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# INFLUENCE OF THE BIOPHOSPHOMAG PREPARATION AND BIOLOGICALLY ACTIVE SUBSTANCES FROM MILK THISTLE SEEDS (*SILYBUM MARIANUM*) ON THE METABOLISM OF LYMPHOCYTES AND THEIR FUNCTIONAL FEATURES

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**Background.** The OVA and patented OVA+ preparations (produced by NULES of Ukraine) were obtained according to O. V. Arnauta *et al*. (2021) by extracting biologically active compounds from the seeds of milk thistle (*Silybum marianum*) using corn oil. Milk thistle seeds are a rich source of flavolignans, flavonoids, vitamins, tannins, macro- and microelements.

The patented preparation biophosphomag (V3), also developed by NULES of Ukraine, is based on artificially phosphorylated casein from cow's milk as a ligand and magnesium ions as a complexing agent (Palonko *et al*., 2022a). These preparations possess protective properties and may enhance cellular adaptation processes (Khyzhniak *et al*., 2022; Palonko *et al*., 2022a,b). According to preliminary data, the preparations exhibit choleretic activity and help stabilize liver and digestive functions. Their potential effects on the metabolism of immune system cells require further study.

Considering that the immune system is adaptive and functions to protect the body by suppressing pathogenic microorganisms and tumor growth through a series of coordinated signals that regulate activation, proliferation, and differentiation of T cell populations,

the aim of this study was to assess proliferative activity, cell viability, and SH-group levels in lymphocytes under the influence of the OVA, OVA+, and V3 protective agents.

**Materials and Methods.** The study evaluated mitochondrial dehydrogenase activity using the MTT assay, glucose levels in the culture medium via the glucose oxidase method, and the concentrations of total and protein-bound SH-groups in T lymphocytes exposed to OVA, OVA+, and V3.

**Results.** A comparative analysis of the effects of OVA, OVA+, and V3 on cultured T lymphocytes of the MT-4 cell line and primary rat T lymphocyte cultures confirmed the safety of these biopreparations within the tested concentration range (0.031–1.0 mg/mL). OVA+ significantly increased mitochondrial dehydrogenase activity (MTT assay), indicating enhanced cell viability compared to the control, while OVA exhibited slight suppression. Glucose levels in the culture medium remained unchanged under OVA and V3 treatment. However, OVA+ treatment resulted in a 1.5-fold ($p < 0.05$) and 2-fold ($p < 0.05$) increase in glucose levels in primary rat T lymphocyte cultures and MT-4 cells, respectively, compared to the control. Furthermore, the levels of total and protein-bound SH-groups in MT-4 cells were significantly elevated under OVA+ treatment, while no changes were observed with OVA or V3.

**Conclusions.** V3, OVA and OVA+ preparations do not exhibit cytotoxic or cytostatic effects on T lymphocytes. The most pronounced effect is exerted by the OVA+ preparation, which causes an increase in the level of glucose in the culture medium and the activity of dehydrogenases, as well as an increase in the content of SH-groups in cells, which indicates its potential in supporting cellular metabolism and protection. At the same time, V3 and OVA demonstrated a neutral effect under the specified conditions.

***Keywords***: T lymphocytes, biological preparations, glucose level, SH-group

## INTRODUCTION

OVA, OVA+, and biophosphomag (V3) alike have adaptogenic properties that provide effective stimulation and correction of metabolic processes. OVA (prepared from the same starting components as OVA+, but the preparation did not have a homogeneous oily appearance) is the initial version of the patented preparation OVA+, i.e., OVA+ is an improved form of the preparation of plant origin (which was patented). During the procedures for obtaining OVA+, the extraction and filtration conditions were carefully observed until a homogeneous oily preparation was obtained. OVA+ contains a large quantity of silymarin, fatty oils, resinous substances, essential oils, mucous substances, lignans, flavonoids, saponins, organic acids, protein structures, alkaloids, vitamins, and selenium. Due to its composition, milk thistle oil is primarily used as a means with a strong hepatoprotective effect, as well as for the normalization of metabolism, haematopoiesis processes, and the functioning of the nervous system (Arnauta *et al.*, 2021). In biophosphomag, inorganic phosphorus is converted into an organic form (Palonko *et al.*, 2022a). The synthesis of the biopreparation is carried out in three stages: casein molecules are additionally enriched with phosphorus by direct phosphorylation, casein peptide bonds are converted into the enol form and magnesium is chelated with casein to obtain a homogeneous powdery preparation. Quality control of the finished biological product is carried out by the quantitative content of phosphorus and magnesium in the finished product (Palonko *et al*., 2022a,b). In our previous studies (Arnauta *et al*., 2021;

Palonko *et al*., 2022a,b) the stimulating effect and correction of metabolic processes of proteins, lipids, and carbohydrates were proven. Hence, it is important to consider the effect of biologics on the cellular link of immunity (T lymphocytes).

