

# Agomelatine reduces brain, kidney and liver oxidative stress but increases plasma cytokine production in the rats with chronic mild stress-induced depression

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**Abstract** Agomelatine (AGOM) as an antidepressant acts both as a melatonin-receptor agonist and a selective serotonin-receptor antagonist. As a potent melatonin derived antioxidant, AGOM might modulate depression-induced lipid peroxidation and pro-inflammatory cytokines in brain, kidney and liver. The present study explores whether AGOM protects against experimental depression-induced brain, kidney and liver oxidative stress, and plasma cytokine production in rats with chronic mild stress (CMS)-induced depression. Thirty-six rats were divided into four groups. The first group was used as an untreated control. The second group received AGOM for 4 weeks. The third group was exposed to chronic mild stress (CMS) of 4 weeks for induction depression. The fourth group received 40 mg/kg AGOM and CMS for 4 weeks. Liver and kidney lipid peroxidation levels were high in the CMS group although they were low in AGOM treatments. AGOM and AGOM + CMS treatments increased the lowered glutathione peroxidase activity and reduced glutathione levels in brain, kidney and liver of CMS group.  $\beta$ -carotene, vitamin A and vitamin E concentrations in the brain, kidney and liver of the four groups were not changed by CMS and AGOM treatments. However, plasma TNF- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-4 levels were high in the CMS and AGOM group and their levels were further increased by the AGOM +

CMS treatment. In conclusions, AGOM induced protective effects against experimental depression-induced brain, kidney, and liver oxidative injuries through regulation of the glutathione concentrations and glutathione peroxidase activity. However, plasma cytokine productions were increased by the AGOM treatment.

**Keywords** Depression · Brain · Liver · Oxidative stress · Cytokine · Glutathione

## Abbreviations

AGOM	agomelatine
CMS	chronic mild stress
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
ROS	reactive oxygen species

## Introduction

Oxidative stress occurs in the body cells during physiological functions including phagocytosis and mitochondrial functions. Accumulation evidences indicated that reactive oxygen species (ROS) play an important role in the pathogenesis of many diseases, particularly in neurological and psychiatric diseases due to the high content of polyunsaturated fatty acids and oxygen consumption and poor antioxidant content of central nervous system (Halliwell 2006; Nazıroğlu 2007). If the oxidative stress is not controlled by antioxidants, it can induce neuron brain damages (Halliwell 2006; Nazıroğlu 2007). ROS are scavenged by enzymatic antioxidants including glutathione peroxidase (GSH-Px) (Nazıroğlu 2009) and non-enzymatic antioxidants, such as reduced glutathione (GSH), vitamin A, and vitamin E (Halliwell 2006). Depression is

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characterized by activation of the inflammatory response system with increased production of pro-cytokines (Andreasson et al. 2007; Cyranowski et al. 2007). It is well known that pro-inflammatory cytokines-induced excessive oxidative stress increase ROS production (Kahya et al. 2015). Oxidative stress increases also psychological stress, which accompanies severe depression (Nazıroğlu and Demirdaş 2015). Additional reports have indicated that lipid peroxidation and antioxidants, such as GSH, GSH-Px, and vitamin E, can act as biomarkers for depression's neuronal oxidative stress (Eren et al. 2007a, b; Kumar et al. 2015). Due to highly relationship between inflammation and intracellular oxidative stress, simultaneous investigation of anti-inflammatory and antioxidant activities of antidepressant is a worthwhile approach in the development of promising potent antidepressive agents.

Melatonin is a strong antioxidant (Espino et al. 2011) and the central nervous system is rich for G protein-coupled melatonin receptors namely MT1 and MT2 (Ekmekcioglu 2006; Tardito et al. 2012). Agomelatine (AGOM) as a melatonin analogue is a selective melatonergic MT1/MT2 dual agonist (Demyttenaere 2011). AGOM is also a selective serotonin-receptor antagonist at 5-HT<sub>2C</sub> receptors (Millan et al. 2003). Hence, AGOM has been licensed for the treatment of major depressive disorder in adults since 2009 in the European Union (Freiesleben and Furczyk 2015). AGOM is a unique antidepressant although there are concerns on AGOM-induced liver injury in human (Voican et al. 2014). Antioxidant role of AGOM was reported in different cells (Gupta and Sharma 2014; Karakus et al. 2013; Inanir et al. 2015) although the results are conflicting.

