Original Research Article: full paper

(2021), «EUREKA: Life Sciences»
Number 4

BIO PROTECTORS' EFFECT ON THE COMPOSITION OF SOME AMINO ACIDS UNDER ALCOHOL-INDUCED OXIDATIVE STRESS

Liliia Kalachniuk[1] ✉
kalachnyuk_liliya@nubip.edu.ua

Petro Fedyshyn[1]

Oleh Smirnov
Laboratory of Molecular Biology and Clinical Biochemistry
Institute of Animal Biology NAAS
38 V. Stusa str., Lviv, Ukraine, 79034

Viktoriia Prys-Kadenko[1]

Roman Palonko[1]

Oleksii Arnauta[1]

[1]*Department of Biochemistry and Physiology of Animals named after Academician M. F. Gulyi*
National University of Life and Environmental Sciences of Ukraine
15 Heroiv Oborony str., Kyiv, Ukraine, 03041

✉**Corresponding author**

Abstract

The studies, which may reveal some elements of regulation between the metabolic processes of proteins (at the level of translation and changes in the amino acid spectrum) as well as catabolism and anabolism of carbohydrates under conditions of pathological deviations of the functioning of the animal organism, are promising, and the search for protective substances of a different nature is necessary.

The aim is to study and analyze a bio protectors' effect on the composition of some amino acids under alcohol-induced oxidative stress.

During the experimental period, changes in the body weight of rats confirm the depressant effect of alcohol on the dynamics of weight gain of animals during their growth and development, and the positive protective effect of betaine and additives (protein+minerals).

The increased activity of alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase in the blood serum of rats in experimental groups of animals with the absence of protectors' substances in the diet indicates a deviation in the functional capacity of the liver. The determined indices of the content of creatinine and urea were increased significantly that points out on possible pathological deviation of the kidney. Under alcohol substances, changes of such biochemical indexes value as lactate dehydrogenase, superoxide dismutase, and catalase, and the content of malonic dialdehyde indicate oxidative stress. In the case of bio protectors' presence, values of biochemical parameters become to ones in the animals of control groups.

It was observed, that betaine has a higher potential for the correction of the above pathological abnormalities than protein-containing additives with minerals in the form of chelate, but the last is perspective for further study and their use as a raw material for the development of more complex bio protectors.

Keywords: alcohol-induced oxidative stress, amino acids, biochemical parameters, bio protectors, rats.

DOI: 10.21303/2504-5695.2021.001985

1. Introduction

Excessive alcohol consumption and/or alcohol-carbohydrate mixtures can damage subcellular components, cells, organs, and the whole organism [1–3]. Mostly, alcohol causes oxidative

stress [4, 5]. Thus, strategies based on reducing oxidative stress can prevent or reduce the development of alcoholic liver disease (ALD).

Therefore, this work aims to study and analyze bio protectors' effect on the composition of some amino acids under alcohol-induced oxidative stress.

2. Materials and Methods

Experiments were performed on male rats weighing 180–220 g, which were divided into 7 groups (control and 1–6 experimental) of 7 animals in each for 28 days. During 7 days of the adaptation period, the rats of the control and experimental groups were kept on drinking water *ad libitum* and dry standard rodent feed. For another 3 weeks, the diet of rats remained the same, except that the animals of the experimental (1–6) groups (**Table 1**).

