Protective effect of honey on learning and memory impairment, depression and neurodegeneration induced by chronic unpredictable mild stress

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ABSTRACT

Introduction: Chronic stress, which has been prevalent in human life, induce structural changes in the hippocampus. Depression and impairment of memory are serious comorbidities of chronic stress. In this study, we evaluated the impact of an Iranian honey pretreatment on memory deficit, depression and the hippocampal neuronal loss in the chronic unpredictable mild stress (CUMS) model.

Methods: Adult male Wistar rats were divided into the control groups that received water or honey (0.2 or 2g/kg) and CUMS groups that subjected different, randomly and unpredictable mild stressors for 4 weeks. Ten days before starting the CUMS procedures, the animals received honey (0.2 or 2g/kg, daily, orally), which was continued until sacrificing. Morris water maze and sucrose performance tests were used to evaluate the spatial learning and memory and depressive-like behavior in the animals respectively. Hippocampus and whole brain samples were collected for further biochemical and histological analysis.

Results: Honey reversed the depression-like behavior and ameliorated the spatial memory deficit induced by CUMS. Also, honey decreased cell death in the hippocampus and reduced the malondialdehyde level in treated animals.

Conclusion: These results revealed that honey diminished learning and memory deficits and depression in chronic stress conditions.

Keywords:
Honey
Chronic mild stress
Learning and memory
Neuronal loss
Oxidative stress

Introduction

Stress is a biological and protective response to a variety of physiological or psychological stimulations. An increase in glucocorticoid level, as well as noradrenaline, are the first reply of the system (Lindau et al., 2016). Chronic stress, which has been more prevalent in daily human life, can lead to some serious health problems. Several studies have revealed the effect of chronic stress on the central nervous system, including structural changes in the hippocampus (Kim et al., 2015; Surget et al., 2011; Yaribeygi et al., 2017). This part of the brain contributes as a key structural system to spatial learning and memory (Devan et al., 1996). Additionally, chronic stress can cause depression, which is severely associated with learning and memory dysfunction (Wu et al., 2017). Intense metabolic activities in neuronal cells particularly have made the brain vulnerable to oxidative stress. Some studies showed that the
hippocampus especially is sensitive to oxidative stress (Braak and Braak, 1991; Noseworthy and Bray, 1998; van Velzen et al., 2017; Wang and Michaelis, 2010). Stress is responsible for the generation of free radicals and chronic inflammation, which is associated with neuronal loss in the hippocampus. Neurodegeneration correlated with learning and memory impairment and Alzheimer’s disease (Chen et al., 2019; Farajdokht et al., 2012; Mohammadi et al., 2014; Srivistava and Kumar, 2015; Takuma et al., 2012). Meanwhile, oxidative stress in the hippocampus is involved in depression (Souza-Monteiro et al., 2019). Diet, including antioxidant ingredients, may attenuate oxidative damages (Kolosova et al., 2006). Besides, the antioxidant can improve cognitive performance, including learning and memory (Baluchnejadmojarad et al., 2012; dos Santos Junior et al., 2005; Ganji et al., 2017; Ikeda-Douglas et al., 2004). Honey, a natural food product, is a valuable source of antioxidants (Chepulis et al., 2009; Gheldof and Engeseth, 2002; Moloudian et al., 2018). Honey in postmenopausal women improved memory performance (Othman et al., 2011). Short and long-term consumption of honey ameliorate the lipid peroxidation and free radicals mediated molecular destruction in the brain tissue (Oyefuga et al., 2012). A previous experimental study showed that antioxidant activity in the brain and memory function improved by honey administration in lead- induced cognitive problems (Abdulmajeed et al., 2016). Also, clinical and experimental investigations showed that honey could improve the symptoms and biomarkers of depression (Al-Rahbi et al., 2014a; Ali and Hendawy, 2018). Tuvalang honey protects memory decline and depressive- like behavior induced by noise stress (Azman et al., 2015; Azman et al., 2016). However, Nigerian honey had no significant effect on spatial working memory (Akanmu et al., 2011). Despite the antioxidant capacity of all types of honey, the botanical origin, climate and environmental factors have a great influence on its antioxidant potency (Maurya et al., 2014; Wang et al., 2016). Recent studies have also shown the changes in the antioxidant capacity of Iranian honey from different flora (Mahmoodi-Khaledi et al., 2017; Moloudian et al., 2018). There are few studies on the impact of Iranian honey on neurocognitive disorders (Akouchekian et al., 2018; Jivad et al., 2015). To our knowledge, there is no study regarding the effect of honey on chronic stress conditions, which is very common in various societies. It has been reported that chronic unpredictable mild stress (CUMS) induces learning and memory impairment and also depression (Liu et al., 2014; Willner, 2017; Wu et al., 2017). Meanwhile, it has been shown that CUMS induces a neuronal loss in the hippocampus (Bakhtiarzadeh et al., 2018; Surget et al., 2011; Xue et al., 2017). In the current study, we tried to evaluate the effect of Iranian honey on learning and memory impairment as well as depression, which were induced by CUMS.