Chronic mild stress (CMS) as an animal model is a valuable model for investigation of pathophysiological changes related to major depression and the effects of antidepressant therapies (Nestler et al. 2002; Eren et al. 2007a, b). In experimental animals, the CMS model attempts to represent most of the abnormalities associated with human depressive disorders. For example, CMS was reported to induce depression, which could be reversed by antidepressant treatment (Willner 1997). The CMS has been using in experimental animal studies for investigating interactions of antioxidant and antidepressant (Eren et al. 2007a, b). The animal model was also preferred in a study examining the effect of AGOM (Dagyte et al. 2011).

AGOM is derived from strong antioxidant melatonin (Freiesleben and Furczyk 2015) and it may induce antioxidant and anti-inflammatory effects on tissue and blood in rat. In addition concern on its hepatotoxicity effect has not been clarified yet. Thus, we aimed to investigate whether or not AGOM has protective effects on the CMS-induced brain, liver, and kidney oxidative damage in rats. With stress and sucrose testing, the rats were given one synergic treatment of AGOM. We then assessed the brain, liver, kidney and plasma in terms of

antioxidant and cytokine levels resulting from the treatments. We will discuss the obtained results with the intention of explaining AGOM's mechanism of action on CMS-induced depression in brain, liver, kidney and blood injury.

## Materials and methods

### Animals and study groups

The study used 36 (10 Weeks) male Wistar albino rats weighing  $180 \pm 30$  g were obtained from the Animal Research Laboratory at Suleyman Demirel University (SDU). The animals were kept at the laboratory and were allowed to feed ad libitum on a commercially available rat chow. The study plan was approved by the Local Experimental Animal Ethical Committee of Suleyman Demirel University (SDU) (Protocol number: 19.02.2014–01). The animals were also maintained and used according to the Animal Welfare Act and the Guide for the Care and Use of Laboratory.

The rats were divided into four groups as follows: The control group ( $n = 8$ ) had no chronic mild stress (CMS) and was not supplemented. They received DMSO (0.1 ml) via intraperitoneal injection (IP) for 4 weeks. In the AGOM group ( $n = 10$ ), the rats received IP 40 mg/kg/day AGOM for 4 weeks of CMS (Karakus et al. 2013; Aygün et al. 2015). The AGOM was dissolved in a DMSO solution (0.1 ml). Stock AGOM (Sigma Chemical Co., St. Louis, MO, USA) was freshly 100 times diluted to its final concentration (40 mg/kg) in 0.9 % w/v sterile saline solution. A total of 28 doses were given to the animals in the groups within 28 days. In the depression group ( $n = 8$ ), experimental depression for 4 weeks was induced in the rats by CMS (Eren et al. 2007a, b). In the CMS + AGOM group ( $n = 10$ ), rats were administrated with IP AGOM (same as the AGOM group) with CMS induction (same as the CMS group).

12 h after the last AGOM dose administration, all rats were killed via cardiac puncture under ether anesthesia in accordance with SDU experimental animal legislation. The blood, brain (cortex), liver and kidney samples were isolated as described in a previous study (Kahya et al. 2015). At the end of the experiments, half of the tissue and blood samples were washed with phosphate buffer (pH 7.2) and immediately used for lipid peroxidation, GSH-Px, and GSH analyses. The remaining tissue and plasma samples were then frozen at  $-33$  °C. Antioxidant vitamins and cytokine analyses were performed within one month.

During the analyses, the tissue samples was placed on ice, cut into small pieces using scissors, and homogenized (2 min at 5000 rpm) in 5 volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), by a ultrasonic homogenization (SONOPULS HD 2070, Bandelin Electronic, Berlin, Germany). All preparation procedures were performed on ice.

### Induction of depression with chronic mild stress (CMS)

Procedures of inducing CMS have been described previously (Eren et al. 2007a; b). The CMS group was exposed to the following stressors in random order: continuous overnight illumination, 40 °C cage tilt, paired housing, damp bedding (300 ml water spilled into bedding), exposure to an empty water bottle immediately following a period of acute water deprivation, stroboscopic illumination (300 flashes/min), and white noise (approx. 90 dB). The stressors were presented in the order shown during the first week and repeated each of the following weeks for a total of 4 weeks. Control animals were left undisturbed in the home cages.

### Sucrose preference tests

Sucrose preference tests, as performed previously (Eren et al. 2007a, b) were used to operationally define anhedonia. Specifically, anhedonia was defined as a reduction in sucrose intake and sucrose preference relative to the intake and preference of the control group. A sucrose preference test consisted of first removing the food and water from each rat's cage (both CMS and control groups) for a period of 20 h. Water and 1 % sucrose were then placed on the cages in pre-weighed glass bottles, and animals were allowed to consume the fluids freely for a period of 1 h. Two baseline preference tests were performed, separated by at least 5 days, and the results were averaged. A preference test was also conducted following the 4-week CMS period.

