

Comparative Anti-hyperlipidemic Effect of Marjoram and Lettuce oils

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Abstract :

Essential oils may counteract and retard the process of lipid oxidation, and elevate antioxidant activity. The purpose of this study was to compare the therapeutic effect of lettuce and marjoram oil in hyperlipidemic rats. Sixty male albino rats were divided into two main groups, the first main group was normal rats and divided into; negative control group (NCG), Marjoram oil group (MOG) and Lettuce oil group (LOG). The second was hyperlipidemic rats that divided into, positive control group (PCG), LipoMarjoram oil group (LipoMOG), LipoLettuce oil group (LipoLOG). Control groups fed on basal diet while in hyperlipidemic rats corn oil replaced with marjoram oil or lettuce oil at the level 10% of basal diet. Serum total cholesterol, triglycerides, total lipids, HDL, VLDLc and LDL-cholesterol were determined. The results of normal groups (LipoMOG & LipoLOG) indicated that, TC and LDL diminished significantly at ($P < 0.001$). Non-significant differences indicated between marjoram and lettuce oil for T.lipids and TG., regarding to the therapeutic effect, LipoLOG revealed the amelioration effect at ($P < 0.01$) by lower TC/LDLc ratio. AI and LDLc/HDLc ratio was improved significantly in both treated and normal rats groups. In conclusion, lettuce oil has an excellent role in reducing deterioration effect of TC and LDLc, while marjoram also may have desirable effects on AI and LDLc/HDLc. Non-significant difference indicated between both oils for T. lipids, TG, VLDL & LDL profiles in both status groups while lettuce oil had the greatest significant therapeutic effect on lipoprotein profiles ratio of hyperlipidemic group.

Key words: Essential oil – lipid profiles - lipoproteins – atherogenic index - rats.

Introduction :

Currently, there is global interest in finding new and safe antioxidants from natural sources, to prevent oxidative deterioration of foods and to minimize oxidative injure of living cells (**Aazzaet al., 2011**). Aromatic plants contain volatile aroma compounds from essential oils like the oregano oil can be considered an effective natural antioxidant (**Arcila-Lozano et al., 2004**). Oregano essential oils obtained from the genera *Origanum* was considered to be nontoxic (**El Babiliet al., 2011**), and was rich in carvacrol, a monoterpenic phenol isomeric with thymol. As, the major components are as follows: Gamma-terpinene, *p*-cymene, alpha-terpinene and alpha-pinene and b - caryophyllene (**Beenaet al., 2013**). Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a predominant monoterpenic phenol occurring in many essential oils of the family Labiatae including, *Origanum* (**Aristatileet al., 2009**). Carvacrol is responsible for the biological activities of oregano (**Baser, 2008**). Feed supplementation with thymol plus carvacrol enhanced performance, increased antioxidant enzyme activities, retarded lipid oxidation, and improved immune response (**Hashemipouret al., 2013**). Therefore, **Alma et al., (2003)** suggested that this essential oil can serve as a good antioxidant. Also, **Aeschbachet al., (1994)** suggested that thymol and carvacrol possess useful antioxidant properties and may become important in the search for 'natural' replacements for 'synthetic' antioxidant food additives.

Also, lettuce is a vegetable plant belonging to Asteraceae family. Lettuce seed oils are characterized by high contents of linoleic and oleic acids. The whole sterol profiles include β -sitosterol (as major component) followed by 7-stigmasterol, campesterol and 5-stigmasterol. It was found that oil has the highest tocopherol content and α -tocopherol is the only constituent in the lettuce seeds oil (**El-Mallah and El-Shami, 2012**). However, some other minor chemical constituents were also isolated and identified from the essential oil of lettuce including β -pinene, α -terpinolene, linalool, 4-terpineol, α -terpineol, o-methylthymol, L-alloaromadendrene and viridiflorene (**Al Nomaaniet al., 2013**). Therefore, the essential oils of lettuce are active candidates to be used as antioxidant. Essential oils may counteract and retard the process of oxidative damage, lipid oxidation and elevate antioxidant activity (**Anthony et al., 2012**).Accordingly, this work was conducted to

evaluate the protective and therapeutic effect on lipids profiles considering lettuce and marjoram oils.

