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IN- VIVO ANTIOXIDANT RELATED EFFECTS OF ORALLY ADMINISTERED AQUEOUS EXTRACT OF LEMON BALM (*MELISSA OFFICINALIS* L.) IN HUMAN

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ABSTRACT

Lemon balm (*Melissa officinalis* L.), belongs to the family Lamiaceae (Labiatae), is one of the important medicinal plant species. The present work aimed to investigate antioxidant related effects of Lemon balm in humans by in vivo study & effects on liver, renal & cardiac function tests. Nine healthy volunteers, each received orally 250 ml of aqueous extract of Lemon balm daily for 5 days. Venous bloods were taken before and one hour after the first dose of aqueous extract (sample I and II respectively) and then one day after the last dose of day five (i.e. day 6, sample III). The first blood taken before the first dose (i.e. sample I), served as control for the next samples of II and III. The following assays were performed: serum total antioxidant status (TAS), erythrocyte reduced glutathione (GSH), malonyldialdehyde (MDA), and serum selected biochemical tests. Oral administration of aqueous extracts of Lemon balm to healthy volunteers, for 5 days, increased significantly serum TAS, erythrocyte GSH and decreased significantly erythrocyte MDA, with no effect on serum biochemical tests for kidney, liver, cardiac and pancreatic, compared to 0 time administration. In conclusion: Lemon balm extract has efficient in vivo antioxidant related effects. As the present findings are obtained in healthy humans with no oxidative stress, this indicates that Lemon balm can improve the base line of the defense mechanisms against possible oxidative stress, with no adverse effects, thus decreasing susceptibility or preventing the progress of pathological conditions related to oxidative stress.

KEYWORDS: **Lemon balm, Total Antioxidant Status, MDA, GSH, Superoxide Dismutase, Serum Biochemical Tests, in Vivo**

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INTRODUCTION

Lemon balm, member of the family Lamiaceae (formerly Labiatae) is a perennial herb. It is commonly referred to as Lemon Balm because of its lemon-like flavor and fragrance. Its wild types are in all Mediterranean countries and South part of the Alps.[1] *M. officinalis* extracts possess antioxidant [2, 3], sedative [4], anti-inflammatory, hepatoprotective, digestive [5, 6], antiviral [2, 7], antilipidaemic [8] properties. Phytochemical studies carried out in *M. officinalis* have demonstrated the presence of numerous constituents, including polyphenolic compounds, essential oils, monotherpenoidaldehides, sesquiterpenes, flavonoids and tannins.[2, 4, 9] All of these may be responsible for the therapeutic efficacy of *M. officinalis* extracts and the prevention of the effects described above. Free radicals and reactive oxygen species are continuously produced in the human body. These oxygen species are the cause of cell damage and the initiation and progression of chronic diseases. Therefore, body systems must be protected from oxidative injury through lines of defense that includes intracellular antioxidants such as reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and catalase, and extracellular antioxidants such as vitamins, micronutrients, carotenoids, polyphenolics and other bioactive compounds. [10, 11] The present study focused on the antioxidant related effects of Lemon balm on normal human volunteers after oral administration of aqueous extract for 5 days & the effects of oral administration of aqueous extract for 5 days on liver, renal & cardiac function tests. These systemic effects are also important to see whether a given plant extract affects laboratory analysis, as many patients may go to the clinical laboratory for analysis after drinking plant extracts that have become a common use in public, although there are no published studies showing their might be effects on laboratory tests.

MATERIALS AND METHODS

Nine healthy volunteers (5men and 4women) with a mean age of 34±18.6 years were recruited in the study after they signed an informed consent according to the ethics committee requirements. Each volunteer received orally 250 ml of aqueous extract of Lemon balm daily for 5 days. Venous blood samples were taken before and one hour after the first dose of aqueous extract (sample I and II respectively) and then one day after the last dose of day five (i.e. day 6, sample III). The first blood sample taken before the first dose (i.e. sample I), served as control for the next samples of II and III.

Ethical Issue : Inclusion And Exclusion Criteria

Individuals were excluded if they had (i) a disease condition, such as liver, renal, or heart dysfunction; (ii) a history of cancer; (iii) allergies to any drug or food ingredient. Furthermore, women were excluded if they were pregnant or lactating. Smokers were excluded. The study was approved by the ethics committee of the university of Jordan on January 12, 2011.

