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RUJMS Original Research

Investigation of Essential Oil of *Melissa Officinalis* for Acute and Sub-Chronic Oral Toxicity

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Abstract

Background**: Medicinal plants continue to play central roles in the healthcare system of a large proportion of the world's population.* ***Aim: *To determine the acute and Sub-chronic oral toxicity of Melissa officinalis essential oil and to serve as criteria to recommend the ethno pharmacological uses of the plant.* ***Methods:*** *In the acute toxicity test, oral administration of 300 and 2000mg/kg of the Melissa officinalis essential oil was evaluated in mice. Sub-chronic toxicity was studied by a daily oral dose of 100 and 200mg/kg for three months. On day 0, 30, 60 and 90, blood samples collected from retro-orbital sinus of the eye rats were used for evaluation of serum biochemistry, hematology and histopathological examination of the heart, lungs, liver, kidney and spleen.* ***Results:*** *The acute toxicity oral study revealed no observable signs of toxicity. In rats, the examination of signs, animal behavior and the changes in body and organs weights showed no abnormalities in the test groups as compared to the controls. There were no significant variations in the hematological parameters of both Melissa officinalis essential oil treated and untreated rats. However, biochemical parameters showed a significant decrease in blood sugar. Melissa officinalis essential oil significantly ($p<0.05$) increased serum urea suggesting degenerative changes in the kidney.* ***Conclusion:*** *We can conclude that Melissa officinalis essential oil is well tolerated in short-term therapies but may have long-term toxic effects on the kidney.*

Keywords: Melissa officinalis; Essential oil; Sub-chronic toxicity; Acute toxicity; Hematological parameters.

Introduction

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both

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world health and international trade. Medicinal plants continue to play central roles in the healthcare system of a large proportion of the world's population [1]. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations [2]. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some cases) and the cultural, spiritual point of view of the people of the countries [2]. The plant *Melissa officinalis* (Lemon balm) is used has been documented in Ancient Greek and Roman times. Also known by the name "cure-all", it has been used as a sedative, antipyretic, antispasmodic, diaphoretic, antihypertensive, aromatic, emmenagogue, and carminative [3]**,** and a treatment for insomnia, sleep disorders, anxiety, depression, neuralgia, migraine, tension headache, nausea, nervous stomach, anorexia, colic, chronic fatigue, shingles, coughs, irregular menstrual periods, toothache, heart conditions, nervous palpitations and high blood pressure [3-9].

Aim of the study: The aim of this study was to evaluate the acute and sub-chronic oral toxicity of the Melissa officinalis essential oil in an animal model.

Subject and Methods

Plant material:

Fresh leaves of *Melissa officinalis* were collected based on ethnopharmacological information from villages around the region Eljadida, middle Morocco in January 2014, with the agreement of the authorities with respect to the United Nations Convention on Biodiversity and with the assistance of traditional medical practitioner. The plant was identified with botanist of the Department of Medicinal and Aromatic Plants, National Institute for Agricultural Research, Morocco. A voucher specimen (No. RAB76712) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat.

Preparation of the essential oil:

The essential oil (0.5 % v/w) was obtained from fresh leaves of *Melissa officinalis* by steam distillation for 3 hours by using a clevenger type apparatus. The extract was stored in a refrigerator at 4°C [10] and protected against light and heat until use.

Animals:

While adult females Swiss mice (20-30g) were used in the LD_{50} calculation

tests, male and female Wistar rats weighing (180-220g) were used in chronic toxicity study.

The animals were obtained from the animal center of Mohammed V-Souissi, Medicine and Pharmacy Faculty Rabat, Morocco. They were housed under standard environmental conditions of temperature (26 ±1°C), relative humidity (60-70%) and light (12h light: 12h dark cycle). All animals had free access to water and standard diet. Before each experiment, the animals fasted overnight with free access to water.

