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Original article

## 8-Cyclopentyl-1,3-dimethylxanthine enhances effectiveness of antidepressant in behavioral tests and modulates redox balance in the cerebral cortex of mice

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### ABSTRACT

The objective of our study was to investigate whether 8-cyclopentyl-1,3-dimethylxanthine (CPT), associated with the adenosine system, enhances the antidepressant efficacy of antidepressant. All experiments were carried out on Albino Swiss mice. Following drugs: CPT (3 mg/kg) and imipramine (15 mg/kg) were administered intraperitoneally (*ip*), 60 min before tests. Two behavioral tests on antidepressant capability – a forced swim test (FST) and a tail suspension test (TST) – were performed. To examine whether co-administration of CPT with antidepressants affects the redox balance, the lipid peroxidation products (LPO), glutathione (GSH), glutathione disulfide (GSSG), nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were determined in the cerebral cortex. The results have demonstrated a CPT-induced enhancement of the antidepressant-like effect of imipramine both in the FST and TST, which may indicate that the adenosine system may be involved in the increasing the effect of antidepressant. Co-administration of CPT with imipramine, such as imipramine alone, decreased the NADP<sup>+</sup> and LPO concentrations and increased the GSH/GSSG ratio in comparison to the control, which may confirm beneficial – but comparable to imipramine – effect on redox balance under environmental stress conditions. An increase in the concentration of GSSG in the cortex of animals treated with imipramine in ineffective dose compared to control and no such changes after combined administration of both drugs may suggest a favorable oxidation-reduction potential resulting from their synergistic antidepressant effect.

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### 1. Introduction

Depression is becoming a civilized issue and involves a rising number of people. According to the World Health Organization, by 2020 depression may become the second most common disease, right after cardiovascular disorders. There are a few hypotheses explaining the emergence of depression. The most popular one points to monoamines (Hasler, 2010). This hypothesis assumes

that the underlying pathophysiologic basis of depression is the depletion of serotonin, norepinephrine, and/or dopamine in the central nervous system (CNS). The ultimate effect can be a neurodegenerative disorder (Lee et al., 2010). It was found that affective disorders cause significant structural changes in the CNS. These changes are related to the structure of the hippocampus and prefrontal cortex and involve the reduction of the number of cells and the weakening of the processes of neuronal plasticity and neurogenesis. It leads to the disappearance of sensitive neurons and glia in the population of cells in these regions (Manji et al., 2003).

The pharmacotherapy of major depressive disorders employs various groups of drugs with a diverse mechanism of action. Tricyclic antidepressants (TCAs) were the first group of medicines used in the treatment and still remain the first-line or, more commonly, the second-line intervention to moderate severe depression. One of their representatives is imipramine. Imipramine and

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others TCAs contribute to an increase of monoamines' concentration in the CNS by non-selective inhibition of their reuptake from neuronal synaptic space.

Yet, the standard pharmacotherapy of depression disorders does not provide satisfactory results, and the effects of treatment usually appear after several weeks. Moreover, a treatment is likely to entail side effects, what results in the patient discontinuing their therapy. On the other hand, many patients suffer from drug-resistant depression and available drugs, also new generation medicines, are ineffective. Due to this fact, the search for some new methods of pharmacotherapy becomes justified. One type of a drug therapy is the treatment with combinations, that is, entirely new chemical compounds.

The results of the research point to the adenosine receptors as a key to the treatment of neurodegenerative disorders, such as depression. The endogenous agonist of these receptors causes the retardation of the release of neurotransmitters (Ammon, 1990). It is confirmed that deficiency of the neurotransmitters, such as acetylcholine, noradrenaline, serotonin and dopamine, is considered to be one of the causes of depression. Therefore, the compounds, which inhibit the activity of the adenosine system, contribute to an increase in the concentration of the above-mentioned neurotransmitters and their severity of transduction (Yacoubi et al., 2001; Karcz-Kubicha et al., 2003). 8-cyclopentyl-1,3-dimethylxanthine is a nonselective phosphodiesterase inhibitor and a selective antagonist of adenosine receptors with the selectivity for the A1 subtype (Ciruela et al., 2006; Okada et al., 2001). As a result, CPT increases neuronal neurotransmission in the CNS. CPT, by blocking A1 receptors, inhibits the effects of endogenous adenosine and increases norepinephrine and serotonin transduction in the CNS (Okada et al., 1997, 2002). Therefore, it can be assumed that CPT, through an increased transduction and serotonergic noradrenergic, can enhance the effect of antidepressants.

The studies have shown that environmental stress is directly implicated in the pathogenesis of neurologic and psychiatric diseases (Schivone et al., 2013; Aschbacher et al., 2013). It was reported that exposure to stress situations can stimulate numerous pathways, leading to increased production of reactive oxygen species (ROS) (Nayanatara et al., 2005). In response to stressful events, oxidative stress was implicated and proposed as a contributing factor in the pathogenesis of depression (Schivone et al., 2013). Oxidative stress refers to elevated intracellular levels of ROS which are produced both during the mitochondrial electron transport of aerobic respiration and through physiologic processes. The excessive production of ROS contributes to the neuronal damage of membrane phospholipids, reduced membrane potential and possible rupture, with release of cell and organelle content into the extracellular space including neurotransmitters (serotonin and noradrenaline) (Khanzode et al., 2003; Takuma et al., 2004). To fight excessive production of ROS, the organism has built protective systems and mechanisms against their harmful effects. However, the brain is considered particularly vulnerable to oxidative injury. This organ is characterized by the high oxygen utilization and the enhanced generation of free radicals as a consequence but the antioxidant defense mechanisms are not effective enough (Pandya et al., 2013).

In this context, it seems very important to search for novel chemical compounds, as well as to the unique combinations of the drugs, which exhibit antioxidant properties. Some reports suggest that antidepressants may have an effect on the CNS's redox balance, however the mechanisms often remain unclear (Purdell et al., 2015; Khanzode et al., 2003; Behr et al., 2012). Earlier studies have shown that the exposure to TCAs leads to an increased generation of ROS and a concomitant reduction of the level of intracellular glutathione, as well as inducing DNA fragmentation (Post et al., 2000). On the other hand, other and more recent studies have

confirmed that imipramine is involved in maintaining the redox balance of the CNS, thus it has a positive therapeutic effect on the treatment of depression (Kumar et al., 2009; Réus et al., 2010; Mokoena et al., 2010). In turn, the scientific literature lacks research information on the impact of CPT on oxidative stress parameters.

These facts mentioned above have become the inspiration for our research. At the outset, the objective of our study was to investigate whether of 8-cyclopentyl-1,3-dimethylxanthine, associated with the adenosine system, enhances the antidepressant efficacy of imipramine. We used imipramine, a drug with a confirmed activity in behavioral tests. Additionally, we wanted to investigate whether the co-administration of CPT with antidepressant under stress conditions affects the redox balance, therefore we examined the oxidative stress markers in the cerebral cortex of mice. The forced swimming test (FST) and tail suspension test (TST) are widely accepted and well-validated tests of studying depressive-like behaviours in mice. (Kondam et al., 2012; Nayanatara et al., 2005; Steru et al., 1985; Porsolt et al., 1977).

