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SAFETY EVALUATION OF *ORIGANUM MAJORANA* VOLATILE OIL

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ABSTRACT

Safety evaluation of *Origanum majorana* volatile oil was investigated. Acute toxicity studies median lethal doses (LD_{50} values) were carried out in mice and rats. Single doses of the tested drug were given orally and clinical signs, symptoms, and mortality were recorded during a 14-day observation period. Chronic toxicity, reproductive and mutagenic activity studies were evaluated in rats and mice. Repeated oral dosing of up to 40 mg/kg was ineffective in rats and mice. Oral teratology study on pregnant rats using higher doses revealed no evidence of the teratogenicity potential of the Origanum *majorana* oil. The tested preparation was also devoid of mutagenic activity in mice at all doses. The results collectively confirmed that the oil under test has a wide safety margin with no evidence of impaired fertility, teratogenicity potential, or mutagenic activity. The results showed that volatile oil derived from *Origanum majorana* is well tolerated with oral LD_{50} of 1.203 g/kg in mice and 1.666 g/kg in rats. A significant decrease in blood

glucose, cholesterol, triglycerides, and total lipid levels was observed after two and three months of drug administration.

KEYWORDS:

Origanum majorana, Volatile oil and Safety profile

INTRODUCTION

Origanum majorana is a member of the mint family Lamiaceae. The products identified as marjoram are the dried leaves and flowering tops of Origanum majorana found worldwide. They obtain phenolic terpenoids (thymol and carvacrol), flavonoids (diosmin, luteolin and apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, thymonin), triacontan, sitosterol, acids (oleanolic acid) and cis sabinene (Maheshwari, 2016). Extracts of Origanum majorana have been reported to decrease the response to acetyl choline, histamine, serotonin and nicotine (Sharma et al, 2016). Origanum majorana leaves were found to strongly inhibit rat intestinal glucosidase (Xiao et al, 2013). The antiviral, bactericidal, antiseptic and antifungal effects of marjoram are attributed to ursolic acid and essential oil and in particular to thymol and carvacrol (Mahezan et al, 2019). The antioxidant and antitumor activities of marjoram have been determined (Farzaneh et al, 2015) and recently, Origanum majorana extracts and its essential oil exerted a potent hypolipidemic effect and were effective in protecting liver, kidney and bone marrow chromosomes from lead intoxication (Aprotosoaie et al,2019). This study was designed to explore short and long-term safety profile of the Origanum majorana volatile oil in rats and mice.

EXPERIMENTAL DETAILS

2.1. Materials and Procedures

Preparation of the aqueous emulsion: Origanum majorana volatile oil was used in this study as a 10 % emulsion, using Cremophor -EL as emulsifier.

1- **Acute Toxicity Studies:**

Using mice and rats, single doses of the Origanum majorana volatile oil and the vehicle at the same volumes were given orally by stomach tube to adult albino Swiss mice or albino rats. Clinical signs, symptoms and mortality were recorded during a 14-day observation period. LD_{50} values were calculated according to Finney's method (Pandey et al, 2006). Animal care in this study followed the regulations of Helsinki declaration and WHA.

2- ***Sub chronic and Chronic Toxicity Studies:***

Three groups, each of 10 mature male albino rats (150-200 g) were used. Origanum majorana volatile oil was given orally by stomach tube daily for a period of 3 months at two dose levels of 20 and 40 mg/kg b/ wt. Meanwhile, rats of the control group were given only Cremophore-EI (the vehicle). The rats were fed with standard feed and provided with water and libitum. All animals were examined daily for well-being. Food consumption in rats were recorded at weekly intervals. After 1, 2 and 3 months from Origanum majorana volatile oil administration, haematological (Vainchenker et al, 2013) and serum analysis (protein, albumin, globulin, and alanine

Aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates (ALP), urea, uric acid, creatinine, glucose, bilirubin, total lipids, cholesterol and triglycerides) were examined (using kits from Diamond Diagnostic, Egypt). Prior to animal sacrifice, a complete physical and ophthalmoscope examination was performed. At the end of experiments, animals were weighed and anesthetized with ether for blood collection. Gross, pathologic changes, weights of several organs and his to pathological finding (Verdijk et al, 2014) were recorded.

3- Reproduction Studies:

Segment 1: Study of Fertility:

A fertility study was carried out in 120 male and female albino rats. *Origanum majorana volatile* oil was given at doses of 20 and 40 mg/kg b/wt. to males and females, for respectively 60 and 14 days prior to mating. The dosed animals were then allowed each to mate with non-dosed counterparts. Dosed female rats were further treated throughout the gestation period. Control animals received the vehicle only. On day 20 of pregnancy, female rats were sacrificed and fetuses were delivered by caesarean section for further examination.