Table 1

Composition of the control diet and experimental diets

Groups of animals →	Control	Experimental					
Diet ↓		1	2	3	4	5	6
Purina rodent chow	+	+	+	+	+	+	+
Additives (protein + minerals)	–	–	–	–	+	–	+
Water	+	–	–	–	–	–	–
EtOH (30 % v/v, 8 g/kg BW)	–	–	+	+	+	+	+
Carbohydrates' mix (in final solution of 35 %)	–	+	–	+	+	–	–
Sulfur-containing bio-protector betaine in the final concentration of 1 %	–	–	–	–	–	+	–

Note: Groups of animals: C – Control; Experimental: 1 – Carbohydrates' mix; 2 – EtOH; 3 – EtOH+Carbohydrates' mix; 4 – EtOH+Carbohydrates' mix+Additives (Protein+Minerals); 5 – EtOH+Betaine; 6 – EtOH+Additives (Protein+Minerals). Additives (Protein+Minerals): proteins 22.5 %, fats 5 %, fiber 4.5 %, ash 5.1 %, moisture 10.2 %, calcium 0.89 %, phosphorus 0.67 %, magnesium 0.24 %, sodium 0.26 %, potassium 1.07 %; vit. A, D3, E, C, K3, B1, B2, B6, B12, nicotinamide, pantothenic acid, biotin, folate, choline, zinc, manganese, copper, iodine, iron.

At the end of the experimental period, the animals were euthanized by deep chloroform anesthesia.

The content of total protein, creatinine, urea, and enzyme activity (alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), γ-glutamyltransferase (GGT, EC 2.3.2.2) was measured by bioassay and biochemistry corresponding standard kits "PLIVA-Lachema Diagnostika" (Czech Republic) [6], and such oxidoreductases as lactate dehydrogenase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC 1.15.1.1) and catalase (EC 1.11.1.6), – according to the described methods [7–9]. The content of TBA-active compounds (malonic dialdehyde, MDA) was determined by reaction with thiobarbituric acid [10].

Student's t-test was used in statistical analysis.

Animal experiments were conducted in compliance with the requirements of the Law of Ukraine "On Protection of Animals from Cruelty" (Article 230 of 2006), "General Ethical Principles of Animal Experiments", approved by the National Congress of Bioethics and by the European Convention on the Protection of vertebrate animals, which are used in experiments and other scientific purposes (Strasbourg, 1986) [11].

3. Results

Changes in the body weight of rats during the experimental period confirm the depressant effect of alcohol on the dynamics of weight gain of animals during their growth and development, and the positive protective effect of betaine and additives (protein+minerals) (**Fig. 1**).

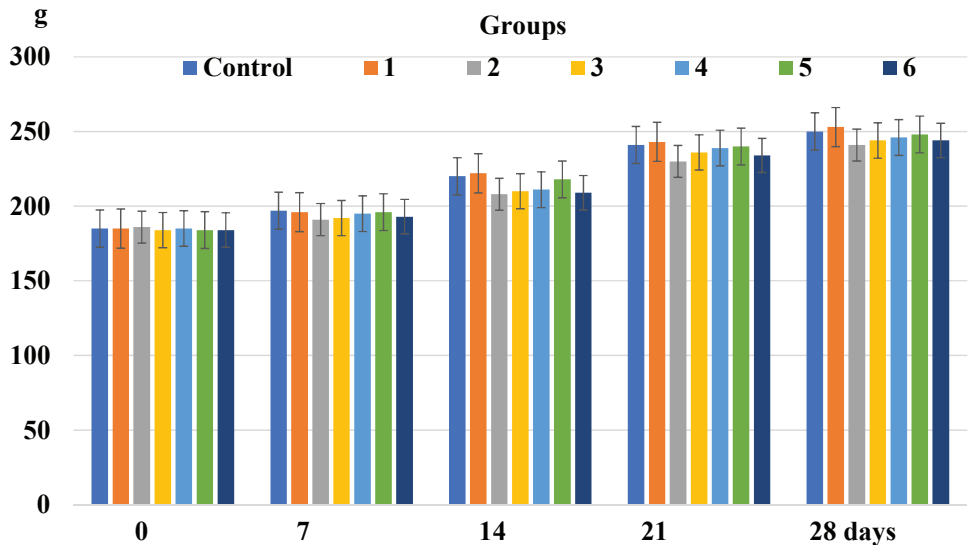

Fig. 1. Body weight of rats in control and 1–6 experimental groups, g (*M*±*m*, *n*=7)

A significant increase of the AST activity in the blood serum of rats of the 2nd experimental group indicates damage to liver cells and the release of this enzyme into the blood through damaged cell membranes. The use of protectors obviously restores the structural and functional state of the cells and, therefore, the activity is likely to decrease (experimental groups 4–6) and approach the level of the control group (**Table 2**). The similar patterns of changes are observed when evaluating the results of determining the activity of ALT and GGT (**Table 2**).

