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Effect of *Melissa officinalis* L. on the level of induced lipid peroxidation in mouse liver

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The aim of this study was to evaluate the effect of *Melissa officinalis* L. aqueous extract on the level of induced lipid peroxidation in mouse liver homogenate. Samples were prepared from homogenized BALB/c mice liver, and subsequently incubated with one of the following lipid peroxidation inducing agents: 0.5 mM H_2O_2; 0.1 mM $FeCl_3$ +ascorbate or H_2O_2 +$FeCl_3$+ascorbate (Fenton reaction), in the presence or absence of extract. *M. officinalis* aqueous extract was prepared by extraction with boiling deionized water in 1:10 ratio (w/v). In the experiments were used two-fold dilutions of the extract containing phenolics equivalent of 21.4 to 1.32 mg gallic acid following preliminary determination of the total phenolic content by Folin-Ciocalteu assay. The levels of lipid peroxidation in mouse liver homogenate, caused by all of the oxidative agents were significantly reduced by all tested dilutions of the extract. *M. officinalis* aqueous extracts could be effective for protection of liver cells from induced lipid peroxidation.

Keywords: Phenols, Mice, Antioxidant, Aqueous extract, *M. officinalis*

INTRODUCTION

Lipid peroxidation is a process in which different types of oxidants (free radicals or non-radical species) deteriorate lipids especially those containing polyunsaturated fatty acids in cells, tissues and organs. The variety of metabolic products (i.e. malondialdehyde and 4-hydroxynonenal) produced in this process are associated with development of several major pathological processes as cancer, diabetes, liver and cardiovascular diseases, etc. (for review see Fruhwirth *et al*. [1], Ayala *et al.* [2]; Griffiths *et al.* [3] and references therein). Modern stressful lifestyle and polluted ecosystems are a rich source of free radicals and reactive oxygen species (ROS) whose harmful effect has to be mitigated using different approaches. Therefore usage of phytochemicals is seen as one of most promising strategies as they could be used not only in drug preparations, foods and beverages but also as packaging materials [4, 5].

Lemon balm (*Melissa officinalis* L.) belongs to the family Lamiaceae and it is well known in phytotherapy (both traditional and conventional), cosmetics and culinary. The species is traditionally used as different infusions in Europe and Asia, however nowadays it is in cultivation worldwide and being sold in different forms (extracts, essential oil, and herbal teas). Along with many other properties (i.e. antiviral, antimicrobial, sedative, digestive, antispasmodic, etc.) the aqueous extracts were reported to have antioxidant activity that could be useful in drug and food industry [6-9]. High levels of phenolic acids found in *M. officinalis*, mainly rosmarinic acid, as well as caffeic acid, protocatechuic, ferulic and syringic acids, contribute to the beneficial effects of its extracts [10-12].

Current work reports on the protective effect of *M. officinalis* L. aqueous extract (MAE) against induced lipid peroxidation in mouse liver homogenate.

EXPERIMENTAL

Chemicals

All reagents were obtained from Sigma–Aldrich (Germany).

Plant material and preparation of extract

M. officinalis L. was obtained from the collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences at vegetative stage. Herbage was dried in an oven at 40 ° C and finely powdered. Extract from lemon balm was prepared in 1:10 ratio (w/v) with deionized water through microwave-assisted

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extraction at p=100W for 5 min. Resulting extract was filtered through filter paper and centrifuged for 10 minutes at 6,000 × g. Supernatant was filtered through PES Millipore 0.22 μm filter (Millipore, USA).

Total phenolics determination

Total phenolic content (TPC) of the extract was determined by Folin-Ciocalteu assay [13]. Briefly, 150 μL samples were introduced into disposable test tubes together with 750 μL of Folin-Ciocalteu's reagent (diluted 1:10 with deionized water) and 600 μL of 7.5% sodium carbonate solution. The tubes were incubated in a water bath at 50 °C for 10 min. Absorbance was measured at 760 nm. TPC was expressed as milligram gallic acid equivalents (GAE) per gram plant material.