**Material and methods**

**Subject and experimental group**

Sixty adults’ male Wistar rats, weighing 230-260g (purchased from Isfahan University of Medical Sciences, School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran) were used in this study. Animals maintained in groups of four per cage under 12h light/ dark cycles with free access to food and water. All experiments were executed in compliance with the NIH guidelines for the care and use of laboratory animals (National Institutes of Health Publication No. 85-23, revised 2010). Procedures were approved by Isfahan University of Medical Sciences Committee of Ethics in Research (IR.MUI.RESEARCH.REC.1398.300). Animals randomly divided into the following groups: control (1, 2 and 3) groups: animals that received water (as a vehicle) or honey (0.2 or 2g/kg/day- orally) until the euthanasia after 46 days of treatment. CUMS (4, 5 and 6) groups: animals that received water or honey (0.2 or 2g/kg/day- orally) from 10 days before the onset of the CUMS procedure, which was lasted for 28 consecutive days and continued until the euthanasia (Abdulmajeed et al., 2016; Al-Rahbi et al., 2014b; Arabmoazzen and Sarkaki, 2015; Najafi et al., 2011). The animals were decapitated after performing the Morris water maze (MVM) test and sucrose performance test (SPT). Hippocampus and whole brain samples were collected for further biochemical and histological analysis, respectively. Adrenal glands were gathered for further analysis of weight changes in different groups (Figure 1).

**Preparation of honey for administration**

Honey was obtained from Dehdar- Taleqan, Alborz province. Regarding the previous study (Guler et al., 2007), the physiochemical properties including proline,
free acidity and electrical conductivity of the honey were measured according to the national Iranian standard No. 92 (evaluated by Hourtash laboratory). Honey (0.2 or 2g/kg), freshly diluted in water was administrated by gavage ones a day for 46 days in different experimental groups (Abdulmajeed et al., 2016; Al-Rahbi et al., 2014b; Arabmoazzen and Sarkaki, 2015; Najafi et al., 2011).

CUMS procedure

Rats in CUMS groups were subjected to different mild stressors for 28 days (Borges Filho et al., 2016; Grippo et al., 2005; Liu et al., 2014; Wu et al., 2017). The procedure of CUMS was performed according to previous studies with some modifications (Borges Filho et al., 2016; Grippo et al., 2005; Liu et al., 2014; Wu et al., 2017). In brief, the procedure consisted of the following stressors: 1- water deprivation (10-12h); 2- food deprivation (18h); 3- overnight illumination; 4- cage tilt 40° (4h); 5- wet bedding (18h, 300ml of water was added to 300g sawdust bedding); 6- clipping the tail (the upper 1/3 of the tail, 1min) and 7- physically restraint (45min). In each day, only one stressor was applied to the animals. The stress sequence was changed every week. For this manner, these stressors were scheduled over a one-week period and repeated randomly throughout 4-week of the experiment. Stressors were applied randomly and at any time of the day to be completely unpredictable.