Table 2

Activity of transferases (AST, ALT and GGT) in the blood of rats, U/L (control and 1–6 experimental groups of animals; *M*±*m*, *n*=7)

Groups of animals →	Control	Experimental					
Enzymatic activity ↓		1	2	3	4	5	6
AST, U/L	98.1±4.9	108.2±3.8	197.6±10.9**	206.3±8.4**	199.8±9.3**	110.1±7.1$^{\#\#}$	170.2±5.8**$^{\#}$
ALT, U/L	79.2±4.7	83.8±5.2	169.3±8.8**	150.3±6.9**	146.4±7.4**	91.3±6.9$^{\#\#}$	120.1±7.2**$^{\#\#}$
GGT, U/L	6.9±0.7	8.4±0.9	19.7±1.1**	21.3±1.3**	20.7±3.1**	9.2±0.6*$^{\#\#}$	15.4±1.1**$^{\#}$

*Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and $^{\#}$p<0.05, and $^{\#\#}$p<0.001 compared with the 2nd experimental group, respectively*

The results (**Table 3**) show that in the blood serum of rats, under the action of the alcohol and alcohol-carbohydrate mixture, the content of total protein is almost unchanged (there is a tendency to increase slightly), and indices of creatinine and urea increase significantly. Such changes of the biochemical indices point out on possible pathological deviation of the kidney. Under the effect of protectors (betaine and Protein additive with Minerals), it was observed a significant reduction of value of creatinine and urea indicators.

Table 3

Nitrogen-containing substances in the blood serum of rats in control and 1–6 experimental groups (*M*±*m*, *n*=7)

Groups of animals →	Control	Experimental					
Biochemical parameters ↓		1	2	3	4	5	6
Total protein, g/L	64.5±3.3	64.5±1.9	64.4±1.3	66.5±1.4	64.5±1.1	64.1±0.8	64.6±0.9
Creatinine, μmol/L	30.5±2.9	34.7±2.2	66.4±3.1**$^{\#\#}$	68.4±2.7**$^{\#\#}$	67.2±1.7**	33.6±2.3$^{\#\#}$	43.6±2.4*$^{\#}$
Urea, mmol/L	3.4±0.6	4.6±0.7	8.2±0.8**	9.1±1.1**	8.3±1.5*	5.1±0.9$^{\#}$	6.1±0.6*

*Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and $^{\#}$p<0.05, and $^{\#\#}$p<0.001 compared with the 2nd experimental group, respectively*

The decreased activity of oxidoreductases (SOD and catalase) in the blood serum of the 2nd and the 3d groups of animals, and the increased concentration of malonic dialdehyde indicates the presence of alcohol-induced oxidative stress (**Table 4**). Under conditions of use protectors in the 4–6 groups of rats, the opposite changes of biochemical indicators value are observed.

Under the influence of alcohol and alcohol-carbohydrates mixture, the activity of LDH in the serum of rats increases almost 2 times (**Table 4**). This indicates a clear manifestation of alcohol-induced steatosis in the liver, the cells are not able to fully utilize the lactic acid, formed from pyruvate in this organ or entered the blood through the plasma membranes of muscle cells, leading to a general depletion of the body. Betaine and protein additives with minerals work in the direction of organism recovery.

