were fasted overnight then sacrificed. Blood samples were immediately collected in clean and dried Wiesserman tubes from the portal vein. The blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum which frozen at -10°C for some analyses.

4- Collection of liver samples:

The liver of sacrificing rats was removed by careful dissection, blotted frees of adhering blood, washed with cold saline solution, and dried between two filter papers. Parts of the livers perfuse with 50 to 100 of ice cold 0.9%NaCL solution for some analyses.

5-Analytical Methods:

Serum (ALT& AST) activity, alkaline phosphates, total protein and albumin were estimated according to Reitman and Frankel (1957), Kind and King (1954), Weichselbaum (1946) and Eastham

(1976), respectively. Serum globulin value was determined by subtracting the albumin from the total proteins according to Coles (1974). Serum creatinine, uric acid and total bilirubin were determined according to Young (1975), Barham and Trinder (1972) and Jendrassik and Grof (1938), respectively.

Serum cholesterol, triglycerides and high density lipoprotein cholesterol, were determined according to Richmond (1973), Buccolo and David (1973) and Grodon and Amer, (1977),respectively .Low density lipoprotein cholesterol (LDL_C) and very low density lipoprotein cholesterol ($VLDL_C$) were calculated according to Friedwald et al ., (1972) and Lee and Nieman, (1996) as follows : LDL_C = Total cholesterol – (HDL_C + $VLDL_C$) while $VLDL_C$ = TG / 5. Athrogenic index was calculated by dividing cholesterol level on HDL_C according to Castelli and Levitar, (1977).

Plasma and liver total antioxidant capacity (TAC), catalase (CAT) ,serum superoxide dismutase (SOD) activity and blood malondialdehyde (MDA) were determined according to Draper and Hadley, (1990), Cohen et al .,(1973):, Beuchamp and Fridovich (1971), and Uchiyama and Mihara (1978), respectively. Liver triglyceride, cholesterol and total lipids were determined according to Young and Pestaner (1975), Abell et al., (1952), and Folch et al., (1957), respectively.

6- Histopathological examination:

Parts of rat livers were collected and immersed in 10% neutral buffered formalin as fixative. The fixative liver samples were sent to Cancer Institute for histopathological examination according to Bancroft et al., (1996).

III -Statistical analysis:

Collected data were presented as mean ±SDM and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Armitage and Berry (1987).

3. Result and Discussion

The chemical composition of marjoram leaves showed higher value of ash, protein ,carbohydrate and fiber in dry weight (DW) as the values were 16.21±0.13 , 12.34±0.47, 66.73±0.55 and 19.69±0.02 (g/100g),respectively than marjoram weight weight (WW) as the values were 14.82± 0.12, 11.29±0.43, 61.02±0.44 and18.00±0.01(g/100g), respectively. The moisture value was 8.56±0.08% while total phenols were 6.64 mg/g as shown in table (1)

Chemical composition of Origanum majorana (Marjoram) depends on the origin of the plants. According to USDA (2009), nutrient values of dried Origanum majorana were 12.66, 7.04, 12.10, 60.56 and 40.3 g/100g of protein, fats, ash, carbohydrates and fiber, respectively.

Phenolic fractionations of marjoram in descending manner were protocathoic, chlorogenic, catechol, coumarin and cinnamic (2499.45, 414.53, 182.38, 145.64 and 105.27, respectively). The values of caffein, vanillic, caffeic, synergic and chrisin were 150.62, 70.06, 57.38, 32.83 and 27.97, (µg/100g), respectively. Marjoram leaves oils fractionations were terpinene-4-ol, P-cymene, myrcene, γ-terpinene and β-pinene as the values in descending manner were 22.78, 13.00, 9.13, 7.70 and 5.53 %. The values of borneol, limonene, α-Pinene, eugenol and sabinene were 6.98, 4.29, 4.23, 2.53 and 1.89% as shown in table (2).

The difference in obtained results is observed even if the same method of extraction is used as it is depended on the origin of the plant. Novak et al., (2000) and Roth (2001) recorded that marjoram (Origanum majorana L.) contains phenolic terpenoids (thymol, carvacrol), flavonoids (diosmetin, luteolin, apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin، thymonin), triacontan, sitosterol, acids (oleanolic acid) and cis-sabinene hydrate. Petr et al. (2008) demonstrated that an online accumulation /mobilization preconcentration technique based on a dynamic pH junction technique and electrokinetic injection was employed for analysis of phenolic acids (sinapic, ferulic, coumarinic, caffeic, syringic, vanillic, and (4-hydroxybenzoic acid) in extracts from Majorana hortensis leaves.