### Lipid peroxidation analyses

Lipid peroxidation levels in the brain, liver and kidney homogenates were measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966) as described in a previous study (Kahya et al. 2015). The values of lipid peroxidation in the brain, liver and kidney samples were expressed as  $\mu\text{mol/g}$  protein.

### Reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and protein assays

The GSH contents of the brain, liver and kidney homogenates were measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of the brain, liver and kidney homogenates were assayed spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan) at 37 °C and 412 nm according to method of the Lawrence and Burk method (1976). Decrease of enzymatic reaction in absorbance at 412 nm against blank was measured spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan). GSH-Px activity and GSH level in the brain, liver and kidney homogenates were expressed as  $\mu\text{mol/g}$  protein. The protein contents in the brain, liver and kidney were measured by method of Lowry et al. (1951) with bovine serum albumin as the standard.

### $\beta$ -carotene, vitamins a and vitamin E analyses in the brain, liver and kidney samples

Vitamins A (retinol) and E ( $\alpha$ -tocopherol) were determined in the brain cortex samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). About 0.25 g of the brain, liver and kidney samples were saponified by the addition of 0.3 ml of saturated (60 %, w/v in water) KOH and two ml of 1 % (w/v in ethanol) pyrogallol, followed by shaking in water bath at 70 °C for 30 min. Two ml of water and 1 ml of n-hexane on ice were added and mixed with the samples that were then rested for 10 min to allow phase separation. Spectrophotometric determinations of vitamin A and vitamin E used absorbance wavelengths of 325 and 532 nm, respectively (UV-1800, Shimadzu, Kyoto, Japan). Calibrations were performed using standard solutions of all-trans retinol and  $\alpha$ -tocopherol in hexane. Vitamin A and E concentrations in the brain, liver and kidney were expressed as  $\mu\text{mol/g}$  tissue.

For  $\beta$ -carotene analyses in the brain, liver and kidney samples, the method of Suzuki and Katoh (1990) was used. Two milliliters of hexane were mixed with 0.25 g of brain,

**Table 1** Effects of agomelatine (AGOM) on sucrose (1 %, w/v) test results in the in rats with chronic mild stress (CMS) (mean  $\pm$  SD)

Weeks	Control ( <i>n</i> = 8)	AGOM ( <i>n</i> = 10)	CMS ( <i>n</i> = 8)	CMS + AGOM ( <i>n</i> = 10)
1st	28.09 $\pm$ 4.56	24.30 $\pm$ 2.14	28.78 $\pm$ 3.52	22.55 $\pm$ 3.90
2nd	38.18 $\pm$ 3.89	37.03 $\pm$ 4.16	36.80 $\pm$ 7.01	37.67 $\pm$ 4.13
3rd	41.30 $\pm$ 5.10	39.91 $\pm$ 3.76	35.18 $\pm$ 3.19 <sup>a,b</sup>	32.61 $\pm$ 2.92 <sup>a,b</sup>
4th	41.93 $\pm$ 3.47	35.28 $\pm$ 2.53 <sup>a</sup>	34.13 $\pm$ 4.66 <sup>a</sup>	34.02 $\pm$ 3.37 <sup>a</sup>

<sup>a</sup>  $p < 0.05$  as compared with group control.

<sup>b</sup>  $p < 0.05$  as compared with AGOM group

**Table 2** Effects of agomelatine (AGOM) on glutathione peroxidase (GSH-Px) activity, lipid peroxidation (LP), reduced glutathione (GSH) levels and vitamin concentrations in brain of rats with chronic mild stress (CMS) (mean  $\pm$  SD)