The animals were treated according to directives of the Official Journal of the European Community about the care and of the use of the animals of laboratory [11, 12].

Acute oral toxicity Study:

Acute oral toxicity study was evaluated according to the OECD guidelines 423 [13]. Following the fasting period of (3-4h), three female mice weighed and the dose was calculated in reference to the body weight. The animals received EOMO starting at 300mg/kg orally by gavage, the animals were observed for toxic symptoms. As no toxic sign observed, another 3 females mice received the highest dose of 2000mg/kg of EOMO orally by gavage.

For each step, surviving animals were weighed and kept under observation for 14 days to register possible mortality, and their behavioral neurological toxicity.

Sub-chronic oral toxicity study:

The chronic oral toxicity study was conducted according to the guidelines [14, 15]. Thirty-six (36) animals of both sexes were randomly divided into three groups of 12 rats each (6 female + 6 male). While the first group was served as a control, group II and III were orally administered 100 and 200mg/kg body weight respectively of the EOMO for three months. Males and females were separated in different cages to avoid breeding. Appearance and overt behavior were recorded daily and blood analysis was realized at D0 and 1, 2 and 3 months. The doses were selected based on LD_{50} values. All animals sacrificed terminally were subjected to detailed necropsy. All organ weight was recorded. Tissues from control and groups were subjected to histopathological evaluation.

Body weight and weight of organs:

Body weight was measured weekly and the organs (heart, lungs, liver, kidney and spleen) were weighed for different groups on the end of this study.

Blood samples:

At D0, months 1, 2 and 3; all animals was fasted overnight and anesthetized afterward with ether for blood collection from the retro-orbital sinus. Blood samples were collected in tubes

containing heparin for biochemical and EDTA for hematology.

Hematology:

Blood samples were collected for the analysis of hematological parameters such as red blood cell (RBC) count, hemoglobin (Hb) levels, hematocrit (HCT), platelets (PLT), White blood cell (WBC) count, differential WBC count [Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E), Basophils (B)], mean corpuscular volume (MCV), mean corpuscular hemoglobin (MH) and mean corpuscular hemoglobin concentration (MCHC).

Biochemical parameters:

The serum was separated by centrifugation at 4000rpm for 10 min, and the levels of Glucose, Creatinine, Urea, Aspartate aminotransferase (AST), Alanine aminotransferase (AlT), total cholesterol, total protein, and triglycerides measured.

Histopathological examination:

At the end of treatment period (three months), the animals were sacrificed for histopathological examination. The following organs were weighted, examined and then fixed in 10% buffered formaldehyde solution: heart, lungs, liver, kidney and spleen. The fixed organs were examined by the histopathological method.

The results were expressed as mean ± S.E.M. Statistical analysis of data was done using one-way analysis of variance followed by student's test. A p value less than 0.05 were considered significant.

Results

Acute oral toxicity study:

Mice treated with 300mg/kg and 2000mg/kg doses of EOMO presented a slight reduction in the locomotors activity and not present signs of toxicants effects which could be detected by an alteration in body weight measured for 14 days. One death was registered in the group treated with 2g/kg, and zero death at the second step (OECD 423).

The animals quickly recorded their normal activity and growth after a period of 24hours. All animals did not show any changes in the general appearance during the observation period. This result indicates that the LD_{50} was higher than 2000mg/kg.

Chronic oral toxicity study

Hematology:

The result of the hematology of both control and rats treated with EOMO is presented in Table 1. The result showed that there was no significant changes in the parameters examined (RBC, Hb, HCT, PLT, WBC,

Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E), Basophils (B), MCV, MCH and MCHC) of the treated groups of rats when compared to negative control [Table-1].

Biochemical parameters:

The biochemical analysis showed a significant decrease in the level of glucose in the rat treated with EOMO at all doses after one, two and three months. However, the urea was significantly increased in rats treated with 200mg/kg of the EOMO after the second month and at the end of treatment for all doses (100 and 200mg/kg. p.o.) [Table-2]. Other parameters examined (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Triglycerides, Cholesterol, Total Protein, and Creatinine) did not record significant alterations in any of the treated groups as compared to the control group.