Since many pathologies are accompanied by immunodeficiency disorders, which affect different components of the innate and adaptive responses, T lymphocytes play a crucial role in maintaining immune homeostasis and are represented by several subpopulations of cells, including helpers, suppressors, and cytotoxic lymphocytes (Sun *et al*., 2023). Appropriate T cell immunity is essential for maintaining host homeostasis and preventing infections. Some pathologies cause an imbalance in the ratio of T lymphocyte subpopulations (e.g., upregulation of suppressors and downregulation of killers) not only due to apoptosis but also metabolic changes (Franzese *et al*., 2024). Recent advances in immunometabolism have shown that cellular metabolism plays a fundamental role in shaping T cell responses. T cell activation and proliferation processes are supported by metabolic reprogramming to meet the increased energy and biomass demand, and deregulation in immune metabolism can lead to autoimmune disorders (Zhang *et al*., 2022). Changes in glucose metabolism of T lymphocytes regulate the processes of growth, functioning, survival and differentiation of activated immune cells and have a profound effect in normal and pathological conditions (Fox *et al.,* 2005; Mu *et al*., 2022). Energy renewal by lymphocytes occurs through glycolysis and mitochondrial oxidative phosphorylation (OxPhos) during their activation (Chang, *et al.,* 2013; Kishore, *et al.,* 2017). CD8+ T cells are an important component of the body's adaptive immune response. During viral or intracellular bacterial infections, CD8+ T cells are rapidly activated and differentiated to exert their immune function by producing cytokines (Cao *et al*., 2023). Metabolic reprogramming differs between B cells and T cells, as well as within regulatory T cell subpopulations (Gerriets *et al*., 2015). For example, subsets of CD4+ T cells where effector T cells and Th17 cells rely on aerobic glycolysis, while memory T cells and T regulatory cells (Treg) rely on fatty acid oxidation to produce energy (Michalek *et al*., 2011). Aerobic glycolysis is also utilized for energy by activated dendritic cells, neutrophils, and pro-inflammatory macrophages (Krawczyk *et al*., 2010). Hempel and colleagues have shown that in the spleens of mice, CD4+ T cells regulate glycolysis and glucose uptake, showing an increase in the ratio of glycolysis to OxPhos upon anti-CD3/CD28 stimulation. In contrast, B cells are regulated by both glycolysis and OxPhos upon stimulation with lipopolysaccharides or anti-B cell receptor stimulation, and maintain the glycolysis/OxPhos ratio at proliferative rest (Hempel *et al*., 1999).

Thorough understanding of the role of metabolism in T cell function could provide insights into mechanisms involved in inflammatory-mediated conditions, with the potential for developing novel therapeutic approaches to treat diseases such as tumor growth, diabetes mellitus and others.

It was previously shown that preparations of biotechnological origin such as biophosphamag (V3), OVA and OVA+ (Palonko *et al*., 2022; Arnauta, *et al.,* 2021) received a positive evaluation in veterinary practice. However, the mechanisms of influence of these substances on the functional activity of immune cells are not known for sure. Considering that the immune system is adaptive and functions to protect the body by suppressing pathogenic microorganisms and tumor growth through a series of coordinated signals that regulate activation, proliferation, and differentiation of T cell populations, the aim of this study was to assess proliferative activity, cell viability, and SH-group levels in lymphocytes under the influence of OVA, OVA+, and V3 protective agents.

## MATERIALS AND METHODS

Cells of lymphocyte origin of the MT-4 line, kindly provided by Dr. M. P. Zavelevich (IEPOR named after R. E. Kavetsky) were cultured under standard conditions in RPMI medium (“Sigma”, USA) with 10 % fetal calf serum (FBS) (“Sigma”) 80 μg/mL gentamicin and cultured in a $CO_2$ incubator at 90–95 % humidity, 5 % $CO_2$ content and a temperature of 37±1 °C (Medcenter Einrichtungen GmbH MMM-Group). Intravital observation, assessment of morphological parameters, and visualization of cell populations were conducted using an inverted AxioVert40 microscope (Carl Zeiss) equipped with AxioVision software.

