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Research article

ANTIOXIDANT STATUS IN THE BLOOD OF PSYCHOSOCIALLY STRESSED RATS TREATED WITH HONEY

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Linden honey represents a unique honey variety valued for its nutritional benefits, distinctive taste and aroma. Phenols, polyphenols, flavonoids, ascorbic acid and phenolic acids in honey have antioxidant activities. This study aimed to investigate the effects of linden honey on the activities of antioxidant enzymes catalase (CAT) and glutathione peroxidase (GPx), as well as on the concentration of malondialdehyde (MDA) in individually housed animals. The investigated parameters were quantified using spectrophotometric method for determination of enzyme activities and MDA concentration in the blood. We found that treatment with linden honey in the socially isolated animals significantly increased the enzyme activities of CAT and GPx, and significantly decreased the concentration of MDA. The modulation of CAT and GPx activities in socially isolated animals treated with linden honey may be very important for understanding the role of honey in the capacity of antioxidant defense system to increase and maintain its stability in psychosocial stress conditions. Our results may be important in biomedical research for understanding the role of honey in the amelioration of oxidative stress.

Keywords: honey, blood, social isolation, enzymes activity, antioxidant status

INTRODUCTION

Social isolation produced by rats' individual housing represents a mild psychosocial stress and resembles perceived isolation observed in depressive disorders [1]. Many studies have shown that social isolation and environmental changes are risk factors in human depression [2]. In addition, COVID-19 pandemic that resulted in quarantine and social isolation has led to the rise in anxiety [3] and also to an increase in posttraumatic

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stress disorder [4]. Chronic social isolation in adult male Wistar rats causes depressive-like and anxiety-like behaviors [5]. It is known that psychosocial stress is associated with increased oxidative stress and decreased antioxidant capacity [6]. For example, the increase in hepatic reactive oxygen species (ROS) amount in individually housed rats might be due to social isolation-induced dysfunctions of antioxidant enzymes [7]. Additionally, isolated rats showed a significant increase in malondialdehyde (MDA) [8] which is the end product of lipid peroxidation and biomarker of free radicals. It is known that long-term isolation induces changes in the activities of endogenous antioxidants. For example, the mentioned stressor changes hippocampal copper, zinc-superoxide dismutase (CuZnSOD) and catalase (CAT) activity [9], as well as hepatic glutathione-dependent defense system [10]. However, very little is known about the ability of natural exogenous antioxidants to reduce increased oxidative stress and decreased antioxidant capacity caused by psychosocial stressors. The doses of honey used in this study have been reported in previous studies [11,12], as well as that it is considered good natural dietary source of antioxidants [13]. It was confirmed that honey contains vitamins including vitamin C as an antioxidant that can capture free radicals [14]. The literature data confirm that the identified compounds in linden honey include monoterpenes (hydrocarbons, ethers, aldehydes, acids, and bifunctional derivatives), isoprenoids, aromatic compounds (phenylpropanoids, phenols) and products degraded from fatty acids to alkaloids [15]. In addition, the results of Gašić and colleagues [16] have shown that samples of honey from Vojvodina and Zlatibor region were distinguished from the honey varieties from the rest of Serbia due to the presence of dicaffeoylquinic acid, ellagic acid, caffeic acid phenethyl ester, and chlorogenic acid. For this reason, in this study we examined the antioxidant status of the blood of psychosocially stressed rats treated with linden honey from the area of Fruška Gora. In addition, the property of linden honey to inhibit lipid peroxidation, as well as to capture hydroxyl and superoxide anion radicals was examined by the "Laboratory for testing natural resources and pharmacologically and biologically active compounds", Novi Sad. Because of the direct involvement of oxidative stress in anxiety-like and depressive-like behavior in stress conditions, detecting the changes of activity of the antioxidant enzymes, as well as concentration of MDA in psychosocially stressed rats treated with linden honey may by very important in the research of the role of linden honey in maintaining antioxidant capacity in pathological conditions.

In this study we examined the activity of the antioxidant enzymes CAT and GPx, and concentrations of MDA in the blood of chronically stressed rats treated with linden honey.

