THE STATE OF PRO- AND ANTIOXIDANT BALANCE OF RATS' BLOOD UNDER CONDITION OF COMBINED CAFFEINE AND ETHANOL INTAKE AND MELATONIN ADMINISTRATION

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The aim of research *– to investigate prooxidant and antioxidant parameters in the blood of rats subjected to alcohol intoxication, caffeine and melatonin administration.*
Materials and methods. *Experiments were performed on 30 male rats weighing 180-200 g. Subacute alcohol intoxication was induced by intragastric administration of 40 % ethanol at a dose of 7 ml/kg of the body weight for 7 days. Caffeine was administered by gavage at a dose of 30 mg/kg of the body weight. The content of malondialdehyde in erythrocytes, oxidatively modified proteins, SH-groups, ceruloplasmin, catalase activity in blood plasma was determined.*
Results. *It was revealed that alcohol intoxication increased malonic dialdehyde and oxidatively modified proteins content 40 % and 37.5 %, respectively. Co-administration of ethanol with caffeine reduced the intensity of oxidative stress in the blood. Rats exposed to ethanol and its combination with caffeine showed decrease in SH-groups in the blood 25 % and 39.7 % below control. Ceruloplasmin and catalase levels were markedly elevated in alcohol-exposed rats besides that catalase activity further increased with the combined ethanol and caffeine exposure. Administration of 5 mg/kg melatonin for 7 days effectively normalized prooxidant and antioxidant markers in ethanol-exposed rats. However, in case of ethanol and caffeine intake, melatonin's impact on catalase and ceruloplasmin levels was less markable.*
Conclusions. *Thus, melatonin showed antioxidant properties, decreasing alcohol-induced oxidative stress in rats' blood, but its impact was reduced in the presence of caffeine.*

Key words: *ethanol, caffeine, melatonin, oxidative stress, antioxidant defense, rats.*

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СТАН ПРО- ТА АНТИОКСИДАНТНОЇ РІВНОВАГИ КРОВІ ЩУРІВ ЗА УМОВ ПОЄДНАНОГО ВВЕДЕННЯ ЕТАНОЛУ ТА КОФЕЇНУ ТА КОРЕКЦІЇ МЕЛАТОНІНОМ

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Мета роботи – *вивчити показники прооксидантної та антиоксидантної систем у крові щурів за умов алкогольної інтоксикації, поєднаної з введенням кофеїну та мелатоніну.*
Матеріали та методи. *Експерименти проведені на 30 самцях щурів масою 180-200 г. Підгостру алкогольну інтоксикацію викликали інтрагастральним введенням 40 %-го етанолу в дозі 7 мл/кг маси тіла протягом 7 днів. Кофеїн вводили внутрішньошлунково в дозі 30 мг/кг маси тіла. Визначали вміст малонового діальдегіду в еритроцитах, окиснювально модифікованих білків, SH-груп, церулоплазміну, активність каталази в плазмі крові.*
Результати. *Встановлено, що алкогольна інтоксикація супроводжувалась зростанням вмісту малонового альдегіду та окисно модифікованих білків на 40 % та 37,5 % вище контрольних показників відповідно. Поєднане введення етанолу та кофеїну супроводжувалось менш вираженими проявами окисного стресу в крові щурів. У тварин, яким вводили як етанол, так і його комбінацію з кофеїном, спостерігалось зниження вмісту у крові SH-груп на 25 % і 39,7 % стосовно контрольних показників відповідно. Вміст церулоплазміну та активність каталази у алкоголізованих щурів виявились значно вищими від рівня контролю, а у щурів, яким вводили комбінацію етанолу з кофеїном, активність каталази зростала. Введення мелатоніну в дозі 5 мг/кг впродовж 7 днів одночасно з етанолом призводило до нормалізації показників прооксидантної та антиоксидантної систем у крові алкоголізованих щурів. Однак у випадку поєднаного введення етанолу та кофеїну нормалізуючий вплив мелатоніну на активність каталази та церулоплазміну був менш суттєвим.*
Висновок. *Мелатонін проявляє антиоксидантні властивості, зменшуючи окисний стрес у крові щурів, викликаний етанолом, але його вплив менш виражений за умов поєднаного введення етанолу та кофеїну.*

Ключові слова: *етанол, кофеїн, мелатонін, окисний стрес, антиоксидантний захист, щури.*

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Introduction

Excessive alcohol consumption is a global issue that poses significant challenges to public health. The World Health Organization identifies alcohol as a major risk factor for non-communicable diseases which contributes to more than 5 % of the global disease burden [1]. Chronic alcohol abuse is a leading cause of liver cirrhosis, neuropsychiatric disorders, cardiovascular diseases, contributing to endothelial dysfunction and atherosclerosis resulting in estimated 3 million deaths annually worldwide [1, 2].