The primary culture of T lymphocytes was obtained from the peripheral blood of rats. The method is based on the „rosette formation” reaction of T lymphocytes. Peripheral heparinized blood (25 units of heparin per 1 mL) of rats was used and applied to a 0.12 EU/mL ficoll-verografin gradient with a density of 1.077 g/ mL Ficoll to obtain leukocytes. The gradient was placed in a test tube, and blood was layered on it at a ratio of 3:2. The tubes were then centrifuged at 400 g for 40 minutes. After centrifugation, plasma and lymphocytes (at least 90 %) remained above the gradient layer.

The lymphocytes were collected with a Pasteur pipette and washed twice with Hanks’ solution by centrifugation at 600 g for 10 minutes. After washing, the lymphocytes were diluted in 1 mL of Hanks’ solution and counted using a hemocytometer.

To separate T- and B lymphocytes, the isolated lymphocyte fraction was incubated with sheep erythrocytes at a ratio of 1:10 (1.5–2 million lymphocytes and 15–20 million erythrocytes). To obtain erythrocytes, defibrinated sheep blood was used, which was obtained from a peripheral vein, then washed three times with 199 medium, and centrifuged at 800 g for 5 minutes. A 0.5% suspension in medium was prepared from the washed erythrocytes. Then, 4.5 mL of medium was added to 0.5 mL of erythrocyte sediment, mixed thoroughly, and 5 mL of 1% suspension was diluted in 5 mL of the medium. The tubes were centrifuged at 400 g for 5 minutes and left at +4 °C for 1 hour. Then, the supernatant containing B lymphocytes was carefully removed and re-centrifuged. The T lymphocyte pellet was carefully resuspended with a Pasteur pipette and left for another 20 minutes at +4 °C to fix the formed rosettes. After that, the tubes were centrifuged under the same conditions, the supernatant was carefully removed, and 1–2 drops of distilled water were added to the precipitate to lyse the erythrocytes. The number of lymphocytes was then counted, and a test was performed to determine the activity of the studied substances against T lymphocytes.

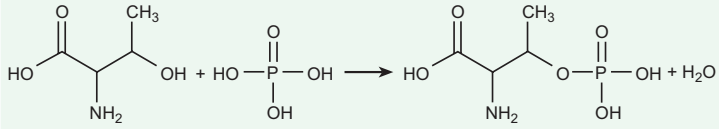
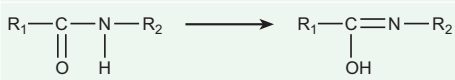
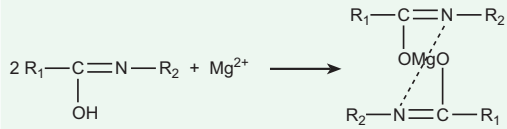
All procedures were performed under sterile conditions in a laminar cabinet (LS, Laminar Systems).

The survival rate of T lymphocytes was determined assessed by routine counting in the Goryaev hematocytometer and in the MTT-colorimetric test. This method assesses proliferative activity based on the intensity of metabolic processes in mitochondrial dehydrogenases (Mosmann, 1983). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a yellow monotetrazolium salt, whose reduction is commonly used to measure cell proliferation and cytotoxicity (Alley *et al.*, 1988). The biochemical essence of the method lies in the ability of mitochondrial dehydrogenases in living cells to cleave tetrazolium rings, forming water-insoluble purple crystals. The regenerative activity of dehydrogenases largely depends on the concentration of intracellular NADH and NADPH, which are associated with extracellular glucose levels (Stockert, 2012). Thus,

compounds affecting the intensity of MTT reduction reflect the activity of mitochondrial respiration and can be used to determine proliferative processes.

The studied biopreparations V3, OVA, OVA+ (**Scheme 1** and **Scheme 2**) were tested in the concentration range of 0.031–1.0 mg/mL. Assessment of cell survival was performed using the trypan blue vital dye. Trypan blue staining (0.4% solution prepared in 0.1 M phosphate-buffered saline (PBS), pH 7.2) was used to quantitatively analyze the ratio of live to dead cells. To do this, 2 samples were taken from each well of the plates, stained with trypan blue, then an equal volume of 0.4% trypan blue solution was added to the cell suspension and the cells were counted in the Goryaev chamber. The obtained results were averaged, taking into account cell dilution and incubation volume.