MATERIALS AND METHODS

Animals

In the experiment adult male rats of Wistar strain, 11-week-old and weighing 300-350 g were used. The animals were maintained under standard vivarium conditions in a temperature-controlled room (22±1.0 °C) and 12 h/12 h light/dark cycle, with

water and food *ad libitum*. Handling of animals was done quickly and carefully to avoid unnecessary discomfort. The rats were habituated to handling and treated as ethically as possible, according to the recommendations of the Ethical Committee of the "Vinča" Institute of Nuclear Sciences, Belgrade, Serbia, which follows the guidelines of the Serbian Society for the Use of Animals in Research and Education. The Committee has approved the planned experiment (06/2023).

Experiment design

Ten (10) animals were randomly divided into two groups. First group, individually housed group (IH group, n=5) of animals was subjected to social isolation with a single animal per cage for 28 days. The visual and olfactory communication among the isolated rats was reduced to a minimal level.

Second group, individually housed group + honey group (IH+HONEY group, n=5), consisted of individually housed animals given 1,5 ml/kg honey orally for 28 days.

The linden honey used in this experiment was collected in 2022 by dr Vojislav Stanić in an apiary located in Vojvodina-Fruška Gora region and stored at room temperature in dark until the experiment. The honey was mixed with distilled water in a ratio of 1:1. The doses of honey used in this study have been reported by Abdulmajeed et al. [11] and Halawa et al. [12].

After the described treatment, blood samples were collected from both groups. Blood was sampled from the rat's tail (into heparinized tubes). After blood collection, the were returned to their cages alive.

Determination of malondialdehyde (MDA), catalase (CAT) activity and glutathione peroxidase (GPx) activity

Determination of MDA level was performed using the spectrophotometric method previously described by Siddique et al. [17]. All sample values were read on the spectrophotometer at 586 nm. Malondialdehyde concentration was expressed as μmol/ml of plasma.

CAT activity was determined by the method of Beutler [18]. All samples were read on the spectrophotometer at 230 nm. The final result for CAT activity was expressed as units per milligram of hemoglobin (U/mg Hb).

Determination of activity was performed using methods previously described by Agergaard and Jensen [19]. All samples were read on spectrophotometer at 366 nm. The final result for GPx activity was expressed as units per gram of hemoglobin (U/g Hb).

Data analysis

The data are presented as means ± S.E.M. The differences in the activity of enzymes (CAT and GPx) between IH group and IH+HONEY group were analyzed by t-test.

Statistical analysis was carried out using the SigmaPlot 10.0 with SigmaStat integration. The statistical significance was accepted at $p<0.05$.

RESULTS

The honey treatment in individually housed animals (IH+HONEY group) resulted in significantly higher CAT activity (26%) (Figure 1a), and GPx activity (31%) (Figure 1b) compared with individually housed animals (IH group). In addition, socially isolated animals treated with honey (IH+HONEY group) had a 35% lower concentration of MDA (Figure 2) compared with individually housed animals (IH group).

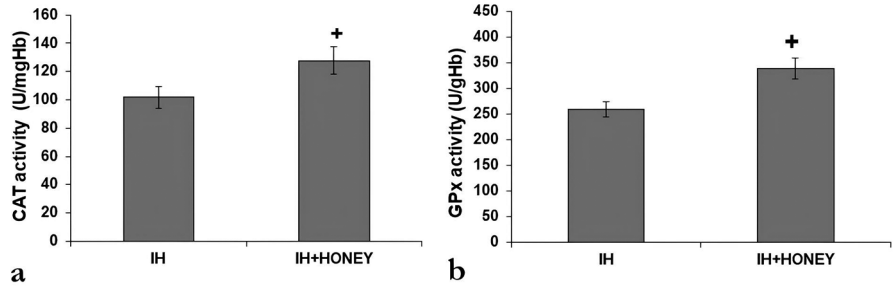

Figure 1. The effects of linden honey on the enzyme activities of catalase (CAT) **[a] and** glutathione peroxidase (GPx) **[b]** in the blood of individually housed animals. The values are means ± S.E.M. of 10 rats. Statistical significance: +$p<0.05$ IH+HONEY group *vs.* IH group (t-test). The final result for CAT activity was expressed as units per milligram of hemoglobin (U/mg Hb) and the final result for GPx activity was expressed as units per gram of hemoglobin (U/g Hb).