Values	Control ( $n = 8$ )	AGOM ( $n = 10$ )	CMS ( $n = 8$ )	CMS + AGOM ( $n = 10$ )
LP ( $\mu\text{mol/g}$ protein)	10.46 $\pm$ 0.75	11.03 $\pm$ 0.89	11.35 $\pm$ 0.91	9.96 $\pm$ 0.93
GSH-Px (IU/g protein)	20.42 $\pm$ 1.23	21.20 $\pm$ 1.30	14.30 $\pm$ 1.40 <sup>a</sup>	19.60 $\pm$ 2.02 <sup>b</sup>
GSH ( $\mu\text{mol/g}$ protein)	9.58 $\pm$ 0.55	9.56 $\pm$ 0.58	9.68 $\pm$ 0.99	9.38 $\pm$ 0.75
Vitamin A ( $\mu\text{mol/g}$ tissue)	1.98 $\pm$ 0.15	1.99 $\pm$ 0.16	1.99 $\pm$ 0.25	1.93 $\pm$ 0.22
$\beta$ -carotene ( $\mu\text{mol/g}$ tissue)	0.94 $\pm$ 0.14	0.95 $\pm$ 0.12	0.99 $\pm$ 0.24	1.01 $\pm$ 0.13
Vitamin E ( $\mu\text{mol/g}$ tissue)	10.80 $\pm$ 0.80	10.70 $\pm$ 0.41	10.40 $\pm$ 0.87	10.50 $\pm$ 0.63

<sup>a</sup>  $p < 0.05$  as compared with group control.<sup>b</sup>  $p < 0.05$  as compared with CMS group

liver and kidney samples. The value of  $\beta$ -carotene in hexane was measured at 453 nm in a spectrophotometer and pure hexane was used as blank. The  $\beta$ -carotene concentrations in the brain, liver and kidney were expressed as  $\mu\text{mol/g}$  tissue.

### Cytokine determinations in plasma

Plasma cytokine [TNF- $\alpha$ , interleukin (IL)-1 $\beta$  and IL-4] levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions as described in previous studies (Senol et al. 2014) (Multimode microplate reader, Infinite® 200 PRO series, Männedorf Switzerland). All ELISA kits were purchased from DRG Inc. (Marburg, Germany). Determinations were made in duplicate, and TNF- $\alpha$ , IL-1 $\beta$  and IL-4 results are expressed in nano gram (ng) and picogram (pg) per milliliter, respectively.

### Statistical analysis

The results are presented as means  $\pm$  standard deviation (SD). The SPSS Statistical program (17.0, SPSS Inc. Chicago, Illinois, USA) was used for the statistical treatment of the data. The Mann-Whitney U-test was used to establish the significance of differences among the four groups. The significance level was set at  $p < 0.05$ .

**Table 3** Effects of agomelatine (AGOM) on glutathione peroxidase (GSH-Px) activity, lipid peroxidation (LP), reduced glutathione (GSH) levels and vitamin concentrations in liver of rats with chronic mild stress (CMS) (mean  $\pm$  SD)

Values	Control ( $n = 8$ )	AGOM ( $n = 10$ )	CMS ( $n = 8$ )	CMS + AGOM ( $n = 10$ )
LP ( $\mu\text{mol/g}$ protein)	11.45 $\pm$ 1.11	10.51 $\pm$ 0.94 <sup>a</sup>	13.14 $\pm$ 1.65 <sup>a,d</sup>	8.52 $\pm$ 1.00 <sup>b,d,f</sup>
GSH-Px (IU/g protein)	26.80 $\pm$ 3.86	29.70 $\pm$ 2.26 <sup>a</sup>	25.80 $\pm$ 1.21 <sup>c</sup>	29.30 $\pm$ 3.48 <sup>c</sup>
GSH ( $\mu\text{mol/g}$ protein)	14.00 $\pm$ 1.29	16.20 $\pm$ 0.68 <sup>b</sup>	13.20 $\pm$ 1.03 <sup>d</sup>	18.40 $\pm$ 2.87 <sup>b,f</sup>
Vitamin A ( $\mu\text{mol/g}$ tissue)	2.96 $\pm$ 0.22	2.98 $\pm$ 0.23	3.19 $\pm$ 0.37	2.90 $\pm$ 0.31
$\beta$ -carotene ( $\mu\text{mol/g}$ tissue)	1.88 $\pm$ 0.29	1.89 $\pm$ 0.24	1.95 $\pm$ 0.29	1.82 $\pm$ 0.23
Vitamin E ( $\mu\text{mol/g}$ tissue)	15.50 $\pm$ 0.15	15.30 $\pm$ 0.59	14.90 $\pm$ 0.90	15.10 $\pm$ 0.90

<sup>a</sup>  $p < 0.05$ <sup>b</sup>  $p < 0.001$  as compared with group control<sup>c</sup>  $p < 0.05$ <sup>d</sup>  $p < 0.001$  as compared with group AGOM<sup>e</sup>  $p < 0.05$ <sup>f</sup>  $p < 0.001$  as compared with group CMS

## Results

### Results of sucrose test

Sucrose (1 %, w/v) test results of study groups is shown in Table 1 and consumption of water with sucrose at 3rd and 4th weeks as ml/kg body weight was significantly ( $p < 0.05$ ) lower in CMS-induced depression group than in basal values. In addition, consumption of water with sucrose at 3rd week as ml/kg body weight was significantly ( $P < 0.05$ ) lower in CMS group than in AGOM groups.

