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ВПЛИВ ЕТАНОЛУ НА БІОПРОДУКТИВНІСТЬ, ФОТОСИНТЕЗ ТА ДИХАННЯ МІКРОВОДОРОСТІ *CHLAMYDOMONAS REINHARDTII*

Деякі органічні сполуки здатні значно стимулювати ріст одноклітинної зеленої водорості Chlamydomonas reinhardtii. Серед них найбільш активними стимуляторами росту є ацетат і одноатомний спирт метанол. Метою даної роботи було вивчення впливу на продуктивність C. reinhartdii іншого спирту – етанолу, який при внутрішньоклітинному окиснені трансформується у ацетат. Отримані результати показали, що етанол стимулює дихання, пригнічує фотосинтез і зупиняє ріст культури. Зроблений висновок, що причиною гальмування росту C. reinhartdii було зниження pH середовища культивування внаслідок окиснення етанолу до оцтової кислоти.

Ключові слова : Chlamydomonas reinhardtii, фотосинтез, дихання, етанол, міксотрофія.

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ВЛИЯНИЕ ЭТАНОЛА НА БИОПРОДУКТИВНОСТЬ, ФОТОСИНТЕЗ И ДЫХАНИЕ МИКРОВОДОРОСЛИ *CHLAMYDOMONAS REINHARDTII*

Некоторые органические соединения способны значительно стимулировать рост одноклеточной зеленой водоросли Chlamydomonas reinhardtii. Среди них наиболее активными стимуляторами роста являются ацетат и одноатомный спирт метанол. Целью данной работы было изучение влияния на продуктивность C. reinhartdii другого спирта – этанола, окисляющегося внутри клеток в ацетат. Результаты показали, что этанол стимулирует дыхание, подавляет фотосинтез и останавливает рост культуры. Сделан вывод, что причиной торможения роста C. reinhartdii было снижение pH среды культивирования в результате окисления этанола до уксусной кислоты.

Ключевые слова : Chlamydomonas reinhardtii, фотосинтез, дыхание, этанол, миксотрофия.

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THE INFLUENCE OF LOW MOLECULAR WEIGHT ORGANIC COMPOUNDS ON ANTIOXIDANT DEFENSE SYSTEMOF THE GASTRIC MUCOSA UNDER ETHANOL-INDUCED GASTRIC LESIONS IN RATS

It was investigated the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa of rats caused by ethanol. It was found that prophylactic injection of this substance at a dose of 1 mg/kg effectively protects the stomach from ethanol injuries. LMOC effectively restored the pro-/ antioxidant equilibrium by reducing the intensity of lipid peroxidation in the gastric mucosa of rats after ethanol injection and increase ofsuperoxidedismutase, catalase activityandactivity of glutathionesystem.

Keywords: ethanol-induced injuries, lipid peroxidation,low molecular weight organic compound.

Introduction. Gastric ulcer is a common disease affecting many people worldwide [1]. The peptic ulcer, characterize by mucosal damage, is predominantly caused by *Helicobacter pylori*, antiplatelet agents such as acetylsalicylic acid [2], nonsteroidal anti-inflammatory drugs (NSAIDs) such as oral bisphosphonates, potassium chloride, immunosuppressive medications [3], serotonin reuptake inhibitors [4], alcohol consumption, and cigarette smoking [5]. The ulcer disease may lead to upper gastrointestinal haemorrhage and perforation [6], which have high morbidity and mortality rates [7]. Thus,the search of the newnontoxic medications is very importanttoday. So the aim of the work was to investigate the preventive effect of low molecular weight organic compound (LMOC) on erosive and ulcerative lesions in the gastric mucosa undere-thanol-induced gastric lesions in rats.

Methods. The animals used in the study were bred and kepton a standard diet in terms of accredited vivarium of Educational and Scientific Center "Institute of Biology" Taras Shevchenko National University of Kyiv in accordance with the "standard rules on arrangement, furnishing and maintenance of experimental biological clinics (vivarium)".