Table 4

Activity of oxidoreductases (LDH, SOD and catalase) and the content of malonic dialdehyde (MDA) in the blood serum and liver tissue of rats (in control and 1–6 experimental groups of animals; *M*±*m*, *n*=7)

Groups of animals →	Control	Experimental					
Biochemical parameters ↓		1	2	3	4	5	6
LDH, U/L	489±18.2	519±26.7	990±28.3**	998±25.9**	734±21.2**##	610±19.1**##	680±19.9**##
SOD, U/mg of protein/min	260±21.2	220±13.1	148.5±15.3**	135.8±13.3**	172.4±17.1*	220.2±14.2#	160.8±12.3*
Catalase, U/mg of protein/min	239.8±11.3	215±7.8	139.3±9.1**	128.7±8.9**	154.3±7.7**	179.7±12.1*#	161.3±6.7*
MDA, nmol/mg of protein	40.9±2.3	47.7±3.5*	56.3±4.1*	66.1±2.4**	50.7±3.1*	42.1±1.8#	45.4±2.1#

*Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and #p<0.05, and ##p<0.001 compared with the 2nd experimental group, respectively*

A study of the level of amino acids, involved in the conversion of methionine (taurine, serine, dipeptide cystine, which consists of two residues of cysteine, and methionine) in the serum of rats, shows a decrease in their content in animals of 2 and 3 experimental groups. With the use of bio-protectors, the content of these amino acids in animals of groups 4–6 is close to the results of the control group (**Fig. 2**).

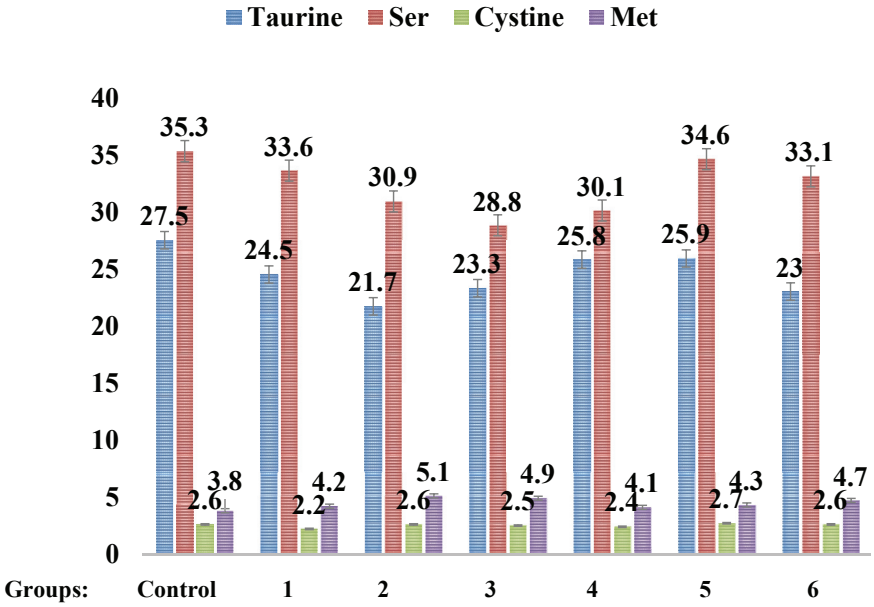

Fig. 2. Level of some amino acids, involved in Met–transformation in the blood serum of rats, μmol/L (in control and 1–6 experimental groups of animals; *M*±*m*, *n*=7; *p<0.05)

The above data of biochemical parameters in the blood serum of rats, kept on diets with components of alcohol-containing substances and bio-protectors, indicate the emergence of a number of pathological abnormalities (including oxidative stress) and will be discussed below.

4. Discussion

In the blood of rats, the activity of ALT is lower than the same AST (**Table 2**). The activity of transferases (ALT, AST, and GGT) in the blood of animals of the experimental groups (2 and 3) was higher than the same indicators of control animals, indicating a deviation in the functional capacity of the liver. Especially, GGT is a diagnostic marker of the liver function. GGT plays a key role in the gamma-glutamyl cycle, glutathione synthesis and degradation, and drug and xenobiotic detoxification. GGT also plays a prooxidant role by regulating effects at different levels in cellular signaling and cellular pathophysiology [12].