Segment II: Study of Embryo toxicity and Teratogenicity:

Three groups of 10 female rats, received the tested drug at doses of 0, 20 and 40 mg/kg b/wt. from day 7 to day 16 of pregnancy. Rats were sacrificed on day 21 of pregnancy. Foetuses were delivered by caesarean section and fatal skeleton and visceral organs were examined according to Bayad (El Ashmawy et al, 2016).

4- *Statistical Analysis:*

The data were analysed by ANOVA as described by Snedecor and Cochran (Armstrong et al, 2000) and mean values of various treatment were compared with control values. Results are present as mean ± S.E. and considered statistically significant if $P < 0.05$.

RESULTS

Acute Toxicity Studies: The acute oral LD5o in mice was 1.203 g/kg b/wt. In rats, it was 1.666 g/kg b/wt. The gross behaviour effects observed in the lethal dose range were hypotonic, ataxia and dyspnoea.

Sub chronic and Chronic Toxicity Studies: Daily administration of *Origanum majorana* volatile oil by gavage at doses of 20 or 40 mg/kg for 1, 2 and 3 months revealed no significant effect on body weights and histological structure of the different organs (unpublished data).

The results concluded that:

-***Origanum majorana*** volatile oil did not induce any significant changes neither in blood haematological parameters ***(Table I)*** nor in protein profile ***(Table II)*** after administration of the drug for 1, 2 or 3 months.

Table 1: haematological parameters of rats given Origanum majorana oil orally daily

For 3 months .Values are means ± S.E. N= 10 animals.

Origanum majorana volatile oil mg/Kg/day By oral gavage tube	**Parameters**			
	H b g /dL	**PCV %**	**RBC**	**WBC**

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Vehicle				
Pre-treatment ±0.7	13.6±0.2	34.2±1.0	6.20±0.3	8.1
1st month ±0.7	14.0±0.2	34.2±1.1	6.10±0.5	7.9
2nd month ±0.6	14.1±0.3	36.2±0.8	6.00±0.3	7.8
3rd month ±0.8	14.1±0.3	34.2±0.8	6.10±0.6	8.0
Origanum majorana Oil (20 mg/Kg)				
Pre-treatment ±0.3	13.6±0.2	33.2±1.0	6.30±0.5	7.8
1st month ±0.4	14.0±0.4	32.2±1.0	6.20±0.3	7.9
2nd month ±0.5	13.8±0.6	34.6±1.0	6.70±0.7	8.1
3rd month ±0.8	14.3±0.2	36.2±1.1	6.30±0.4	8.2
Origanum majorana Oil (40 mg/Kg)				
Pre-treatment ±0.7	14.2±0.2	34.7±1.0	6.20±0.3	7.81
1st month ±0.7	14.0±0.2	35.2±0.70	6.40±0.3	7.61
2nd month ±0.7	14.9±0.2	36.2±0.09	6.60±0.3	8.10
3rd month ±0.7	14.4±0.2	36.4±1.0	6.50±0.3	8.20

H b = Haemoglobin g/dl, PCV%= packed cell volume %,RBC=Red Blood cell (x 10^6 /C mm.), WBC= White blood cell (X10^3 / C mm)

Table II: Serum protein profile in rats given Origanum majorana oil Orally Daily for 3

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Months .Values are Means ± S.E., N= 10 Animals

Origanum majorana volatile oil mg/Kg/day By oral gavage tube	**parameters**		
	protein g /dL	**Albumin g /dL**	**Globulin g /d L**
Vehicle			
Pre-treatment ±0.1	6.2±1.0	4.20±0.3	2.1
1st month ±0.5	5.9±1.1	3.90±0.5	1.9
2nd month ±0.4	6.2±0.8	4.10±0.3	2.0
3rd month ±0.6	6.0±0.5	4.28±0.6	1.8
Origanum majorana Oil (20 mg/Kg)			
Pre-treatment ±0.3	6.8±0.3	4.20±0.2	1.7
1st month ±0.1	6.1±0.2	4.30±0.1	1.9
2nd month ±0.4	6.2±0.5	4.40±0.3	1.8
3rd month ±0.2	6.0±0.4	3.90±0.4	1.8
Origanum majorana Oil (40 mg/Kg)			
Pre-treatment	6.2±1.0	4.30±0.3	1.9 ±0.1
1st month	6.02±0.70	4.40±0.3	1.7 ±0.7

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2nd month	6..2±0.20	3.90±0.3	2. 3 ±0.7
3rd month	6.1±0.1	3.90±0.3	2. 2 ±0.7

Origanum majorana volatile oil administration for 1, 2 or 3 months did not induce any liver pathological, functional or metabolic changes as demonstrated by serum biochemical analysis (Table Ill}. Origanum majorana volatile oil administration did not cause any kidney excretory fun

Table III : Effect of Oral Administration of Origanum majorana oil on serum AST, ALT and ALP in Rats .Values are Means ± S.E., N= 10 Animals.