### Results of lipid peroxidation in brain, liver and kidney

Lipid peroxidation level as malondialdehyde (MDA) acts as a biomarker of oxidative stress (Placer et al. 1966). Research reports that patients with depression have high levels of lipid peroxidation due to activation of inflammatory and mitochondrial redox systems (Demyttenaere 2011) although AGOM induced antioxidant role through inhibition of melatonin receptors and mitochondrial redox systems (Kumar et al. 2015). We evaluated the inhibitory activities against oxidative stress and the scavenging activities of AGOM in the brain (Table 2), liver (Table 3) and kidney (Table 4) of rats with CMS-induced

**Table 4** Effects of agomelatine (AGOM) on glutathione peroxidase (GSH-Px) activity, lipid peroxidation (LP), reduced glutathione (GSH) levels and vitamin concentrations in kidney of rats with chronic mild stress (CMS) (mean  $\pm$  SD)

Values	Control ( $n = 8$ )	AGOM ( $n = 10$ )	CMS ( $n = 8$ )	CMS + AGOM ( $n = 10$ )
LP ( $\mu\text{mol/g}$ protein)	10.62 $\pm$ 1.27	9.85 $\pm$ 1.05	12.88 $\pm$ 1.27 <sup>a,c</sup>	10.91 $\pm$ 1.23 <sup>e</sup>
GSH-Px (IU/g protein)	18.40 $\pm$ 1.29	20.00 $\pm$ 0.91 <sup>a</sup>	17.20 $\pm$ 1.19 <sup>d</sup>	20.60 $\pm$ 1.42 <sup>f</sup>
GSH ( $\mu\text{mol/g}$ protein)	10.97 $\pm$ 0.80	14.17 $\pm$ 1.12 <sup>b</sup>	9.33 $\pm$ 0.48 <sup>b,d</sup>	11.31 $\pm$ 1.09 <sup>b,d,f</sup>
Vitamin A ( $\mu\text{mol/g}$ tissue)	2.08 $\pm$ 0.17	1.95 $\pm$ 0.23	1.95 $\pm$ 0.23	2.00 $\pm$ 0.19
$\beta$ -carotene ( $\mu\text{mol/g}$ tissue)	1.56 $\pm$ 0.18	1.53 $\pm$ 0.18	1.56 $\pm$ 0.28	1.60 $\pm$ 0.24
Vitamin E ( $\mu\text{mol/g}$ tissue)	10.60 $\pm$ 0.93	10.70 $\pm$ 0.89	14.90 $\pm$ 0.90	10.80 $\pm$ 1.05

<sup>a</sup>  $p < 0.05$ <sup>b</sup>  $p < 0.001$  as compared with group control<sup>c</sup>  $p < 0.05$ <sup>d</sup>  $p < 0.001$  as compared with group AGOM<sup>e</sup>  $p < 0.05$ <sup>f</sup>  $p < 0.001$  as compared with group CMS

depression. The results showed that lipid peroxidation levels in the liver and kidney in the CMS group were significantly ( $p < 0.05$ ) higher than the control. Hence, oxidative stress levels in the rats' liver and kidney were increased by depression induction. However, AGOM treatment decreased the lipid peroxidation levels of the rats' liver, and kidney. The lipid peroxidation levels in the liver ( $p < 0.001$ ) and kidney ( $p < 0.05$ ) in the AGOM and AGOM + CMS groups were markedly lower than in the CMS group.