*Scheme 1.* **Obtaining biophosphomag (V3) according to R. I. Palonko *et al.*** (2022a)

| | |
|---|---|
| **Step 1.** Direct phosphorylation of casein |  |
| **Step 2.** Conversion of casein peptide bonds to the enol form |  |
| **Step 3.** Chelation of magnesium with casein |  |

*Scheme 2.* **Obtaining OVA and OVA+ according to O. V. Arnauta *et al.*** (2021)

| OVA | OVA+ |
|---|---|
| **Stage 1.** Extraction of crushed milk thistle fruits with refined corn oil for 10 days in a dark glass container at room temperature. Proportion of components – 1 L (920 g) of oil: 200 g of milk thistle fruits | **Stage 1.** Extraction of crushed milk thistle fruits with refined corn oil for 14 days with slow rotation at 37 °C. The proportion of components – 1 L (920 g) of oil: 200 g of milk thistle fruits |
| | **Stage 2.** Sequential filtration of the oil extract to obtain a homogeneous liquid through filters of 5 μm, 0.45 μm, and 0.22 μm |

To determine the activity of mitochondrial dehydrogenases in the MTT test, cells were cultured in 96-well plates ($3 \cdot 10^4$ cells per well in volume 200 μL) for 24 hours in RPMI-1640 medium (Sigma, USA) containing 10 % FBS (Sigma, USA) under standard conditions (37 °C, 5 % $CO_2$, and 90–95 % humidity) with test substances (Biophosphomag (V3), OVA, and OVA+) in the concentration range of 0.031–1.0 mg/mL. Also, cells were cultured in 12-well plates ($3 \cdot 10^5$ cells per well) in a volume of 1000 μL for 2 days with the same agents at a concentration of 0.125 mg/mL.

Four hours before the end of the incubation period with the test compounds, MTT was added to the culture medium in a volume of 20 μL per well in the 96-well plates and 50 μL per well in the 12-well plates, to a final concentration of 0.6 mg/mL. After incubation with MTT, the plates were centrifuged for 10 minutes at 450 g using a K-26

centrifuge equipped with plate supports. The culture medium was then removed, and the formazan crystals formed in the cells were dissolved in 100 μL of dimethyl sulfoxide in the 96-well plates. A multiwell spectrophotometer (Labsystems Multiscan MS) was used to evaluate and visualize the results of the MTT test at a wavelength of 540 nm.

Determining the level of glucose in the incubation medium of cells was performed using a standard set based on glucose-oxidase reaction, which we modified for culture medium of cells. Initial cell concentration was about $1 \cdot 10^5$ cells/mL in the sample volume of 200 μL. Determination was performed according to the protocol of the manufacturer "Felicit-Diagnostics" (Ukraine) (Nikolaienko *et al.*, 2015).

The activity of mitochondrial dehydrogenases and glucose levels were calculated by dividing the corresponding optical density values obtained in the MTT test and glucose oxidase method by the concentration of live cells counted in the Goryaev chamber in the corresponding parallel wells under the influence of the studied drugs.

Determination of the total content of SH groups in the MT-4 cells was carried out by adding 0.5 mL of 30 mM Tris-HCI with 1 mM EDTA (pH 8.0) to 0.075 mL of cell lysate and adding 0.05 mL of 1.25% sodium dodecyl sulfate (SDS). The mixture was incubated for 15 minutes at room temperature. Next, for detection, 0.025 mL of Elman's reagent was added and incubated for 30 minutes in the dark. Extinction was measured at λ = 412 nm against a control sample (0.575 mL of 30 mM Tris-HCI with 1 mM EDTA with the addition of 0.05 mL of 1.25% SDS and 0.025 mL of Ellmann's reagent), as described earlier (Maslii, 2023). Determination of the content of non-protein SH groups was carried out by adding 0.025 mL of 10.5% TCA (trichloroacetic acid) to 0.075 mL of homogenate. Samples were incubated for 10 minutes at room temperature, followed by centrifugation for 15 minutes at 400 g. The supernatant was neutralized by adding 1M NaOH to pH 7.0. For detection, 0.525 mL of 30 mM Tris-HCI with 1 mM EDTA (pH 8.0) and 0.025 mL of Ellman's reagent were added and incubated for 30 minutes in the dark. Extinction was measured at λ = 412 nm against a control sample (0.625 mL of 30 mM Tris-HCI with 1 mM EDTA and the addition of 0.025 mL of Ellmann's reagent).