Transferases are involved in the conversion of alanine to pyruvate, from which glucose is synthesized through a series of reactions in the glucose-alanine cycle, and previous experimental data [13] also indicate an increase in serum glucogenic amino acids (for example, alanine, glutamate, glutamine), indicating their use in many processes (some of them are neutralization of ammonia, antioxidant reactions, conversion into intermediates of the tricarboxylic acid cycle, and the formation and accumulation of ATP) throughout the body, especially the liver, in adaptation under the influence of toxic substances. Along with this, indicators of transferase activity may indicate pathomorphological abnormalities in the tissues of the kidneys, as, for example, in the work with microscopic examination of histo-preparations of the kidneys of rats, which are kept on an alcoholic carbohydrate diet [14].

Preventive use of protective substances (betaine and protein-containing additives enriched with minerals in chelated form) restores the activity of the studied enzymes and normalizes redox processes and the intensity of transamination in the body of alcohol-intoxicated animals.

Alcohol intoxication increases the level of biosynthesis of triacylglycerols (TAG) and cholesterol in the liver, which, in turn, affects their content in the blood serum of rats [15]. The increase in the blood serum TAG in rats consuming ethanol is most likely due to increased levels of lipoproteins, such as LDL. The high content of TAG induces the synthesis of apolipoprotein B-100 (apo B) in liver cells. In turn, apoB in combination with TAG forms very-low-density lipoproteins (VLDL), which are released into the blood. An increase in the content of TAG in the blood of rats with the development of alcoholic steatosis of the liver leads not only to an increase in LDL, but also to a decrease in HDL [14, 16].

Such processes cause probable changes in the activity of oxidoreductases, namely: its increase for LDH and decrease for SOD and catalase. Along with this, the concentration of MDA in the liver tissues increases (**Table 4**).

In animals with an excessive carbohydrate load in the diet (experimental groups), chronic hyperglycemia is observed, which can cause the development of pre-diabetic conditions. It is confirmed by the results of histological studies that indicate a certain correlation between the development of pathological conditions of the liver tissue from blood glucose levels [12].

Betaine and a protein supplement with minerals in chelated form normalize metabolic processes, especially the conversion of methionine and its intermediates, involved in antioxidant defence mechanisms (**Fig. 3**) [17].

It should be noted, that in the 2nd group of animals the level of amino acids decreased unevenly. The amount of cystine and methionine decreased slightly compared to the control group, while the level of taurine decreased by almost 13 % compared to the control group, and the level of serine – by 15 %. It is likely, that the decrease in serine levels is due to the fact that it is a precursor to methionine (which is part of S-adenosylmethionine or SAM) and cysteine (which is part of glutathione – a tripeptide consisting of residues of γ-glutamic acid, cysteine, and glycine). Decreased levels of taurine (synthesized from cysteine) – an amino acid with antioxidant properties, may indicate that this amino acid is an antioxidant system of the "first action". In this case, we can assume that serine was used for the synthesis of cysteine and that for the synthesis of taurine. Also, these results may indicate the "secondary" nature of SAM as an antioxidant system compared to glutathione and the amino acid taurine, as it was not activated with a significant decrease in the number of amino acids – taurine and serine.

According to the results of the study [18–20], theoretically, betaine is a more promising bio protector than S-adenosylmethionine. It is planned to study the immunomodulatory properties of betaine, the protein-containing additives with minerals in chelated form, and biophosphomag (synthesized on the basis of additionally phosphorylated casein with magnesium in chelated form), and other biological products *in vitro*.