#### Results of glutathione peroxidase (GSH-Px) activity and reduced glutathione (GSH) level in brain, liver and kidney

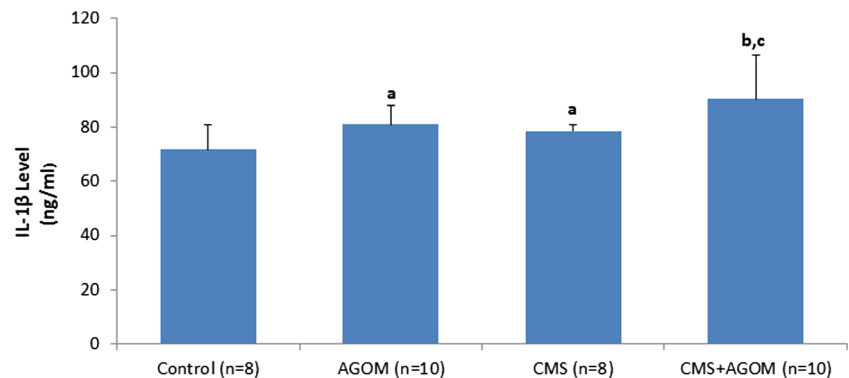
Tables 2, 3 and 4 present the mean GSH-Px activities and GSH results for the four groups in terms of the brain, liver and kidney, respectively. The results showed that the brain, liver and kidney GSH-Px activities ( $p < 0.05$  and  $p < 0.001$ ) and GSH ( $p < 0.05$ ) levels were significantly lower in the CMS group compared to the control group. However, GSH and GSH-Px values were markedly ( $p < 0.05$  and  $p < 0.001$ ) increased in the AGOM and AGOM + CMS groups by the AGOM treatments.

#### Results of antioxidant vitamin concentrations in brain, liver and kidney

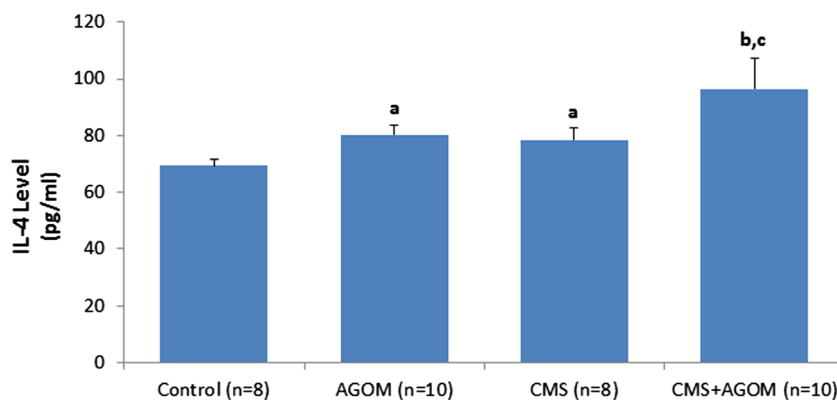
Tables 2, 3 and 4 also provide the mean vitamin A,  $\beta$ -carotene and vitamin E concentrations in the four groups' brain, liver, and kidney. The vitamin A,  $\beta$ -carotene and vitamin E concentrations were not changed in the four groups by AGOM treatment and CMS exposure.

#### Results of cytokines in plasma

Figures 1, 2, and 3 show the mean IL-1 $\beta$ , IL-4, and TNF- $\alpha$  levels in the plasma of the four groups. Mean IL-1 $\beta$  levels as ng/ml in the control, AGOM, CMS, and CMS + AGOM groups were 71, 80, 78, and 90, respectively. Mean TNF- $\alpha$  levels as ng/ml in the four groups were 64, 92, 80, and 106, respectively. Mean IL-4 levels as pg/ml in the four groups were 69, 80, 78, and 96, respectively. The IL-1 $\beta$  ( $p < 0.05$ ), IL-4 ( $p < 0.05$ ), and TNF- $\alpha$  ( $p < 0.001$ ) levels significantly increased in the plasma of rats with CMS-induced depression and these levels further increased with AGOM and CMS + AGOM treatments ( $p < 0.05$  and  $p < 0.001$ ).

**Fig. 1** The effects of agomelatine (AGOM) on plasma IL-1 $\beta$  level in rats with chronic mild stress (CMS) (mean  $\pm$  SD). <sup>a</sup> $p < 0.05$  and <sup>b</sup> $p < 0.01$  as compared with group control. <sup>c</sup> $p < 0.05$  as compared with groups AGOM and CMS

**Fig. 2** The effects of agomelatine (AGOM) on plasma IL-4 level in rats with chronic mild stress (CMS) (mean  $\pm$  SD). <sup>a</sup> $p < 0.05$  and <sup>b</sup> $p < 0.01$  as compared with group control. <sup>c</sup> $p < 0.05$  as compared with groups AGOM and CMS