Materials and Methods:

Materials:

Sixty adult male albino rats, Sprague Dawley strain, with an average weight ranged between (120 ± 5 g) were obtained from Research Institute of Ophthalmology, Giza, Egypt. Seeds of lettuce and marjoram herb were purchased fresh from Ministry of Agriculture, Cairo, Egypt. Then the oils were extracted in the labs of National Nutrition Institute, Ministry of Health, Cairo, Egypt according to the method given by **Schefferet al., (1977)**. Cholesterol (white crystalline powder), bile salt, casein, vitamins, minerals and cellulose were obtained from El-Gomharyafar Pharm. and Chem. Ind. Comp., Cairo, Egypt. Spectrum peak pick, induced by infra red (IR) and ultra violet (UV) was analyzed in Micro Analytical Center of AlbsateinResearchCenter, Cairo.

Methods:

1. Induction of hyperlipidemia:

The induction of hyperlipidemia for rats occurred by feeding on hyperlipidemic diet which was done by formulated basal diet with 1% cholesterol, 2% sheep fat and 0.5% cholic acid to enhance the enteral absorption of lipids (**Pan et al., 2016**).

2. Experimental design :

Rats were housed in wire cages under the normal laboratory condition and fed on basal diet for a week as adaptation period. Body weight for each rat recorded at the beginning of the experiment and once a week for 4 weeks, and at the end of experimental period each rat weight recorded. Rats were randomly divided into two main groups as follow: The first main group based on normal rats that divided into three groups (each of 10 rats); negative control group (NCG): Rats fed on basal diet only consisted of casein (12%), corn oil (10%), vitamin mixtures (1%), salt mixture (4%), fiber (cellulose) (5%), starch (67.5%), choline chloride (0.2%) and L.methionine (0.3%) (**Reeves et al., 1993**). Marjoram or lettuce oils(groupMOG and group LOG) replaced corn oil in diets. Second main group of infected rats with hyperlipidemia was divided into three groups (each of 10 rats), positive control group (PCG): Hyperlipidemic rats fed on lipidimic diet only along the experimental period. LipoMarjoram oil group (LipoMOG) and LipoLettuce group

(LipoLoG) prepared by replacing corn oil with marjoram oil or lettuce oil. All diets prepared at equal nutritional value diet.

3. Biological evaluation:

At the end of experiment, rats fasted overnight and blood samples were collected from the aortic vein. Body weight was recorded weekly throughout the feeding period which lasted after 4 weeks. The body weight gain (BWG%), feed efficiency ratio (FER) were determined according to **Chapman *et al.*, (1959)** using the following equations.

$$\text{BWG (g)} = \frac{\text{Final weight} - \text{Initial weight}}{28}$$

$$\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Feed Intake (g)}}$$

Feed intake (FI) was also calculated daily.

4. Biochemical analysis :

Serum total cholesterol was determined according to the enzymatic method described by **Allain (1974)**. Enzymatic calorimetric determination of triglycerides was carried out according to **Fossati and Prencipe, (1982)**. Determination of total lipids in serum was colorimetrically determined according to **Schmitt and Drevon (1964)**. HDL-cholesterol was determined in serum according to **Lopez, (1977)**. VLDLc and LDL-cholesterol concentrate were estimated according to (**Friedewald *et al.*, 1972**) as follow: VLDL-c (mg/dl) = Triglycerides/5, then LDL-c (mg/dl) = Total cholesterol - (HDL-c + VLDL-c). Atherogenic index was calculated as TC / HDLc ratio according to (**Olatunji and Soladoye, 2007**).

$$\text{Atherogenic Index} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

5. Histopathological Examination:

Samples of liver were taken and fixed in 10% neutral buffered formalin for 24 hours. Paraffin sections 6 μm thick were prepared and stained with Hematoxylin and Eosin (H & E) for the examination by light microscopy (**Shih *et al.*, 2015**). The histopathology was carried out in histology lab at Faculty of Veterinary Medicine, Cairo University, Egypt.

6. Statistical Analysis :

All data was analyzed by using Computer Program Statistical Package for Social Sciences (SPSS) version 10, 1998. Results are

reported as mean \pm SD. A significant difference was statistical analyzed by independent sample t-test between normal and Hyperlipidemic rats.

Results:

Results of spectrum peak picking by infra red division and instrument properties by ultra violet for both lettuce and marjoram oils were shown in figure (1&2) which reflected the fatty acid contents of the both tested oils.