## 2. Methods

### 2.1. Animals

All experiments were carried out on naive adult male Albino Swiss mice (25–30 g) purchased from the licensed breeder (Kołacz, Warszawa, Poland). The animals were kept in cages at room temperature ( $20 \pm 2$  °C) under a 12 h day/12 h night cycle in constant environmental conditions (humidity, noise). They had free access to food and water except for the short time that they were removed from their cages for testing. The animals were used after 7 days of acclimatization to laboratory conditions. Each experimental group consisted of 10 animals. Each mouse was used only once. Procedures involving mice and their care in all the experiments of the present study were approved by the Local Ethics Committee at the Medical University of Lublin and were performed in accordance with binding European standards related to the experimental studies on animal models.

### 2.2. Drugs and chemicals

The following substances were used in the study: CPT (3 mg/kg, Sigma-Aldrich, Poznań, Poland) and imipramine hydrochloride (15 mg/kg, Sigma-Aldrich, Poznań, Poland). For the assessment of oxidative stress parameters, the commercial kits were used: concentration of nicotinamide adenosine dinucleotide phosphate – NADP<sup>+</sup>, NADPH (BioChain, Newark, USA); lipid peroxidation – LPO (based on the concentration of malondialdehyde (MDA) and 4-hydroxyalkenals (4HAE), OxisResearch, Burlingame, USA) and concentration of glutathione – GSH, GSSG, GSH/GSSG ratio (Calbiochem, Darmstadt, Germany).

### 2.3. Experimental design

Imipramine was dissolved in saline (0.9% NaCl), whereas CPT was suspended in 1% aqueous solution of Tween 80 (POCH, Gliwice, Poland). The tested drugs were administered *ip* 60 min before behavioral test. The selected doses, as well as the procedure of administration, were based on those reported in the literature and on our previous experiments (Poleszak et al., 2006; Szopa et al., 2016; Herbet et al., 2016). All solutions were prepared just before the experiment. The animals from the control group received injections of vehicle (0.9% NaCl). The volume of the vehicle or drug solutions for *ip* administration was 10 ml/kg. Five (1–5) groups of mice were administered in the following order: 1. saline

and saline (stress-naïve group), 2. saline and saline (control group), 3. CPT and saline, 4. imipramine and saline, 5. CPT and imipramine. Group 1 was not subjected to the stress factor and was used only for biochemical studies. Other animals, 60 min after injection were subjected to behavioral tests: Forced Swim Test (FST), Tail Suspension Test (TST) and Spontaneous Locomotor Activity.

#### 2.4. Forced swim test

The procedure was carried out on mice according to Porsolt et al. (1977). After the administration of drugs, the animals were individually placed into the glass cylinder (height 25 cm, diameter 10 cm) filled with water ( $25 \pm 1$  °C) and left for 6 min. Each animal was considered as immobile when it floated in the water in an upright position and made only small movements to keep its head above the water. The total immobility time of animals was measured during the last 4 min of the 6-min long period of the test. The immobility time (in seconds) was scored in real time by two blind observers. The results were expressed as the arithmetic mean  $\pm$  standard error of the mean (SEM) for each experimental group.

#### 2.5. Tail suspension test

The procedure was performed with the use of mice according to Steru et al. (1985). After the administration of drugs, each animal was suspended by the tail to the vertical bar in wooden box ( $30 \times 30$  cm). The animals were fastened by adhesive tape fixed 2 cm from the end of the tail for 6 min. The test lasted for 6 min and the total immobility time of animals was recorded during the last 4 min. The immobility of animals was stated as it stopped moving body and limbs, making exclusively the movements necessary to breathe. The immobility time (in seconds) was scored in real time by two blind observers. The results were expressed as the arithmetic mean  $\pm$  standard error of the mean (SEM) for each experimental group.

#### 2.6. Spontaneous locomotor activity

The procedure was performed to avoid the risk of obtaining false positive/negative results in the FST, which might be caused by the impact of tested compounds on the locomotor activity. The spontaneous locomotor activity was measured using an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, Columbus, OH, USA). This apparatus consists of four transparent cages with lids ( $43 \times 43 \times 32$  cm), a set of four infrared emitters (each emitter has 16 laser beams), and four detectors monitoring animal movements. After ip administration of saline/ tested drugs, each animal was placed individually into the cages for 10-min long period. Spontaneous locomotor activity was assessed between the 2nd and the 6th min, which referring to the time interval analyzed in the FST. The results were expressed as the arithmetic average distance that a mouse travelled (in cm)  $\pm$  SEM for each experimental group.

#### 2.7. Measurement of redox equilibrium parameters

Stress-naïve group (1) and the animals, which were used in the FST (2–5), were decapitated and the samples of cerebral cortex were collected. The samples were washed with 20 ml of saline and stored at  $-75$  °C until the time of biochemical analysis. The homogenates were prepared from frozen brain samples, using extraction buffer (phosphate buffered saline, PBS, Thermo Fisher Scientific). In these homogenates all measurements were performed. The experimental procedures were performed according to the instructions supplied with the respective kit.

##### 2.7.1. The NADP<sup>+</sup> and NADPH assay

The determination of NADP<sup>+</sup> and NADPH was based on the reaction by enzyme catalyst. This method applies to a cyclic glucose dehydrogenase, consisting in the reduction of NADPH involving MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The intensity of the colour of the reduced product, measured at a wavelength of 565 nm, is proportional to the NADP<sup>+</sup> and NADPH concentrations.

##### 2.7.2. Lipid peroxidation

The principle of the method is based on the reaction of a chromogenic reagent R1 (N-methyl-2-phenylindole) with MDA and 4-hydroxyalkenals at 45 °C. Two molecules of R1 react with one molecule of MDA or 4-hydroxyalkenals to form a chromophore with an absorbance maximum at 586 nm. Measuring the concentration of MDA in combination with 4-hydroxyalkenals in methane sulfonic acid was used as an indicator of lipid peroxidation.

##### 2.7.3. GSH and GSSG levels

GSH and GSSG concentrations were determined by an enzymatic reaction using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) and reagent M2VP (1-methyl-2-vinyl-pyridine-trifluoro-methanesulfonamide sulfonate). In this method, GSH reacts with Ellman's reagent to form a product determined with the spectrophotometer at a wavelength of  $\lambda = 412$  nm.

#### 2.8. Statistics

The results obtained in the FST, TST, locomotor activity, and biochemical assays (groups 2–5) were subjected to statistical analysis using two-way ANOVA with Bonferroni's *post hoc* test. The statistical significance among the 1–2 groups was determined by Student's *t*-test. All results are presented as the mean  $\pm$  standard error of the mean (SEM). *P* values less than or equal to 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