2.3.1. Preparation of Aqueous Extract of Lemon balm

This was prepared as usually used by the Jordanian public in dealing with this plant. Dried leaves were purchased from the local herbal store in Amman, Jordan. 50 g of leaves was boiled with 2.5 L water for 10-15 min, and then left covered soaking for 3-4 hrs at room temperature, then 250 ml of soaked aqueous extract was given orally to each individual daily for 5 days.

2.3.2. Blood Samples

Three blood samples were collected in gell clot activator tubes from each healthy volunteer (sample I before drinking the aqueous extract , sample II after one hour of the first dose (drinking aqueous extract) on day one and sample III at day 6 (i.e. one day following the last dose of day five). Gell tubes were centrifuged for 10 min at 3000 xg at room temperature to separate and collect serum. Then 2 ml of distilled water added to the cells under the gell in tubes and the tubes were centrifuged for 5 min at 3000 xg and the supernatant (hemolysate) was collected. All samples (serum and hemolysate) were stored frozen at - 20°C until analysis.

2.3.3. Determination of Serum Total Antioxidant Status (TAS)

Serum total antioxidant status measured by TAS kit from Randox. The results were expressed as mmol/L.

2.3.4. Determination of Erythrocyte MDA

Erythrocyte MDA was determined as a measure of lipid peroxidation according to stocks and dormandy's method (1971) using thiobarbituric acid (TBA) as modified by srour et al. (2000).[12] All MDA concentrations were expressed as nmol/gHb.

2.3.5. Determination of Erythrocyte Reduced Glutathione (GSH)

Erythrocyte reduced glutathione (GSH) was determined using Ellman's method [13] with slight modification as described elsewhere.[14] All GSH concentrations were expressed in mg/g Hb.

2.3.6. Determination of Erythrocyte Superoxide Dismutas (SOD) Activity

Erythrocyte superoxide dismutas (SOD) was measured using kit from Randox.[15] The results were expressed as U/gHb.

2.3.7. Determination of Serum Biochemical Parameters

The kits for determination of serum biochemical parameters were purchased commercially from Roche and 902 Hitachi analyzer used to perform the following biochemical parameters: serum sodium (Na), potassium (K), urea nitrogen (BUN), creatinine (CREA), uric acid (UA), albumin (ALB), total protein (TP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine phosphokinase (CPK) and amylase (AMYL).

2.4. *Statistical Analysis*

All data are reported as the mean ± S.D., statistical analysis was performed using SPSS statistics 17. The results were compared by paired *t*-test. The results with a value of $P \leq 0.05$ were considered significant.

RESULTS

The results of the in vivo study are shown in table 1. As shown in table 1, oral administration of aqueous extracts of Lemon balm to healthy volunteers for 5 days increased significantly serum total antioxidant status (TAS) (from 1.23 to 1.37), erythrocyte reduced glutathione (GSH) (from 0.80 to 1.05) and decreased significantly erythrocyte malonyldialdehyde (MDA) (from 27.7 to 22.9) at day 6 (i.e. one day following the last dose of day five) of administration, compared to 0 time administration. Lemon balm had no significant effect on any of the following serum parameters that stayed within the reference ranges (sodium, potassium, urea nitrogen (BUN), creatinine, uric acid (UA), albumin, total protein, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK), amylase (AMYL)) that have been measured at 0 time, 1 hour after the first dose of day 1, or at day 6 (i.e. one day following the last dose of day five) (Table 1).

Table 1

Results of the in vivo study of oral administration of Melissa officinalis L.aqueous extract to healthy volunteers. Each value represents the mean value ± S.D., (n =9), *P value ≤ 0.05, compared to 0 time administration. NM indicates not measured.