In regarding to the obtained results of marjoram oil fractionations , Zargari, (1996) reported that the plant leaves also contain many substances as B-penin, P-cimen, terpinolon, antol, stragole, 2, 5 dimethyl estirin, and B-sprijen. Rodrigues et al. (2002) recorded the most prominent component of dried leaves, flowers, and commercial samples was terpinen-4-ol in four of the samples analyzed; only in one sample was α-terpineol present as the major compound. Pavol and Ivan (2006) mentioned that the content of essential oil into the dry marjoram is 1.2%. A wide range of secondary metabolites (terpinen-4ol: 6%, α-terpinene: 28%, γ-terpinene: 16%, sabinene: 2%, limonene: 6%, cineole: 7% a linalool: 2%) presents its composition (Vera and Chane-Ming 1999). The essential oil of marjoram has in acidic solution sabinene hydrate rearranges to give terpinen-4-ol and small amounts of -terpinene, γ-terpinene and p-cymene (Fischer et al., 1987).

The liver injured non treated group showed

a significant increase weight gain percent and FER at p<0.05 compared to normal control group. The treated group with silymarin showed a significant decrease in final weight, weight gain, weight gain percent and FER at p<0.05, 0.01 &0.001 compared with normal control group. The treated group with marjoram leaves showed non significant difference in the values of final weight, weight gain and food intake while FER value was significantly increase at p<0 compared to normal control group. The treated groups with marjoram oil and extract showed a significant decrease in final weight, weight gain and weight gain percent at p<0.05, &0.001 and a significant increase in the values of FER compared to normal control group. The treated groups with silymarin, marjoram leaves, oil and extract showed a significant decrease in final weight, weight gain and weight gain percent compared to non treated group. The treated group with silymarin showed also a significant decrease in FER while the treated group with marjoram leaves showed a significant decrease weight gain percent compared to non treated group as shown in table (3).

The metabolism of carbon tetrachloride produced free radicals in the cell can which act directly on biological structures. Cleavage of the carbon-chloride bond results in the production of chloroform and lipids radical, which react with molecular oxygen. This initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum .The peroxidation process leads to the release of soluble products affect on cell membranes. CCL4-induced cirrhosis in rats led to prolonged oxidative stress in the intestine (Lee et al., 2005 and Abd El-Ghany, 2006). Chronic ingestion of high levels of alcohol may cause oxidative stress that result in the formation (through alcohol metabolism) of excess free radicals, acetaldehyde, lipid and protein oxidation, and their reactive products (Okamoto and Okabe, 2000). The chemical compositions of marjoram leaves and lots of esters like in marjoram oils containing have a bactericidal activity; it inhibits the growth of micro organisms improve final weight, weight gain and FER in liver injured groups which treated with marjoram leaves, oil and extract (El-Ashmawy et al., 2007 and Petr et al .,2008).

The liver injured non treated group showed a significant increase in serum amino transferase enzymes (AST&ALT), alkaline phosphatase (ALP), albumin, creatinine, uric acid and total bilirubin at p< 0.05 ,0.01&0.001 and a significant decrease in serum globulin at p< 0.001 compared to normal control group.

The treated group with silymarin showed a significant increase in serum alanine amino transferase enzyme (ALT), albumin, creatinine and uric acid at p< 0.05 ,0.01&0.001 and a significant decrease in serum globulin compared at p< 0.001 while the treated group with marjoram leaves showed a significant increase in serum alkaline phosphatase (ALP), creatinine and uric acid at p< 0.05& 0.01 and a significant decrease in serum alanine aminotransferase enzyme (ALT) and globulin at p< 0.01 compared to normal control group. The treated group with marjoram oil showed a significant increase in albumin at p< 0.05 and a significant decrease in serum (ALT), alkaline phosphate (ALP), globulin and total bilirubin at p< 0.05 &0.01 while The treated group with marjoram extract showed a significant increase in serum aspartate transferase enzymes (AST) and albumin at p< 0.05 &0.01 and a significant decrease in serum (ALT), alkaline phosphate (ALP), globulin and uric acid at p< 0.05 ,0.01&0.001 compared to normal control group.