Body weight and weight of organs:

No significant changes were observed in body weight [Table-3]. Also, there were no significant differences between the control and treated groups in the organ weights as shown in Table 3.

Histopathology:

Histopathological studies of the organs (heart, lungs, liver, kidney and spleen) of animals that were exposed to chronic treatment with *Melissa officinalis* Essential Oil at different doses (100 and 200mg/kg) showed that only the kidney had histopathological modifications, as compared to control animals.

Some incidental and spontaneous lesions were observed, with less frequency and not dose dependent. As shown figure 1, the kidney of rats treated with EOMO showed when compared to control rat kidney [Fig. 1].

As shown figure1 (E), the kidney of rats treated with EOMO showed glomerular ischemic lesion with sickle cell anemia and minimal focal tubular necrosis when compared to control rat kidney.

Discussion

In aromatherapy, the essential oils are believed to possess anti-inflammatory and psychotropic effects and to be useful for treating nervous breakdown and depression [22, 23]. Oil compositions of *M. officinalis* L. have already been reported [22, 23]. Thus, it has been shown that Nerol, Isopulegol, Citral, Caryophyllene, Caryophyllene oxide, and Citronella account for 80% of *M. officinalis* L. essential oils, but in our study, these compounds represent 84.08%. These differences in chemical composition of essential oil may be due to both developmental and environmental factors that influence plant metabolism.

Following oral administration of *M. officinalis* L. extract at the doses of 300 and 2000 mg/kg, p.o., no toxicity and no significant changes in the body

weight between the control and treated groups were demonstrated at these doses.

This result indicates that the LD_{50} was higher than 2000mg/kg. These results were previously reported by Bounihi et al., [22]. In a chronic oral toxicity study, it appeared that the EOMO at the doses used did not produce any marked changes in rats, as evidenced by the absence of toxic symptoms, no changes in body weight.

As shown in Table 3, the groups treated with EOMO at the doses of 100 and 200mg/kg/day showed no significant changes in the body weights when compared with the control group.

Furthermore, no behavioral alterations were recorded in the treated rats.

The organ weight did not show significant changes between the treated and the control groups [Table-4]. However, slight changes were found in the weights of organs that may due to the variation in the size of organs in each animal [16].

Hematopoietic system is one of the most sensitive targets for toxic compounds and important index of physiological and pathological status. Also, blood profile usually gives vital information on the response of the body to injury or stress [17]. The hematological parameters showed no significant differences between the control and the treated groups, indicating that EOMO had no toxicological effects on the circulating blood cells on their production.

The evaluation of biochemical parameters was performed in order to evaluate the liver, renal, lipid and glycemic profiles of experimental compared to control animals, in order to give insight into pathological changes and nature of the disease.

The liver is the major organ involved in drug biotransformation. High levels of AST and ALT are reported in liver diseases or hepatotoxicity [18]. However, ALT is more specific to liver and thus a better parameter for detecting liver injury as AST is also associated with diseases of other organs such as heart and muscle [19]. In this study, the liver profile parameters (ALT, AST, TP) revealed normal functioning of the liver. Renal dysfunction can be assessed by concurrent measurements of Urea and Creatinine and their normal levels reflect at reduced likelihood of renal problems [20]. The increase in serum Urea levels observed at different doses (100 and 200mg/kg) on the end of treatment while there was a significant elevation of Urea values from the second month to thirsted month at higher dose 200mg/kg when compared to the negative group.

This may be associated with kidney dysfunction most likely by renal filtration mechanism and probably indicates that chronic exposure of EOMO at different doses for up to 90 days may interfere with the capacity of the kidney to excrete this metabolite as suggested by [24]. The lipid profile parameters (TGY and Chol) are indications that the extracts do not

present any risk of hypercholesterolemia or atherosclerosis at all doses.