Animals

White BALB/c mice with a weight of 30-40 grams were used. At the time of sacrifice, the mice were anesthetized with ether. The experiments were performed according to the "Principles of Laboratory Animal Care" (NIH publication No. 85-23) and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services).

Preparation of liver homogenate

The liver was perfused *in situ* with ice-cold 0.15 M KCl. The gall bladder was removed and the liver was isolated, washed, cut with scissors and again washed with ice-cold 0.15 M KCl. The resulting biological material was filtered through gauze and 0.15 M KCl was added to a final volume of 10 ml. The liver was homogenized with a teflon pestle homogenizer and subsequently centrifuged at 3000 × g at 0-4°C for 10 min. After filtration through gauze, the amount of protein of the resulting supernatant from the non-nuclear homogenate was measured.

The protein quantification was performed by the method of Lowry *et al.* [14].

Measurement of lipid peroxidation

Lipid peroxidation (LPO) was determined by the amount of the thiobarbituric acid reactive substances (TBARS), formed in fresh preparations, according to the method of Hunter *et al.* [15]. The hepatic homogenate was diluted with 0.05 M potassium phosphate buffer, pH 7.4, to a final amount of 1 mg protein / ml solution. For each tested concentration of the MAE, 4 samples were prepared containing 500 μl of MAE and 500 μl of liver homogenate. After incubation for 1 hour in a water bath in a shaker at 37 °C, the samples from each dilution were divided into 4 groups: only with extract; with extract and H_2O_2 (10 μl of 0.5 mM H_2O_2); with extract and Fe^{3+} (10 μl of 0.1 mM $FeCl_3$) and ascorbate (50 μl of 0.5 mM ascorbate); with extract and Fe^{3+} (10 μl of 0.1 mM $FeCl_3$), ascorbate (50 μl of 0.5 mM ascorbate) and H_2O_2 (10 μl of 0.5 mM H_2O_2). Additionally, double samples of the homogenate alone, as well as of the pro-oxidants with hepatic homogenate were also prepared, and used for comparison with the samples treated with the extracts. After incubation on a water bath in a shaker at 37 °C for 30 min, 0.6 ml of 40% trichloroacetic acid / 5N HCl / 2% thiobarbituric acid in a 2:1:2 ratio was added to each sample. The so prepared samples were boiled in a water bath for 15 min. After cooling and centrifuging at 3000 × g. for 5 min, the samples were measured spectrophotometrically at wavelengths of 532 nm and 600 nm. The amount of thiobarbituric acid reactive substances was expressed as nmol malondialdehyde (MDA) / mg protein and was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. The experiment was performed three times with double replicates of all samples.

Statistics

Obtained data for the lipid peroxidation in the different induction systems was tested for variance and results from different extract treatments were compared to controls using Dunnett's t test (p=0.05).

RESULTS AND DISCUSSION

The polyphenolic compounds are considered as the main antioxidants in plants [16]. Pereira *et al.* [17] made a comparison between the antioxidant effect of quercetin, gallic acid, quercitrin and rutin, which are some of the most common phenolic compounds found in plant extracts, including *M. officinalis*. They found that highest antioxidant activity is exhibited by quercetin followed by gallic acid, quercitrin and rutin.

Since the polyphenols are considered the compounds directly linked to the antioxidant activity in plants, in our study we measured the total phenolic content in the MAE. The total phenolic content in the aqueous extract of *M. officinalis* was 169.11 ± 0.3 mg GAE / per gram plant material. In the experiments were used two-fold dilutions of the MAE containing phenolics ranging from 21.4 to 1.32 mg GAE.

On Fig. 1 it can be seen that the level of naturally occurred LPO in mouse liver homogenate is reduced around three times after treatment with

any of the subsequent dilutions of the MAE. There was no significant difference in the degree of effectiveness in the tested concentrations of the MAE.