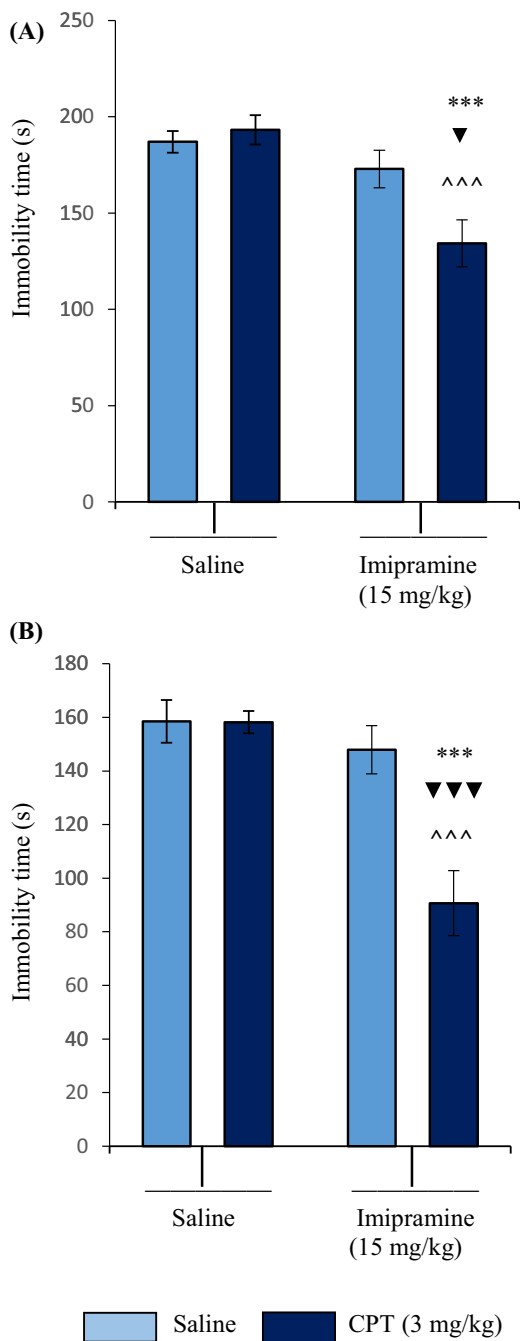
### 3. Results

#### 3.1. Assessment of behaviour in the FST

The effect of combined administration of CPT and imipramine on the total duration of the immobility time in mice is shown in Fig. 1A. CPT (3 mg/kg) injected in combination with imipramine (15 mg/kg) significantly reduced the immobility time in the FST in mice (Fig. 1A and Table 1). Imipramine and CPT given alone had no effect on the immobility time. The two-way ANOVA has demonstrated a significant effect of imipramine [ $F(1, 34) = 15.62$ ,  $p = 0.0004$ ], no effect of CPT [ $F(1, 34) = 3.07$ ,  $p = 0.0888$ ], and a significant interaction between imipramine and CPT [ $F(1, 34) = 5.88$ ,  $p = 0.0208$ ].

#### 3.2. Assessment of behaviour in the TST

The effect of the combined administration of CPT and imipramine on the total duration of the immobility time in mice is shown in Fig. 1B. CPT injected in combination with imipramine significantly reduced the immobility time in the TST in mice (Fig. 1B and Table 1). CPT and imipramine and given alone had no effect on the immobility time. The two-way ANOVA has demonstrated a significant effect of imipramine [ $F(1, 34) = 10.33$ ,  $p = 0.0029$ ], a significant effect of CPT [ $F(1, 34) = 10.54$ ,  $p = 0.0026$ ], and a significant interaction between imipramine and CPT [ $F(1, 34) = 19.48$ ,  $p < 0.0001$ ].



**Fig. 1.** The effect of combined administration of CPT and imipramine in: the FST (A) and the TST (B) in mice. Tested drugs and saline were administered *ip* 60 min before the tests. The values represent mean  $\pm$  SEM. Significance: \*\*\**p* < 0.001 versus control group; ▼*p* < 0.05, ▼▼▼*p* < 0.001 versus group received only imipramine; ^^^*p* < 0.001 versus group received only CPT (two-way ANOVA followed by Bonferroni's *post hoc* test).

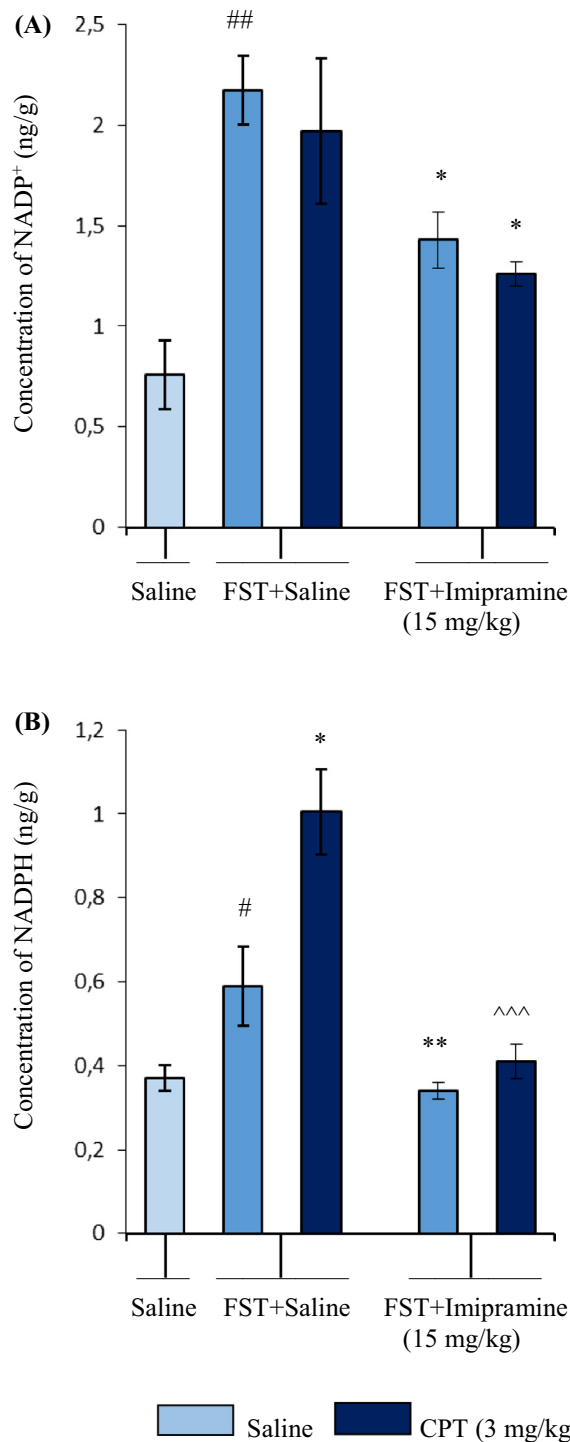
**Table 1**  
Effect of treatments on spontaneous locomotor activity in mice.

Treatment (mg/kg)	Distance travelled between 2nd and 6th min (cm)
Saline + saline	441.3 $\pm$ 27.17
CPT 3 + saline	397.6 $\pm$ 76.27
Imipramine 15 + saline	531.6 $\pm$ 55.83
CPT 3 + imipramine 15	445.6 $\pm$ 78.41

Tested drugs and saline were administered *ip* 60 min before the test. Distance travelled was recorded between the 2nd and the 6th min of the test. Data are presented as the means  $\pm$  SEM. The results were considered statistically significant if *p* < 0.05 (two-way ANOVA followed by Bonferroni's *post hoc* test).

**3.3. Assessment of behaviour in the spontaneous locomotor activity test**

The effect of the combined administration of CPT and imipramine on the spontaneous locomotor activity in mice is presented in Table 1. CPT and imipramine administered either alone or



**Fig. 2.** The effect of combined administration of CPT and imipramine on NADP<sup>+</sup> (ng/g) (A) and NADPH<sup>+</sup> (ng/g) (B) concentration in cerebral cortex of mice. The values represent mean  $\pm$  SEM. Significance: #*p* < 0.05, ##*p* < 0.01 versus stress-naïve group (Student's *t*-test). Significance: \**p* < 0.05, \*\**p* < 0.01 versus control group; ^^^*p* < 0.05 versus group received only CPT (two-way ANOVA followed by Bonferroni's *post hoc* test).

combination did not cause any statistically significant increase of the locomotor activity of mice (Table 1). The two-way ANOVA demonstrated no effect of imipramine [ $F(1, 27) = 1.24$ ,  $p = 0.2749$ ], no effect of CPT [ $F(1, 27) = 1.09$ ,  $p = 0.3058$ ], and no interaction [ $F(1, 27) = 0.12$ ,  $p = 0.7360$ ].