The studywas carried out on 30 white laboratory Wistar rats. The research was conducted in accordancewith the Law of Ukraine dated 21.02.2006 № 3447-IV "On protection of animals from abuse" and the ethical standards and rules of working with laboratory animals (Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington DC, 1996) [8]. All animals selected for the experiment were subjected to veterinary examination, were acclimated for five days, and then randomly divided into groups, numbered and marked appropriately.

For examine of the preventive actionof LMOC (sodium 2-(2-hydroxyphenoxy) acetyl)-L-prolinate)rats were divided into 3 groups of 10 animal seach: 1stgroup was intact rats, 2nd and 3rd – rats, which had ethanol-induced ulcer at ion of the gastric mucosa (GM). Rats of 2nd group were injected with normal saline at a volume 2 ml/kg 30 minutes before ulcerogenic factor action, they were the control for the 3rd group. Rats of the 3rd group were treated with LMOC which was injected at thedoseof 1 mg/kg(2 ml/kg of saline solution) 30 minutes before ethanol action (compound was syn the size dat Lomonosov Moscow State University).

Erosive and ulcerative lesions of GM of rats in the 2nd and 3rd groups were caused by intra gastric ethanol infusion. After 1 hour from ethanol exposure the rats were sacrificed.To assess the state of GM of rats after ulcerogenic factor action stomach was removed, cutalong the lesser curvature, turn mucous out and thoroughly was hed with saline. The area and number of ulcers was measure dusing experimental gastroscope.

In the homogenateof GM of rats the content of lipid peroxidation(LP) products(the concentration of hydrogen peroxide, dien conjugates, thiobarbituric acid (TBA)- active products and Shiff's bases) wasmeasured by standard biochemical methods [9-12]. Antioxidant protection of the GM under condition of ethanol administration was assessed by the superoxide dismutase, catalase and glutathione system activity [13-15]. To study the influence of NMOS on glutathione system we examined the content of reduced (GSH) and oxidized glutathione (GSSG), glu-

tathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT) activity [14, 16-19]. All obtained datawere subjected to statistical analysis using software package "Statistics, 8.0". Shapiro-Wilks criterion was used for the analysis of data distribution type. As the data were normally distributed, we used Levan criterion forevaluation the equality of variance and Student's t-test for independent samples. We calculated mean values (M) and standard error of mean (m). Significant difference was considered at $p \leq 0,05$ [20].

Results. After an hourfrom ethanol exposure in the group of rats which were injected with water simultaneously with ethanol ulcerative lesions of gastric mucosa were reported in 100% of animals. The number of ulcers per one stomach was 13.1±1.8, and the total area of the ulcerative lesionswas equal to256.1±68.0 mm^2. Prophylactic administration of LMOC had no effect on the number of ulcerative lesions in animals. However, the average size of lesions was 83±18.61 mm^2, so it was established the significant reduction in lesion area by 67.6 % ($p<0.05$) compare tothe control group (Fig.1).

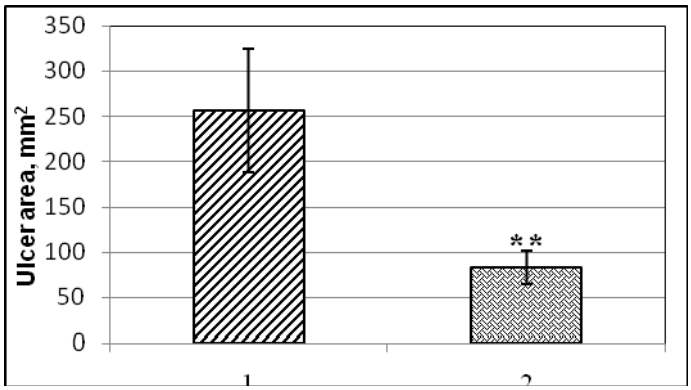

Figure 1. The ulcer area in the gastric mucosa of rats caused by the introduction of ethanol under treatment with low molecular weight organic compound (10 rats in each group, M±m):
1 – saline + ethanol;
2 – low molecular weight organic compound + ethanol

After the ethanol introduction it was established a significant increase of the intensity of LP in the GM of rats. The content of diene conjugates, TBA-active products and Shiff's bases was increased by 5.2 times, 2.3 times and 4.93 times accordingly compared with intact control (Table 1). This indicates the active accumulation of reactive oxygen species(ROS) after administration of ethanol. Indeed, hydrogen peroxide content inGMwas exaggeratedby 4.87 times. As a result of intensification of LPin the GM of rats superoxide dismutase and catalase activity was raised by 4.28 times ($p<0.001$) and 2.33 times ($p<0.01$) accordinglycompared with intact controls (Table 2).