The content of protein SH groups was determined by subtracting the content of non-protein SH from the total content of SH groups (C (protein) = C (total) – C (non-protein)).

All experiments were carried out in three biological and five to seven analytical replicates. The data were processed by standard methods of variation statistics using Microsoft Excel 2007 (Microsoft, Redmond, WA). Mean values and standard errors are presented in **Table**. Statistical analyses were performed using an unpaired *t*-test to compare group means. The differences among biopreparations and concentrations were assessed using a two-way ANOVA. The analysis was conducted using GraphPad Prism (version 10.4.1; GraphPad Software, San Diego, CA, USA). A p-value of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

The proliferative parameters of MT-4 cells (T lymphocyte cell line) were investigated using the MTT test following treatment with the V3, OVA, and OVA+ agents. The $NAD^+$/NADH redox couple is known as a regulator of cellular energy metabolism, that is, of glycolysis and mitochondrial oxidative phosphorylation. By contrast, $NADP^+$ together with its reduced form, NADPH, is involved in maintaining redox balance and supporting the biosynthesis of fatty acids and nucleic acids (Yang & Sauve, 2016). Thus,

compounds affecting the intensity of MTT reduction reflect the activity of mitochondrial respiration and can be used to determine proliferative processes.

In initial experiments, no cytotoxic or cytostatic effects were observed in MT-4 cells with OVA, OVA+, and V3 within the concentration range of 0.031–1.0 mg/mL, relative to the corresponding control (**Fig. 1**).

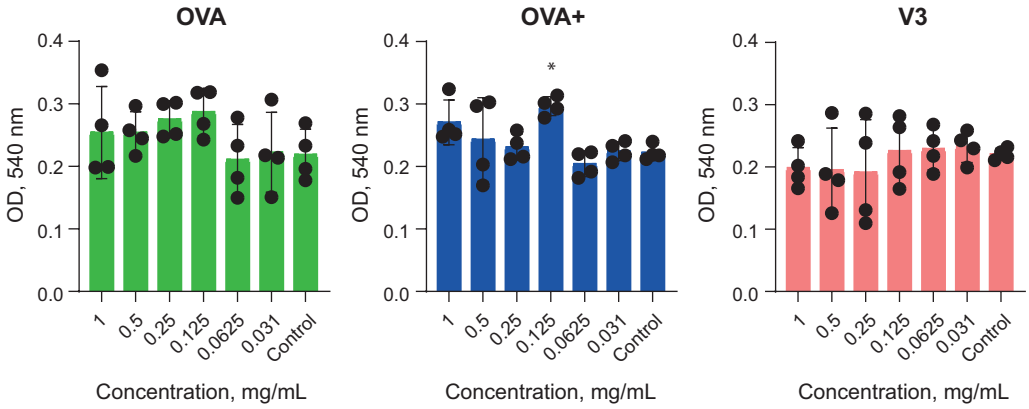



**Fig.1.** Data of MTT-test in cells of the MT-4 line under the action of OVA, OVA+ and V3 in the concentration range (0.031–1.0 mg/mL) under culturing cells for 1 day with biopreparations in standard conditions
**Note:** * – p <0.05 compared to control

According to the data presented (**Fig. 1**), at a concentration of 0.125 mg/mL, OVA+ and OVA biological preparations exhibited a growth-stimulating effect on T lymphocytes of the MT-4 cell line, therefore, further studies of the effect of these preparations on the level of glucose in the incubation medium, the content of SH-groups, as well as the effect on the primary culture of rat T lymphocytes were carried out by adding the biological preparations to the incubation medium at a concentration of 0.125 mg/mL and extending the incubation to 2 days. Under the influence of OVA, OVA+ and V3 metabolic activity and cellular viability were assessed using the MTT test and routine cell counting when stained with trypan blue. An increase in proliferation of MT-4 cell line and primary culture of rat T lymphocytes was detected both by counting the cell concentration and by assessing in the MTT test under the influence of OVA+ (**Fig. 2**) relative to the corresponding control. Along with the increase in the total cell concentration under the influence of OVA+, a slight decrease in the percentage of dead cells of the MT-4 line was observed (**Fig. 2**). The percentage of dead cells when treating MT-4 cells with OVA was also lower compared to the control sample. However, for T lymphocytes of primary culture of rats under the influence of the OVA biopreparation, an increase in the content of dead cells was detected compared to the control, and under the influence of OVA+, the percentage of dead cells did not differ from the corresponding control. As for the V3 biopreparation, no differences in indicators with the control were recorded in either the primary culture of T lymphocytes or the MT-4 cell line.