In comparing to non treated group, the treated group with silymarin showed a significant decrease in serum ALT &AST, ALP, creatinine and total bilirubin while treated group with marjoram extract showed a significant decrease in serum ALT, ALP, creatinine ,uric acid and total bilirubin. The values of ALT &AST, creatinine, uric acid and total bilirubin were decreased in treated group with marjoram leaves and also in treated group with marjoram oil beside ALP. All treated group showed a significant increase in globulin as shown in table (4).

ALT and AST are the enzymes increased in liver disease as hepatocellular damage. Severe or chronic hepatic diseases decrease synthesis of protein as well as plasma protein concentration. The alteration in serum albumin is found more commonly in chronic liver disease. Therapeutic/prophylactic administration of an antioxidant leads to an increase in the level of albumin in the serum (Christina et al., 2006). Silymarinis a flavonolignan and consists of a mixture of mainly three flavonoids, silybin (silibinin), silydianin and silychristin. Silibinin is the major component (70–80%) found in silymarin and is thought to be the most biological active compound. Pharmacological studies revealed that silymarin is non-toxic even at higher physiological doses, which suggests its safe use for the treatment of various diseases (Katiyar, 2005). The results of the present study demonstrated that O.majorana has no harmful effect on hepato-renal functions and these results agreed with the results of Aristatile et al., (2009). Carvacro is a predominant monoterpenic phenol which occurs in many essential oils of the family Labiatae including Origanum .Oral administration of carvacrol of marjoram for 21 days could afford a significant hepatoprotective and antioxidant effect against galactosamine -induced rats hepatotoxicity.

Kawachi et al., (2000) and El-Ashmawy et al., (2007) recorded that the treatment with O. majorana alcoholic extracts and its volatile oil significantly reduced enzyme activities and serum levels of urea, and creatinine as alcoholic extracts and volatile oil showed a better hepatoprotective effect .

The liver injured non treated group showed a significant increase in serum cholesterol, triglycerides , LDL-c , VLDL-c and CHO/HDLc at p< 0.001 and also liver triglycerides, cholesterol and total lipids at p< 0.05 ,0.01&0.001 and a significant decrease in serum HDL-c at p< 0.05 compared to normal control group. The treated group with silymarin showed a significant decrease in serum cholesterol, triglycerides ,HDL-c, LDL-c and VLDL-c, at p< 0.05 ,0.01&0.001 and a significant increase in liver triglycerides at p< 0.001 while the treated groups with marjoram leaves showed a significant increase in liver triglycerides at p< 0.01 compared to normal control group. The treated groups with marjoram oil and extract showed a significant decrease in serum triglycerides , VLDL-c and liver triglycerides at p< 0.05 ,0.01&0.001 but a significant increase in liver cholesterol at p< 0.05 compared to normal control group.

The treated groups with silymarin, marjoram leaves, oil and extract showed a significant decrease in serum cholesterol, triglycerides, LDL-c, VLDL-c and CHO/HDLc and a significant increase in serum HDL-c while the treated group with silymarin showed a significant decrease in liver cholesterol and total lipids. The treated group with marjoram leaves showed a significant decrease in liver triglycerides, cholesterol and total lipids but the treated group with marjoram oil and extract showed a significant decrease in liver triglycerides and a significant increase in liver cholesterol compared to non treated group as shown in table (5).

Soltan and Abdel - Wahab (2006) recorded that hyperlipidaemia appeared in rats fed on high fat diet and group fed on high fat diet supplemented with plant products. A significant increase in serum total cholesterol, triacylglycerol, very-low-density lipoprotein and low-density lipoprotein cholesterol, total liver cholesterol and triacylglycerol, and liver glutathione in hypercholesterolaemic group compared to the control group. Marjoram was effective in reducing the values of the biochemical parameters of lipid profiles. The liver injured non treated group showed a significant decrease in both blood and liver total antioxidant capacity (TAC), catalase (CAT) and serum superoxide dismutase (SOD) activity at p< 0.01&0.001 but a significant increase in both blood and liver malondialdehyde (MDA) at p< 0.001 compared to normal control group.The treated groups with silymarin showed a significant increase in serum CAT ,liver TAC and liver MDA at p< 0.05, 0.01&0.001 but a significant decrease in serum SOD activity at p< 0.01 and liver total CAT at p< 0.01 while the treated groups with marjoram leaves showed a significant increase in serum CAT and liver MDA at p< 0.05&0.01 but a significant decrease in serum SOD ,liver CAT and liver SOD at p< 0.05&0.001 compared to normal control group. The treated groups with marjoram oil and extract showed a significant decrease in serum CAT, serum SOD , liver TAC , liver CAT and liver SOD p< 0.05, 0.00&0.001 and a significant increase in serum MDA at p< 0.01 compared to normal control group.
The treated groups with silymarin, marjoram leaves, oil and extract showed a significant increase in serum TAC and liver CAT but a significant decrease in liver MDA while the treated group with silymarin showed a significant decrease in serum MDA and a significant increase in serum CAT, liver TAC and in both serum and liver SOD and compared to non treated group.