***Table1*. Lipid peroxidation in the gastric mucosa of rats with erosive and ulcerate velesions caused by ethanol and prophylactic injection of low molecular weight organic compound (M+m)**

	Intact control	Saline group	Low molecular weight organic compound group
H_2O_2, μmol × mgof $protein^{-1}$	2.1±0.2	10.23±0.92***	10.51±0.94***
Dieneconjugates, nmol × mgof $protein^{-1}$	71.3±6.4	371.3±35.3***	239.6±21.9***/##
TBA-active products, nmol × mgof $protein^{-1}$	44.2±4.4	101.7±9.94***	63.6±5.95*/#
Shiff's bases, c.u. ×mgof $protein^{-1}$	1.2±0.1	5.91±0.58***	4.02±0.35*/#

*, *** – $p<0.05$, $p<0.001$ compared with intact control, #, ## – $p<0.05$, $p<0.01$ compared with saline group

LMOC decreased the content of diene conjugates by1.55 times ($p<0.01$), TBA-active products – by 1.6 times ($p<0.05$), Shiff's bases–by 1.47 times ($p<0.05$) in the GMof rats compared with the control group. The superoxide dismutase activity was increased by 1.65 times ($p<0.01$) under influence of LMOC compared with intact animals. However, comparing the group of rats injected with saline and LMOC, it should be noted that LMOCdiminished the enzyme activity compared with control group. Given the lower superoxide dismutase and higher catalase activity in rats treated with LMOC, the concentration of hydrogen peroxide in both groups was similar, suggesting acceleration of ROS scavenging by LMOC.

Examining of glutathione system activity it was established that GSH significantly decreased by 29 % ($p<0.05$) in the GM of the group of animals exposed to ethanol compared with intact control (Table 3). In contrast, oxidized glutathione content increased, indicating the use of its reduced form for the scavenging of ROS. This effect may be due to both increased use of glutathione by GP and its use for inactivation of ROS. Indeed, in our study found that the GP activity exceeded by 38 % ($p<0.05$) values of intact animals. One of the likely causes of a GSH decrease may also be a reduction in the GR activity, which restores GSSG to GSH, by 27 % ($p<0.05$) compared with intact rats. This enzyme uses NADPH as a reducing equivalent, and NADPH content is significantly reduced under conditions of ethanol-induced oxidative stress [21]. As a result of ethanol effect the GR activity decreased by 54 % ($p<0.001$) versusintact control. After the introduction of ethanol the GT activity was reduced by 43 % ($p<0.05$). This effect may be explained by depletion of glutathione systemunder the accumulation of excessive amounts of ROS.

***Table* 2. The antioxidant enzymes activity in the gastric mucosa of rats with erosive andulcerative lesions caused by ethanol and prophylactic injection of low molecular weight organic compound(M ± m)**

	Intact control	Saline group	Low molecular weight organic compound group
Superoxidedismutaseactivity, c.u. × min^{-1} × mgof $protein^{-1}$	0.16±0.01	0.686±0.050***	0.264±0.024*/###
Catalase activity, nmol × min^{-1} × mgof$protein^{-1}$	314.7±29.8	733.4±63.8**	1110.4±109.7***/##

, * – p<0.01,p<0.001 comparedwithintactcontrol, ##, ### – p<0.01, p<0.001 compared with saline group

Under conditions of LMOC treatment GSH content decreased by 52 % (p<0.01) compared to the intact control, and by 46.2 % (p<0.05) compared to the saline group. The content of the oxidized form of glutathione under conditions of prophylactic administration of LMOC was higher by 35 % (p <0.05) versus the intact rats, but decreased by 26.5% (p <0.05) compared with the control. Given increased activity of GT by 16 % (p <0.05) compared to the control in rats treated with LMOC, this suggested a more intensive use of glutathione in the inactivation ROS under LMOC treatment. This finding was confirmed by a decrease in the content of LP products under conditions of administration of the investigated compound. The GR activity was reduced by 54,3% (p<0.001) versus the intact rats, and was less by 37,3% (p<0.05) compared with the saline group. The high content of oxidized glutathione form gave the evidence of glutathione catabolism decrease under the treatment with LMOC.