#### 3.4. NADP<sup>+</sup> and NADPH concentrations in cerebral cortex

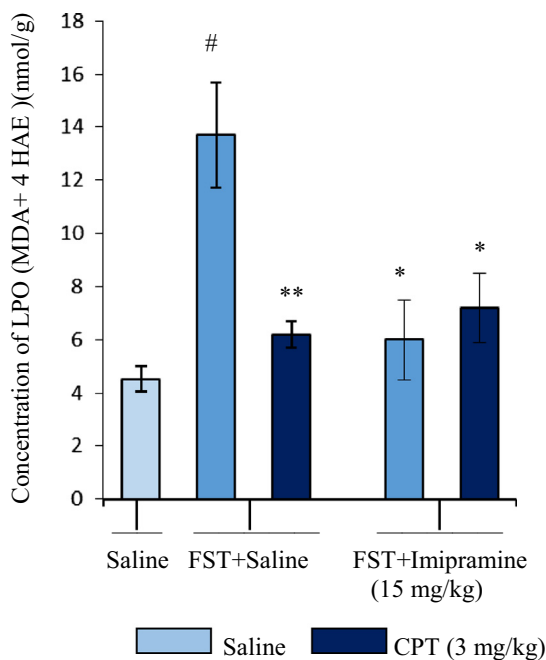
It was observed that the NADP<sup>+</sup> and NADPH concentrations were significantly higher in the group of mice exposed to environmental stress (FST) in comparison to the stress-naïve control group (Fig. 2A).

In the groups of mice treated with CPT there were no statistically significant changes in the concentration of the NADP<sup>+</sup> as compared to the control. However, in the group of animals receiving imipramine and concomitant CPT and imipramine, a significant decrease of NADP<sup>+</sup> concentrations, compared to the control group, was observed. In the groups of mice receiving CPT, a significant increase in the concentration of NADPH was observed as compared to the control (Fig. 2B). However, in the group pre-treated with imipramine a significant decrease in NADPH concentration was noted. In turn, the co-administration of CPT and imipramine caused a decrease in the concentration of NADPH as compared to CPT.

#### 3.5. Lipid peroxidation in cerebral cortex

We have noticed that the level of MDA + 4HAE in mice exposed to FST significantly increased, when compared to the stress-naïve control group (Fig. 3).

Our studies have shown a significant decrease of MDA + 4HAE in the cerebral cortex of animals receiving CPT and imipramine either alone or in combination as compared to the control.

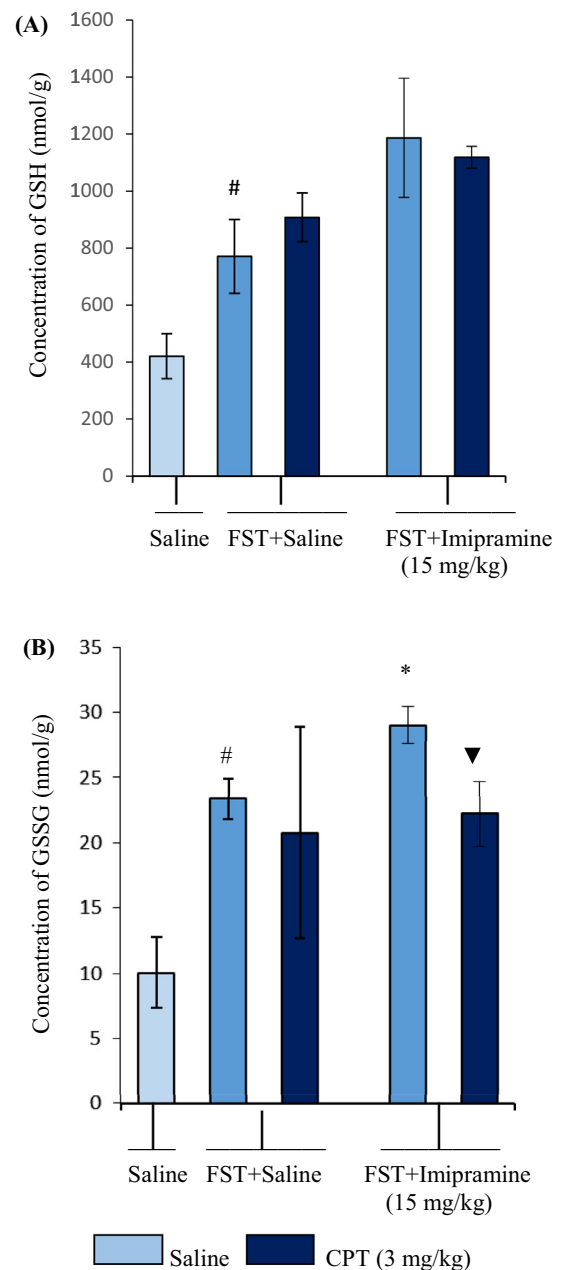


**Fig. 3.** The effect of combined administration of CPT and imipramine on LPO (MDA + 4 HAE) (nmol/g) concentration in cerebral cortex of mice. The values represent mean  $\pm$  SEM. Significance: <sup>#</sup> $p < 0.05$  versus stress-naïve group (Student's *t*-test). Significance: <sup>\*</sup> $p < 0.05$ , <sup>\*\*</sup> $p < 0.01$  versus control group (two-way ANOVA followed by Bonferroni's *post hoc* test).

#### 3.6. GSH and GSSG levels in cerebral cortex

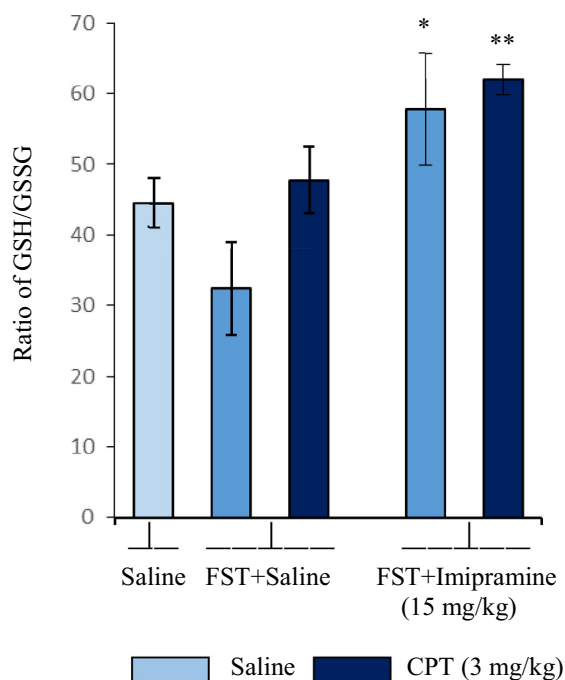
Data obtained in the samples of cerebral cortex of mice subjected to FST show a statistically significant increase in GSH and GSSG. However, the levels of GSH/GSSG ratio in mice exposed to FST did not change substantially, in comparison to the group not subjected to the stress factor (Fig. 4A).

