

Effect of Various Levels of (*Origanum majorana*) on Liver Function of Intoxicated Rats by Carbon Tetrachloride.

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Abstract:

Origanum majorana is a bushy tender perennial herb known to have antioxidant levels hepatoprotective antibacterial. Because *Origanum majorana* (LOM) has antihypertensive and antiplatelet aggregation properties, this exploratory study aims to investigate the effect of various levels of (*Origanum majorana*) on liver function of intoxicated rats by carbon tetrachloride. Before the research, the rats were kept in a cage (animal home) for one week. The rats were categorized into two groups, with the first (n=6) given only basal food. As a negative control group, a 28-day baseline diet was used (C-ve). The second group rats intoxicated with carbon tetrachloride (n= 18 rats), 5%, 10% LMO and positive control. The results indicated that liver enzymes, total bilirubin levels, and total protein decreased significantly ($p<0.05$). Additionally, triglycerides, total cholesterol, and LDL-c significantly reduced ($p<0.05$) in rats treated with 5% and 10% marjoram, whereas HDLc levels increased significantly. Marjoram is a herb that can be utilized under certain situations and the direction of a physician in the diets of individuals with liver disease

Keywords: Marjoram, liver functions, hepatoprotective, intoxicated rats.

1. INTRODUCTION

Origanum Majorana, also identified as sweet marjoram, is a plant in the Lamiaceae family that is evergreen and herbaceous. There are approximately 900 species of *Origanum*. Several species of these plants are extensively used to flavor alcohol, food, and perfumery products Vera et al. (1999). Recently, sudorific, stomachic, expectorants, emmenagogues, stimulants, antiseptics, hepatoprotectives, and nephroprotective have been employed to treat a variety of diseases (Soltan et al. 2016). As a perennial herb, marjoram is bushy and can reach a height of up to 1 foot tall. It originated in Asia but was brought to Europe, where it became popular among the Romans and Greeks (Calonne, 2022; Gough, 2002). In Marathi and Hindi, it is known as Marwa. Its leaf tips are square, with small, grey-green leaves that can be fuzzy, and it has square stems. Bunches of white or pink flowers grow from knot-like buds. The essential oil extracted from the branches and leaves has a pleasant and calming perfume that is slightly peppery, woody, spicy, and nutty. (Petr et al., 2008) describe it as having a more mellow aroma and qualities than oregano, which is closely related but maybe overwhelming (fresh, sweet, warm, herbaceous, and a touch woody). The leaves are traditionally used to treat insomnia, diabetes, asthma, catarrh, and nervousness. Studies have proven that leaf extracts are effective antioxidants, hepatoprotectives, and antibacterial, an antihypertensive and antiplatelet aggregation agent. *Origanum*

extracts and essential oils have been extensively studied in academia and the food industry (Yadav et al., 2020). Kelly, (2004) Chronic liver damage occur when steatosis leads to chronic hepatitis, cirrhosis, fibrosis, and hepatocellular cancer. Since oxidative stress is critical to the genesis and progression of liver disease (Moreno et al., 2021; Tejada et al., 2021), antioxidants were investigated as therapeutics and pharmacological adjuvants to counteract liver damage (Gopikrishna et al., 2021; Tejada et al., 2021; Vitaglione et al., 2010).

- **THE PURPOSE OF THE STUDY:**

This study aimed to investigate the effect of various Levels of (*Origanum majorana*) on liver function of intoxicated rats by carbon tetrachloride

2. MATERIALS

2.1. Plant

The Mint family (Lamiaceae - mints, months), Genus (*Origanum* L.), and species (*Origanum majorana* L. - sweet marjoram) were obtained from a local market as dried material.

2.2. Carbon tetrachloride (CCl₄)

Carbon tetrachloride (CCl₄) was given by El-Gomhoria company for chemical industries (Cairo, Egypt). They gave it to them in a 10% liquid solution. It was given out in white plastic water bottles that could hold one litre, as per (Passmore & Eastwood, 1986), and it was given out as a toxic substance ingredient for liver disease. It is diluted using paraffin oil obtained from the drugstore during the induction (Passmore & Eastwood, 1986).

