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THE GLUTATHIONE SYSTEM STATE IN RATS EXPOSED TO ETHANOL INTOXICATION, ITS COMBINATION WITH CAFFEINE AND MODIFIED PHOTOPERIOD

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Key words: *ethanol, caffeine, light exposure, melatonin, glutathione, glutathione peroxidase, glutathione-S-transferase*

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Resume. Objective. *to study the content of reduced glutathione (GSH), activities of glutathione peroxidase (GP) and glutathione-S-transferase enzymes (GST) in blood and liver of rats exposed to subacute alcohol intoxication, its combination with caffeine intake or constant light exposure, and the possibility of their correction with melatonin.*

Materials and methods*. Experiments were performed on 42 male rats weighing 180-200 g kept under standard conditions of the vivarium and artificial equinox. Alcohol intoxication was induced by intragastric administration 40 % ethanol at a dose of 7 ml/kg of body weight for 7 days. Caffeine was administered by gavage at a dose of 30 mg/kg of body weight.*

Results. *Ethanol poisoning and its combination with caffeine intake or exposure to constant light resulted in a decrease in GSH level and GP activity in RBCs and liver, besides the combination of ethanol+light resulted in more pronounced depletion of the parameters. The combination of ethanol with caffeine resulted in less reduction of GSH level in the blood (by 25%) but more depletion of GSH in the liver (by 45% vs. control) than in ethanol-treated rats. There was an elevation of GST activity in the liver of all groups of alcoholized animals. Administration of 5 mg/kg melatonin for 7 days limited depletion in GSH and prevented the changes in GP and GST activities in the blood and liver of all groups of animals.*

Conclusions. *Melatonin administration prevented ethanol-induced toxicity in rats exposed to ethanol and its combination with caffeine or constant light for 7 days by limiting the depletion in GSH and preventing the changes in GP and GST activities in the blood and liver of all groups of animals.*

СТАН ПОКАЗНИКІВ ГЛУТАТІОНОВОЇ СИСТЕМИ ЩУРІВ ЗА УМОВ ІНТОКСИКАЦІЇ ЕТАНОЛОМ, ПОЄДНАННЯ ЇЇ ІЗ ВВЕДЕННЯМ КОФЕЇНУ АБО ЗМІНЕНИМ ФОТОПЕРІОДОМ

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Ключові слова: *етанол, кофеїн, освітлення, мелатонін, глутатіон, глутатіонпероксидаза, глутатіон-S-трансфераза.*

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Резюме. Мета роботи. *дослідити вміст відновленого глутатіону (ГSH), активність глутатіонпероксидази (ГП) та глутатіон-S-трансферази (ГST) у крові та печінці щурів за умов підгострої алкогольної інтоксикації, поєднання її з уведенням кофеїну або постійним освітленням та можливість їх корекції мелатоніном.*

Матеріал і методи. *Експерименти проводили на 42 самцях щурів масою 180-200 г, які утримувалися за стандартних умов віварію і штучного рівнодення. Алкогольну інтоксикацію викликали внутрішньошлунковим уведенням 40% етанолу в дозі 7 мл/кг маси тіла впродовж 7 діб. Кофеїн уводили внутрішньошлунково в дозі 30 мг/кг маси тіла.*

Результати. *Отруєння етанолом та його поєднання з прийомом кофеїну або постійним освітленням призводило до зниження рівня ГSH та активності ГП в еритроцитах і печінці, причому, комбінація етанол+світло призводила до більш вираженого зниження показників. Поєднання етанолу з уведенням кофеїну викликала менш виражене зниження рівня ГSH у крові (на 25%), але більш виражене зниження цього показника в печінці (на 45% нижче контролю), ніж у щурів, які отримували лише етанол. Спостерігалось підвищення активності ГST у печінці всіх груп алкоголізованих тварин. Уведення 5 мг/кг мелатоніну протягом 7 днів запобігало зниженню вмісту ГSH і змінам активності ГП і ГST у крові та печінці усіх груп тварин.*

***Висновки**. Уведення мелатоніну зменшує токсичні ефекти у щурів за умов впливу етанолу та його комбінації з кофеїном або постійним світлом протягом 7 днів, зменшуючи виснаження відновлення глутатіону та запобігаючи змінам активності глутатіонпероксидази та глутатіон-S-трансферази у крові та печінці всіх груп тварин.*

Introduction. Enhancement of free radical oxidation and the development of oxidative stress is the main pathogenetic mechanism of ethanol toxicity [1]. Ethanol-induced oxidative stress is the result of combined impairment of antioxidant defense and the production of reactive oxygen species by mitochondrial electron transport chain, alcohol-inducible cytochrome P450 (CYP) 2E1 and activated phagocytes. The glutathione system plays an important role in antiradical and antiperoxide protection of cells [1,2]. The coordinated action of all its components (reduced glutathione, glutathione peroxidase, glutathione-S-transferase, glutathione reductase) helps to maintain the optimal level of redox potential in cells [3].