***Table* 3. Glutathione content and enzymes activity of glutathione antioxidant defense system in the gastric mucosa of rats under condition of erosive and ulcerative lesions caused by ethanol and prophylactic injection of low molecular weight organic compound(M±m)**

	Intact control	Saline group	Low molecular weight organic compound group
Reducedglutathione, nmol of GSH ×mgof$protein^{-1}$	33.26±0.82	23.31±0.12*	15.94±1.04**/#
Oxidized glutathione,nmol of GSSG × mgof $protein^{-1}$	39.49±1.71	72.44±0.79**	53.36±2.83*/#
Glutathione transferaseactivity,nmolof conjugate glutathione with 1-chloro-2,4-dinitrobenzene × min ×mgof$protein^{-1}$	135.88±13.06	78.29±7.26**	90.93±8.78*/#
Glutathione reductase activity, nmolNADPH × min ×mgof$protein^{-1}$	1156.7±49.3	844.05±16.1*	528.7±48.5***/#
Glutathione peroxidaseactivity,nmol of GSH × min × mgof $protein^{-1}$	0.78±0.05	1.08±0.06*	0.8±0.03#

The GP activity did not differ from the intact control and significantly decreased by 26% (p<0.05) compared with the control group. These data indicate a resumption of the normal GP activity under conditions of ethanol administration and prophylactic administration of LMOC.

Thus, the results of the study showed that the LMOC reduces the intensity of LP in the GM of rats after administration of ethanol and possessesthe antioxidant effect by increasing superoxide dismutase, catalase and glutathione systemactivity.

Conclusions: 1.LMOC significantly reduces the intensity of LP in GM of rats under conditions of ethanol-induced injury. 2.LMOC increased the superoxide dismutase, catalase activity and activity of glutathione system.

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ВПЛИВ НИЗЬКОМОЛЕКУЛЯРНОЇ ОРГАНІЧНОЇ СПОЛУКИ НА СИСТЕМУ АНТИОКСИДАНТНОГО ЗАХИСТУ СЛИЗОВОЇ ОБОЛОНКИ ШЛУНКА В УМОВАХ ЕТАНОЛ-ІНДУКУВАНОГО УРАЖЕННЯ ШЛУНКА У ЩУРІВ

Було досліджено профілактичний ефект низькомолекулярної органічної сполуки на ерозивно-виразкові ураження в слизовій оболонці шлунка щурів, викликані етанолом. Встановлено, що профілактичні ін'єкції цієї речовини в дозі 1 мг/кг ефективно захищали шлункові дуражень, викликани хетанолом. Сполука ефективно відновила про-/ антиоксидантну рівновагу шляхом зменшення інтенсивності перекисного окислення ліпідів у слизовій оболонці шлунка щурів після введення етанолу та підвищення супероксиддисмутазної, каталазної активності та активност іглутатіонової системи.

Ключові слова:етанол-індуковані ураження, перекисне окиснення ліпідів, низкомолекулярна органічна сполука.

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ВЛИЯНИЕ НИЗКОМОЛЕКУЛЯРНОГО ОРГАНИЧЕСКОГО СОЕДИНЕНИЯ НА СИСТЕМУ АНТИОКСИДАНТНОЙ ЗАЩИТЫ СЛИЗИСТОЙ ОБОЛОЧКИ ЖЕЛУДКА В УСЛОВИЯХ ЭТАНОЛ-ИНДУЦИРОВАННЫХПОРАЖЕНИЙ ЖЕЛУДКА У КРЫС

Было исследовано профилактический эффект низкомолекулярного органического соединения на эрозивно-язвенные поражения в слизистой оболочке желудка крыс, вызванные этанолом. Установлено, что профилактические инъекции этого вещества в дозе 1 мг/кг эффективно защищали желудок от поражений, вызванных этанолом. Соединение эффективно восстановило про-/антиоксидантное равновесие путем уменьшения интенсивности перекисного окисления липидов в слизистой оболочке желудка крыс после введения этанола и повышение супероксиддисмутазной, каталазнойактивности и активности глутатионовой системы.