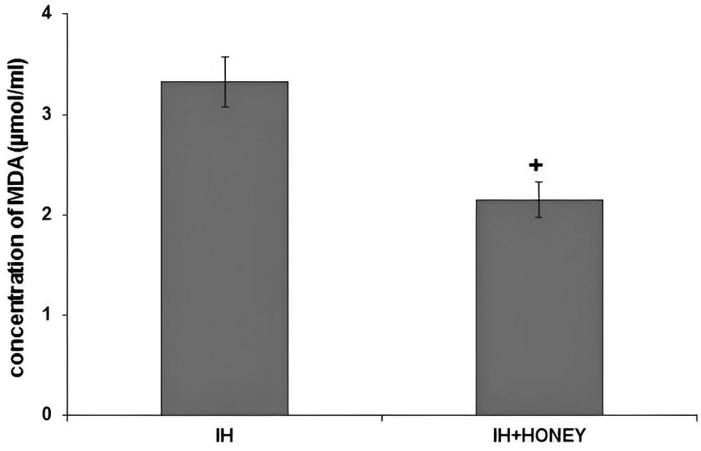

Figure 2. The effects of linden honey on the concentrations of malondialdehyde (MDA) in blood of individually housed animals. The values are means ± S.E.M. of 10 rats. Statistical significance: +$p<0.05$ IH+HONEY group *vs.* IH group (t-test). The final result for the concentration of MDA was expressed as µmol/ml of plasma.

DISCUSSION

In our previous study we confirmed that social isolation significantly increases the concentration of MDA in the left adrenal medulla [20]. In addition, the literature data confirm that social isolation influence the occurrence of oxidative stress [21]. Also, Bove et al. [7] observed that isolated rats showed significant elevations in ROS and increased concentration of MDA in the liver, which confirms that social isolation triggers oxidative stress. It is known that the increased lipid peroxidation in isolated rats can be a consequence of the dysfunction of antioxidant enzymes, which confirms that the antioxidant defense system is insufficient to keep the levels of ROS low [7]. Several studies have confirmed that honey supplementation has therapeutic effects under pathological conditions [22,23]. In the present study we observed that in response to linden honey antioxidant enzymes underwent modulation. We found that socially isolated animals treated with linden honey showed increased CAT and GPx activity compared with individually housed animals. It is known that CAT and GPx protect tissues from highly reactive hydroxyl radical derived from H_2O_2 [24]. Our results are in accordance with the reports of Oyefuga and colleagues [25] who found that supplementation with honey significantly decreased the lipid peroxidation and increased antioxidant enzymes activity in the brain. The results presented in this study indicated that linden honey increases antioxidant activity and that it is possibly involved in the protection from oxidative stress. The literature data have shown that the effect of honey to prevent of oxidative damage might be due to its phenolic and non-phenolic antioxidant content [26]. It is known that phenols, polyphenols, flavonoids, catalase, ascorbic acid and phenolic acids in honey have antioxidant activities [27,28]. "The laboratory for testing natural resources and pharmacologically and biologically active compounds" Novi Sad confirmed that the linden honey used in our experiment has the ability to capture hydroxyl radicals IC_{50}=1.116 mg/ml ± 0.157.

The ability of honey to neutralize hydroxyl radicals was tested using the spectrophotometric method previously described by Pintać et al. [29]. Recent studies have confirmed that honey contains CAT, as well as that the antioxidant defense mechanism of honey can be attributed to CAT [30]. Also, as reported by Gheldoff and Engeseth [31], honey contains selenium. Costa-Silva et al. [32] found that increased GPx might be due to the presence of selenium in honey which is a cofactor for GPx activity. In addition, Halawa et al. [12] have suggested that honey exerts its protective role through its antioxidant mechanism and through restoring glutathione activity in rats. Namely, honey is a good natural source of sulfhydryl (SH) groups. Also, it is known that the most important endogenous antioxidant is glutathione, as well as that the SH group of glutathione is important as a direct scavenger of ROS [33]. Abdulmajeed et al. [11] found that administration of honey improves antioxidant activities as shown by increased brain SOD and glutathione-dependent defense system. An important result of this study is that honey treatment in animals exposed to social isolation significantly decreases concentration of MDA in blood. Decreased levels of