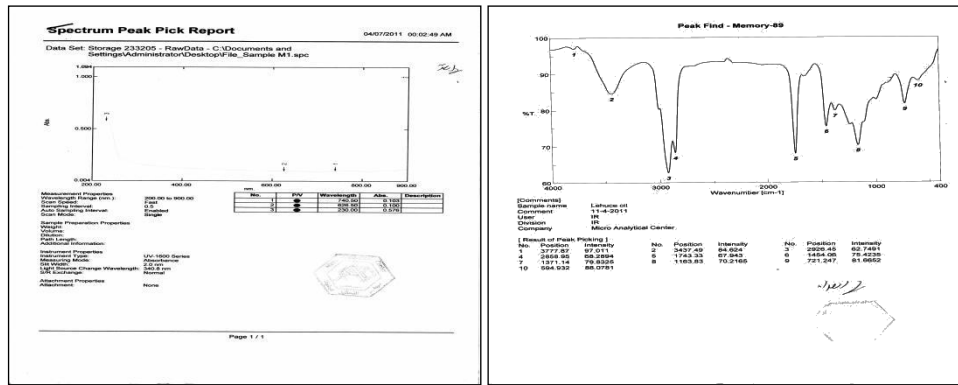


Figure (1): Spectrum peak pick of lettuce oil (IF and UV)

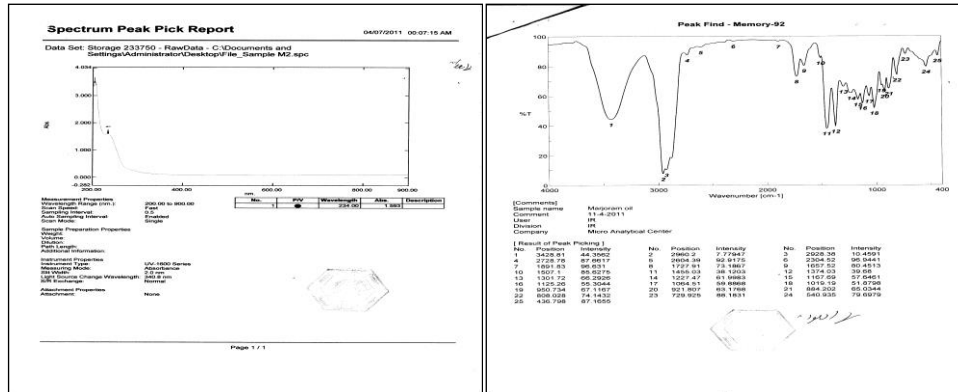


Figure (2): Spectrum peak pick of marjoram oil (IF and UV)

1. Effect of marjoram and lettuce oil on BWG%, FI and FER% in hyperlipidemic rats:

Effect of marjoram and lettuce oil on BWG, FI and FER in hyperlipidemic rats are recorded in table (1).

Table (1): Effect of marjoram and lettuce oil on BWG, FI and FER of hyperlipidemic rats

	Normal rats			T. value	Hyperlipidemic rats			T. value
	NCG	MOG	LOG		PCG	MOG	LOG	
BWG g/day	2.76± 0.74	2.74± 0.34	3.09± 0.85	-0.80 NS	10.78± 1.29	5.61± 0.96	5.52± 3.71	0.05 NS
% change		-0.60	12.08			-47.95	-48.80	
FI (g/day)	13.00± 1.41	14.20± 2.28	13.60± 0.89	0.68 NS	14.40± 0.89	9.00± 1.22	9.40± 1.52	-0.59 NS
% change		9.23	4.62			-37.5	-34.72	
FER ratio	0.212± 0.06	0.193± 0.07	0.227± 0.08	-0.46 NS	0.75± 0.15	0.623± 0.17	0.587± 0.13	1.61 NS
% change		19.23	30.77			7.96	0.88	

* P<0.05, Values are expressed as Mean ±SD (n=10).

*Sig.: Significant difference was calculated by independent sample t-test between Normal rats and Hyperlipidemic rats for each oil.

*% change: Calculated compared to the plain basal diet.

As shown in table (1), the mean value of BWG, FI (g/day) and FER ratio for marjoram and lettuce oil did not differ significantly among normal or hyperlipidemic groups as compared to control groups. As expected BWG was high for rats that fed on the hyperlipidemic diets; accordingly calculated FER was higher, but FI was not always higher for hyperlipidemic group.