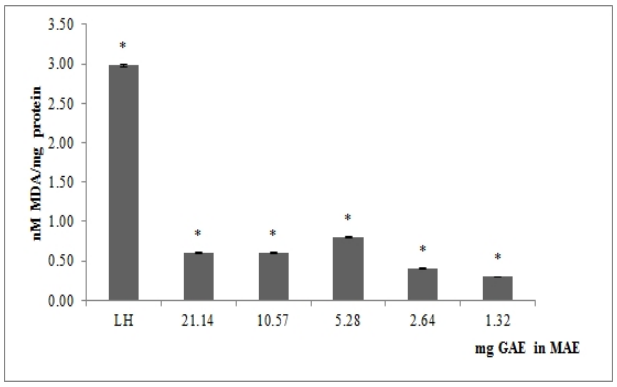

Fig. 1. Effect of *Melissa officinalis* aqueous extract (MAE) on naturally occurred LPO in mouse liver homogenate. LH - liver homogenate, *- $p<0.05$ (n=3)

The experiments with pro-oxidants showed that the highest level of LPO in mouse liver homogenate, presented as amount of produced MDA, is obtained after treatment with $FeCl_3$+ascorbate (Fig. 3) or H_2O_2 + $FeCl_3$ + ascorbate (Fenton reaction) (Fig. 4), while H_2O_2 alone does not produce such a strong effect (Fig. 2).

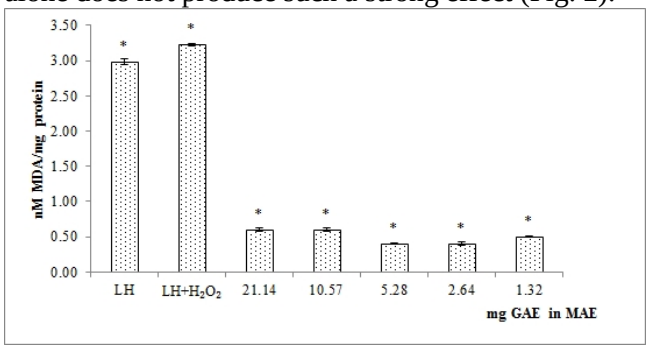

Fig. 2. Effect of *Melissa officinalis* aqueous extract (MAE) on LPO in mouse liver homogenate induced by H_2O_2. LH - liver homogenate, *- $p<0.05$ (n=3).

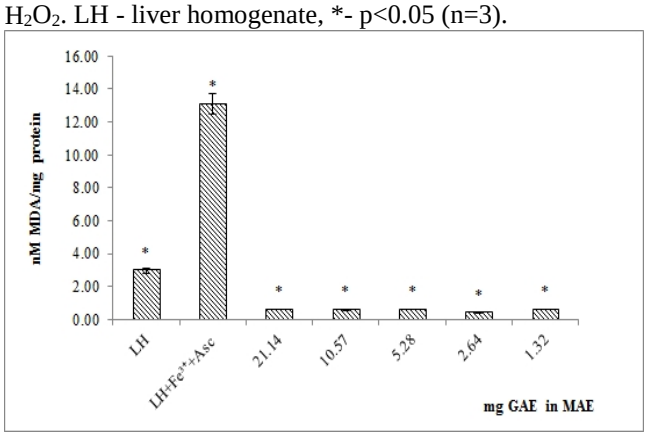

Fig. 3. Effect of *Melissa officinalis* aqueous extract (MAE) on LPO in mouse liver homogenate induced by Fe^{3+} and ascorbate. LH - liver homogenate, *- $p<0.05$ (n=3).