In the groups of mice, which received CPT alone and simultaneously with imipramine, no statistically significant changes in the GSH concentration were observed as compared to the control. In the group of mice receiving CPT there were no significant changes in the concentration of GSSG as compared to the control group. It should be mentioned, that a high standard deviation was recorded



**Fig. 4.** The effect of combined administration of CPT and imipramine on GSH (nmol/g) (A) and GSSG (nmol/g) (B) concentration in cerebral cortex of mice. The values represent mean  $\pm$  SEM. Significance: <sup>#</sup> $p < 0.05$  versus stress-naïve group (Student's *t*-test). Significance: <sup>\*</sup> $p < 0.05$  versus control group; <sup>▼</sup> $p < 0.05$  versus group received imipramine (two-way ANOVA followed by Bonferroni's *post hoc* test).





**Fig. 5.** The effect of combined administration of CPT and imipramine on GSH/GSSG ratio in cerebral cortex of mice. The values represent mean  $\pm$  SEM. Significance: \* $p < 0.05$ , \*\* $p < 0.01$  versus control group (two-way ANOVA followed by Bonferroni's post hoc test – group 4 vs 1,2,3).

in this group. Imipramine administered to mice increased the concentration of GSSG compared with the control. However, the concomitant administration of CPT with imipramine caused a decrease of the GSSG concentration in the cortex of mice compared with the group of animals receiving only imipramine. In the cortex of mice treated with CPT alone no statistically significant changes in the GSH/GSSG ratio were observed as compared to the control (Fig. 5). In turn, imipramine, administered to rodents both separately and in a combination with CPT, increased the GSH/GSSG ratio compared to the control.

#### 4. Discussion

The presented study evaluates the impact of selective antagonist of adenosine A1 receptor – CPT, on the antidepressant activity of the classic antidepressant – imipramine. For this purpose, the antidepressant potential of active substances was assessed by two behavioral tests, commonly used in experimental pharmacology – the FST and TST. To exclude false negative/positive results obtained in these tests, the spontaneous locomotor activity of animals was recorded.

The results have shown that the simultaneous use of CPT and imipramine in ineffective doses, 3 mg/kg and 15 mg/kg accordingly, leads to a statistically significant shortening of the immobility time both in the FST and TST versus all experimental groups. It should be noted that the observed changes in mice behaviour were not due to the severity of their spontaneous motor activity. It was demonstrated that other neurotransmission systems, such as dopaminergic, glutamatergic and serotonergic ones, as well as the corticotrophin system, can be modulated by adenosine transduction (Okada et al., 2001; Yamato et al., 2002). In the behavioral despair animal models adenosine and its analogues generate depressant-like effects (Hunter et al., 2003). Moreover, these agents inhibit any therapeutic effect of classical antidepressants. It should be emphasized that different therapeutic strategies used

to treat depression also affect adenosine neurotransmission. TCAs (such as nortriptyline, chlorimipramine or desipramine) were proved to attach to adenosine receptors and induce a dose-dependent decrease in the ecto-nucleotidase activity, resulting in the reduced levels of extracellular adenosine in the cortical synapses (Barcellos et al., 1998). The obtained potentiation of the antidepressant effect of imipramine by CPT is probably related to the synergistic inhibition of these two compounds on the adenosine system activity, which results in the increased noradrenergic, serotonergic (Sebastiao and Ribeiro, 1996), and glutamatergic neurotransmission (Di Iorio et al., 1996). CPT, being an antagonist of adenosine A1 receptors – located on the serotonergic neurons in the structures of CNS, such as dorsal nucleus and locus coeruleus – leads to the severity of adrenergic and serotonergic neurotransmission, which may explain the intensified antidepressant effect of imipramine by CPT (Okada et al., 2002; Mossner et al., 2000).

The studies of last decade have shown that the oxidative stress is increased in major depressive disorders and in bipolar disorder (Andreazza et al., 2008). The excessive oxidative stress triggers a cascade of neurodegenerative events, which results in apoptotic injury. The brain is much more vulnerable to free oxygen radicals than other tissues, since it utilizes 20% of the oxygen consumed by the body (Sarandol et al., 2007). Moreover, the brain contains great amounts of polyunsaturated fatty acids and a low concentration of antioxidant enzymes. Scientific research has revealed that the treatment with antidepressants can reverse the increased oxidative stress observed in individuals with depression (Behr et al., 2012; Réus et al., 2010). Antidepressant drugs and substances may affect the oxidation-reduction balance, for example by the putting impact on concentration of GSH, lipid peroxidation, microglial NADPH oxidase activation, modulation mRNA levels of antioxidant enzymes and oxidative stress response genes (Abdel Salam et al., 2013; Réus et al., 2010; Mokoena et al., 2010). Moreover, they can mutually modulate their activities in this field. The antioxidant effects of antidepressant drugs seem to vary, depending on the dose, treatment regimen, and duration (Behr et al., 2012). Therefore, the aim of this study was also to evaluate the oxidative stress markers in the cerebral cortex of mice pre-treated with CPT and antidepressant under stress conditions. The FST is a widely-accepted model of depressive-like behaviours and studying physical stress. In addition, the stress in the form of forced swimming may activate the free radical processes (Kondam et al., 2012; Nayanatara et al., 2005).

In order to confirm that the acute stress (FST) causes a disturbance in redox equilibrium, we have additionally examined the oxidative stress markers in the cerebral cortex of mice that were not stressed in the form of swimming. We have noticed that in the group of mice subjected to FST there was an increase of NADP<sup>+</sup> and NADPH in comparison to the stress-naïve group. It is established that NADPH is a key component in cellular antioxidant systems (Ying, 2008). NADPH can be used for a one-electron reduction of drugs causing the ROS generation. However, NADPH is an indispensable factor in quenching ROS through the glutathione regeneration mechanism (Śliwińska et al., 2012). NADPH enhances the cellular antioxidant capacity by acting as a substrate for glutathione reductase to reduce GSSG to GSH. Over double increased NADP<sup>+</sup> levels in the group of mice subjected to acute swimming stress may confirm the generation of free radicals, whereas the increased concentration of NADPH may indicate an increased body requirement for NADPH. It should be emphasized here that physical exhaustion while swimming is most closely associated with the performance of radicals and the lipid peroxidation and it may induce redox stress independent of depression-like alterations. Oxidative stress can be attributed to physical fatigue and ROS leads to functional disturbances (Fan et al., 2016). In all organisms, in response to a oxidative stress, is observed adaptive

response, which is manifested by increased resistance and changes at the molecular and biochemical level (Crawford and Davies, 1994). It should be mentioned that in our experiment we used acute environmental stress, not expecting the adaptive alterations in redox state or receptor expression, as in case of chronic exposure to stress in depression.