Thus, according to the presented MTT test data, the absorption indicators calculated per concentration of live cells in the primary culture of rat T lymphocytes was higher than in the culture of MT-4 cells in the control and under the influence of OVA and OVA+ biological preparations, while under the influence of V3, this index did not differ significantly in both cell cultures. This effect may be due to the fact that although MT-4

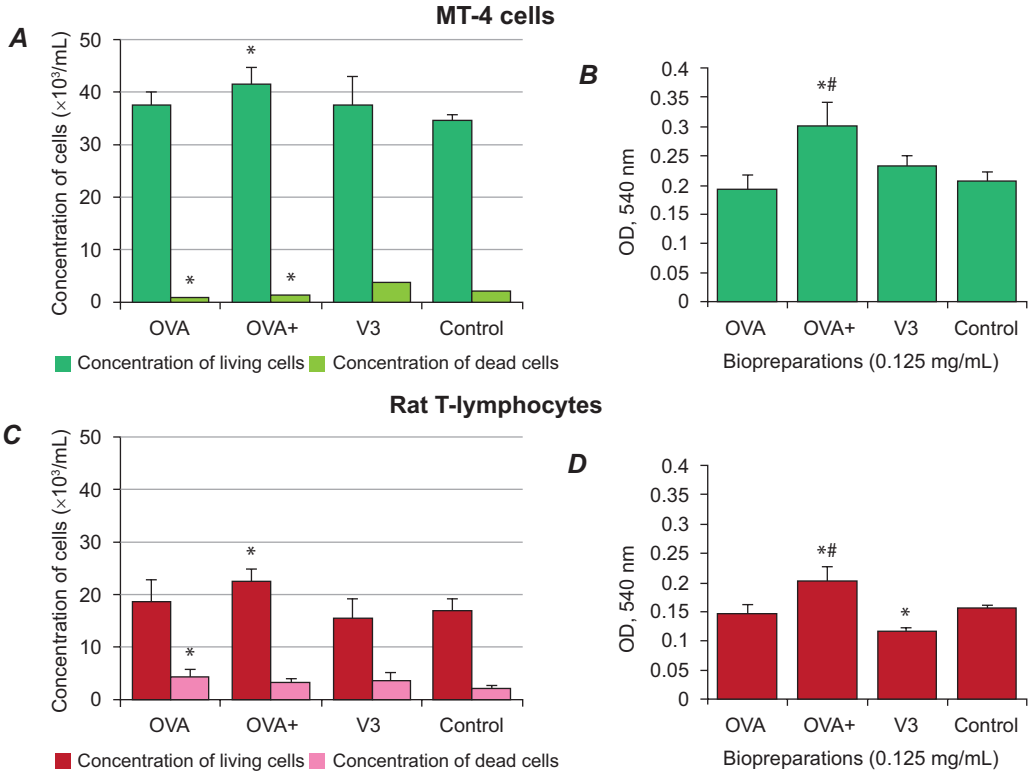



**Fig. 2.** Survival rate of MT-4 cell line (***A***, ***B***) and primary culture of rat T lymphocytes (***C***, ***D***) by routine counting (***A***, ***C***) and in the MTT test (***B***, ***D***) under the action of OVA, OVA+, and V3 biological preparations at concentrations of 0.125 mg/mL

**Note:** * – p <0.05 compared to control; # – p <0.05 compared to OVA

cells have phenotypic features of T lymphocyte origin, they have a transformed phenotype. Therefore, they may rely more on glycolysis for ATP production, as described by the "Warburg effect". It is known that most cancer cells use aerobic glycolysis for growth; this effect is also a key process that supports T cell activation and differentiation. (Liberti & Locasale, 2016). Activated T lymphocytes and macrophages are known to have a high affinity for glucose (Wieman *et al*., 2007), and the use of glycolysis inhibitors (2-deoxy-D-glucose) prevents the activation of macrophages *in vitro* and blocks inflammation *in vivo*. Paradoxically, despite glycolysis not being the most efficient way to produce ATP, activated immune cells predominantly rely on glycolysis. In contrast, oxidative phosphorylation, which involves mitochondrial bioenergetics, is a more complex and potentially slower process. Lymphocytes, which are expected to generate ATP, rapidly switch to glycolysis (O'Neill & Pearce, 2016; Luengo, 2021). Several scientific studies have demonstrated increased glycolysis in LPS-activated macrophages, dendritic cells (DCs), T lymphocytes, and B lymphocytes. Although less efficient in terms of energy production, aerobic glycolysis generates metabolic intermediates that are used in anabolic pathways required to sustain cell growth and to produce daughter cells. (O'Neill & Pearce, 2016). Accordingly, the utilization of glucose by T lymphocytes in response to test compounds is a key indicator that may provide insight into their metabolic activity (see **Table**).