Origanum majorana volatile oil mg/Kg/day By oral gavage tube	**Parameters**		
	AST (U/L)	**ALT (U /L)**	**ALP (U /L)**
Vehicle			
Pre-treatment	48.2±3.0	17.1±1.3	103.4 ±3.01
1st month	53.0±3.1	18.1±1.5	110.3 ±4.05
2nd month	49.4±2.3	20.3±0.9	106.0 ±6.04
3rd month	51.2±2.5	18.2±0.6	98.5 ±8.06
Origanum majorana Oil (20 mg/Kg)			

Pre-treatment ±0.01	51.2±2.0	19.17±0.3	108.2
1st month ±0.01	48.2±2.3	16.17±0.3	98.6
2nd month ±0.01	46.2±2.7	18.17±0.3	1 00.1
3rd month ±0.01	47.9±2.8	16.17±0.3	98.41
Origanum majorana Oil (40 mg/Kg)			
Pre-treatment ±0.02	53.2±5.4	18.17±0.01	11 0.6
1st month ±0.04	49.2±3.6	16.18±0.03	105.5
2nd month ±0.06	46.2±5.6	16.99±0.02	108.4
3rd month ±0.05	48.2±6.1	16.80±0.03	95 .7

Table IV : Effect of Oral Administration of Origanum majorana oil on serum Urea, Uric acid and Creatinine in Rats .Values are Means ± S.E., N= 10 Animals.

Origanum majorana volatile oil mg/Kg/day By oral gavage tube	**parameter**		
	Urea (mg/dL)	**Uric acid (mg /dL)**	**Creatinine (mg /dL)**

Vehicle			
Pre-treatment	27.2±3.0	1.6±1.3	0.6 ±0.01
1st month	25.4±3.1	1.7±1.5	0.7 ±0.05
2nd month	2 5.0±2.3	1.6±0.9	0.6 ±0.04
3rd month	28.9±2.5	1.8±0.6	0.5 ±0.06
Origanum majorana Oil (20 mg/Kg)			
Pre-treatment	32.2±2.0	1.9±0.3	0.7 ±0.01
1st month	29.2±2.3	2.0±0.3	0.5 ±0.01
2nd month	30.2±2.7	1.9±0.3	0.6 ±0.01
3rd month	28.8±2.8 ±0.01	1.7±0.3	0.51
Origanum majorana Oil (40 mg/Kg)			
Pre-treatment	31.8±5.4	2.17±0.01	0.6 ±0.02
1st month	27.9±3.6 ±0.08	1.80±0.03	0.6
2nd month	29.2±5.6 ±0.04	1.9±0.02	0.5
3rd month	25.9±6.1 ±0.01	1.7.±0.03	0 .5

Origanum majorana volatile oil significantly reduced blood glucose but did not significantly changes serum indirect bilirubin and total bilirubin ***(Table V)***.

Table V : Effect of Oral Administration of Origanum majorana oil on Blood glucose , total Bilirubin and direct Bilirubin Levels in Rats .Values are Means ± S.E., N= 10 Animals.

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Origanum majorana volatile oil mg/Kg/day By oral gavage tube	parameters		
	Glucose (m g/dl)	Direct bilirubin (mg /dl)	Total bilirubin (mg /dl)
Vehicle			
Pre-treatment	86.2±1.0 ±0.01	0.22±0.3	0.65
1st month	83.0±1.1 ±0.05	0.24±0.5	0.63
2nd month	8 6.4±6.3 ±0.04	0.20±0.3	0.60
3rd month	88.2±0.5 ±0.06	0.19±0.6	0.58
Origanum majorana Oil (20 mg/Kg)			
Pre-treatment	84.2±2.0 ±0.01	0.16±0.3	0.71
1st month	79.2±2.3 ±0.01	0.14±0.3	0.66
2nd month	64.2±2.7 ±0.01	0.14±0.3	0.67
3rd month	74.7±2.8 ±0.01	0.15±0.3	0.71
Origanum majorana Oil (40 mg/Kg)			
Pre-treatment	79.2±5.4 ±0.02	0.18±0.01	0.64
1st month	64.2±5.6 ±0.04	0.16±0.03	0.68
2nd month	61.2±4.6 ±0.06	0.14±0.02	0.58
3rd month	64.2±6.8	0.14±0.03	0.59

±0.05

-***Origanum majorana*** volatile oil significantly reduced blood cholesterol, triglycerides and total lipids after two and three months from its administration **(Table VI).**

Table VI: Effect of Oral Administration of Origanum majorana oil on Lipid profile in Rats .Values are Means ± S.E., N= 10 Animals.