The EOMO show hypoglycemic activity, this might be due to impaired insulin action or inadequate insulin secretion [25].

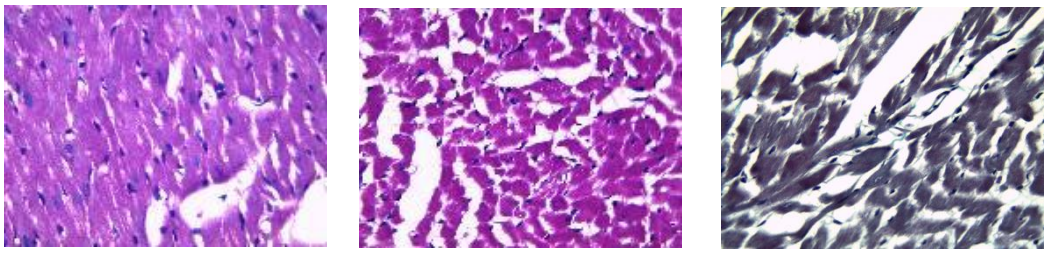
The biochemical parameters of kidney damage as a result of sub chronic exposure of the rats to EOMO for three months were confirmed by the histopathological findings of this organ.

The lesions include glomerular ischemic lesion with sickle cell anemia and minimal focal tubular necrosis when compared to control rat kidney. These changes will interfere with the ability of the kidney to carry out its normal excretory roles.

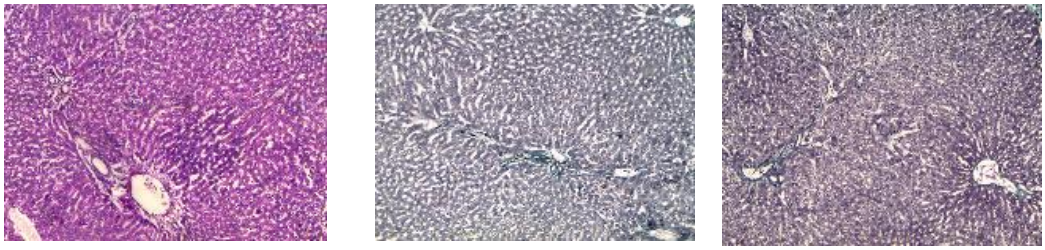
This may have contributed to the high levels of Urea seen in the blood of rats treated with EOMO on the end of this study. Also, the kidney is highly susceptible to toxicants because a high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules [25].

(T) **(MO_1)** **(MO_2)**

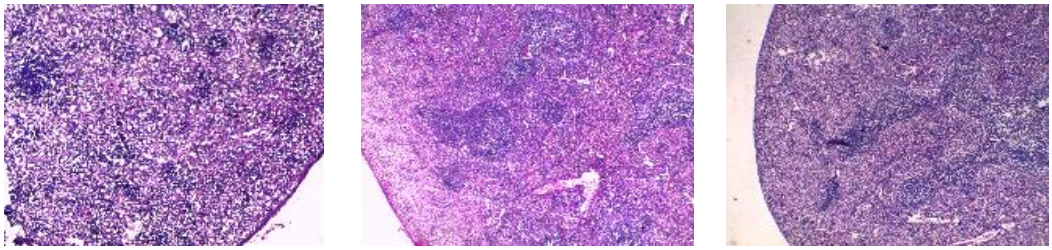
(A)