The treated group with marjoram leaves showed a significant decrease in serum MDA and a significant increase in serum CAT and liver TAC and SOD but the treated group with marjoram oil showed a significant decrease in serum MDA compared to non treated group as shown in table (6).
The results agreed with El-Ashmawy et al., (2007) who recorded revealed a significant increase in lipid peroxidation and decrease in the level of glutathione in the liver in the ethanol-treated group. ethanol induced oxidative stress is the result of the combined impairment of antioxidant defenses and the production of reactive oxygen species by the mitochondrial electron transport chain, the alcohol-inducible cytochrome P450 and activated phagocytes However, co-administration of the extracts of protective plants resulted in minimizing the hazard effects of ethanol toxicity on liver tissue. Marjoram volatile oil (essential oil) extract are useful herbal remedies, especially for controlling oxidative damages Ipek et al., (2005) and Mustafa et al .,(2008) reported that liver GHS and CAT levels were reported to decrease and the MDA level were increased in animals subjected to liver injury Silymarin can protect against genomic defects in liver. Carvacrol protects hepatocytes similar to silymarin without any undue effect on their genetic make up.

The marjoram extracts demonstrated varying degrees of efficacy in each screen. The extract was the most effective at reducing iron(III), scavenging 1,1-diphenyl-2-picrylhydrazyl radicals, inhibiting ascorbate-iron(III)-catalyzed hydroxyl radical-mediated brain phospholipid peroxidation, and site-specific hydroxyl radical-mediated 2-deoxy-d-ribose degradation (Damien et al .,2004).

Table (1): Chemical composition of marjoram leaves (g/100g).

Ash		Fat		Protein	
W.W	D.W	W.W	D.W	W.W	D.W
14.82± 0.12	16.21± 0.13	4.32± 0.11	4.72++ 0.12	11.29± 0.43	12.34± 0.47
Carbohydrates		Fibers		Moisture %	Total Phenols mg/g
W.W	D.W	W.W	D.W		
61.02± 0.44	66.73± 0.55	18.00± 0.01	19.69± 0.02	8.56± 0.08	6.64

Table (2): Phenolic and oil fractionations of marjoram leaves.

Phenolic fractionation(µg/100g)				*Oils fractionation (%)*			
Caffeic	57.38	Coumarin	145.64	α-Pinene	4.23	P-Cymene	*13.00*
Vanillic	70.06	Cinnamic	105.27	β-Pinene	5.53	γ-Terpinene	*7.70*
Synergic	32.83	Catechol	182.38	Myrcene	9.13	Limonene	*4.29*
Chrisin	27.97	Protocathoic	2499.45	Borneol	6.98	Sabinene	*1.89*
Caffein	*150.62*	*Chlorogenic*	*414.53*	*Eugenol*	*2.53*	*Terpinene-4-ol*	*22.78*

Table (3): Body weight gain (g), food intake (g/day) and food efficiency ratio (FER) of normal control and liver injured rat groups.