Origanum majorana volatile oil mg/Kg/day By oral gavage tube	**Parameters**		
	Total Lipids (g/ dl)	**Cholesterol (mg /dl)**	**Triglycerides (mg /dl)**
Vehicle			
Pre-treatment ±0.01	6.52±0.12	83.5±1.3	71.4
1st month ±0.02	6.42±0.1	85.7±1.5	68.3
2nd month ±0.04	6.29±0.12	81.4±0.9	74.7
3rd month ±0.06	6.41±0.12	87.8±0.6	73.5
Origanum majorana Oil (20 mg/Kg)			
Pre-treatment ±3.01	6.22±0.13	88.5±1.6	68.4
1st month ±2.41	5.92±0.17	79.5±1.2	71.4
2nd month	5.90±0.15	81.5±1.4	60.9

±3.11			
3rd month ±2.10	4.39±0.12	74.5±1.7	50.9
Origanum majorana Oil (40 mg/Kg)			
Pre-treatment 62.2±2.01	6.14±0.12	84.5±3.3	
1st month ±3.21	5.00±0.10	81.3±2.3	59.0
2nd month ±2.13	4.72±0.14	75.3±1.9	55.4
3rd month ±4.01	4.10±0.12	73.5±2.8	51.4

Significantly different compared with controls (pre-treatment) p ‹ 0 .05

DISCUSSION

The pharmacological actions of *Origanum majorana have* been investigated at our laboratories and by others (3). Our studies revealed that marjoram oil is a potent hypocholesterolemic, hypolipidemic in addition of protecting vital organs and chromosomes from oxidative stress (Banerjee et al, 2017).

In this study, acute and long-term safety of *Origanum majorana* oil was evaluated. Acute toxicity studies of marjoram oil indicated safety margin that is sufficiently wide to exclude the occurrence of toxic and lethal effects during clinical use even with relative overdosing.

Daily administration of marjoram oil in rats for 1, 2 and 3 months revealed no significant effect on body weights or histological structure of the different organs. Moreover, long-term (3

months) marjoram oil administration had no hazard effects on the hemopoietic system as indicated by the non-- significant effects on blood haematological parameters. In addition, it had no harmful effects on liver and kidney functions as it restored histological structures and serum levels of AST, ALT, ALP, urea, uric acid and creatinine (Banerjee et al, 2017).

Repeated oral administration of marjoram oil for 2 and 3 months significantly decreased blood total Cholesterol (TC), triglycerides (TG) and total lipids as Reported. *Origanum majorana* oil decreased TC and low-density lipoprotein- Cholesterol (LDL-C) and enhanced the high – density lipoprotein- cholesterol (LDL-C).

This is advantageous for treatment of hypercholesterolemia. Especially among Egyptians where low HDL-C is the prevalent lip rote in abnormality. High levels of TC and, more importantly, LDL-C are major coronary risk factors (Kappelle et al, 2011). Recent studies also showed that triglycerides were independently related to coronary heart disease (Lin et al, 2010). And most of the anti-hyper cholesterol emic drugs did not decrease TG levels, but marjoram lowered it significantly. This effect may be related to the increase in endothelium bound lipoprotein lipase which hydrolyses the triglycerides into fatty acids.

Marjoram oil also significantly decreased blood glucose levels after administration for 1, 2 and 3 months. This is concordant with the finding that a marjoram strongly inhibited rat intestinal alpha glycosidase , and in turn, reduced the release of glucose from carbohydrates, resulting in a dose related delay in (or reduction of) postprandial increase in blood glucose and TG and increased insulin binding in muscle . The pathogenesis of diabetes mellitus (OM) and the possibility of its management by oral administration of hypoglycaemic agents have been extensively studied in

recent years. Drugs of plant origin are considered to be less toxic and almost free from side effects than synthetic drugs. The oil under test may be of value in treatment of diabetes and hyperlipidaemia as previously recorded (Lin et al, 2010).

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