2. Effect of marjoram and lettuce oil on serum T. lipids, TG & TC for normal & hyperlipidemic rats:

Table (2) show the comparative effects of marjoram and lettuce oil supplements for normal and hyperlipidemic rats.

Table (2): Effect of marjoram and lettuce oil on serum lipid fractions in normal & hyperlipidemic rats

Parameters	Normal groups			T. value	Hyperlipidemic rats			T. value
	NCG	MOG	LOG		PCG	MOG	LOG	
T. Lipids	2.29± 0.08	1.94± 0.23	1.52± 0.23	2.676NS	3.60± 0.45	2.75± 0.31	2.55± 0.24	1.058 NS
% change		-15.28	-33.62			-23.61	-29.17	
TG	59.91± 5.08	46.73± 4.30	45.73± 3.17	0.602NS	77.89± 4.87	63.51± 4.21	62.77± 6.36	0.218 NS
% change		-21.10	-23.67			-18.46	-19.41	
TC	84.04± 2.37	77.31± 1.82	70.71± 1.75	12.73 ***	104.14± 8.64	86.56± 5.11	83.03± 5.56	2.361 NS
% change		-8.01	-15.86			-16.88	-20.27	

* P<0.05, Values are expressed as Mean ±SD (n=10).

*Sig.: Significant difference was calculated by independent sample t-test between Normal rats and Hyperlipidemic rats for each oil.

*% change: Calculated compared to the plain basal diet.

It could be cleared from the table, the levels of T.lipids, TG and TC (mg/dl) in serum of normal and hyperlipidemic rats. For normal rats T.lipids and TG showed non-significant differences among marjoram and lettuce groups. Otherwise, TC was improved significantly in normal rats serum at $P < 0.001$ in lettuce group than marjoram group which had higher %change (by -15.86 vs. -8.01), respectively. Regarding to the therapeutic effect for hyperlipidemic rats considering total lipids, triglycerides and TC there were non-significant differences between marjoram and lettuce oils.

3. Effect of marjoram and lettuce oil on serum lipoproteins in normal and hyperlipidemic rats:

The comparative therapeutic effect among marjoram and lettuce oil supplements as fed to normal hyperlipidemic rats is listed in table (3).

Table (3): Effect of marjoram and lettuce oil on serum lipoproteins in normal and hyperlipidemic rats

Parameters	Normal groups			T. value	Hyperlipidemic rats			T. value
	NCG	MOG	LOG		PCG	MOG	LOG	
HDLc	47.83± 1.83	51.59± 1.56	52.62± 1.88	-1.067NS	36.42± 3.36	43.29± 3.23	52.40± 6.39	-2.387NS
% change		7.86	10.01			18.86	43.88	
LDLc	24.23± 3.65	16.38± 2.28	8.95± 1.52	10.52***	53.20± 10.10	30.57± 4.79	29.95± 3.96	0.193NS
% change		-32.40	-63.06			-42.54	-43.70	
VLDLc	11.98± 1.02	9.35± 0.86	9.15± 0.63	0.602NS	15.58± 0.97	12.70± 0.84	12.55± 1.27	0.218NS
% change		-21.95	-23.62			-18.49	-19.45	

* $P < 0.05$, Values are expressed as Mean \pm SD (n=10).

*Sig.: Significant difference was calculated by independent sample t-test between Normal rats and Hyperlipidemic rats for each oil.

*% change: Calculated compared to the plain basal diet.

It could be observed from the table that lettuce oil showed the best improvement considering elevation of HDLc level by 10.01% and 43.88% for LOG and LipoLOG, respectively, provided that there was non-significant difference between them. On the other hand, significant differences was mentioned ($P < 0.001$) in serum LDLc between both oils for normal groups, but with non significant differences for treated groups.

Serum VLDLc did not differ significantly either in normal or in treated groups.

4. Effect of marjoram and lettuce oil on AI, TC/LDLc and LDLc/HDLc as calculated for normal & hyperlipidemic rats:

Table (4) show the comparative effect among marjoram and lettuce oil for normal and hyperlipidemic rats considering AI, TC/LDLc and LDLc/HDLc.