In recent years, the consumption of caffeinated alcohol drinks has considerably increased worldwide [4]. Besides experimental studies evidencing the antioxidant effect of caffeine [5,6], there is currently abundant evidence pointing to its ability to stimulate apoptosis, mutagenic action, and prooxidant effect at certain concentrations [6,7].

Melatonin (N-acetyl-5-methoxytryptamine) is a pineal gland hormone which has an extremely wide range of physiological functions, one of which is potent antioxidant action evidenced by numerous in vitro and in vivo studies [8]. Melatonin secretion by the pineal gland is controlled by an endogenous circadian rhythm, rising at night and suppressed by light [9]. Some studies showed the impact of evening caffeine intake on human circadian rhythms resulting in decreased nighttime melatonin levels [10]. Furthermore, alcohol intake is usually combined with circadian disruption. Disbalance in melatonin secretion leads to a shift in the pro- and antioxidant balance in organs and tissues and, therefore, decreases the body's vulnerability to various toxins, including ethanol [8].

Objective. To study the content of reduced glutathione (GSH), activities of glutathione peroxidase [EC 1.11.1.9] (GP), and glutathione-S-transferase enzymes (GST) [EC 2.5.1.18] in blood and liver of rats exposed to subacute alcohol intoxication, its combination with constant light or caffeine intake and the possibility of their correction with melatonin.

Material and methods. The study was performed in compliance with the Rules of the work using experimental animals (1977) and the Council of Europe Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (Strasbourg, 1986). It was conducted according to directions of the International Committee of Medical Journals Editors (ICMJE), as well as "Bioethical expertise of preclinical and other scientific research performed on animals" (Kyiv, 2006).

Experiments were performed on 42 male Wistar rats weighing 180-200 g which were randomly grouped and kept in polycarbonate cages (3-4 rats per cage) in a room under controlled environmental conditions (temperature 21±1 °C and 12:12 h light/dark cycle, with lights on 8:00 a.m.). Animals received food and water ad libitum.

Subacute alcohol intoxication was induced by intragastric administration 40 % ethanol at a dose of 7 ml/kg of body weight for 7 days. Caffeine was administered intragastrically by gavage at a dose of 30 mg/kg of body weight which is equivalent to a dose of 6 mg/kg in humans and corresponded to moderate coffee consumption (5 cups of coffee or about 400 mg of caffeine). The light exposure was caused by a constant fluorescent light of 1500 lux intensity for 24 hours a day. Melatonin ("Vita-melatonin", JSC "Kyiv Vitamin Plant") was given by gavage at a dose of 5 mg/kg of body weight at 20^{00} for 7 days along with alcohol intoxication. The control group of animals received equivolume amount of water.

Rats were randomly assigned into 7 groups: group 1- untreated control; group 2 - induced subacute alcohol intoxication; group 3- alcohol intoxication + melatonin administration; group 4 - alcohol intoxication + caffeine administration; group 5 - alcohol intoxication + caffeine + melatonin; group 6 - alcohol intoxication + constant light exposure; group 7 - alcohol intoxication + constant light exposure + melatonin.

Animals were decapitated under light ether anesthesia on the 7^{th} day after the beginning of the experiment. Blood samples were collected in presence of anticoagulant EDTA (1 mg/ml of blood). Erythrocytes were washed three times with five volumes of saline solution and centrifuged at 3000 rpm for 10 min. The liver samples were excised, minced, rinsed with cold 50 mm Tris-HCl buffer (pH=7.4) to remove the blood and homogenized in a glass homogenizer with a motor-driven teflon pestle on ice to prepare 5 % homogenates. The homogenates were centrifuged for 10 min at 900g.

The spectrophotometric assay method for GSH involves oxidation of GSH by the 5.5'-dithio-bis (2-nitrobenzoic acid) to form the yellow derivative 5'-thio-2-nitrobenzoic acid, measured at 412 nm [11]. The activity of GPx was determined by the amount of oxidized glutathione measured spectrophotometrically at 262 nm which is formed from reduced one during H_2O_2 detoxification by glutathione peroxidase [11]. The activity of the enzyme was expressed in nanomol of oxidized glutathione produced in 1 min per 1 mg of protein.