Pathogenesis of ethanol-induced cell damage involves generation of reactive oxygen species (ROS) during its metabolism in hepatocytes by cytosolic alcohol dehydrogenase pathway and microsomal ethanol-oxidizing system. Both pathways result in acetaldehyde production, which is highly reactive toxic intermediate leading to ROS generation, triggering oxidative stress, mitochondrial damage, initiating apoptosis and cascade of events leading to cell death [2, 3, 4].

Chronic alcohol intake enhances oxidative stress also by depleting endogenous antioxidant system, such as decrease in reduced glutathione, impairing the cellular defense mechanisms against ROS [5]. Alcohol-induced activation of inflammatory pathways, including nuclear factor-kappa B, further amplifies oxidative stress and promotes tissue injury [6].

Caffeine, commonly consumed in various forms such as coffee, tea, and energy drinks, has become an integral part of modern lifestyle, with a global average daily intake of approximately 200 mg per person [7]. Caffeine (1,3,7-trimethylxanthin) has drawn attention for its potential health benefits, particularly its antioxidant properties. Studies suggest that caffeine intake may reduce the risk of certain chronic diseases, including neurodegenerative disorders and certain types of cancer by decreasing oxidative stress-induced cell death [8, 9]. Caffeine is recognized for its antioxidant properties, related to its ability to scavenge free radicals and inhibit free-radical oxidation of biomolecules protecting cells from oxidative stress [10, 11]. Moreover, caffeine has been reported to enhance endogenous antioxidant defense by upregulating the expression and activity of antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase [11]. But there are some evidences about caffeine's pro-oxidant effects, particularly at high concentrations [11, 12].

Alcoholic energy drinks which combine alcohol with high doses of caffeine and other stimulants are quite popular nowadays. The co-administration of ethanol and caffeine raises questions about their potential interactions that may either exacerbate or mitigate their respective effects on the human body [13].

Melatonin, a pineal gland hormone, besides its well-established role in regulating circadian rhythms possesses potent antioxidant properties, capable of scavenging free radicals and modulating antioxidant enzyme activity [14, 15]. Melatonin easily crosses cell membranes, including the blood-brain barrier, that makes it promising candidate to suppress oxidative stress associated with chronic alcohol consumption. In the context of chronic alcohol intake along with caffeine, investigating the efficacy of melatonin as a therapeutic agent to correct oxidative disbalance is relevant.

The aim of research

To investigate indicators of prooxidant system (malonic dialdehyde and oxidatively modified proteins) and antioxidant system (HS-groups, ceruloplasmin, catalase) in the blood of rats exposed to subacute alcohol intoxication, its combination with caffeine intake, and melatonin administration.

Research materials 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 performed according to directions of 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 (4 rats per cage) in a room under controlled environmental condition (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 of 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 corresponds to moderate coffee consumption (5 cups of coffee or about 400 mg of caffeine). Melatonin («Vita-melatonin», JSC «Kyiv Vitamin Plant») was given by gavage at dose of 5 mg/kg of body weight for 7 days along with alcohol intoxication. The control group of animals received equivolume amount of water.

Rats were randomly assigned into 5 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.

Animals were decapitated under light ether anesthesia on the 7th day after beginning of the experiment. Blood samples were collected in the presence of anticoagulant EDTA (1 mg/ml of blood). Blood plasma was obtained by blood centrifugation at 3000 rpm for 10 min. Erythrocytes were washed three times with five volumes of saline solution and centrifuged at 3000 rpm for 10 min.

Malonic dialdehyde (MDA) content was assayed in erythrocytes by spectrophotometric measurement of trimethine colored complex formed in reaction with thiobarbituric acid at high temperature and acidic pH [16]. The content of oxidatively modified proteins (OMP) in blood plasma was assayed by reaction of amino acids carbonyl derivatives with 2.4-dinitrophenyl hydrazine which results in formation of hydrazones having specific absorption spectrum. Aldehydes derivates were determined at 370 nm [17]. The content of SH-groups was determined by the reaction with 5,5-dithiobis-2-nitrobenzoic acid (Ellmans' reagent) [18]. The ceruloplasmin content in the blood plasma

was measured spectrophotometrically by determination of phenylenediamine oxidation products and expressed in mg/l of blood plasma [19]. The catalase activity was determined by a method based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate with a maximum absorption at λ=410 nm [19]. Total protein content was assayed by Lowry using 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 to considered sufficient for valid conclusions to be made. Data are illustrated as mean±SEM (n=6 animals per group). $P<0.05$ was considered as statistically significant differences.

Results and their discussion

We observed (table 1) that alcohol intoxication was accompanied by a 40 % increase in the blood malonic dialdehyde content in rats compared to the control group. Alongside the increase of lipid peroxidation, there was an intensification of free radical-induced damage to protein molecules, leading to 37.5 % increase in OMP content in the blood plasma above the control level.