In the study, it was observed that in the cortex of animals subjected to environmental stress, after receiving CPT with imipramine, a significant decrease in the concentration of the NADP<sup>+</sup> was revealed as compared to the control group. In the group of mice treated with imipramine, the levels of NADP<sup>+</sup>, as well as NADPH, were lower *versus* the control. In case of NADPH, the co-administration of CPT and imipramine has led to the reduction of this parameter, but only in comparison with CPT group. We have observed in our experiment a reduction in NADP<sup>+</sup> levels compared to controls in both the group of mice receiving only imipramine and also the one treated simultaneously with CPT. These data may suggest the intensification of the reduction processes and at the same time indicate no differences in the impact of imipramine given alone and in combination with CPT. In our research, a significant decrease in the concentration of NADPH was observed in the cerebral cortex of mice treated with imipramine compared to the control group. One of the possible explanations could be a relationship with consumption of NADPH in antioxidative mechanisms of imipramine under stress conditions. It may be speculated that marked decreases in NADPH result from the engagement in ROS reduction. Moreover, NADPH is also a substrate for NADPH oxidase in ROS production (Kovac et al., 2015). It can be presumed therefore, that the above data is the result of NADPH oxidase stimulation and, in consequence, of the decrease in NADPH. Interestingly, we have noticed that in the group receiving CPT the level of NADPH was significantly higher than in the control group, which may indicate high reduction potential of CPT. Here, in the group of animals receiving CPT and imipramine simultaneously there were no changes neither compared to the control nor to imipramine, but only when compared to CPT group, the significant decrease was noted. On one hand, no difference in comparison to control and imipramine does not indicate that the co-administration of imipramine with CPT is more beneficial than imipramine alone for an improve of redox potential. However, on the other hand, it should be noted that no significant decrease in NADPH was observed after co-administration of imipramine and CPT, which was reported after administration of imipramine alone. Against this backdrop, the effect of peer support appears to be positive.

Lipid peroxidation is one of the main events induced by oxidative stress. Stress, which accompanies depression, may increase the MDA + 4 HAE, marker level of lipid peroxidation (Bilici et al., 2001). The consequence of lipid peroxidation is a damage of neuronal membrane phospholipids, depolarization of cell membranes, and the reduction of hydrophobicity of the lipid interior of the membrane. Extensive lipid peroxidation is shown to cause membrane disorganization, by peroxidizing mainly the polyunsaturated fatty acids and phospholipids leading to alterations in the ratio of polyunsaturated fatty acids to other fatty acids. This process causes also retardation of the activity of enzymes and membrane transport proteins. Moreover, lipid peroxidation products, MDA and 4HAE, decrease the NADH-dependent mitochondrial respiratory chain (Korolczuk et al., 2016). Thus, lipid peroxidation is considered as a serious consequence of free radical toxicity, leading to profound changes in the membrane structure and function that may even cause cellular death (Nayanatara et al., 2005). In our work, we have noticed that in mice exposed to FST, the lipid peroxidation was very significantly increased compared to the stress-naïve control group, which may be an evidence of increasing ROS generation and respiratory chain impairment. In turn, a decrease in the concentration of MDA + 4 HAE in the cerebral cortex of mice

treated with the tested substances was observed. Nevertheless, the reduction of this parameter in the single drug groups, as well as in the group receiving combination, was similar. Admittedly, in the group of mice receiving only CPT, there was a stronger reduction in MDA + 4HAE ( $p < 0.01$ ) than in the other groups ( $p < 0.05$ ). However, the combined administration of both drugs did not show any significant improvement compared to control or imipramine administered alone. Anyway, the fact that lipid peroxidation was lower in all treated groups compared with the control may suggest a reduction in the number of ROS, which contributes to reducing the damage caused by oxidative stress (Kumagai et al., 2004). Similar results were reported by other authors, who observed an increase in MDA + 4 HAE in animals with induced depression and a decrease of lipid peroxidation after antidepressant administration (Eren et al., 2007; Réus et al., 2010; Mokoena et al., 2010). The research by Khanzode et al. also showed a decrease of MDA + 4 HAE after the administration of fluoxetine and citalopram in the serum of patients with depression (Khanzode et al., 2003).

The most important factor in the non-enzymatic antioxidant defense and a key indicator of oxidative stress is glutathione. It exists in either reduced (GSH) or oxidized (GSSG) state, and the maintenance of adequate levels of GSH is essential for preventing oxidative damage to the brain and for the reconstruction of damaged cell components (Gawryluk et al., 2011). On the other hand, a high level of GSSG in the cells induces a conformational change in the protein which ultimately leads to the impairment of their function. In our study, we have noticed a statistically significant increase in GSH and GSSG levels in mice exposed to FST in comparison to the group not subjected to the stress factor (Fig. 4A). Increase in GSSG, like the previous results, may confirm an increase in ROS generation under acute swimming stress. However, in our study we have not noticed a change in the GSH/GSSG ratio in the brain of mice subjected to environmental stress. The ratio reduced to oxidized glutathione is a known indicator of oxidative stress. As a result of oxidative stress, this ratio rapidly decreases, whereas the growth of the GSH/GSSG ratio reduces oxidative damage (Ahrens et al., 1995). Hence, this result does not confirm the occurrence of oxidative stress and may be indicative of the effectiveness of the antioxidant system. Data obtained from cortex of mice subjected to FST and receiving tested drugs alone or in combination have not revealed significant changes in the concentration of GSH in comparison to the control group. Our experiments have shown that the imipramine administered to mice increased the concentration of GSSG compared with the control. In the group receiving CPT no change in the concentration of GSSG was reported as compared to the control, although a large standard error was noted. In turn, the co-administration of imipramine with CPT caused a decrease of the GSSG concentration in the cortex of mice compared with the group of animals receiving only imipramine and did not cause any changes compared to the control. Imipramine attaches to adenosine receptors and reduces levels of extracellular adenosine in the cortical synapses (Barcellos et al., 1998). Similarly, CPT, by blocking A1 receptors, inhibits the effects of endogenous adenosine (Okada et al., 2002). Adenosine hyperpolarizes neurons by activating K<sup>+</sup> conductance and inhibits Ca<sup>2+</sup> influx into the nerve terminal, which probably account for its ability to reduce the release of excitatory amino acids (Rudolphi et al., 1992). However, adenosine is released under conditions of oxidative stress and the A1 adenosine receptor contributes to the cytoprotective action of adenosine under conditions of ROS generate in the brain (Nie et al., 1998). Therefore, both imipramine and CPT may potentiate antidepressant effects by affecting the adenosine system. On the other hand, they may contribute to extenuation of defense against ROS. Observed in our study increase of GSSG concentration in the group of mice receiving imipramine may be due to the fact that imipramine in the ineffective dose does

not have antidepressant effect and therefore does not prevent the formation of ROS, but perhaps it weakens the internal defense system in the above-mentioned mechanism. The lack of such a change in the group of mice receiving both drugs simultaneously may result from their synergistic antidepressant effect, which was demonstrated in our experiment. However, further research is required to explain the role of the A1 receptor in defense against oxidative stress under environmental stress conditions. In our work, an increase was observed in the GSH/GSSG ratio in the cerebral cortex of mice treated with imipramine alone and in combination with CPT as compared to the control. The increased rate of GSH/GSSG observed in the groups of animals receiving imipramine and CPT with imipramine may indicate an increased production of ROS and, at the same time, retention of the capacity for antioxidant defenses. The obtained data do not indicate significant differences in the impact of imipramine given alone and in combination with CPT. Zafir et al., after inciting stress in rodents, observed a decrease in the GSH concentration, and after the administration of imipramine, venlafaxine or fluoxetine they reported an increase in the GSH concentration in the animals' brains (Zafir et al., 2009). Moretti et al. confirmed an increased concentration of GSH in the cerebral cortex and hippocampus of mice exposed to stress and administered ascorbic acid and fluoxetine (Moretti et al., 2012).