Sucrose performance test

The SPT is a common test to identify the depression in the animals (Belovicova et al., 2017). This test was accomplished after 28 days of exposure to CUMS. For the adaptation of the animals, before the test, rats were treated with sucrose solution (1% w/v), two bottles containing sucrose solution were placed in each cage for 24h. On the second day, one bottle of sucrose solution was replaced with tap water. After adaptation, animals were deprived of food and water for 24h. On the test day, a water bottle and a sucrose solution bottle were placed in each cage for each animal. Rats were free to access the water or sucrose. Each bottle contained a 100ml solution. The volumes of consumed sucrose solution and water in 24h were recorded. The sucrose preference was calculated as: the sucrose preference (%)= sucrose consumption/ (sucrose consumption+ water consumption) (Liu et al., 2014).

Morris water maze test

To study the effect of honey supplementation on spatial learning and memory, the MVM test was performed at 29th day of the experiment. The procedure was designed as described previously with some modifications (Liu et al., 2014; Morris, 2015; Sadeghi et al., 2017; Wu et al., 2017). In brief, the cylindrical tank, which was 150cm in diameter and 60cm in height, was filled with water (24±1°C) to a depth of 35cm and surrounded by
a variety of extra-maze cues. The test consisted of four consecutive acquisition sessions followed by memory retention (probe trial- day 5). The pool was divided into four quadrants and the round platform was placed in the center of the South- East (target) quadrant 3cm underneath the water. Rats were placed into the pool facing the wall and were given 90s to find the hidden platform based on the surrounded cues during each trial. If the rat failed to locate the platform, it was guided to find the platform. The animals were allowed to stay on the platform for 30s. Four acquisition trials were performed in each day. Each animal participated in 16 trials within four days. The time that rats spent to find the hidden platform was measured as the latency. In each trial, the latency and distance of the travel in orbit were tracked by the video camera positioned above the center of the pool and the tracking software was Neuro Vision. To evaluate the memory retention, in a probe trial in which the platform was removed, the rat was placed in the pool for 90s and the time that the animal spent in the target quadrant was recorded.

**Tissue sampling**

Hippocampi and adrenal glands were collected from the deep anesthetized and decapitated animals. Hippocampus samples were immediately frozen in liquid nitrogen and then stored at -80°C for further biochemical analysis. The adrenal glands were removed and weighed. For histological analysis, rats were transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde.

**Nissl staining and histological analysis**

A series of 7µm thick coronal sections were obtained from the paraffin- embedded brain samples. Sections from areas between Bregma -2.76 to Bregma 3.24 were collected for the current study (Paxinos and Watson, 2006). Nissl staining was performed to investigate the neuronal density in the CA1 and CA3 regions of the hippocampus. The Nissl staining procedure was explained previously (Yazdi et al., 2020). The CA1 and CA3 regions of the hippocampus were identified under Olympus BX- 51 microscope and Digital images (200x magnification) were captured by DP72 camera. Cell density in the hippocampus was quantified by counting all the cells in each microscopic field. Image J software version 1.5i was used for cell counting. Three slides per animal and three animals from each group were used for the analysis.

**Biochemical analysis**

For analysing the malondialdehyde (MDA) and total antioxidant capacity (TAC) in the hippocampus, the same weight of the tissues in different experimental groups were collected. For MDA assay, the tissues were homogenized with PBS and MDA lysis buffer then centrifuged at 6000g for 10min and the supernatants were collected. A TBA solution was added and the mixture heated for 60min at 95°C. The absorbance of the supernatant was read at 532nm using the spectrophotometer. For TAC assay, the tissues were homogenized by PBS and centrifuged at 12000g for 15min. The supernatant was mixed with TAC buffer, Cu2+ and chromogen solutions. The plate was incubated for 50min at room temperature. The absorbance of the specimens was read at 450nm. The MDA and TAC concentrations were determined by the standard curve and reported as nmol/g tissue. All the procedures were under standard conditions, according to the Kiazist´s company instructions on the reagent kits.