GST activity assay is based on spectrophotometric measurement of reduced glutathione conjugate with 1-chloro-2,4-dinitrobenzene, formed in the reaction under the action of the enzyme. The activity of the enzyme was expressed in nmoles of the conjugate formed in 1 min per 1 mg of protein [12].

Total protein content was assayed by Lowry using a standard reagent kit for clinical diagnostics ("Filisit-Diagnostics" Co., Ltd.).

The results were statistically processed using the STATISTICA 10 software (StatSoft Inc.). A Shapiro-Wilk test was performed to verify normality of data distribution and then Mann-Whitney test was used, which was considered sufficient for valid conclusions to be made. Data are illustrated as mean±SEM (n=6 animals per group). P<0.05 was regarded as statistically significant differences.

Results and their discussion. In this study, we demonstrated that the model of subacute ethanol stress caused a decrease in reduced glutathione content in erythrocytes and liver by 42% and 37% below the control group (Table). It could be due to the direct oxidation of glutathione SH groups by acetaldehyde and its free radical metabolites.

Table

The content of GSH, GP and GST activities in rats in terms of alcohol intoxication combined with light exposure, caffeine, and melatonin intake (M±m, n=6)

	Blood		**Liver**		
Groups/ Indices	**GSH, µMol/ml**	**GP, µMol/min• g Hb**	**GSH , µMol/g tissue**	**GP, nMol/min• mg protein**	**GST , nMol/min• mg protein**
Control	0.875±0.079	121.5±9.2	7.24±0.42	86.2±6.4	152.8±8.8
Ethanol	0.506±0.046*	91.1±7.8*	4.49±0.62*	48.3±5.4*	197.1±11.2*
Ethanol + melatonin	0.794±0.064	130.5±9.1	6.82±0.59	88.6±8.3	175.7±7.8*
Ethanol + caffeine	0.653±0.039*#	95.7±6.24*	4.01±0.39*	67.0±6.6*#	228.8±12.3*#
Ethanol + caffeine + melatonin	0.995±0.085	108.7±8.3	7.18±0.418	101.9±8.1*	178.1±9.3*
Ethanol + light	0.457±0.062*	79.5±10.1*	3.01±0.52*#	56.9±7.4*	207.8±9.2*
Ethanol + light + melatonin	0.996±0.066*	121.8±11.0	6.98±0.46	74.2±6.3	181.1±11.4*

*Note: * – statistically significant difference compared to the control group (p≤0,05);*
 # – statistically significant difference compared to ethanol treated group (p≤0,05).

Combined ethanol and caffeine intake showed the value of GSH in the blood decreased less (by 25.3% lower vs. the control group), which could be the evidence of caffeine antioxidant effect. However, in the liver of such animals, GSH content was 55.4% below the control group, which is much lower than in animals treated with ethanol only. It could result from glutathione system depletion due to more pronounced manifestations of oxidative stress caused by activation of microsomal oxidative system in hepatocytes, involved in both ethanol and caffeine metabolism when combined in high concentrations.

A study of glutathione enzymes in alcohol intoxication and its combination with caffeine showed a decrease in glutathione peroxidase activity in the blood (by 25.1% and 21.2%, respectively) and liver (by 44% and 22% below control). Such a decrease in the enzyme activity could be caused by GSH deficiency in cells and the compensatory increase in catalase activity in response to oxidative stress.

Glutathione-S-transferase activity, a sensitive marker of endogenous intoxication in the body, in the liver of alcoholic rats appeared to be by 29% higher than in control. Caffeine administration caused an increase in GST activity in the liver of alcoholized animals by 49% above the control which was statistically higher than in animals treated with ethanol alone. These data are consistent with the studies showing the ability of caffeine to stimulate the synthesis of antitoxic enzymes (in particular, GST) in rats' liver and kidneys [5, 6].

The combination of alcohol intoxication with constant light exposure caused more pronounced changes in the glutathione system compared to animals kept under normal light conditions. Thus, the GSH level in the blood and liver of such animals was 48% and 58% below the control value, respectively, indicating depletion in reducing capacity of the antioxidant system caused by decreased melatonin synthesis under constant lighting. Moreover, decreased levels of reduced glutathione could be caused by increased GST activity in the liver, which exceeded the control by 36% and was higher than in the group of animals exposed to ethanol alone. Combination of ethanol poisoning with constant light resulted in a decrease in GP activity by 35% and 34% below the control level.