MDA in individually housed animals treated with linden honey, found in our study, confirm that honey is involved in the reduction of oxidative stress in psychosocial stress conditions. The reduced MDA levels are probably the consequence of increased CAT and GPx activity. In addition, the "Laboratory for testing natural resources and pharmacologically and biologically active compounds" Novi Sad, confirmed that the linden honey we used in our experiment has the ability to inhibit lipid peroxidation (24.5% ± 3.2 at the concentration of honey 0.179 mg/ml; spectrophotometric method previously described by Pintać et al. [29]). Our results confirm that linden honey increases the antioxidant activity and can protect from oxidative stress. Also, the latest results confirmed that linden honey is a source of numerous vitamins, minerals, organic and inorganic acids [34]. For example, Farkas et al. [34] showed that linden honey samples displayed significantly higher levels of caffeic, chlorogenic, ferulic and *p*-coumaric acids compared to acacia and goldenrod honeys. In addition, apigenin, chrysin and galangin were measured in both Hungarian and Serbian linden honey samples [34]. These results suggest that the geographical origin can strongly influence the composition of characteristic compounds which can contribute to the health benefits of honey [34].

CONCLUSIONS

In the present study we found that treatment with linden honey in socially isolated animals significantly increased the enzyme activities of CAT and GPx, and significantly decreased the concentration of MDA. The modulation of CAT and GPx activities in socially isolated animals treated with linden honey may be very important for understanding the role of honey in the capacity of the antioxidant defense system to increase and maintain its stability in psychosocial stress conditions. The understanding of the influence of honey on redox balance regulation, might contribute to the development of therapeutic strategies, aiming at reduction of oxidative stress in pathological conditions.

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Authors' contributions

LjG participated in the experiment, in the statistical analysis, designed and wrote the manuscript. VS participated in the experiment, participated to draft the statistical analysis, helped to draft the manuscript. VS helped to draft the statistical analysis. DJ participated in the experiment. SP helped to draft the manuscript. BB participated in the analysis of the results. SBP helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Statement

The Ethical Committee for the care and use of laboratory animals of the Institute of Nuclear Sciences "Vinča", Belgrade, Serbia has approved the planned experiment. The opinion (06/2023) of the Ethical Committee is that the use of laboratory animals is ethically justified for conducting the experiment. The Ethical Committee of the "Vinča" Institute of Nuclear Sciences "Vinča", Belgrade, Serbia, follows the guidelines of the Serbian Society for the Use of Animals in Research and Education.

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ANTIOKSIDATIVNI STATUS U KRVI PACOVA IZLAGANIH PSIHOSOCIJALNOM STRESU TRETIRANIH MEDOM

Ljubica GAVRILOVIĆ, Vesna STOJILJKOVIĆ, Vojislav STANIĆ, Dragoljub JOVANOVIĆ, Snežana PEJIĆ, Branka BOROVIĆ, Snežana B. PAJOVIĆ

Lipov med predstavlja jedinstvenu vrstu meda cenjenu zbog svoje nutritivne prednosti, specifičnog ukusa i mirisa. Fenoli, polifenoli, flavonoidi, askorbinska kiselina i fenolne kiseline u medu imaju antioksidativno dejstvo. Ovo istraživanje je imalo za cilj da ispita uticaj lipovog meda na aktivnosti antioksidativnih enzima katalaze (CAT) i glutation peroksidaze (GPx), kao i na koncentraciju malondialdehida (MDA) kod individualno smeštenih životinja. Ispitivani parametri su kvantifikovani spektrofotometrijskim metodama. Ustanovili smo da tretman lipovim medom, kod socijalno izolovanih životinja, značajno povećava aktivnost enzima CAT i GPx, ali da značajno smanjuje koncentraciju MDA. Modulacija aktivnosti CAT i GPx u krvi socijalno izolovanih životinja tretiranih lipovim medom, može biti veoma važna za razumevanje uloge meda u sposobnosti antioksidativnog odbrambenog sistema da poveća i održi njegovu stabilnost u uslovima psihosocijalnog stresa. Naši rezultati mogu biti važni u biomedicinskim istraživanjima za razumevanje uloge meda u smanjenju oksidativnog stresa.