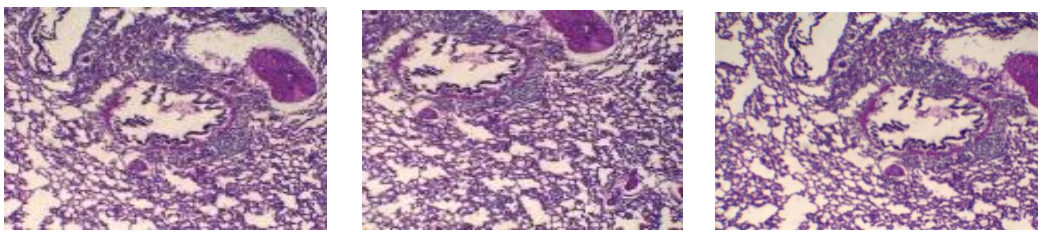


(B)



(C)





(D)

(E)

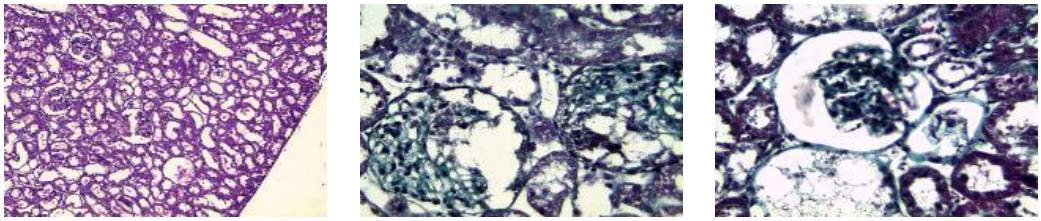


Figure 1: Photomicrographs of the sections of the heart (A), liver (B), spleen (C), lungs (D), kidney (E) of control (T) and EOMO administered at the dose 100mg/kg (MO_1) and the dose 200mg/kg (MO_2).

Table 1: Hematological values of rats in the chronic toxicity study of the essential oil of *Melissa officinalis* L

		Essential oil of *Melissa Officinalis* L.					
		Treatment time					
		1 month		**2 months**		**3 months**	
Parameters	Control	100mg /kg	200mg /kg	100mg /kg	200mg /kg	100mg /kg	200mg /kg
RBC	7.89 ± 0.73	7.7 ± 0.83	7.69 ± 0.81	7.66 ± 0.53	8.14 ± 0.72	8.08 ± 0.5	8.38 ± 0.78
Haemoglobin	14.58 ± 0.73	14.55 ± 1.26	14.26 ± 1.01	13.71 ± 1.34	14.25 ± 0.94	14.8 ± 0.74	14.91 ± 0.64
Haematocrit	39.75 ±	40.08 ±	40.93 ±	40.75 ±	41.65 ±	42.65 ±	42.26 ±

	2.19	2.38	1.55	2.27	2.22	3.53	2.97
Platelets	623 ± 63	583 ± 61	574 ± 97	620 ± 53	586 ± 83	578 ± 90	586 ± 82
WBC	10.54 ± 2.1	10.53 ± 2.34	11.04 ± 1.97	11.07 ± 2	11.85 ± 1.60	10.94 ± 1.67	11.74 ± 2.34
Neutrophils	20.75 ± 1.89	20.7 ± 2.22	20.51 ± 1.99	21.03 ± 2.1	20.18 ± 1.85	21.08 ± 2.18	20.23 ± 2.07
Lymphocytes	70.51 ± 2.66	69.98 ± 4.06	69.86 ± 3.01	69.81 ± 2.77	68.03 ± 4.07	67.1 ± 3.61	66.91 ± 3.93
Monocytes	2.03 ± 0.16	2.00 ± 0.06	1.98 ± 0.07	2.05 ± 0.16	2.00 ± 0.14	2.00 ± 0.1	2.03 ± 0.13
Eosinophils	1.7 ± 0.23	1.7 ± 0.27	1.7 ± 0.26	1.73 ± 0.24	1.73 ± 0.25	1.75 ± 0.22	1.75 ± 0.24
MCV	51.47 ± 1.15	51.8 ± 0.69	51.7 ± 0.94	51.02 ± 1.15	51.37 ± 0.96	51.92 ± 1.18	51.5 ± 0.94
MCH	18.33 ± 0.59	17.76 ± 0.53	18.11 ± 0.95	17.78 ± 0.86	17.73 ± 0.72	18.35 ± 0.48	18.21 ± 1.02
MCHC	34.17 ± 0.37	34.1 ± 0.66	34.12 ± 0.26	34.02 ± 0.87	34.47 ± 0.78	34.1 ± 0.39	34.00 ± 0.77