**Glucose level (μmol/mL) in the culture medium of MT-4 cells and T lymphocytes primary rat culture under the action of test compounds (0.125 mg/mL) for two days**

| Samples/Culture | OVA | OVA+ | (V3) | Control | Control/RPMI-1640 |
|---|---|---|---|---|---|
| MT-4 | 3.78±0.73^ | 9.08 ±0.66* | 5.76±1.03 | 4.47±0.83 | 11.3 |
| T lymphocytes primary rat culture | 6.79±2.33 | 8.44±0.67* | 6.13±1.97 | 5.79±1.23 | 11.3 |

**Note:** * – P <0.05 compared to control; ^ – P <0.05 OVA vs OVA+

It is known that the active components of redox reactions in the body are thiol groups, as powerful reducing agents. The most common carrier of SH-groups is glutathione, which participates in the detoxification processes of reactive oxygen species and free radicals (Ulrich & Jakob, 2019). The effector functions of T cells are triggered through the regulation of metabolic activity, an important role in which belongs to the glutathione system, and in the case of energy deficiency, activated T cells are able to reprogram metabolism to glycolysis and glutaminolysis. Under the action of bioreparations, we investigated the content of SH groups in the MT-4 cells. As shown by the data presented, under the influence of OVA+, the content of both total SH groups and protein groups increased, while incubation of cells with OVA and V3 did not change these indicators compared to the control (**Fig. 3**)

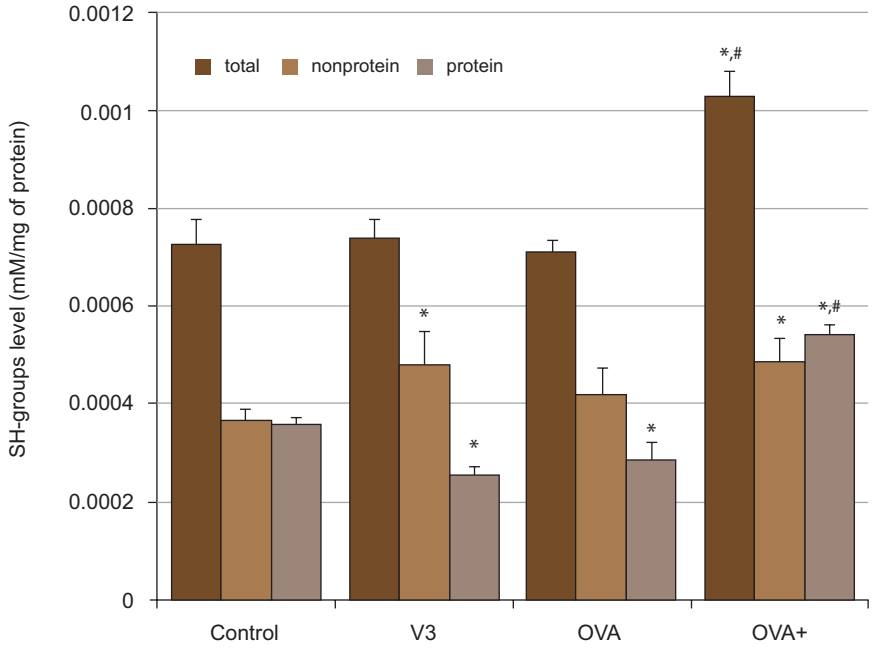



**Fig. 3.** Level of SH-groups (total, nonprotein, protein) in the MT-4 cells under the influence OVA, OVA+, and V3 biopreparations at concentrations of 0.125 mg/mL