Table (4): Effect of marjoram and lettuce oil on AI, TC/LDLc and LDLc/HDLc of normal and hyperlipidemic rats

Parameters	Normal groups			T. value	Hyperlipidemic rats			T. value
	NCG	MOG	LOG		PCG	MOG	LOG	
AI	1.76±	1.50±	1.34±	6.162 **	2.86±	2.00±	1.59±	3.17*
	0.08	0.05	0.10		0.36	0.17	0.24	
% change		-14.77	-23.86			-30.07	-44.41	
TC/LDLc	3.46±	4.72±	7.90±	-5.859 **	1.96±	2.83±	2.77±	-1.57**
	0.51	0.60	1.56		0.29	0.33	0.77	
% change		36.42	128.32			44.39	41.33	
LDLc/HDLc	0.51±	0.32±	0.17±	8.424 **	1.46±	0.71±	0.57±	2.904*
	0.09	0.05	0.10		0.36	0.15	0.13	
% change		-37.25	-66.67			-51.37	-60.96	

* P<0.05, Values are expressed as Mean ±SD (n=10).

*Sig.: Significant difference was calculated by independent sample t-test between Normal rats and Hyperlipidemic rats for each oil.

*% change: Calculated compared to the plain basal diet.

The data illustrated in the table showed that lipoprotein profiles ratios were improved significantly in both normal and hyperlipidemic groups that supplemented with different oils of marjoram or lettuce. For normal groups, there was significant (P<0.01) difference between supplemented oils in different ratios by higher percent in LOG by -23.86, 128.32 and -66.25% for AI, TC/LDLc and LDLc/HDLc, respectively. On the same line, the significant improvement at (P<0.05) was detected in treated groups. Finally, in comparison with different

supplemented oils, it could be assumed that marjoram oil had improved significance ($P < 0.01$) the lipids profile in normal groups in particular AI and LDLc/HDLc; while lettuce oil had a greatest therapeutic effect in hyperlipidemic groups.

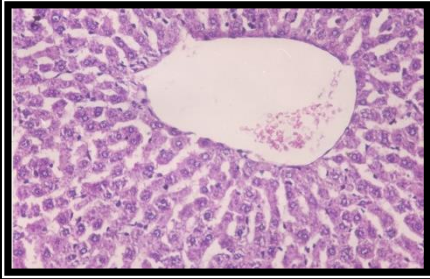
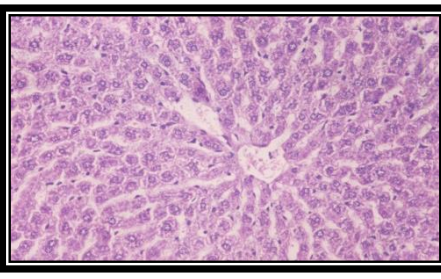
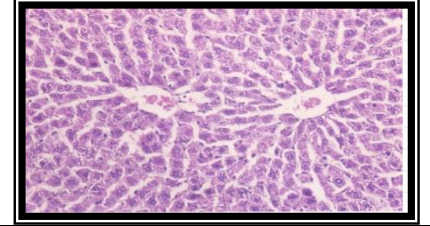
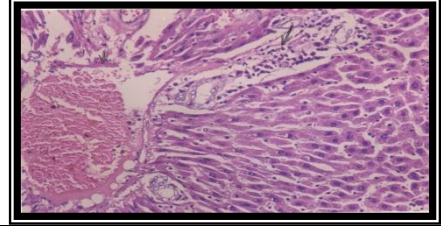
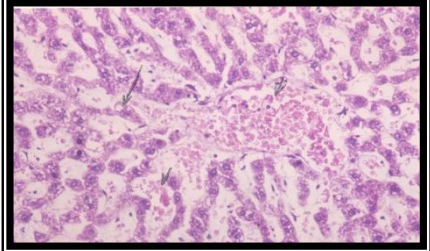
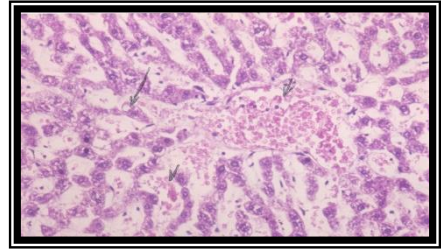
5. Effect of marjoram and lettuce oil on histopathological changes in liver of hyperlipidemic rats:

Histopathological changes associated with lettuce and marjoram oils were presented in table (5). For NCG, the examination revealed normal hepatic tissues (Photo 1). For MOG and LOG groups, the examination revealed no changes in hepatic tissue (Photo 2&3). As for positive group the histopathological examination revealed mild degenerative changes of hepatic cells as congestion of hepatoportal blood vessel and portal infiltration with mononuclear cells (Photo 4). For LipoMOG and LipoLOG groups it revealed moderate degenerative changes of hepatocytes in liver and activation of kaupffer cells (Photo 5&6).