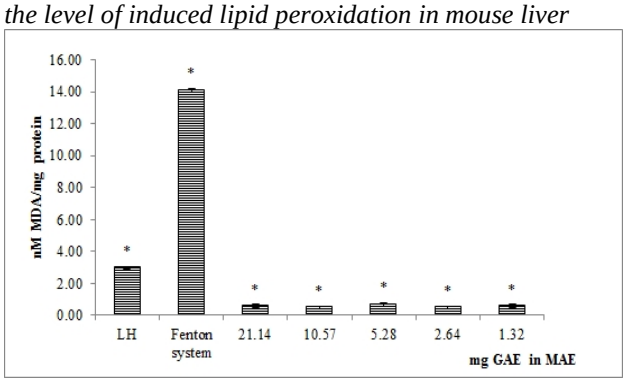

Fig. 4. Effect of *Melissa officinalis* aqueous extract (MAE) on LPO in mouse liver homogenate induced by Fenton's reaction. LH - liver homogenate, *- $p<0.05$ (n=3).

In the treatments with the different pro-oxidants, all tested concentrations of the extract significantly reduced the amount of MDA to levels even below the control of liver homogenate ($p<0.05$). The strongest effect was observed in the experiment with Fenton reaction where the decrease of MDA in the samples treated with the MAE extract had thirteen times lower MDA levels than those treated with the pro-oxidant alone. There were no significant differences between the antioxidant activities of the different concentrations of MAE, which suggested than even in very low concentration, the aqueous extract of *M. officinalis* can protect the liver cells from lipid peroxidation. Similar results were registered by Mimica-Dukic *et al.* [18] who found that treatment with *M. officinalis* essential oil possesses very strong inhibition of LPO, particularly in the Fenton system of induction.

Hohmann *et al.* [19] found that aqueous methanol extract of *M. officinalis* has considerable concentration-dependent inhibition of lipid peroxidation enzyme-dependent and enzyme-independent lipid peroxidation systems.

In an experiment with induced cerebral lipid peroxidation by iron sulfate (10 μM), sodium nitroprusside (5 μM) or 3-nitropropionic acid (2 mM), Pereira *et al.* [17] found that the aqueous extract of *M. officinals* has a strong effect on TBARS production induced by all tested pro-oxidants. Moreover, the effect of the aqueous extract was superior to those of methanolic and ethanolic extracts of the plant.

Our results suggested that the aqueous extract of *M. officials* protects the liver cells, both from naturally occurred and induced by pro-oxidants lipid peroxidation. The effect is very strong even in very low amounts of polyphenols (1.32 mg GAE), which are considered the main substances responsible for the antioxidative effect of the plant.

CONCLUSION

It can be concluded that the aqueous extract of *M. officinalis* effectively protects the liver cells from artificially induced and naturally occurred lipid peroxidation *in vitro*.

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ЕФЕКТ НА *Melissa officinalis* L. ВЪРХУ НИВОТО НА ИНДУЦИРАНА ЛИПИДНА ПЕРОКСИДАЦИЯ В ЧЕРЕН ДРОБ НА МИШКИ

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(Резюме)

Целта на това изследване е да се оцени ефектът на воден екстракт от *Melissa officinalis* L. върху нивото на индуцирана пероксидация на липидите в хомогенат от миши черен дроб. Пробите са приготвени от хомогенизиран BALB/c миши черен дроб, който в последствие е инкубиран с един от следните агенти, индуциращи липидна пероксидация: 0.5 mM H_2O_2; 0.1 mM $FeCl_3$ + аскорбат или H_2O_2 + $FeCl_3$ + аскорбат (реакция на Фентън) в присъствие или отсъствие на екстракт. Воден екстракт от *M. officinalis* L. е приготвен чрез екстракция с кипяща дейонизирана вода в съотношение 1:10 (w/v). В експериментите е използвано двукратно разреждане на екстракта, съдържащ фенолен еквивалент от 1.4 до 1.32 mg галова киселина след предварително определяне на общото фенолно съдържание по метода на Folin-Ciocalteu. Нивата на липидната пероксидация на хомогената от миши черен дроб, предизвикана от всички оксидативни агенти, са понижени съществено при всички разреждания на екстракта. Водните екстракти на *M. officinalis* могат да са ефективни за протекция на чернодробните клетки от индуцирана липидна пероксидация.