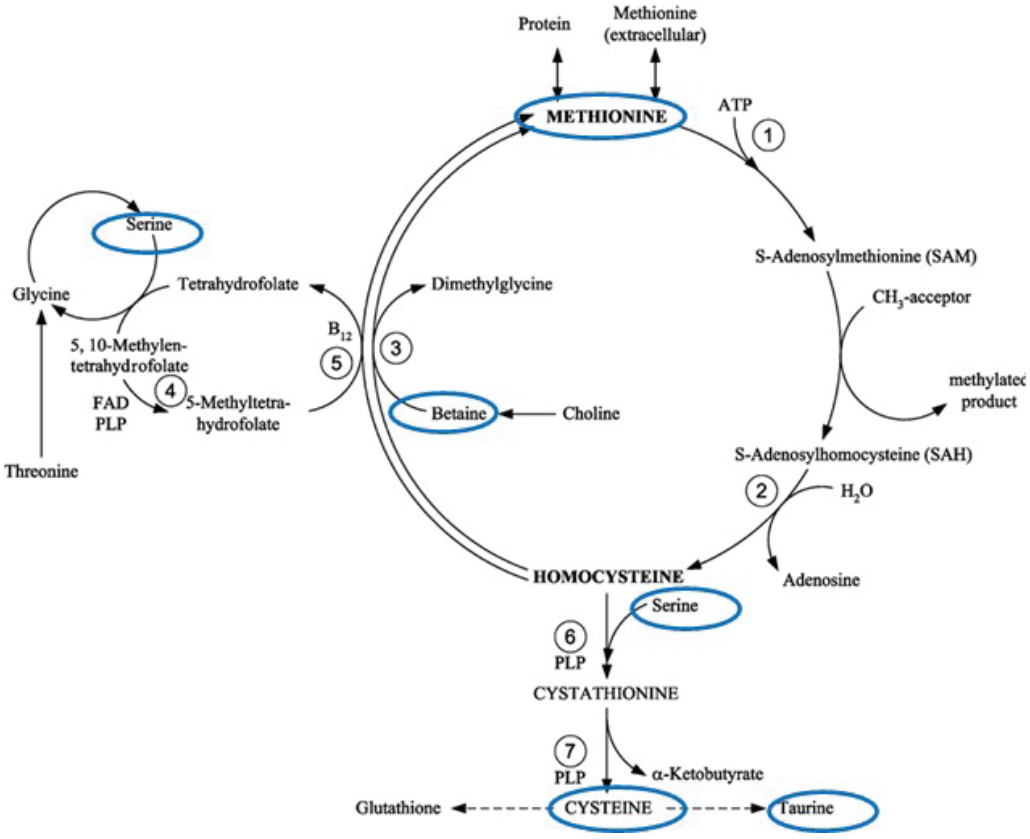

Fig. 3. Methionine cycle and intermediates (adapted [17])

5. Conclusion

The determined biochemical indicators show metabolic changes and individual pathomorphological abnormalities in the rats under conditions of intoxication of alcohol and the alcohol-carbohydrate mixture.

In the blood serum of rats in the experimental groups of animals with the absence of protectors' substances in the diet compared to control, the increased activity of ALT (169.3±8.8 U/L, $p<0.001$), AST (197.6±10.9 U/L, $p<0.001$), GGT (19.7±1.1 U/L, $p<0.001$) indicates a deviation in the functional capacity of the liver as well as the increased indices of the content of creatinine (66.4±3.1 μmol/L, $p<0.001$) and urea (8.2±0.8 mmol/L, $p<0.001$) points out on a possible pathological deviation of the kidneys.

The oxidative stress is demonstrated through changes of such biochemical indexes value compared to control as the increasing LDH-activity (990±28.3 U/L, $p<0.001$) and the content of malonic dialdehyde (56.3±4.1 nmol/mg of protein, $p<0.05$), and the decreasing enzyme activity of SOD (148.5±15.3 U/mg of protein/min, $p<0.001$), and catalase (9.3±9.1 U/mg of protein/min, $p<0.001$) under conditions of alcohol substances presence in the diet of rats.