2.3. Rats

At 14-16 weeks of age, twenty-four (24) adult male Sprague-Dawley rats weighing 150-160 g B.Wt were studied. The animals were housed in sanitary conditions in plastic cages with trainless metal roofing. For adaption, rats were given the basal diet for seven days prior to the study. A smallmouth bottle connected with a metallic tube and a piece of plastic tubing at the mouth provided Ad libitum water. As previously indicated, rats were acclimatized on a 12-hour light/12-hour night condition for seven days prior to the beginning of the research to allow for acclimation.

3. METHODS

3.1. Preparation of plant

Marjoram (LMO) dried plant leaves were acquired from a local market in al Baha, Saudi Arabia. All plant ingredients were powdered in a mixer and stored in glass with a dark stopper vial in a cold, dry location until usage. All herbs and plants must be stored in a cold, dark and dry atmosphere, according to Russo (2001), to prevent the oxidation of their contents. Carbon tetrachloride (CCl₄) in 50% V/V

paraffin oil (2ml / kg b. wt.) was given to 20 male albino rats subcutaneously twice a week for two weeks to cause chronic liver injury, as described by Jayasekhar et al., (1997). After the injection of CCl₄, blood was taken by a retro-orbital approach to check for liver disease and to evaluate liver function.

3.2. Diets

Table (1): The composition of basal and Experimental diet:

Component (g)	Basal diet	Basal diet+5% myrrh	Basal diet+10% myrrh
Test ingredients	---	5	10
Casein	20	20	20
Lipids	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5
Vitamin mix	1	1	1
Cellulose	5	5	5
Choline chloride	2	2	2
Sucrose	10	10	10
Corn starch	Up to 100	Up to 100	Up to 100

Source: (Campbell, 1963a, 1963b), (Hegsted et al., 1941), Reeves et al., (1993).

Rats groups and feeding.

The study used 24 Sprague Dawley white male albino rats ranging between 150 – 160 grams. Each of the four groups contained six rats. The following are the rat groups:

- **Group (1):** In the negative control experiment, rats were fed a standard diet (control"-").
- **Group (2):** The control positive group (control "+") consisted of rats fed a standard diet and administered with carbon tetrachloride (CCl₄).
- **Group (3)** was given a standard diet plus 5% LMO.
- **Group (4)** was given a standard diet plus 10% LMO.

Blood sampling

At the conclusion of the trial, rats were slaughtered under ether anaesthesia (28 days). The retro-orbital approach was used to collect blood samples in a clean, dry centrifuge tube. They were allowed to coagulate at room temperature before centrifuging for fifteen min at 1500 rpm. Serum was obtained using a wash and dry syringe, placed in Wasserman tubes, and preserved at -10 °C until biochemical analysis. The livers, spleens, lungs, hearts, and kidneys of the rats were then separated and

washed in saline before being weighed and dried. The weight values of the mentioned organs were calculated using the procedure outlined below. According to Drury & Wallington (1967), organs were kept in formalin (10% V/V) before histological analysis.

Biological evaluation:

The feed efficiency ratio (FER), food intake (consumption), bodyweight gain percent (BWG percent), and feed efficiency ratio, according to Chapman D.G, (1959) were calculated using the following equations:

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

$$FER = \frac{Gain\ in\ body\ weight\ (g / day)}{Food\ Intake\ (g / day)}$$

$$\text{The organs' relative weight} = \frac{\text{Organs weight}}{\text{Animal body weight}} \times 100$$

Biochemical analysis

3.3. The function of liver enzymes

Activity of aspartate aminotransferase (AST): The activity of the AST enzyme was determined using a spectrometer and specialized kits (BioMerieux) in accordance with the manufacturer's instructions Reitman and Frankel (1957). Activity of serum alanine aminotransferase (ALT): The colorimetric technique described was used to test the activity of the ALT enzyme. (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) concentrations were determined using the Roy technique (1970), a colorimetric ALP assay. Serum total bilirubin was determined calorimetrically, as previously described by Doumas et al., (1973). Total cholesterol in serum was determined using a spectrophotometer calibrated to 578 nm (Ratliff & Adams, 1973).