**Statistical analysis**

GraphPad Prism 5 software was used for statistical analysis. Data are presented as mean±SEM. The normal distribution of the data was determined by the Kolmogorov-Smirnov test. To analyze the MVM test, the moving distance and escape latency of 4 trials per day per animal was calculated by repeated measures analysis of the variance (ANOVA) followed by Bonferroni’s multiple comparisons post-test. The significant changes between the experimental groups in other tests were determined by one-way ANOVA followed by the Tukey post-test. The P values of less than 0.05 were considered statistically significant.

**Results**

**Honey physiochemical properties**

The honey contained proline, 569.4mg 100g-1 (standard: <180), free acidity, 13.2 meq kg-1 (standard: >40) and electrical conductivity, 0.37mS cm-1 (standard: >0.8).

_Honey supplementation prevented the adrenal weight_
increase in CUMS rats

The weight of adrenal gland increases in conditions of chronic stress (Ulrich-Lai et al., 2006). To clarify the impact of CUMS and honey supplementation on the adrenal gland, the weight of the adrenal was measured. One-way ANOVA showed that CUMS significantly increased the adrenal weight \( F(5,38)=16.54, \ P<0.0001 \). Tukey post-test showed that CUMS increased the adrenal weight compared to the control group \( P<0.001 \). It also showed that the adrenal weight in the honey treated groups at both doses decreased compared to the CUMS group \( P<0.001 \). However, honey in the control groups had no significant effect on adrenal weight (Figure 2).

Honey supplementation reversed the depression-like behavior of CUMS rats

The SPT is used to evaluate the depressive-like behavior in animals (Belovicova et al., 2017). One-way ANOVA indicated a main significant change in the sucrose performance \( F(5,54)=27.42, \ P<0.0001 \). Tukey post-test showed that after 4 weeks of CUMS exposure, the percentage of sucrose performance was significantly decreased in the stressed rats, compared to the control \( P<0.001 \), Figure 3). Tukey post-test also showed that honey alone at both doses had no impact on sucrose intake. Chronic and daily treatment with honey, significantly increased the amount of sucrose performance compared to the stressed animals \( P<0.001 \). These data showed that honey supplementation could restore the depression, which is induced by chronic stress.

Honey supplementation improved spatial learning and memory impairment in CUMS rats

Figures 4A and B show the moving distance of the animals in the tank and escape latency, respectively. In all groups, the trend of learning became progressively shorter across training sessions. In the spatial cognitive ability test, training days in moving distance \( F(3,162)=87.18, \ P<0.0001 \) and escape latency \( F(3,162)=70.85, \ P<0.0001 \) parameters, showed significant effect on decreasing learning deficits induced by CUMS. Also, treatment with honey in moving distance \( F(5,54)=20.12, \ P<0.0001 \) and escape latency \( F(5,54)=8.26, \ P<0.0001 \) parameters, showed significant impact on attenuating the learning problems induced by CUMS. However, there was no significant effect of training days and treatment factors interaction on moving distance \( F(15,162)=1.29, \ P=0.2111 \) and escape latency \( F(15,162)=1.13, \ P=0.33 \) parameters. Also, Bonferroni multiple comparison showed that the animals in the CUMS group had a significantly longer moving distance and escape latency compared to the control groups (moving distance: \( P<0.05 \) at day 1 and \( P<0.001 \) at days 2, 3 and 4; escape latency: \( P<0.01 \) and \( P<0.05 \) at second and third days, respectively), which is due to the learning impairment induced by CUMS. Data analysis showed a significant decrease in moving distance in honey treated group (dose 2g/kg) in all the training days \( P<0.01 \) at day 1 and \( P<0.001 \) at days 2, 3 and 4). Also, on the third day honey, at a lower dose significantly decreased the moving distance \( P<0.01 \), Figure 4A).

Meanwhile, latency to find the hidden platform significantly reduced by honey administration (dose 2g/
FIGURE 4. Effect of honey administration on spatial learning impairment induced by CUMS. Morris water maze test was done to evaluate the impact of honey on spatial learning performance. (A) Average travel distance and (B) Escape latency were compared in the different experimental groups. Ctr: control group, CUMS: chronic unpredictable mild stress group. Data are expressed as means±SEM and calculated by repeated measures analysis of the variance (ANOVA) followed by Bonferroni’s multiple comparisons posttest, *P<0.05, **P<0.01, ***P<0.001.