## 5. Conclusions

To conclude, the results obtained in our study demonstrated a CPT-induced enhancement of the antidepressant-like effect of imipramine both in the FST and TST. The observed significant changes may correspond with the modifications in the levels of monoamines and glutamate in the CNS. Our observations indicate that the adenosine A1 receptors inhibition may be involved in increasing the antidepressant-like effect of imipramine and other antidepressant drugs which acts via multiple monoamine neurotransmissions.

An increase of NADP<sup>+</sup>, NADPH, MDA + 4 HAE, GSH and GSSG may suggest an increasing generation of ROS under acute swimming stress. The co-administration of CPT and imipramine, such as imipramine alone, resulted in the decrease in NADP<sup>+</sup> and LPO concentrations and the increase in the GSH/GSSG ratio *versus* the control group, which may confirm beneficial, but comparable to imipramine, effect on redox balance under environmental stress conditions. An increase in the concentration of GSSG in the cortex of animals treated with imipramine in ineffective dose compared to control and no such changes after combined administration of both drugs may suggest a favorable oxidation-reduction potential resulting from their synergistic antidepressant effect.

Therefore, the combination of selective antagonists of adenosine receptors with tricyclic antidepressants, such as imipramine, may afford new pharmacological opportunities of the treatment of depression.

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## Compliance with ethical standards

All procedures were conducted in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and Polish legislation acts concerning animal

experimentations. The experimental procedures and protocols were approved by the First Local Ethics Committee at the Medical University of Lublin.

## Conflict of interest

The authors declare no conflict of interest.

## References

- Abdel Salam, O.M.E., Mohammed, N.A., Sleem, A.A., Farrag, A.R., 2013. The effect of antidepressant drugs on thioacetamide-induced oxidative stress. *Eur. Rev. Med. Pharmacol. Sci.* 17, 735–744.
- Ahrens, B., Müller-Oerlinghausen, B., Schou, M., Wolf, T., Alda, M., Grof, E., Grof, P., Lenz, G., Simhandl, C., Thau, K., Vestergaard, P., Wolf, R., Möller, H.J., 1995. Excess cardiovascular and suicide mortality of affective disorders may be reduced by lithium prophylaxis. *J. Affect. Disord.* 33, 67–75.
- Ammon, H.P.T., 1990. Biochemical mechanism of caffeine tolerance. *Arch. Pharm.* 324, 261–267.
- Andreazza, A.C., Kauer-Sant'anna, M., Frey, B.N., Bond, D.J., Kapczinski, F., Young, L.T., Yatham, L.N., 2008. Oxidative stress markers in bipolar disorder: a meta-analysis. *J. Affect. Disord.* 111, 135–144.
- Aschbacher, K., O'Donovan, A., Wolkowitz, O.M., Dhabhar, F.S., Su, Y., Epel, E., 2013. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology* 9, 1698–1708.
- Barcellos, C.K., Schetinger, M.R., Dias, R.D., Sarkis, J.J., 1998. In vitro effect of central nervous system active drugs on the ATPase-ADPase activity and acetylcholinesterase activity from cerebral cortex of adult rats. *Gen Pharmacol.* 31, 563–567.
- Behr, G.A., Moreira, J.C., Frey, B.N., 2012. Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder. *Oxid. Med. Cell. Longev.* <https://doi.org/10.1155/2012/609421>.
- Bilici, M., Efe, H., Köroğlu, M.A., Uydu, H.A., Bekaroğlu, M., Değer, O., 2001. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J. Affect. Disord.* 64, 43–51.
- Ciruella, F., Casadó, V., Rodrigues, R.J., Luján, R., Burgueño, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S.R., Mallol, J., Cortés, A., Canela, E.I., López-Giménez, J.F., Milligan, G., Luis, C., Cunha, R.A., Ferré, S., Franco, R., 2006. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1–A2A receptor heteromers. *J. Neurosci.* 26, 2080–2087.
- Crawford, D.R., Davies, K.J., 1994. Adaptive response and oxidative stress. *Environ. Health. Perspect.* 10, 25–28.
- Di Iorio, P., Battaglia, G., Ciccirelli, R., Ballerini, P., Giuliani, P., Poli, A., Nicoletti, F., Caciagli, F., 1996. Interaction between A1 adenosine and class II metabotropic glutamate receptors in the regulation of purine and glutamate release from rat hippocampal slices. *J. Neurochem.* 67, 302–309.
- Eren, I., Naziroğlu, M., Demirdas, A., 2007. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. *Neurochem. Res.* 32, 1188–1195.
- Fan, H., Zhangbin, T., Hua, Y., Huang, X., Gao, Y., Wu, Y., Liu, B., Zhou, Y., 2016. Deep sea water improves exercise and inhibits oxidative stress in a physical fatigue mouse model. *Biomed. Rep.* 4 (6), 751–757.
- Gawryluk, J.W., Wang, J.F., Andreazza, A.C., Shao, L., Young, L.T., 2011. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int. J. Neuropsychopharmacol.* 14, 123–130.
- Hasler, G., 2010. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? *World Psychiatry* 9, 155–161.
- Herbet, M., Szopa, A., Wośko, S., Serefko, A., Izdebska, M., Gawrońska-Grzywacz, M., Piątkowska-Chmiel, I., Janas, M., Gieroba, R., Korga, A., Poleszak, E., Dudka, J., 2016. The positive synergism of CPT and MK-801 in behavioral tests and in reduction of environmental stress and redox signaling changes in mice cerebral cortex. *CNS Neurol. Disord. Drug. Targets.* <https://doi.org/10.2174/1871527315666161010152414>.
- Hunter, A.M., Balleine, B.W., Minor, T.R., 2003. Helplessness and escape performance: glutamate-adenosine interactions in the frontal cortex. *Behav. Neurosci.* 117, 123–135.
- Karcz-Kubicha, M., Antoniou, K., Terasmaa, A., Quarta, D., Solinas, M., Justinova, Z., Pezzola, A., Reggio, R., Müller, C.E., Fuxe, K., Goldberg, S.R., Popoli, P., Ferré, S., 2003. Involvement of adenosine A1 and A2A receptors in the motor effects of caffeine after its acute and chronic administration. *Neuropsychopharmacology* 28, 1281–1291.
- Khanzode, S.D., Dakhale, G.N., Khanzode, S.S., Saoji, A., Palasodkar, R., 2003. Oxidative damage and major depression: the potential antioxidant action of selective serotonin reuptake inhibitors. *Redox Rep.* 8, 365–370.
- Kondam, A., Kate, N.N., Lakshmi, G., Suresh, M., Chandrashekar, M., 2012. Effect of forced swim stress on wistar albino rats in various behavioral parameters. *Int. J. Med. Res. Health Sci.* 1, 7–12.
- Korolczuk, A., Caban, K., Amarowicz, M., Czechowska, G., Irla-Miduch, J., 2016. Oxidative stress and liver morphology in experimental cyclosporine A-induced hepatotoxicity. *Bio. Med. Res. Int.* <https://doi.org/10.1155/2016/5823271>.