3.4. Determination of triglycerides:

Jacobs & Van-Denmark, (1960) determined the triglyceride concentration by an enzymatic colorimetric technique. The determination of HDL was performed in accordance with (Jacobs & Van-Denmark, 1960). VLDL and LDL were determined using the method reported by Lee (Lee, 2009).

3.5. Histopathological analysis:

Liver specimens were collected immediately following the experiment's conclusion, fixed in 10% neutral formalin, dried in ethyl alcohol, cleaned in xylene, and embedded in paraffin wax. Staining with hematoxylin and eosin was used to create 4-6 thick slices (Maniatopoulos et al., 1988).

4. Analytical statistics

The data were analyzed statistically using the automated SPSS program (Statistic Program statistical software, SAS Institute, Sigmastat, Cary, NC). The impact of various treatments was established using a one-way ANOVA (variance analysis) test along with Duncan's multiple tests, with $p < 0.05$ indicating statistical significance across groups (Snedecor and Cochran, 1967).

5. RESULTS AND DISCUSSION

5.1. Biological effects

The effect of various concentrations of LMO on body weight gain (BWG%) of change in liver disorder rats is shown in Table (2). After four weeks, both normal and liver disease rats gained weight, as shown in table (2). Bodyweight gain in the normal group rats was 35.78 ± 0.973 gm/100gm. While liver disorder rats given LMO at various levels (5%, and 10%, positive control) showed 10.24 ± 0.624 , 34.04 ± 0.579 , and 33.58 ± 0.455 gm/100gm, respectively. The findings revealed that there were no significant differences ($p < 0.05$) between all sample groups.

The normal rat group's food intake was 17.19 ± 0.041 gm/100gm. While in liver disorder rats, groups with LMO at different levels (5%, and 10%, positive control), showed 15.05 ± 0.038 , 17.61 ± 0.025 , and 19.09 ± 0.015 gm/100gm, respectively, results illustrated strong significance ($p < 0.05$) when compared to control negative.

5.2. Feed efficiency ratio (FER)

The values for the normal rats group were 0.130.007. While in liver disorder rat's groups with LMO at different levels (control positive, 5%, and 10%) were 0.06 ± 0.003 , 0.11 ± 0.004 , and 0.11 ± 0.006 . Results showed that there were no statistically significant differences ($p < 0.05$) between sample groups. This finding is consistent with the hypothesis (Albano, 2006). Final weight, weight gain, weight gain %, and FER were all significantly lower ($p < 0.05$) in the LMO oil-treated group, as were serum ALT, Total

bilirubin ALP, ALP, uric acid, creatinine, MDA, and liver triglycerides, but there was a significant rise ($p < 0.05$) in liver cholesterol and globulin. In the silymarin, oil, LMO leaves, and extract-treated groups, there was a significant decrease ($p < 0.05$) in the final weight, weight gain percent, weight gain, and FER, as well as a significant decrease ($p < 0.05$) in serum cholesterol, LDL-c, triglycerides, VLDL-c, and CHO/HDLc, and a significant increase in serum HDL-c, but a significant increase in liver CAT and serum TAC.

Table (2): The effect of feeding different amounts of LMO on body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER) in rats injected with LMO in rats injected with (CCl₄).

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
FI (g)	17.19±0.041 ^d	15.05±0.038 ^g	17.61±0.025 ^c	19.09±0.015 ^a
BWG (g)	35.78±0.973 ^a	10.24±0.624 ^d	34.04±0.579 ^a	33.58±0.455 ^a
FER (g)	0.13±0.007 ^a	0.06±0.003 ^e	0.11±0.004 ^b	0.11±0.006 ^b

The values represent arithmetic means and the standard error of the mean. Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at $p = 0.001$, whereas those with the same letters are non-significant.