Ethanol poisoning at a dose of 7 ml/kg body weight combined with caffeine administration at a dose of 30 mg/kg body weight for 7 days resulted in 25 % increase in MDA content in erythrocytes and 32 % increase in the blood plasma OMP content compared to the control level. These indicators were likely lower than in alcoholized animals that did not receive caffeine, confirming the antioxidant effect of caffeine.

Table 1

Some indicators of pro- and antioxidant system in rats' blood in terms of alcohol intoxication combined with caffeine and melatonin intake (M±m, n=6)

Groups	MDA nMol/ml	OMP, mMol/g prot.	SH-groups, mMol/g prot.	Ceruloplasmin, mg/l	Catalase, μmol/ min l
Control	11.6±1.15	0.76±0.06	5.21 ± 0.30	110.0 ± 12.1	12.9 ± 0.82
Ethanol	16.0±1.66*	1.07±0.08*	3.75 ± 0.29*	200.4 ± 16.2*	15.8 ± 1.54*
Ethanol + melatonin	13.2±1.06	0.87±0.10	5.11 ± 0.43	129.2 ± 11.9*	11.5 ± 1.27
Ethanol + caffeine	14.3±0.96*#	1.02±0.09*	3.14 ± 0.32*#	165.8 ± 9.1*#	17.3 ± 1.28*
Ethanol + caffeine + melatonin	10.4±1.05	0.91±0.15	3.89 ± 0.31*	147.3 ± 8.3*	18.5 ± 1.01*

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

It has been established that subacute alcohol intoxication was accompanied by a disbalance in the indicators of antioxidant defense in the rats' blood. SH-groups, also known as thiol groups, play a crucial role in the context of oxidative stress as they are key components of glutathione, active sites of many antioxidant enzymes and transition-metal ion chelators. The content of SH-groups in the blood plasma of animals with subacute alcohol intoxication was 28 % lower than the control level, whereas the combined administration of ethanol and caffeine caused a more pronounced decrease in this indicator (40 % lower than the control).

The content of the antioxidant enzyme ceruloplasmin in the blood plasma of animals treated with ethanol was 82 % higher than the control group, while in animals receiving a combination of ethanol and caffeine, the increase in this indicator was significantly less and exceeded the control level 50.7 %.

Ethanol poisoning was associated with an increase in catalase activity in the blood 23 % higher than the control, while the combined administration of ethanol and caffeine caused even greater increase in this parameter (35 % higher than the control). These findings align with literature sources demonstrating caffeine's ability to increase catalase and superoxide dismutase activity under physiological and pathological conditions [29, 34].

In the study, we demonstrated (table 1) that administration of 5 mg/kg melatonin during subacute ethanol stress and its combination with caffeine intake for 7 days limited the rise in lipid peroxidation products and oxidatively modified proteins. Thus, the content of MDA exceeded the level of control in the blood plasma of alcohol-exposed animals 15 % only. The melatonin administration prevented statistically changes in OMP content in the blood plasma of animals treated with ethanol, as well as its combination with caffeine intake.

Administration of melatonin to ethanol-exposed rats proved to be quite effective in normalizing the level of SH-groups, ceruloplasmin and catalase activity in the blood. This suggests that melatonin has a potential role in restoring antioxidant defense mechanisms compromised by alcohol intake. But as for animals exposed to combination of ethanol and caffeine the impact of melatonin was less effective: the level of SH-groups and ceruloplasmin remained 25 % below and 34 % above the control level respectively; catalase activity exceeded control group 44 % that is even higher than for animals exposed to combination of ethanol and caffeine only. Thus, while melatonin still demonstrated effectiveness in normalizing antioxidant markers, the magnitude of improvement was lower compared to animals exposed to ethanol alone. This suggests a complex interaction between melatonin and caffeine in modulating oxidative stress responses.

Conclusions

1. The co-administration of caffeine with ethanol demonstrated a mitigating effect on oxidative stress markers compared to animals exposed to ethanol alone. This suggests potential antioxidant role of caffeine in counteracting alcohol-induced oxidative stress.

2. Melatonin administration in terms of subacute ethanol exposure, both alone and in combination with caffeine intake, showed protective effect against the rise in lipid peroxidation products and oxidatively modified proteins.

3. Melatonin appeared to be effective in normalizing the levels of SH-groups, ceruloplasmin, and catalase activity in the blood of rats exposed to ethanol alone but was less pronounced in animals exposed to a combination of ethanol and caffeine.

Prospects for further research

To study the state of metabolic biochemical indicators in blood plasma of rats exposed to subacute alcohol intoxication and its combination with constant light exposure.

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