FIGURE 5. Effect of honey treatment on memory improvement in CUMS induced animals. (A) Animal travel orbit in the probe test (B) Analysis of the probe test data indicated that honey at the higher dose (2g/kg) significantly increased the time spent in the target quadrant compared to the CUMS group. Ctr: control group, CUMS: chronic unpredictable mild stress group. Data are expressed as mean±SEM and were analyzed by one-way ANOVA followed by Tukey posttest, *P<0.05.
kg) at second \((P<0.05)\), third \((P<0.01)\) and forth \((P<0.05)\) days of the training trial (Figure 4B). To evaluate the spatial memory performance, the platform was removed at day 5 of the test, and the rat was placed in the pool for 90s. The time spent in the target quadrant was recorded for further analysis. Our results showed a significant decrease \((P<0.05)\) in time spent in the target quadrant in stressed rats that is a result of memory deficit induced by CUMS. Conversely, honey supplementation at dose 2g/kg significantly enhanced \((P<0.05)\) the time spent in removed platform quadrant compared to the CUMS group. Data indicate that honey prevented memory impairment in stressed animals (Figure 5).

**Honey supplementation ameliorated the neuronal loss in the hippocampus induced by CUMS**

Neuronal damage occurs in the hippocampus under chronic stress (Bakhtiarzadeh et al., 2018; Surget et al., 2011; Xue et al., 2017). The number of neurons in CA1 and CA3 of the hippocampus was counted in the Nissl stained sections. Consistent with previous reports, one-way ANOVA, \([\text{CA1}}: F(5,42)= 4.85, P=0.001; \text{CA3}}: F(5,42)= 10.57, P<0.0001\] with Tukey post-test showed a significant decrease in cell number in both CA1 \((P<0.001)\) and CA3 \((P<0.001)\) regions of the hippocampus in CUMS induced animals compared to the control group. Tukey post-test also showed that neuronal death significantly attenuated in the honey treated animals at dose 2g/kg in both CA1 and CA3 regions \((P<0.01 \text{ in CA1 and } P<0.001 \text{ in CA3})\), compared to the CUMS group. However, honey at dose 0.2g/kg had a protective effect just in CA1 region \((P<0.05, \text{ Figure } 6)\).

**Honey supplementation decreased the MDA level in the hippocampus of the stressed animals but had no effect on TAC level**

Evaluation of the level of MDA and TAC in the hippocampus showed an increase in MDA and decrease in TAC level in stressed animals compared to control \((P<0.05)\). Honey treatment at both doses reduced MDA level in stressed group to that of the control group; however, the changes were not significant compared to stress group (Figure 7A). Meanwhile, honey at dose 0.2g/kg in stressed treated group increased the TAC level in the hippocampus while it wasn’t significantly (Figure 7B).