In the case of bio protectors' (betaine or protein-containing additives with minerals in the form of chelate) presence in the diet, the values of biochemical parameters become close to the ones in the animals of the control groups.

Potential bio protectors for the correction of the above pathological abnormalities may be betaine and protein-containing additives with minerals in the form of chelate, due to their significant effect on protein, lipid, and carbohydrate metabolism. These preparations are perspective for further study and use, as they are easily modified and can be used as a raw material for the development of more complex bio protectors.

Acknowledgements

The research was performed within the research topics No. state registration 0120U102130 and 0116U001883

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Ding, W.-X., Yang, L. (2019). Alcohol and drug-induced liver injury: Metabolism, mechanisms, pathogenesis and potential therapies. Liver Research, 3 (3-4), 129–131. doi: https://doi.org/10.1016/j.livres.2019.11.006

[2] Elagina, A. A., Lyashev, Y. D., Lyashev, A. Y., Pronyaeva, T. V., Chahine, A. R. (2020). Correction of Lipid Metabolism Disorders in Diabetes Mellitus with Peptide Drugs. Bulletin of Experimental Biology and Medicine, 168 (5), 618–620. doi: https://doi.org/10.1007/s10517-020-04764-2

[3] Nagy, L. E., Ding, W.-X., Cresci, G., Saikia, P., Shah, V. H. (2016). Linking Pathogenic Mechanisms of Alcoholic Liver Disease With Clinical Phenotypes. Gastroenterology, 150 (8), 1756–1768. doi: https://doi.org/10.1053/j.gastro.2016.02.035

[4] Li, Q., Cui, J., Fang, C., Liu, M., Min, G., Li, L. (2017). S-Adenosylmethionine Attenuates Oxidative Stress and Neuroinflammation Induced by Amyloid-β Through Modulation of Glutathione Metabolism. Journal of Alzheimer's Disease, 58 (2), 549–558. doi: https://doi.org/10.3233/jad-170177

[5] Vergani, L., Baldini, F., Khalil, M., Voci, A., Putignano, P., Miraglia, N. (2020). New Perspectives of S-Adenosylmethionine (SAMe) Applications to Attenuate Fatty Acid-Induced Steatosis and Oxidative Stress in Hepatic and Endothelial Cells. Molecules, 25 (18), 4237. doi: https://doi.org/10.3390/molecules25184237

[6] Ostapchenko, L. I., Kalachniuk, L. H., Garmanchuk, L. V., Kuchmerovska, T. M., Arnauta, O. V., Arnauta, N. V., Smirnov, O. O. (2019) Theoretical and methodical fundamentals of the study of metabolic processes in human and animals using blood indicators (manual). Kyiv: NPE Yamchynskyi O.V., 296.

[7] Kalachnyuk, L., Basarab, I., Mel'nychuk, D., Mel'nychuk, S., Kalachnyuk, M., Koshman, O., Kalachnyuk, G. (2011). Oxidation of lactate and ldh localization in the substructures of cell under effect of exogenous factors. Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohiy im. Gzhytskoho, 13 (4 (50)), 80–86.

[8] Chevari, S., Chaba, I., Sekey, Y. (1985). Rol' superoksiddismutazy v okislitel'nyh protsessah kletki i metod opredeleniya ee v biologicheskih materialah. Laboratornoe delo, 11, 678–681.

[9] Korolyuk, M. et. al. (1988). Metod opredeleniya aktivnosti katalazy. Laboratornoe delo, 1, 16–19.

[10] Stal'naya, I. D., Garishvili, T. G. (1977). Metod opredeleniya malonovogo dial'degida s pomosch'yu tiobarbiturovoy kisloty. Sovremennye metody v biohimii. Moscow: Meditsina, 66–68.