Ключевые слова: этанол-индуцированные поражения, перекисное окисление липидов, низкомолекулярное органическое соединение.

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VERTEBRATES FROM THE METHOLITHIC SITE LASPI VII (CRIMEA, UKRAINE)

The article deals with the results of studying the remains of vertebrates from the Mesolithic site Laspi. It is established that a relatively small taxonomic diversity is inherent to this locality; it is mainly represented by four basic species: Great Bustard, Wels catfish (recently became extinct in the Crimea), Zander and the European hare, and four other species that have seemingly been hunted occasionally, as their bones are present, but few: Grey Partridge, Spotted Crake, European hamster and European hedgehog. In those days the basis of the local people's ration was bustard, and the successful hunting for this bird was probably the reason for the Mesolithic people to stop on this territory. Analysis of species diversity suggests that the animals were hunted in the winter. It is established that the rivers of the region, destroyed by an earthquake in 1790, were deep enough for catfish to live there.

Key words: mesolit, Crimea,vertebrates.

Introduction: In the territory of Ukraine, as well as in many other regions of the Palearctic, formation of the modern climatic zones with species richness close to the modern one began during the Mesolithic. Research into the history and trends of wildlife is extremely important for understanding the anthropogenic transformation of ecosystems. It is not possible without studying fossils. The historical aspect of description of the animal world is a basis for understanding the real ties that bind the living world into a single functioning system [10]. Information about findings of the fossil vertebrates, with their careful studying and interpretation, is rather important.

Taxonomic richness of the Crimean peninsula is much higher than that of the neighboring areas due to the great diversity of terrain and climate. The main game animals in the mountainous part of the peninsula were wild boar (25% of bones from archaeological sites belonging to this species), roe deer (20%), red deer (14%), and also rabbit (10 %). Saiga and carnivorous mammals were hunted much less frequently (5% and 10%, respectively). Bones of marine mammals (seals and dolphins) were found in some Crimean localities, which are remote from the sea (Zamil-Koba and Fatma-Koba) [2].

At the end of the Mesolithic basis of the population on the Southern coast of the Crimea were Tauri tribes associated with the Kizil-Koba culture [7]. Locations of most Mesolithic sites in the Crimea and in the rest of Ukraine were determined by their proximity to water and suitability for hunting, fishing and gathering [3].

Location. Laspi VII site was discovered in 1973, and excavated in 1974-76 under the guidance of Dmitri Telegin (60 m^2 were dug out). The site is located in a rock canopy, with several large stones in front of it serving as a wind barrier [Буров]. Radiocarbon dating indicates the age of 5670-7135 BC [Телегін]. The sea level was lower then and the site was further from the sea by 0.5-1.5 m [2].

Skeletal remains in Laspi VII are located in five layers that were signified by the letters of the Cyrillic alphabet during the field work: А (top layer), Б, В, Г and Д (bottom layer). Here we use the Latin alphabet, the letters of which correspond to Cyrillic. Top layer – A ("А" in Cyrillic) beneath layers: B ("Б"), C ("В"), D ("Г"), E ("Д"). Thin layers of clay, ash and the shellfish remains are deposited between bonyferous horizons. The type of sedimentation indicates that they are naturally transferred from the higher area (eastern part of the cliff).

Laspi VII is repeatedly mentioned in the papers (mainly by archaeologists). In addition to information about the instruments, we know about the discovery of a large number of mollusk shells (*Helix*, *Dreissena*) [2]. Most of the tools are different flint cores. But there are also bone harpoons (except for Laspi in the Crimea they were found only at Murzak-Koba and Kara-Koba). Spindly darts present at the Laspi site are also known from Shang-Koba and Fatma-Koba) [7]. Birds from this site are described by Tsvelykh and Taykova [8]. In our work we used other, previously non-published, material from the Laspi VII. Tsvelykh & Taykova did not mention all the birds in their paper, because those bones