Table 2: Biochemical values of rats in the chronic toxicity study of the essential oil of *Melissa officinalis* L

		Essential oil of *Melissa Officinalis* L.					
		Treatment time					
		1 month		**2 months**		**3 months**	
Parameters	**Control**	**100mg /kg**	**200mg /kg**	**100mg /kg**	**200mg /kg**	**100mg /kg**	**200mg /kg**
ASAT (U/I)	248 ± 27.81	250 ± 28.24	245 ± 26.21	246 ± 26.37	246 ± 27.19	252 ± 18.8	246 ± 29.87
ALAT (U/I)	67.83 ± 4.3	65.83 ±4.7	66.33 ±3.88	66.66 ± 4.84	67.83 ± 4.95	67.33 ± 4.96	66.83 ±3.37
Triglycerides (g/l)	0.66 ±0.17	0.66 ±0.25	0.65 ± 0.2	0.67 ± 0.005	0.67 ±0.36	0.66 ±0.15	0.67 ± 0.09
Cholesterol (g/l)	0.86 ± 0.02	0.86 ± 0.15	0.86 ± 0.16	0.82 ± 0.04	0.82 ± 0.09	0.8 ± 0.13	0.8 ± 0.2
Total protein (g/dl)	66.83 ± 4.26	67.16 ±3.65	67.16 ±3.06	68.16 ± 3.65	67 ± 3.34	68.5 ± 1.51	69.83 ± 1.83
Urea (mg/l)	0.26 ± 0.05	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.01	0.33 ± 0.04*	0.39 ± 0.03*	0.38 ± 0.03*
Creatinine (mg/l)	5 ± 0.63	5.16 ± 0.4	5.16 ± 0.75	5.5 ± 0.54	5.33 ± 0.51	5.66 ± 0.51	5.5 ± 0.54
Glucose (g/l)	1.52 ± 0.12	1.13 ±0.14*	1.16 ± 0.2*	1.21 ± 0.2*	1.20 ± 0.2*	1.13 ± 0.13*	1.13 ± 0.2*

Table 3: Body Weights of rats in the chronic toxicity study of the essential oil of Melissa officinalis L

Organs	Control	Essential Oil of *Melissa officinalis* L.	
		100mg/kg	200mg/kg
Lungs	1.81 ± 0.05	1.81 ± 0.07	1.81 ± 0.06
Heart	0.88 ± 0.09	0.88 ± 0.08	0.87 ± 0.08
Liver	10.43 ± 0.24	10.44 ± 0.07	10.76 ± 0.12
Kidney	0.85 ± 0.03	0.83 ± 0.01	0.85 ± 0.02
Spleen	0.72 ± 0.02	0.73 ± 0.03	0.75 ± 0.04

	Group		
	Control	EOMO 100mg/kg .p.o.	EOMO 200mg/kg .p.o.
0	192.17 ± 13.62	192.45 ± 10.81	191.62 ± 13.53
1	239.27 ± 13.62	250.96 ± 30.05	256.81 ± 17.41
2	263.07 ± 19.21	288.88 ± 38.37	290.67 ± 24.83
3	286.32 ± 25.15	309.04 ± 41.23	310.37 ± 27.41

Table 4: Organ Weights of rats in the chronic toxicity study of the essential oil of Melissa officinalis L

Conclusion

Thus, we can conclude that, *Melissa officinalis* essential oil is well tolerated in short term therapies but may have long term toxic effects on the kidney.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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