[11] Zakon Ukrainy «Pro zakhyst tvaryn vid zhorstokoho povodzhennia. Vidomosti Verkhovnoi Rady Ukrainy (VVR), 2006, No. 27, st. 230. Available at: https://zakon.rada.gov.ua/laws/show/3447-15

[12] Owczarek, D. (2016). Diet and nutritional factors in inflammatory bowel diseases. World Journal of Gastroenterology, 22 (3), 895. doi: https://doi.org/10.3748/wjg.v22.i3.895

[13] Prys-Kadenko, V. O., Stadnyk, Yu. V., Kalinina, A. S., Balykina, K., Kalachniuk, L. H. (2017). Indicators of protein-carbohydrate exchange for hepatostateatosis. Scientific Messenger LNUVMB, 19 (78), 194–196. Available at: http://nbuv.gov.ua/j-pdf/nvlnuvmbvn_2017_19_78_42.pdf

[14] Kalachniuk, L. H., Smirnov, O. O., Prys-Kadenko, V. O., Arnauta, O. V., Palonko, R. I., Fedyshyn, P. M. (2020). Adaptability of living organisms. «Intellektuelles Kapital - die Grundlage für innovative Entwicklung '2020», «Intellectual capital is the foundation of innovative development» («Heritage of European science»). Section - Biology and Ecology. Karlsruhe, 16. Available at: https://www.sworld.com.ua/index.php/secciisge3-9/32826-sge4-055

[15] Echeverría, F., Valenzuela, R., Bustamante, A., Álvarez, D., Ortiz, M., Soto-Alarcon, S. A. et. al. (2018). Attenuation of High-Fat Diet-Induced Rat Liver Oxidative Stress and Steatosis by Combined Hydroxytyrosol- (HT-) Eicosapentaenoic Acid Supplementation Mainly Relies on HT. Oxidative Medicine and Cellular Longevity, 2018, 1–13. doi: https://doi.org/10.1155/2018/5109503

[16] Romero, F., Shah, D., Duong, M., Stafstrom, W., Hoek, J. B., Kallen, C. B. et. al. (2014). Chronic Alcohol Ingestion in Rats Alters Lung Metabolism, Promotes Lipid Accumulation, and Impairs Alveolar Macrophage Functions. American Journal of Respiratory Cell and Molecular Biology, 51 (6), 840–849. doi: https://doi.org/10.1165/rcmb.2014-0127oc

[17] Methionine, SAMe, Homocysteine, and the Methionine Cycle. Available at: https://benbest.com/health/Meth.html#homo

[18] Singhal, N. K., Sternbach, S., Fleming, S., Alkhayer, K., Shelestak, J., Popescu, D. et. al. (2020). Betaine restores epigenetic control and supports neuronal mitochondria in the cuprizone mouse model of multiple sclerosis. Epigenetics, 15 (8), 871–886. doi: https://doi.org/10.1080/15592294.2020.1735075

[19] Figueroa-Soto, C. G., Valenzuela-Soto, E. M. (2018). Glycine betaine rather than acting only as an osmolyte also plays a role as regulator in cellular metabolism. Biochimie, 147, 89–97. doi: https://doi.org/10.1016/j.biochi.2018.01.002

[20] Zeisel, S. (2017). Choline, Other Methyl-Donors and Epigenetics. Nutrients, 9 (5), 445. doi: https://doi.org/10.3390/nu9050445

Received date 28.05.2021
Accepted date 03.07.2021
Published date 30.07.2021

How to cite: *Kalachniuk, L., Fedyshyn, P., Smirnov, O., Prys-Kadenko, V., Palonko, R., Arnauta, O. (2021). Bio protectors' effect on the composition of some amino acids under alcohol-induced oxidative stress. EUREKA: Life Sciences, 4, 50–57. doi: https://doi.org/10.21303/2504-5695.2021.001985*