Groups / Variables	Normal control	*Liver injured groups*				
		Non treated	Treated with silymarin	*Treated with marjoram*		
				leaves	oil	*extract*
Initial weight	138.20 ± 7.82 ^{a}	137.60 ± 8.35 ^{a}	142.00 ± 5.94 ^{a}	141.00 ± 8.00 ^{a}	137.20 ± 9.78 ^{a}	*141.20 ±9.88 ^{a}*
Final Weight(g)	214.80 ±18.93 ^{a}	223.80 ±28.37 ^{a}	184.60 ±16.95 ^{b*}	224.00 ±21.81 ^{a}	168.20 ±10.52 ^{b*}	*174.20 ±18.70 ^{b*}*
Weight Gain (g)	76.60 ±11.14 ^{a}	86.20 ±10.11 ^{a}	42.60 ±1.14 ^{b**}	83.00 ±18.51 ^{a}	31.00 ±2.92 ^{c***}	*33.00 ±5.49 ^{c***}*
Weight Gain %	55.42 ±11.95 ^{b}	62.77 ±1.68 ^{a*}	30.25 ±2.76 ^{c***}	59.01 ±13.38 ^{b}	22.67 ±2.48 ^{d***}	*23.17 ±6.65 ^{b*}*
Food Intake(g/d))	18.52 ±0.87 ^{a}	17.98 ±2.51 ^{a}	16.44 ±1.81 ^{a}	17.64 ±1.44 ^{a}	15.27 ±1.01 ^{a}	*15.77 ±1.40 ^{a}*
FER	*0.07 ±0.002 ^{b}*	*0.09 ±0.003 ^{a*}*	*0.05 ±0.004 ^{c**}*	*0.08 ±0.01 ^{a*}*	*0.10 ±0.01 ^{a***}*	*0.10 ±0.03 ^{a***}*

Significant with normal control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (4): Some liver and renal function parameters of normal control and liver injured rat groups at the end of the study.

Groups / Variables	Normal control	*Liver injured groups*				
		Non treated	Treated With silymarin	*Treated with marjoram*		
				leaves	oil	*extract*
AST (µ /ml)	8.20± 1.84 ^{c}	12.20± 0.84 ^{a**}	7.40± 0.55 ^{c}	9.80± 0.84 ^{c}	10.40± 1.55 ^{c}	*12.20± 2.84 ^{ab**}*
ALT (µ /ml)	23.20± 2.84 ^{c}	48.20± 0.84 ^{a***}	37.20± 0.84 ^{b**}	11.40± 0.89 ^{e**}	14.20± 3.84 ^{e*}	*19.40± 1.89 ^{d*}*
ALP (µ /ml)	301.40± 83.71 ^{b}	455.02± 63.78 ^{a**}	351.80± 43.94 ^{b}	410.58± 99.96 ^{a*}	198.20± 22.84 ^{c***}	*154.2± 18.84 ^{c}*

T.P g/dl	5.82± 1.70 a	5.20± 0.47 a	6.44± 0.09 a	5.02± 0.22 a	5.30± 0.51 a	*5.36± 0.42 a*
Albumin g/ dl	2.10± 0.45 b	3.69± 0.32 a*	3.44± 0.11 a*	2.98± 0.56 a b	3.26± 0.21 a*	*3.30± 0.22 a**
Globulin g/dl	3.72± 0.28 a	1.51± 0.32 d***	3.00± 0.07 b*	2.04± 0.59 c**	2.04± 0.36 c**	*2.06± 0.27 c***
Creatinine mg/dl	0.37± 0.02 c	0.49± 0.02 a*	0.44± 0.05 b*	0.44± 0.04 b*	0.40± 0.05 bc	*0.38± 0.03 bc*
Uric Acid mg/dl	2.35± 0.18 c	5.15± 0.46 a***	5.03± 0.32 a***	4.13± 0.6 b**	2.17± 0.31 c	*1.45± 0.16 d**
T.Bilirubin mg/dl	*0.51± 0.02 b*	*0.59± 0.09 a**	*0.49± 0.03 b*	*0.45± 0.05 b*	*0.42± 0.02 c***	*0.52± 0.04 b*

Significant with normal control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (5): Some serum and liver lipid profile of normal control and liver injured rat groups at the end of the study.