5.3. The effect on the organs' relative weights

The results presented in table (3) demonstrate the impact of various LMO concentrations on organ weight and organ weight/body weight in normal and liver disease rats following four weeks of feeding. The liver in normal rats' groups with LMO was 3.79±0.083gm/100gm. While liver disorder rats' groups at a different level of LMO (5%, and 10%, positive control of LMO) showed a relative decrease weight of the liver 3.49±0.077, 4.09±0.092, and 3.64±0.045 gm/100gm, respectively. The normal rat group (control sample) had a relative heart weight of 0.91±0.041 gm/100gm. In comparison, liver disorder rats' groups at various levels of LMO (positive control, 5%, and 10%) showed that 0.49±0.021, 0.87±0.017, and 0.83±0.018 gm/100gm, respectively. The results indicated that all of the groups were significantly ($p = 0.001$) more than the control negative group. Relative spleen in the normal rat group, the weight value was 0.62±0.059 gm/100gm. While liver disorder rats group fed diet at various levels of LMO (5%, and 10% positive control of LMO) showed that 0.49±0.053, 0.62±0.018, and 0.58±0.019 gm/100gm, respectively. The results indicated that all of the groups were significantly ($p = 0.001$) more than the control negative group. According to Srihari et al. (2008), it is an agent for a liver injury that concluded that LMO is used medicinally to treat liver disorders. It has been used for anemia, dropsy, stomach, depression, melancholia, hysteria, generalized seizures, and cholecystography.

Table (3): The impact of different concentrations of LMO on the relative organ weight of CCl₄-intoxicated rats.

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
Liver (g)	3.79±0.083 ^b	3.49±0.077 ^c	4.09±0.092 ^a	3.64±0.045 ^c
Spleen (g)	0.62±0.059 ^a	0.49±0.053 ^d	0.62±0.018 ^a	0.58±0.019 ^b
Heart (g)	0.91±0.041 ^a	0.49±0.053 ^d	0.87±0.017 ^a	0.83±0.018 ^a

The values represent arithmetic means and the standard error of the mean. Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at $p = 0.001$, whereas those with the same letters are non-significant.

5.4. The impact on hepatic enzymes (AST, ALT, and ALP)

Table (4) illustrates the impact of feeding various amounts of LMO on liver enzymes in the blood of CCl₄-intoxicated rats after four weeks of feeding, alanine aminotransferase (ALT), including aspartate amino transaminase (AST), and alkaline phosphatase (ALP). In contrast, liver disorder rats' groups at different levels of LMO (5%, and 10%, positive control) showed total Lipids values $130.6 \pm 124.6 \pm 2.3 \pm 1.79$, and 118.3 ± 2.4 mg/dl respectively.

ALT normal rate group's values were 36.5 ± 1.6 mmol/L. While in liver disorder, rats' groups at different levels of LMO (5%, and 10%, positive control) were 70.5 ± 2.4 , 65.5 ± 2.8 , and 46.7 ± 2.2 mg/dl respectively. The results indicated that all of the groups were significantly ($p = 0.001$) more than the control negative group.

ALP normal rate group's values were 84.5 ± 1.9 mmol/L. While in liver disorder, rats' groups fed a diet with different levels of LMO (5%, and 10%, positive control) were 159.4 ± 2.7 , 154.7 ± 2.5 , and 146.3 ± 2.8 mg/dl, respectively. The results indicated that rats fed 10% LMO had significantly higher levels of positivity ($p = 0.001$) when compared with control positive rats. According to Sikander et al. (2013), the impact of a different extracts of *O. vulgare* leaves isolate on CCl₄-induced hepatotoxicity had been examined in normal and hepatotoxic rats. Rats were separated into six groups to test OV's hepatoprotective activity: control, *O. vulgare*, carbon tetrachloride (CCl₄; 2 ml/kg), and three treatment groups received CCl₄ and *O. vulgare* orally for 15 days at concentrations of 50, 100, and 150 mg/kg body weight. To test liver function, researchers measured ALT (alanine aminotransferase), ALP (alkaline phosphatase),

and AST (aspartate aminotransferase) in serum, as well as LPO (lipid peroxide), CAT, SOD, GST, GR, GPx, and GSH in liver tissue. In comparison to controls, CCl₄ treatment resulted in morphological and biochemical evidence of liver injury. In the presence of OV, CCl₄-induced hepatotoxicity was significantly reduced.