Table (5): Histopathological results for control and studied groups

Organ	Histopathology	Studied groups					
		Normal groups			Hyperlipidemic groups		
		NCG	MOG	LOG	PCG	Lipo-MOG	Lipo-LOG
Liver	Fatty degeneration	N	N	N	++	+	+
	Congestion	N	N	N	++	+	+
	Inflammatory cell	N	N	N	++	+	+
	dilated of blood vessels	N	N	N	++	+	+

Normal= N, Mild = +, Moderate= ++, and Severe = +++

	
<p>Photo (1): Liver of NCG showing normal histology of hepatic lobule (H and E X 200)</p>	<p>Photo(2): Liver of MOG showing apparent normal hepatocytes (H and E X 200).</p>
	
<p>Photo(3):Liver of LOG showing apparent normal hepatocytes (H and E X 200).</p>	<p>Photo(4):Liver of PCG showing congestion of hepatoportal blood vessel and portal infiltration with mononuclear cells (H and E X 200).</p>
	
<p>Photo(5): Liver of Lipo-MCG showing no dilatation and congestion of central vein and hepatic sinusoids as well as vacuolation of hepatocytes (H and E X 200).</p>	<p>Photo(6): Liver of Lipo-LOG showing no dilatation and congestion of central vein and hepatic sinusoids as well as vacuolation of hepatocytes (H and E X 200).</p>

Discussion

No significant differences in body weight changes was observed, in agreement with the studies of **Bayramogluet al., (2013)**. While, there was higher significant ($P < 0.01$) difference in LipoMOG than MOG in BWG% and FER%, as a study of **Bukovskaet al., (2007)** who found that administration of the medium dose of oregano oils significantly accelerated the body weight gain recovery. Also **Bampidiset al., (2005)** concluded that dietary oregano may be used as a natural herbal growth promoter as improved FER.

Importantly, dietary consumption of phytosterols and certain monounsaturated fatty acids, including the oleic acid, as in lettuce oil, has been shown to reduce cholesterol absorption and plasma cholesterol concentrations (**Park and Carr, 2013**). Moreover, **Silbernagelet al., (2013)** supported the recommendations for the use of phytosterols as LDL cholesterol-lowering agents. Phytosterols (PS) supplementation is increasingly accepted as a dietary strategy to lower plasma cholesterol concentrations. **Lottenberget al., (2012)** supported the hypothesis that polyunsaturated fatty acids and phytosterols can act as signaling molecules and alter the expression of genes involved in cholesterol transport and metabolism. Phytosterols (PS), such as campesterol and b-sitosterol, are naturally derived plant components with a similar chemical structure to cholesterol. Compared with cholesterol, campesterol carries an extra methyl group and b-sitosterol an extra ethyl group at the C-24 position. **De Smetet al., (2012)** suggested that plant sterols/stanols compete with intestinal cholesterol for incorporation into mixed micelles as well as into chylomicrons. Next, the focus shifted toward cellular processes. All these processes ultimately lowered intestinal cholesterol absorption. In other study of **Wolańska and Kłosiewicz-Latoszek, (2012)** it was evidenced that lettuce consumption increases the total cholesterol end-products excretion and improves antioxidant status due to the richness in antioxidants (vitamins C, E and carotenoids). **Nicolle et al., (2004)** concluded that lettuce showed a beneficial effect on lipid metabolism on tissue oxidation. Therefore, regular consumption of lettuce should contribute to protection against cardiovascular diseases. **Myriiet al., (2012)** proved that phytosterols like stanols/sterols can be considered to be effective and safe

cholesterol-lowering functional food ingredients, and are recommended as dietary modifiers of serum lipids (**Talati et al., 2010**).