## Discussion

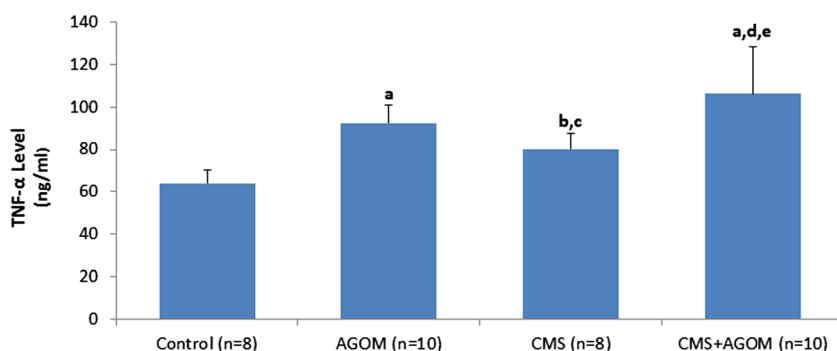
We found that CMS-induced depression increased lipid peroxidation levels in the liver and kidney as well as TNF- $\alpha$ , IL-1 $\beta$ , and IL-4 levels in plasma. However, GSH-Px activity and GSH level were decreased by induction of CMS depression. Therefore, CMS-induced depression in the animals was characterized by increased oxidative stress and cytokine production along with decreased antioxidant levels. AGOM administration decreased lipid peroxidation although GSH-Px activity, GSH, TNF- $\alpha$ , IL-1 $\beta$ , and IL-4 levels increased. We have thus shown that AGOM-based treatments modulated the balance of pro- and antioxidant in rats and ringdoves by down-regulating the levels of oxidative stress while up-regulating GSH redox system. To the best of our knowledge, the current study is the first to compare AGOM with particular reference to its effects on cytokine production and antioxidant redox systems in CMS-induced oxidative injury in rats.

Accumulative evidences indicate that depression is characterized by activation of the inflammatory response system with increased production of pro-cytokines (Andreasson et al. 2007; Cyranowski et al. 2007; Nazıroğlu and Demirdaş 2015). It is well known that activation of phagocytic cells by pro-inflammatory cytokines leads to excessive production of ROS, which leads to an increase the levels of lipid peroxidation as MDA through oxidation of cell membrane polyunsaturated fatty acids, and causes loss of fluidity the biological membrane. As a result of those alterations, the biological

membranes induce cytokine production (Halliwell 2006). On the other hand, excessive ROS production induces phospholipase A2 activation, which changes receptor functions in the cell membranes, induces immune cells, and leads to secretion of interleukins from T cells (Sierra-Honigmann and Murphy 1992). Liver cytochrome P450 enzyme is important physiological enzyme in detoxification of ROS products in body. 90 % of AGOM is metabolized through the liver by the hepatic cytochrome P450 (Freiesleben and Furczyk 2015). Up to 80 % of AGOM is eliminated in urine as pharmacologically inactive metabolites, the main metabolites being hydroxylated and demethylated AGOM (Freiesleben and Furczyk 2015). In the current study, liver and kidney lipid peroxidation and cytokine levels are increased in CMS-induced rats due to inflammatory characterization of depression. In the current study, liver and kidney lipid peroxidation and plasma cytokine levels are increased in the rats by induction of depression although liver and kidney lipid peroxidation levels are decreased by AGOM treatment and it might be modulation of hepatic cytochrome P450 (Freiesleben and Furczyk 2015). Brain lipid peroxidation level did not change in the four groups statistically. Adaptive antioxidant responses of brain were accompanied by liver antioxidant down-regulation.

Existing evidence indicates that excessive generation of ROS might contribute to the onset of symptoms in CMS-induced depression (Eren et al. 2007a; b). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant redox system, such as a decrease in GSH levels

**Fig. 3** The effects of agomelatine (AGOM) on plasma TNF- $\alpha$  level in rats with chronic mild stress (CMS) (mean  $\pm$  SD). <sup>a</sup> $p < 0.001$  and <sup>b</sup> $p < 0.05$  as compared with group control. <sup>c</sup> $p < 0.05$  and <sup>d</sup> $p < 0.001$  as compared with group AGOM. <sup>e</sup> $p < 0.001$  as compared with group CMS