Table (4): The effects of different LMO concentrations on the serum aspartate amino transaminase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) enzyme levels in Ccl₄-intoxicated rats. (n=6 rats)

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
AST (U/L) *	65.6±1.8 ^e	130.6 ± 2.1 ^a	124.6 ± 2.3 ^b	118.3 ± 2.4 ^c
ALT (U/L) *	36.5 ± 1.6 ^e	70.5 ± 2.4 ^a	65.5 ± 2.8 ^b	46.7 ± 2.2 ^c
ALP (U/L) *	84.5 ± 1.9 ^e	159.4 ± 2.7 ^a	154.7 ± 2.5 ^b	146.3 ± 2.8 ^c

The values represent arithmetic means and the standard error of the mean. Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at p = 0.001, whereas those with the same letters are non-significant.

5.5. Impact on liver function

Table (5) represents the effect of feeding various levels of LMO on total protein and total bilirubin in both normal and liver-diseased rats after 4 weeks of feeding. Total protein in normal rats' groups was (6.68 ± 1.3^a) mg/dl. At the same time, liver disorder rats' groups at various levels (positive control, 5%, and 10% LMO) showed total protein values (4.65 ± 1.6, 6.54 ± 1.8, and 6.55 ± 1.2) mg/dl, respectively.

Total bilirubin values in normal rate group values were (0.66 ± 0.01) mmol/L. While in liver disorder rats' groups fed a diet with different levels LMO were 0.99 ± 0.012, 0.83 ± 0.013, and 0.80 ± 0.012 mg/dl, respectively at various levels LMO (5 %, and 10 %, positive control). These findings agree with that of (Ravishah et al., 2012). By enhancing antioxidant defense potential, hydroethanolic extracts of *Origanum vulgare*, *Mentha pepita*, and *Pimpinella anisum* were demonstrated to synergistically defend the liver and kidney tissues against toxicity caused by combination anti-TB treatment. Patients receiving anti-tuberculosis drugs may utilize these extracts as a dietary supplement in polyherbal preparations. More research in other animal models with varying doses will be required to clarify the particular processes behind the benefits of this polyherbal mixture.

Table (5): Effects of different concentrations of LMO on total protein and total bilirubin levels in CCl₄-intoxicated rats' serum.

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
Total protein (mg/dl)	6.68 ± 1.3 ^a	4.65 ± 1.6 ^b	6.54 ± 1.8 ^a	6.55 ± 1.2 ^a
Total bilirubin (mg/dl)	0.66 ± 0.01 ^b	0.99 ± 0.012 ^a	0.83 ± 0.013 ^b	0.80 ± 0.012 ^b

The values represent arithmetic means and the standard error of the mean. Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at p = 0.001, whereas those with the same letters are non-significant.

5.6. Impact on total cholesterol and triglycerides

After four weeks of feeding, the effect of varying levels of LMO on T-Cholesterol and Triglycerides in both normal and liver-diseased rats is shown in Table 6. T-Cholesterol in normal rat groups was 88.98 ± 1.4 mg/dl. In contrast, liver disorder rats groups at different levels of LMO (positive control, 5%, and 10% turmeric) showed T-Cholesterol values 88.98 ± 1.4, 105.95 ± 1.6, and 101.97 ± 1.8 mg/dl, respectively. Triglycerides values in normal rat groups were 43.35 ± 1.5 mmol/L. While in liver disorder rats groups at different levels of LMO (positive control, 5%, and 10% LMO) were 56.60 ± 1.9, 52.60 ± 1.4, and 49.50 ± 1.2 mg/dl, respectively.

Table (6): Impact of various LMO concentrations on triglyceride and total cholesterol levels in the serum of CCl₄-intoxicated rats.