In the study, we demonstrated (Table) that administration of 5 mg/kg melatonin during subacute ethanol stress and its combination with caffeine intake or constant light for 7 days limited the decrease in reduced

glutathione level in the liver of all studied groups. In the blood of rats exposed to combined ethanol and caffeine or altered photoperiod, the content of GSH was even higher than in the control group (by 13% and 24%, respectively).

Melatonin helped to restore the reduced glutathione peroxidase activity in the blood and liver of all studied groups of animals up to the control level. Moreover, in the liver of rats treated with a combination of ethanol and caffeine GP activity exceeded the control level by 19%.

Melatonin administration caused glutathione-S-transferase activity in the liver of animals treated with ethanol and its combination with caffeine or constant light exposure to be significantly lower than in untreated animals, but it remained above the control level (by 15%, 17% and 18%, respectively). The normalizing impact of melatonin on glutathione system activity could be due to its direct antiradical effect, as secondary antioxidant capacity, stimulating the activity of antioxidant enzymes by affecting the expression of relevant genes [8].

Conclusions. Thus, ethanol poisoning and its combination with caffeine intake or light exposure could result in a significant disbalance in the glutathione antioxidant system resulting in decreased GSH level and GP activity and increased GST activity in the blood and liver. The combination of ethanol with caffeine or constant light exposure potentiated the toxic effects of ethanol in the liver. Melatonin administration at a dose of 5 mg/kg body weight prevented ethanol-induced toxicity in rats exposed to ethanol and its combination with caffeine or constant light by limiting the depletion in GSH and preventing the changes in GP and GST activities in the blood and liver of all group of animals.

Prospects for further research. To study the state of the antioxidant system in blood plasma, liver, and kidneys of rats exposed to subacute alcohol intoxication, its combination with constant light exposure, and caffeine intake.

References

1. Birková A, Hubková B, Cižmárová B. Bolerázska B. Current view on the mechanisms of alcohol-mediated toxicity. Int J Mol Sci. 2021 Sep;22(18):9686. DOI: 10.3390/ijms22189686.

2. Harjumäki R, Pridgeon CS, Ingelman-Sundberg M. CYP2E1 in alcoholic and non-alcoholic liver injury. Roles of ROS, reactive intermediates and lipid overload. Int J Mol Sci. 2021 Jul 30;22(15):8221. DOI: 10.3390/ijms22158221.

3. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C. Changes in glutathione content in liver diseases: an update. Antioxidants. 2021 Feb 28;10(3):364. DOI: 10.3390/antiox10030364.

4. Ikram M, Park TJ, Ali T, Kim MO. Antioxidant and neuroprotective effects of caffeine against Alzheimer's and Parkinson's disease: insight into the role of Nrf-2 and A2AR signaling. Antioxidants. 2020 Sept 22;9(9):902. DOI: 10.3390/antiox9090902.

5. Martini D, Del Bo C, Tassotti M, Riso P, Del Rio D, Brighenti F, et al. Coffee consumption and oxidative stress: a review of human intervention studies. Molecules. 2016 Jul 28;21(8):979. DOI: 10.3390/molecules21080979.

6. GülçİnI. In vitro prooxidant effect of caffeine. Journal of Enzyme Inhibition and Medicinal Chemistry. 2008 Oct;23(1):149-52. DOI: 10.1080/14756360701306404.

7. Galano A, Reiter RJ. Melatonin and its metabolites vs oxidative stress: from individual actions to collective protection. J Pineal Res. 2018 June 5;65(1):e12514. DOI: 10.1111/jpi.12514.

8. Cipolla-Neto J, Amaral FG. Melatonin as a hormone: new physiological and clinical insights. Endocrine Reviews. 2018 Dec;39(6):990-1028. DOI: 10.1210/er.2018-00084.

9. Burke TM, Markwald RR, McHill AW, Chinoy ED, Snider JA, Bessman SC, et al. Effects of caffeine on the human circadian clock in vivo and in vitro. Sci Transl Med. 2015 Sep 16;7(305):305ra146. DOI: 10.1126/scitranslmed.aac5125.

10. Davydova NV. The biochemical mechanisms of antioxidative action of the Rhodiola rosea extract [dissertation abstract]. Kyiv; 2004. 148 p.

11. Habig WH, Jakoby WB. Glutathione S-transferases (rat and human). Methods in enzymology. 1981;77:218-31. DOI: 10.1016/S0076-6879(81)77029-0.

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