Groups / Variables	Normal control	*Liver injured groups*				
		Non treated	Treated With silymarin	*Treated with marjoram*		
				leaves	oil	*extract*
Serum CHO (mg/dl)	106.00± 14.00 b	136.40± 14.55 a***	88.20± 9.84 c***	111.20± 12.84 b	117.20± 13.84 b	*103.00± 13.71 b*
Serum T.G (mg/dl)	94.40± 8.89 b	162.80± 13.84 a***	74.00± 9.71 c*	95.60± 10.55 b	67.00± 6.71 c**	*66.40± 7.89 c**
Serum HDLc (mg/dl)	54.60± 5.89 a	32.80± 3.84 c***	46.60± 4.55 b*	57.40± 6.89 a	59.00± 10.71 a	*48.40± 5.89 ab*
Serum LDLc (mg/dl)	32.52± 4.72 c	71.04± 8.93 a***	26.80± 0.57 d**	34.68± 3.47 c	44.80± 7.27 b*	*41.32± 5.29 b**
Serum VLDLc (mg/dl)	18.88± 2.18 b	32.56± 3.17 a***	14.80± 1.14 c*	19.12± 2.11 b	13.40± 4.14 c*	*13.28± 0.18 c**
CHO/HDLc	1.94± 0.01 b	4.15± 0.06 a***	1.89± 0.01 b	1.93± 0.03 b	1.98± 0.02 b	*2.12± 0.03 b*
Liver T.G (mg/dl)	67.94± 8.82 d	99.64± 11.71 a***	90.56± 10.75 ab***	84.22± 8.83 c**	41.62± 7.79 e***	*41.12± 3.94 e****
Liver CHO (mg/dl)	36.68± 5.98 c	48.64± 6.93 a*	32.68± 3.98 c	38.64± 4.82 c	58.76± 6.92 b**	*63.24± 7.70 b****
Liver T. Lipids (mg/dl)	*151.64± 22.79 b*	*217.11± 27.90 a***	*158.18± 25.99 b*	*168.68± 24.81 b*	*198.52± 17.73 ab*	*192.96± 18.98 ab*

Significant with normal control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (6): Some antioxidant parameters of blood and liver tissue of normal control and liver injured rat groups at the end the study

Groups / Variables	Normal control	*Liver injured groups*				
		Non treated	Treated with silymarin	*Treated with marjoram*		
				leaves	oil	*extract*
Blood TAC (mmol/L)	2.21± 0.18 ^{a}	1.31± 0.06 ^{c*}*	2.35± 0.02 ^{a}	2.23± 0.13 ^{a}	1.62± 0.24 ^{ab}	*1.97± 0.19 ^{ab}*
Serum CAT (µ /ml)	543.50± 99.46 ^{b}	243.50± 77.04 ^{c**}	773.68± 180.20 ^{a**}	619.55± 132.71 ^{a*}	242.31± 92.14 ^{c**}	*236.67± 67.16 ^{c**}*
Serum SOD (µ /ml)	0.52± 0.04 ^{a}	0.22± 0.01 ^{c***}	0.33± 0.05 ^{b**}	0.20± 0.01 ^{c***}	0.40± 0.11 ^{b*}	*0.24± 0.09 ^{c**}*
Serum MDA (n mol/ml packed cells)	0.71± 0.03 ^{c}	2.12± 0.06 ^{a***}	0.94± 0.20 ^{c}	0.81± 0.17 ^{c}	1.43± 0.22 ^{b**}	*2.14± 0.88 ^{a*}*
Liver TAC ((mmol/g)	2.72± 0.35 ^{b}	1.32± 0.15 ^{c**}	3.06± 0.40 ^{a*}	2.40 ± 0.29 ^{b}	1.26± 0.05 ^{c*}	*1.53± 0.26 ^{c*}*
Liver CAT (µ /ml)	1.81± 0.10 ^{a}	0.23± 0.05 ^{d***}	0.35± 0.03 ^{c**}	0.83± 0.12 ^{b*}	0.41± 0.09 ^{c**}	*0.35± 0.08 ^{c**}*
Liver SOD (µ /ml)	355.55± 88.99 ^{a}	142.16± 32.81^{d***}	386.29± 87.96 ^{a}	256.56± 35.66 ^{b*}	143.86± 42.69 ^{d**}	*133.45 ± 14.77^{d***}*
Liver MDA (nmol/mgptn)	*19.75± 0.80 ^{c}*	*54.51± 0.86 ^{a***}*	*34.21± 0.91 ^{b***}*	*29.02± 0.61 ^{b**}*	*21.34± 0.76 ^{c}*	*20.89± 3.69 ^{c}*

Significant with normal control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Histopathological examination

The histological examination of liver sections of rat from the normal group revealed the normal histological structure of hepatic lobule (Pict. 1). Examined sections from the non-treated group showed vacuolar degeneration of hepatocytes and kupffor cells activation with focal area of hepatic necrosis associated with leucocytic cells infiltration (Pict. 2). However, liver of rat from the group treated with silymarin, marjoram leaves, oil and extract showed no histopathological changes (Pict. 3, 4, 5& 6). The histopathological results were agreed with the results of Marja et al., (1999) who found that alcoholic extracts of O. majorana significantly reduce micronucleus, total aberrant cells and different types of chromosomal aberrations, fragment, ring chromosome, centric attenuation, gap and stickiness. Volatile oil significantly reduces micronucleus and fragment, gaps, ring chromosome and stickiness. The results suggest that marjoram leaves, oil and extract affords a significant hepatoprotective and hypolipidemic effect against carbon tetrachloride and ethanol induced-liver injury in rats (Figure 1).