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
Total cholesterol (mg/dl)	88.98 ± 1.4 ^d	105.95 ± 1.6 ^a	101.97 ± 1.8 ^b	98.90 ± 1.2 ^c
Triglycerides(mg/dl)	43.35 ± 1.5 ^d	56.60 ± 1.9 ^a	52.60 ± 1.4 ^b	49.50 ± 1.2 ^c

(The abbreviation (U/L) * stands for the unit per liter. The values represent arithmetic means and standard error (±). Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at p = 0.001, whereas those with the same letters are non-significant.

After four weeks of feeding, the impact of various concentrations of LMO on HDL, LDL, and VLDL in normal and liver-diseased rats is shown in Table 7. HDL values in normal rat groups were 75.99±1.2 mmol/L. While in liver disorder rats, groups fed a diet with different levels, LMO was 63.96±1.1, 74.75±1.3, and 72.10±1.3 mg/dl at LMO (5%, and 10% positive control) mg/dl, respectively. LDL values in the normal rat group were 16.35±1.2 mmol/L. While in liver

disorder rats groups at different levels of LMO (5%, and 10%, positive control) were 18.64 ± 1.4 , 16.70 ± 1.3 , and 16.90 ± 1.3 mg/dl respectively. VLDL values in normal rat groups were 8.67 ± 1.1 mmol/L. While in liver disorder rats, groups with oral at different levels of LMO were 11.32 ± 1.6 , 10.52 ± 1.8 , and 9.90 ± 1.1 mg/dl at levels (5% and 10% positive control) mg/dl, respectively. The results revealed a large number of significant variances. ($p < 0.05$) between all groups when compared with the control negative. The findings of this investigation corroborated previous findings.

The effects of administering hypercholesterolemic male rats the natural herbs LMO and ginger, or a combination of the two, on their cholesterol levels were explored by (Amarowicz et al., 2009). The experiment included 70 male albino rats divided into two groups, the first of which consisted of ten rats (control group). In comparison, the second main group, 60 rats, have been diseased with cholesterol through nutrition on a high-cholesterol diet, then divided into six equal subgroups and fed for six weeks, with one serving as a positive control group (Group 1), while groups (2, 3) were fed adding LMO at rates of 5% and 10%, respectively, and groups (4, 5) were fed adding ginger at rates of 5% and 10%, respectively (5%, 10%, respectively). Group 6 was fed with a 10% LMO and ginger mixture added (1:1 w/w). The effects of each herb on blood fats (triglycerides, cholesterol, high and low-density lipoprotein-cholesterol (HDL-c and HDL-c), as well as liver enzymes, were studied (AST and ALT).

Table (7): Effects of different concentrations of LMO on serum lipoprotein fractions (HDLc, LDLc, and VLDL) in Ccl4-intoxicated rats.

Parameters	Control (-)	Control (+)	LMO treatment	
			5%	10%
HDLc. (mg/dl)	75.99 ± 1.2^a	63.96 ± 1.1^c	74.75 ± 1.3^b	72.10 ± 1.3^c
LDLc. (mg/dl)	16.35 ± 1.2^c	18.64 ± 1.4^a	16.70 ± 1.3^c	16.90 ± 1.3^c
VLDLc. (mg/dl)	8.67 ± 1.1^d	11.32 ± 1.6^a	10.52 ± 1.8^b	9.90 ± 1.1^c

The values represent arithmetic means and the standard error of the mean. Using a one-way ANOVA test means that distinct letters (a, b, c, d) in the same column differ substantially at $p = 0.001$, whereas those with the same letters are non-significant (n=6 rats).

CONCLUSIONS AND RECOMMENDATIONS

The findings demonstrate that Marjoram (LMO) has a good effect in improving the enzymatic liver in hepatic rats, with the improvement rate increasing in the 10% (LMO) group because it was discovered to have a synergistic effect in protecting the liver tissues against toxicity by

enhancing antioxidant defense capability. Patients so may utilize these extracts as a dietary supplement in polyherbal preparations. It could be recommended that:

- Marjoram powder is recommended for hepatic patients.
- The various amount of LMO powder, particularly that of 5% is useful for hepatic patient
- Various doses of LMO powder may be recommended for decreasing LDL and atherogenic index readings.

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