On the other side, the essential marjoram oil showed a higher antioxidant activity (**Handlet et al., 2008**). Moreover, **Terenina et al., (2011)** concluded that carvacrol and thymol are the main antioxidant components of oregano oil. Total phenolic contents were found to be rosmarinic acid and acetin that have free radical scavenging activities and reducing/antioxidant capacities (**Ozkan et al., 2010**). Carvacrol is responsible for the main biological activities of oregano (**Baser, 2008**), cholesterol-reducer, antioxidant effects (**Akkolet et al., (2009)**). Finally, our results are in accordance with the results of **Aristatilet et al., (2009)** who suggested that carvacrol affords a significant hypolipidemic effect in rats.

Conclusion :

Lettuce oil had a deterioration reduction effect on TC and LDLc. Moreover, marjoram also had desirable effects on AI and LDLc/HDLc in normal rats. Non-significant differences indicated between both oils for lipid profiles in hyperlipidemic status provided that lettuce oil had a more therapeutic effect on lipoprotein profiles ratio.

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التأثير الخافض لدهون الدم المقارن بين زيت البردقوش و زيت الخس

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الملخص العربي :

قد تقاوم الزيوت العطرية أو تؤخر عملية أكسدة الدهون ورفع النشاط المضاد للأكسدة. لذلك تهدف الدراسة لمقارنة التأثير العلاجي الخافض لدهون الدم لكلا من زيت الخس وزيت البردقوش في الفئران المصابة بارتفاع دهون الدم. تم تقسيم 60 فأر ذكور بيضاء سلالة سبراجو داوولي إلى مجموعتين رئيسيتين، المجموعة الرئيسية الأولى و هي الفئران الطبيعية وتنقسم إلى مجموعة ضابطة سلبية، مجموعة ضابطة لزيت البردقوش، ومجموعة ضابطة لزيت الخس. والمجموعة الثانية و هي الفئران المصابة بارتفاع دهون الدم و تنقسم إلى، مجموعة ضابطة موجبة، و مجموعة زيت البردقوش المصابة، و مجموعة زيت الخس المصابة. تغذت المجموعات الضابطة على الغذاء الأساسي فقط بينما المجموعات المعالجة مع استبدال زيت الذرة بزيت البردقوش أو زيت الخس بنسبة 10٪ من الغذاء الأساسي وقد تم قياس الكوليسترول الكلي والدهون الثلاثية والليبيدات الكلية والليوبروتينات السيرم. أظهرت نتائج المجموعات السليمة إنخفاض مؤشر الكوليسترول الكلي والليوبروتينات المنخفضة الكثافة بشكل كبير ($P < 0.001$) بمجموعة الخس بالمقارنة بمجموعة البردقوش. بينما لم تتواجد معنوية بين مجموعات زيت البردقوش وزيت الخس بمعدل الدهون الكلية و الجليسيريدات الثلاثية. و فيما يخص التأثير العلاجي، أظهرت مجموعة زيت الخس المصابة تأثيراً مخفضاً لمعدل الليوبروتينات منخفضة الكثافة وكذلك الكوليسترول الكلي. بينما تحسن بدرجة معنوية كلا من مؤشر الأكسدة (AI) ومعدل الليوبروتينات المنخفضة الكثافة الى الليوبروتينات المرتفعة الكثافة في كلا من المجموعات المعالجة والسليمة. تتلخص النتائج في أن زيت الخس له تأثير ممتاز خافض لمعدل الكوليسترول الكلي والليوبروتينات المنخفضة الكثافة، بينما زيت البردقوش كان له أيضا تأثير مخفض في الفئران السليمة لمعدل أكسدة الدهون (مؤشر AI) و معدل الليوبروتينات المنخفضة الكثافة الى الليوبروتينات المرتفعة الكثافة. كما لم تتواجد فروق معنوية بين كل من زيت الخس والبردقوش في التأثير العلاجي لدهون الدم الكلية والجليسيريدات الثلاثية والليوبروتينات منخفضة الكثافة والليوبروتينات مرتفعة الكثافة للمجموعات السليمة، بينما مجموعة زيت الخس كانت ذات التأثير العلاجي الأفضل لمعدل الليوبروتينات الدم في المجموعات المصابة.

الكلمات المفتاحية: الزيوت العطرية – مؤشرات دهون الدم – الليوبروتينات – مؤشر أكسدة الدهون – الفئران.