and decreased activity of antioxidant defense enzymes, including GSH-Px (Eren et al. 2007a, b; Nazıroğlu and Demirdaş 2015). Accumulation evidences support that the oxidative process significantly contributes to CMS-induced depression oxidative toxicity (Eren et al. 2007a, b). Results of recent reports indicate that the production of ROS is a key contributing factor in the pathology of depression (Demyttenaere 2011). AGOM treatment markedly, attenuates depleted antioxidant system due to excessive oxidative stress. It has been also reported that activation of melatonin receptors up-regulate gene expression of the antioxidant enzyme (Gupta and Sharma 2014). Melatonin receptor agonists have been reported to exert direct ROS scavenging activity and indirect antioxidant activity via its stimulatory actions on the antioxidant system (Kharwar and Haldar 2012). It was reported that modulation of melatonin receptor pathway through melatonin agonist induces ROS scavenger effect (Shirazi et al. 2013). Increased mitochondrial membrane depolarization through overload calcium ion ( $\text{Ca}^{2+}$ ) entry induces excessive ROS production in cells including neurons (Balaban et al. 2016). It has been reported that AGOM modulated intestinal and neuronal mitochondrial function and  $\text{Ca}^{2+}$  entry (de Mello et al. 2015; Kumar et al. 2015), which resulted in improvement of antioxidant redox pathway in animals. We observed decreased lipid peroxidation levels in the liver and kidney of experimental depression-induced rats through AGOM treatment. However, GSH-Px activity and GSH levels were increased in brain, liver and kidney of the rats with CMS by AGOM treatment. Hence, AGOM treatment in the current study shows antioxidant effects through stimulation of melatonin receptors and regulation of mitochondrial function; this may be due to activation of the GSH enzyme redox system. Antioxidant vitamin concentrations in brain, liver and kidney did not change in the four groups. Adaptive antioxidant responses of antioxidant vitamins were accompanied by GSH-Px enzymatic activity up-regulation.

There are limited studies on antioxidant effects of AGOM. Gupta and Sharma (2014) reported similarly that administration of agomelatine did not show any significant effect on the levels of MDA and GSH in brain striatum of mice. Increased lipid peroxidation and decreased GSH levels were decreased in ileum of rats with prenatal valproic acid-induced autism spectrum disorder by AGOM (Kumar et al. 2015). In a study with PC-12 neuronal cell line conducted by our groups (Akpınar et al. 2014), the decreased lipid peroxidation levels were observed low in the AGOM group. In addition, the same study showed that GSH level and GSH-Px activity were high in the AGOM group, indicating an antioxidant role of AGOM in the neurons.

Accumulating evidence suggests that depression is associated with increased neuroinflammation and phagocytic activation (Cyranowski et al. 2007). A high level of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-4, and IL-1 $\alpha$ ,

exist in the plasma of patients with depression (Andreasson et al. 2007; Cyranowski et al. 2007). The generation of ROS and the activation of the NADPH oxidase redox system induced by depression play a central role in the pathogenesis of depression (Bakunina et al. 2015), and these radicals stimulate the production of cytokines (Vaváková et al. 2015). A role for TNF- $\alpha$  and IL-1 $\alpha$  as mediators of the depression in cytochrome P450 activity in brain and liver during central nervous system inflammation is reported by Nicholson and Renton (2001) and they observed that the production of the cytokine production within the brain did not participate in the signaling process in the brain that leads to the concomitant loss of CYP1A activity in the liver (Nicholson and Renton 2001). AGOM is mostly metabolized through the liver by the hepatic cytochrome P450 (Freiesleben and Furczyk 2015). Reports show that TNF- $\alpha$ , IL-4, and IL-1 $\alpha$ , levels contribute to brain-cell failure through depression-induced oxidative stress, microglia and phagocytic cell activations (Nicholson and Renton 2001; Bakunina et al. 2015; Vaváková et al. 2015). To our knowledge, there is no report on the effects of AGOM and melatonin in plasma TNF- $\alpha$ , IL-4, and IL-1 $\alpha$  levels in rats with CMS-induced depression. In the current study, CMS-induced increase of plasma TNF- $\alpha$ , IL-4, and IL-1 $\alpha$  levels was further increased through modulation of the antioxidant redox system with AGOM treatments due to activation of hepatic cytochrome P450.

In conclusion, we used an experimental animal model to demonstrate that AGOM administration can prevent depression-induced brain, liver and kidney toxicity but it stimulates cytokine production. In addition, the mechanisms underlying the protective properties of AGOM include the antioxidant. Therefore, AGOM can reduce lipid peroxidation levels in the brain, liver, and kidney of rats with depression by virtue of their inherent antioxidant properties. Toxic effect of AGOM on liver might be induced by increase of plasma cytokine production. In consequence, AGOM treatment may have a beneficial effect in managing depression as well as their oxidant complications.

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**Author's contributions** AD and MN formulated the hypothesis and was responsible for writing the report. GÖÜ was responsible for the animal experiments.

**Compliance with ethical standards**

**Financial disclosure** There is no financial disclosure for the current study.

**Conflict of interest** None of the authors have any conflicts to disclose. All authors approved the final manuscript.

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