**Discussion**

Previous reports have shown the relationship between chronic stress and hippocampal atrophy (Krugers et al., 2010; Price and Duman, 2020; Tyrtyshnaia et al., 2019). Various mechanisms are behind the hippocampal neuronal loss induced by CUMS, such as oxidative stress and related inflammation, insulin signaling pathway, norepinephrine, some other hormones and various neurotransmitters (Espinoza-Garcia et al., 2017; Finlay et al., 1995; Latt et al., 2018; Mehta et al., 2017b; Mora et al., 2012; Natarajan et al., 2017). In addition, the hypothalamic–pituitary–adrenal axis is the other pathway that is highly activated by the chronic stress (Mohammadi et al., 2014; Surget et al., 2011; Yang et al., 2017). Over-exposure of the glucocorticoids in the animals has been implicated in the hippocampal neuronal loss (Latt et al., 2018; MacMaster and Kusumakar, 2004). In the present study, behavioral, histological and biochemical approaches were applied to investigate the protective effect of honey on CUMS induced memory deficits and depression. To evaluate the induction of chronic stress in the animals, the adrenal glands were weighed. Previous studies showed that adrenal gland weight increased under chronic stress (Rostamkhani et al., 2012; Ulrich-Lai et al., 2006). Also, our data demonstrated that adrenal weight in stressed rats increased significantly compared to the control group. However, honey protected the adrenal gland from weight elevation. This result might be in line with the previous study, which showed that administration of antioxidants after the establishment of the stress in animals decreased the adrenal weight (Airapetians et al., 1986). However, further studies are needed to realize the exact underlying mechanism. One of the comorbidities of chronic stress is depression, which can be investigated by the SPT. Reduced sweet solution intake is the index of depression in this test. Meanwhile, CUMS procedure has long been used as a model of depression (Liu et al., 2014; Stemmelin et al., 2010; Willner, 2017; Willner, 1997; Wu et al., 2017). We performed the SPT after four weeks induction of a various range of stress to the animals. Our results demonstrated that honey supplementation at both doses significantly attenuated the depression-like behavior. Previous studies also revealed the antidepressant efficiency of the honey administration (Azman et al., 2015; Sheas et al., 2019). It is reported that chronic
FIGURE 6. Effect of honey administration on neuronal loss in the hippocampus. (A) Nissl staining assessed the amount of cell death in different experimental groups in CA1 and CA3 regions of the hippocampus. (B, C) Quantitative analysis of cell density. Honey at high dose (2g/kg) significantly decreased cell loss in both CA1 and CA3 regions. However, a low dose of honey administration significantly attenuated the neuronal loss in CA1. Ctr: control group, CUMS: chronic unpredictable mild stress group, H: honey. Data are expressed as mean±SEM and were analyzed by one-way ANOVA followed by Tukey posttest. Scale bar: 50μm, n= 3; *P<0.05, **P<0.01, ***P<0.001.
stress elevated plasma levels of corticotropin-releasing and adrenocorticotropin hormones, as well as interleukin-1β, tumor necrosis factor-α, interleukin-6, kynurenine and decreased serotonin level in the hippocampus. Meanwhile, chronic stress increased indoleamine-2,3-dioxygenase and caspase 3 as well as 9 activity. However, brain derived neurotrophic factor, nerve growth factor level, and Na+/K+ ATPase activity strongly decreased in the hippocampus of the depressed animals, which was induced by CUMS. Treatment with chrysin, as a flavonoid which is found in honey, modified these alterations occasioned by chronic stress (Borges Filho et al., 2016; Jesse et al., 2015). Also, a recent report has shown pinocembrin, a flavonoid isolated from honey, mitigated depressive-like behaviors via a decrease in the concentration of reactive oxygen species and MDA, but increased the superoxide dismutase activity, suggesting that it could protect oxidative stress in CUMS mice. Also, pinocembrin inhibited cell apoptosis and regulated inflammatory factors expression such as interleukin-10, transforming growth factor-β in the hippocampus of CUMS animals. Moreover, CUMS inhibited the Nrf2/HO-1 signaling pathway and activated the phosphorylation of NF-κB which were converted by pinocembrin (Wang et al., 2020). Therefore, the antidepressant-like effect of honey administration in the current study might be due to the regulation of above mentioned neurochemical factors. More analysis is needed to clarify the exact underlying mechanism.