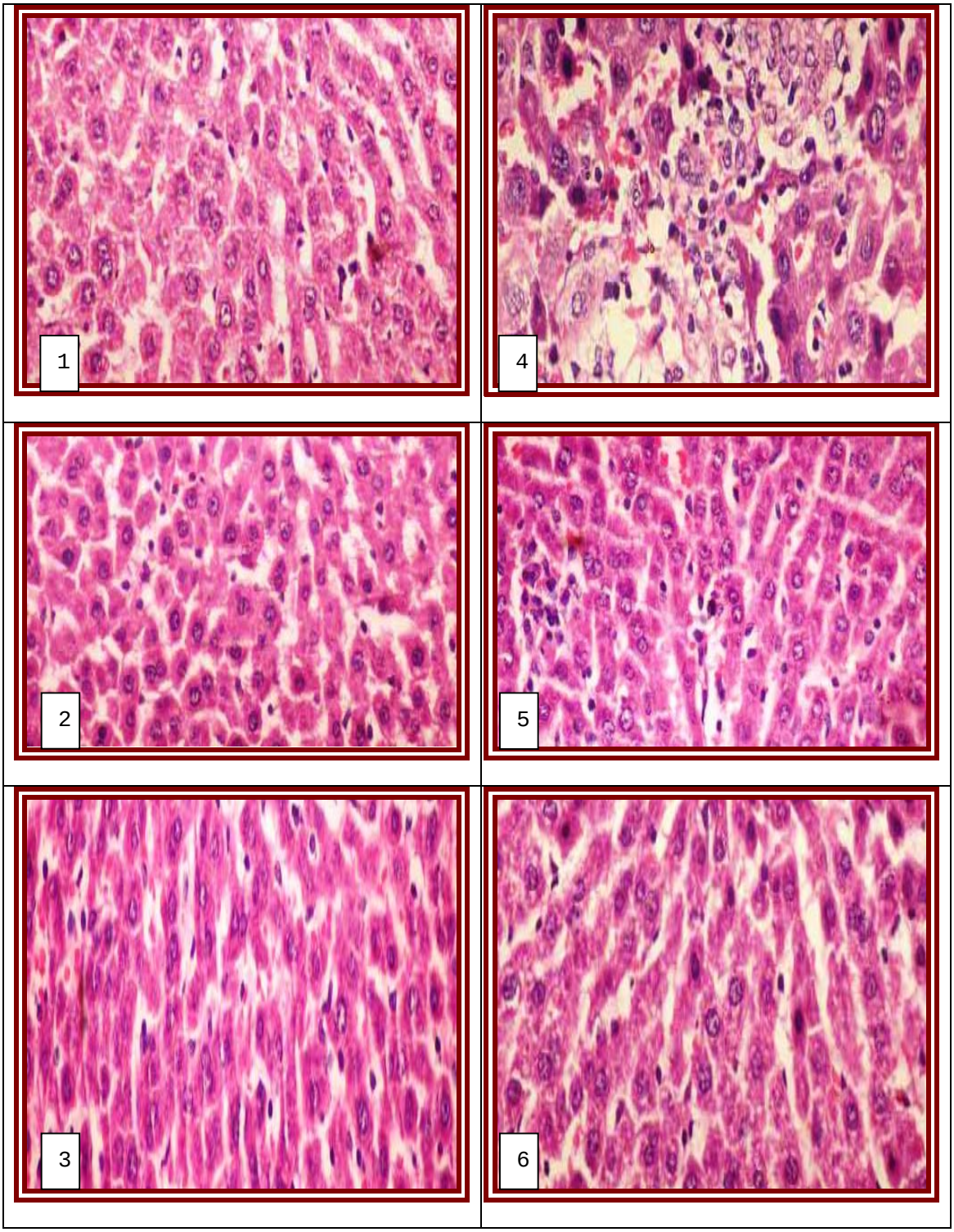

Figure 1. Histopathological examination

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11/2/2010