Measured parameter	Sample I	Sample II	Sample III
Serum TAS (mmol/L)	1.23±0.2	1.36±0.2*	1.37±0.22*
Erythrocyte GSH (mg/gHb)	0.80±0.15	NM	1.05±0.14*
Erythrocyte SOD (U/gHb)	1132.0±139.0	NM	1210.3±119.2
Erythrocyte MDA(nmol/gHb)	27.7±4.8	NM	22.9±3.4*
Serum K (ref value=3.7-5.2 mmol/L)	4.38±0.40	4.4±0.2	4.34±0.38
Serum Na (ref value=135-145mmol/L)	146.0±2.2	144.±1.2	145.4±1.7
Serum BUN (ref value=6-20 mg/dL)	13.5±3.5	13.0±2.8	9.7±2.0
Serum CREA(ref value= 0.6-1.3 mg/dL)	0.74±0.13	0.72±0.13	0.65±0.16
Serum UA (ref value= 3.5-7.2 mg/dL)	4.92±1.0	5.0±1.0	5.4±1.3
Serum ALB(ref value= 34-54 g/L)	45.2±2.2	44.6±2.5	46.6±3.8
Serum TP(ref value = 60-859/L)	77.6±3.9	76.1±2.1	79.7±4.4
Serum ALP(ref value=55-142 U/L)	112.9±71.5	110.3±67.2	112.0±78.3
Serum AST(ref value=8-40U/L)	15.8±5.7	16.2±5.0	16.4±5.4
Serum ALT(ref value= 7-55 U/L)	17.4±13.3	17.5±14.1	18.9±15.7
Serum CPK(ref value=38-176 U/L)	90.5±65.7	93.0±64.4	80.5±35.8
SerumLDH(refvalue=200-450 U/L)	307.3±48.0	317±48.2	304.4±39.7
Serum AMY(refvalue=40-140 U/L)	46.8±15.2	45.9±14.7	43.3±16.6

DISCUSSION

The present in vivo study on humans showed that oral administration for 5 days of aqueous extracts of Lemon balm increased significantly the serum total antioxidant status (TAS), erythrocyte reduced glutathione (GSH) and decreased significantly erythrocyte Malonyldialdehyde (MDA). Lemon balm had no significant effect on any of the following serum parameters that stayed within the reference ranges (sodium, potassium, urea nitrogen (BUN), creatinine, uric acid (UA), albumin, total protein, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK), amylase (AMYL)). This result agrees with the findings of Moeko Noguchi-Shinohara et al [16], the only published study in litrature, who evaluated the safety, tolerability and pharmacokinetics of single dose of *Melissa officinalis* extract which contained 500 mg rosmarinic acid. They showed that rosmarinic acid supplementation did not affect liver, kidney, or blood cell function parameters, and no adverse event was reported by any of the participants due to the study treatment, indicating a single dose of Melissa officinalis extract containing 500 mg rosmarinic acid per day appears to be safe and tolerable in humans, but further studies may needed to investigate the safety of long-term rosmarinic acid use. This result also confirms a similar finding for biochemical tests by others.[14,17]As the present findings are obtained in healthy humans with no oxidative stress induction, this indicates that Lemon balm can improve the base line of the defense mechanisms against possible oxidative stress, thus decreasing susceptibility to diseases related to oxidative stress. Absence of Lemon balm effect on serum LDH of healthy volunteers may indicate in vivo anti-hemolytic activity for this plant and/or absence of adverse in vivo hemolytic activity. Absence of the effect of oral administration of aqueous extract of Lemon balm on serum biochemical tests that stayed within the reference ranges for kidney function (BUN,CREA), liver function enzymes and tests (ALT, AST, ALP, albumin, total protein), cardiac enzymes (CPK) and pancreatic amylase, indicates absence of adverse effects on these organs. However, the importance of the present in vivo study on healthy humans also related to the lifestyle factors such as diet, physical activity, alcohol consumption, cigarette smoking and others, that have been suggested to seriously influence the oxidative stress response in humans, tipping the balance of oxidative burden/antioxidant response to one side or the other, thus a diet rich in vegetables and in natural antioxidants, favoring the antioxidant side, has been found to be preferred by long-lived healthy individuals.[18,19] Measurement of various biochemical laboratory tests in serum after oral

intake of Lemon balm was also important, to see whether a given plant extract affects laboratory analysis, as many patients may go to the clinical laboratory for analysis after drinking herbal extracts that have become a common habit in public, as there are no published studies showing their might be effects on laboratory tests.

CONCLUSION

The positive antioxidant related effects of Lemon balm on healthy humans with no adverse effects on major body systems may indicate that this plant by improving the base line of the body defense mechanisms might be helpful in preventing the occurrence or progress of pathological conditions related to oxidative stress.

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