Many studies displayed the negative impact of chronic stress on learning and memory. Morris water maze test is one of the most widely used tests as hippocampal-dependent spatial learning and memory performance (Liu et al., 2014; Wu et al., 2017). In the present study, we found that impairment in both learning and memory performance induced by CUMS was significantly reversed by honey supplementation, which was consistent with previous studies (Abdulmajeed et al., 2016; Arshad et al., 2020; Azman et al., 2015; Azman et al., 2016; Othman et al., 2011). Generally, the evidence indicated that honey supplementation attenuated depression and learning and memory comorbidities induced by CUMS. It has been reported that the hippocampal neurodegeneration is highly related to depression and some cognitive disorders such as learning and memory impairment (Lee et al., 2002; Sapolsky, 2000; Setti et al., 2017). Meanwhile, there is growing evidence that different antidepressant drugs make proliferative and neurogenesis changes in the hippocampus regions (Stemmelin et al., 2008). To characterize the underlying mechanism of the positive effect of honey supplementation, at the next step, we investigated the cell density in the hippocampus. Previous experimental studies have revealed the neurodegeneration in the hippocampus of the animals, which were under chronic stress (Al-Rahbi et al., 2014c;
Mehrpouya et al., 2015; Surget et al., 2011; Takuma et al., 2012). Our results also showed the neurodegeneration in both CA1 and CA3 regions of the hippocampus in the CUMS group compared to control. Neuroprotection is explained as any activity that conserves neuronal function and structure (Casson et al., 2012). Neuronal loss significantly decreased in the hippocampus of the treated animals. It is reported that honey ingredients have a protective effect on neurons (Jafari Anarkooli et al., 2014; Saad et al., 2015; Takashima et al., 2019).

Also, an experiment has been suggested that honey has a positive effect at the neuronal morphometric level (Adli, 2018). In parallel with the previous studies, our results confirmed the neuroprotective effect of honey supplementation. Hippocampal damage induced by chronic stress also occurs due to mitochondrial dysfunction and apoptosis by activating ROS/JNK signaling pathway (Zhang et al., 2020). Effect of honey administration on these mechanisms should be investigated in the future. Meanwhile, oxidative stress pathway is one of the most important mechanisms that may lead to neuronal loss under chronic stress (Manoli et al., 2000; Salim, 2017; Schiavone et al., 2013; Xu et al., 2019; Zhang et al., 2020). In the next part of the study, we investigated the effect of honey supplementation on two oxidative stress markers, malondialdehyde and total antioxidant capacity. MDA is the most commonly used lipid marker of oxidative stress. The other common assay is the TAC, which represents changes in small molecules and protein antioxidant based capacity (Marrocco et al., 2017; Popović et al., 2019). Several studies showed the MDA and TAC changes in the hippocampus in chronic stress circumstances (Che et al., 2015; Hu et al., 2016; Jin et al., 2015; Mehta et al., 2017a). Our results also showed the increase and decrease of MDA and TAC in the hippocampus of the stressed rats, respectively. Honey at both doses attenuated lipid peroxidation by decreasing the MDA level of the hippocampus to that of the control group. Meanwhile, honey at dose 0.2g/kg in stressed treated group increased the TAC level in the hippocampus while it wasn’t significantly. Oxidative stress occurs due to the imbalance between reactive oxygen species formation and enzymatic or non-enzymatic antioxidants (Marrocco et al., 2017). The influence of the honey antioxidants on the oxidative stress pathway might also be through some different oxidative species or biomarkers. A previous study by Azman and colleague (2016) showed that honey reduced the brain oxidative stress through the effect on MDA, protein carbonyl and superoxide dismutasebut not via glutathione peroxidase, glutathione reductase, catalase and TAC.

According to the result of the current study, honey supplementation diminished learning and memory impairment and depression that might be mediated via its neuroprotective activity. It seems that the reduction in the neuronal loss might be due to the attenuation in lipid peroxidation induced by honey supplementation in the hippocampus. However, in the future, investigation of other biomarkers of the oxidative pathway, such as the enzymatic or non- enzymatic antioxidants, DNA/RNA damage markers, protein oxidation or nitration markers could help us to find more about the underlying mechanisms of the honey supplementation on neuroprotection.

**Conclusion**

In conclusion, learning and memory impairment and also depression which are two major comorbidities of the chronic stress, attenuated by Iranian honey supplementation. It seems that these findings are partly mediated via the honey neuroprotective activity in the hippocampus tissue of the stressed animals that might be due to a reduction in lipid peroxidation activity. Further studies are needed to find out the other related mechanisms of honey supplementation on the neuronal cells in the hippocampus.

**Acknowledgment**

This work was supported by the Isfahan University of Medical Sciences, Isfahan, Iran [grant number 198028].

**Conflict of interest**

None.

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