- Kovac, S., Angelova, P.R., Holmström, K.M., Zhang, Y., Dinkova-Kostova, A.T., Abramov, A.Y., 2015. Nrf2 regulates ROS production by mitochondria and NADPH oxidase. *Biochim. Biophys. Acta* 4, 794–801.
- Kumagai, T., Matsukawa, N., Kaneko, Y., 2004. A lipid peroxidation-derived inflammatory mediator. Identification of 4-hydroxy-2-nonenal as a potential inducer of cyclooxygenase-2 in macrophages. *J. Biol. Chem.* 279, 48389–48396.
- Kumar, A., Garg, R., Gaur, V., Kumar, P., 2009. Nitric oxide mechanism in protective effect of imipramine and venlafaxine against acute immobilization stress-induced behavioral and biochemical alteration in mice. *Neurosci. Lett.* 467, 72–75.
- Lee, C.H., Lee, S.K., Lee, J.Y., 2010. Deciphering neuropharmacology: from basic neurobiology to new drug development. *Arch. Pharm. Res.* 10, 1463–1466.
- Manji, H.K., Quiroz, J.A., Sporn, J., 2003. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol. Psychiatry* 53, 7–742.
- Mokoena, M.L., Harvey, B.H., Oliver, D.W., Brink, C.B., 2010. Ozone modulates the effects of imipramine on immobility in the forced swim test, and nonspecific parameters of hippocampal oxidative stress in the rat. *Metab. Brain. Dis.* 25, 125–133.
- Moretti, M., Colla, A., de Oliveira Balen, G., dos Santos, D.B., Budni, J., de Freitas, A.E., Farina, M., Severo Rodrigues, A.L., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. *J. Psychiatr. Res.* 46, 331–340.
- Mossner, R., Albert, D., Persico, A.M., Hennig, T., Bengel, D., Holtmann, B., Schmitt, A., Keller, F., Simantov, R., Murphy, D., Seif, I., Deckert, J., Lesch, K.P., 2000. Differential regulation of adenosine A1 and A2A receptors in serotonin transporter and monoamine oxidase A-deficient mice. *Eur. Neuropsychopharmacol.* 10, 489–493.
- Nayanatara, A.K., Nagaraja, H.S., Anupama, B.K., 2005. The effect of repeated swimming stress on organ weights and lipid peroxidation in rats. *J. Physiol. Sci.* 1, 3–9.
- Nie, Z., Mei, Y., Ford, M., Rybak, L., Marcuzzi, A., Ren, H., Stiles, G.L., Ramkumar, V., 1998. Oxidative stress increases A1 adenosine receptor expression by activating nuclear factor kappa B. *Mol. Pharmacol.* 53, 663–669.
- Okada, M., Kawata, Y., Kiryu, K., Mizuno, K., Wada, K., Tasaki, H., Kaneko, S., 1997. Effects of adenosine receptor subtypes on hippocampal extracellular serotonin level and serotonin reuptake activity. *J. Neurochem.* 69, 2581–2588.
- Okada, M., Nutt, D.J., Murakami, T., Zhu, G., Kamata, A., Kawata, Y., Kaneko, S., 2001. Adenosine receptor subtypes modulate two major functional pathways for hippocampal serotonin release. *J. Neurosci.* 21, 628–640.
- Okada, M., Zhu, G., Yoshida, S., Iwasa, H., Kaneko, S., 2002. Mechanisms of interaction between adenosine receptor subtypes on hippocampal serotonin release. *Nihon Shinkei Seishin Yakurigaku Zasshi* 22, 61–69.
- Pandya, C.D., Howel, K.R., Pillai, A., 2013. Antioxidant as potential therapeutics for neuropsychiatric disorders. *Pro. Neuropsychopharmacol. Biol. Psychiatry* 6, 214–223.
- Poleszak, E., Wlaż, P., Kędzierska, E., Nieoczym, D., Wyska, E., Szymura-Oleksiak, J., Fidecka, S., Radziwoń-Zaleska, M., Nowak, G., 2006. Immobility stress induces depression-like behavior in the forced swim test in mice: effect of magnesium and imipramine. *Pharmacol. Rep.* 58, 746–752.
- Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Théor.* 229, 327–336.
- Post, A., Crochemore, C., Uhr, M., Holsboer, F., Behl, C., 2000. Differential induction of NF-kappaB activity and neural cell death by antidepressant in vitro. *Eur. J. Neurosci.* 12, 4331–4337.
- Purdel, N.C., Miulescu, R.D., Neamtu, M.C., Avramescu, E.T., Ilie, M., Manda, G., Margină, D.M., 2015. Studies regarding the protective effects exhibited by antidepressants on cell models. *Rom. J. Morphol. Embryol.* 56, 781–788.
- Réus, G.Z., Stringari, R.B., de Souza, B., Petronilho, F., Dal-Pizzol, F., Hallak, J.E., Zuardi, A.W., Crippa, J.A., Quevedo, J., 2010. Harmin and imipramine promote antioxidant activities in prefrontal cortex and hippocampus. *Oxid. Med. Cell. Longev.* 3, 325–331.
- Rudolph, K.A., Schubert, P., Parkinson, F.E., Fredholm, B.B., 1992. Neuroprotective role of adenosine in cerebral ischaemia. *Trends Pharmacol. Sci.* 13, 439–445.
- Sarandol, A., Sarandol, E., Eker, S.S., Erdinc, S., Vatanserver, E., Kirli, S., 2007. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. *Hum. Psychopharmacol.* 22, 67–73.
- Schiavone, S., Jaquet, V., Trabace, L., Krause, K.H., 2013. Severe life stress and oxidative stress in the brain: from animal models to human pathology. *Antioxid. Redox. Signal.* 18 (12), 1475–1490.
- Sebastiao, A.M., Ribeiro, J.A., 1996. Adenosine A2 receptor mediated excitatory action on the nervous system. *Prog. Neurobiol.* 48, 167–189.
- Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85, 367–370.
- Szopa, A., Poleszak, E., Wyska, E., Serefko, A., Wośko, S., Wlaż, A., Pieróg, M., Wróbel, A., Wlaż, P., 2016. Caffeine enhances the antidepressant-like activity of common antidepressant drugs in the forced swim test in mice. *Naunyn-Schmiedeberg Arch. Pharmacol.* 389, 211–221.
- Śliwińska, J., Dudka, J., Korga, A., Burdan, F., Matysiak, W., Jodłowska-Jędrych, B., Mandziuk, S., Dawidek-Pietryka, K., 2012. Tirapazamine-doxorubicin interaction referring to heart oxidative stress and Ca<sup>2+</sup> balance protein levels. *Oxid. Med. Cell. Longev.* <https://doi.org/10.1155/2012/890826>.
- Takuma, K., Baba, A., Matsuda, T., 2004. Astrocyte apoptosis: implications for neuroprotection. *Prog. Neurobiol.* 72, 111–127.
- Yacoubi, E.Y.M., Ledent, C., Parmentier, M., Bertorelli, R., Ongini, E., Costentin, J., Vaugeois, J.M., 2001. Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice. *Br. J. Pharmacol.* 134, 68–77.
- Yamato, T., Yamasaki, S., Misumi, Y., Kino, M., Obata, T., Aomine, M., 2002. Modulation of the stress response by coffee: an in vivo microdialysis study of hippocampal serotonin and dopamine levels in rat. *Neurosci. Lett.* 332, 87–90.
- Ying, W., 2008. NAD<sup>+</sup>/NADH and NADP<sup>+</sup>/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid. Redox. Signal.* 10 (2), 179–206.
- Zafir, A., Ara, A., Banu, N., 2009. In vivo antioxidant status: a putative target of antidepressant action. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 220–228.