**Note:** * – p <0.05 compared control; # – p <0.05 compared OVA+

The features and general patterns of changes in the content of protein and nonprotein -SH- and -S-S groups under the action of various biocompounds may have potential

diagnostic value in model test systems of antigen-antibody immune reactions *in vitro*, which significantly expands the analytical and diagnostic capabilities of modern immunoassays. Studies of glucose, glutamine, and fatty acid metabolism as fuels for the basic functions of lymphocytes and macrophages have been conducted over the past decades to understand the relationship between metabolic diseases and the immune system. Furthermore, the development of methods for metabolite analysis has contributed to the recent advancements in this field of research and has allowed for the creation of new strategies to treat chronic inflammatory diseases, cancer, and viral infections (Chapman & Chi, 2022; Diniz *et al*., 2023). Based on the results of previous studies (Arnauta *et al*., 2021; Palonko *et al*., 2022a,b) and the data presented here, it can be assumed that OVA and biophosphomag drugs can be used as immunosuppressants, while OVA+ may serve as an immunostimulant.

## CONCLUSION

The V3, OVA, and OVA+ biopreparations did not exhibit any cytotoxic or cytostatic effects on T lymphocytes. Among them, OVA+ showed the most pronounced activity, as evidenced by an increased glucose accumulation in the culture medium, enhanced mitochondrial dehydrogenase activity, and elevated SH-group levels. These findings suggest a potential metabolic and protective effect of OVA+, whereas V3 and OVA demonstrated neutral effects under the studied conditions.

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest**: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Human Rights**: this article does not contain any studies with human subjects performed by any of the authors.

**Animal studies**: all international, national, and institutional guidelines for the care and use of laboratory animals were followed

## AUTHOR CONTRIBUTIONS

Conceptualization, [L.G.; L.K]; methodology, [T.D.; I.S.]; validation, [P.F; N.S.; O.P.]; formal analysis, [V.L.].; investigation, [L.G.; T.D.; I.S.; P.F.; N.S.; V.L.; Y.P.] resources, [I.S., P.F] data curation, [L.G.; L.K]; writing – original draft preparation, [L.G.]; writing – review and editing, [T.D.; O.P]; visualization, [I.S.] supervision, [L.G.]; project administration, [L.K.; S.H.]; funding acquisition, [V.L.; N.S.].

All authors have read and agreed to the published version of the manuscript.

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## ВПЛИВ ПРЕПАРАТУ БІОФОСФОМАГ І БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН ІЗ НАСІННЯ РОЗТОРОПШІ ПЛЯМИСТОЇ (*SILYBUM MARIANUM*) НА МЕТАБОЛІЗМ ЛІМФОЦИТІВ ТА ЇХНІ ФУНКЦІОНАЛЬНІ ОСОБЛИВОСТІ

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**Обґрунтування.** Препарати OVA та запатентовані OVA+ (виробництва НУБіП України) було отримано згідно з О. В. Арнаутою та ін. (2021), способом екстракції біологічно активних сполук із насіння розторопші плямистої (*Silybum marianum*) з використанням кукурудзяної олії. Насіння розторопші є багатим джерелом флаволігнанів, флавоноїдів, вітамінів, танінів, макро- та мікроелементів.

Запатентований препарат БіоФосфоМаг (V3), також розроблений НУБіП України, базується на штучно фосфорильованому казеїні коров'ячого молока як ліганді та на іонах магнію як комплексоутворювачі (Palonko *et al.*, 2022a). Ці препарати мають захисні властивості й можуть посилювати процеси клітинної адаптації (Khyzhnyak *et al.*, 2022; Palonko *et al.*, 2022a,b). За попередніми даними, препарати проявляють жовчогінну активність, сприяють стабілізації функцій печінки і травлення, а потенційний вплив на метаболізм клітин імунної системи потребує подальшого вивчення.

Беручи до уваги, що імунна система є адаптивною та функціонує для захисту організму через пригнічення патогенних мікроорганізмів і росту пухлини за допомогою серії скоординованих сигналів, які регулюють активацію, проліферацію та диференціацію популяцій Т-клітин, метою цього дослідження було оцінити проліферативну активність, життєздатність клітин і рівень SH-груп у лімфоцитах під впливом захисних агентів OVA, OVA+ та V3.

**Матеріали та методи.** У дослідженні оцінювали активність мітохондріальної дегідрогенази за допомогою МТТ-тесту, рівні глюкози в культуральному середовищі за допомогою глюкозооксидазного методу та концентрації загальних і зв'язаних з білком SH-груп у Т-лімфоцитах, що зазнали впливу OVA, OVA+ та V3.

**Результати.** Порівняльний аналіз впливу OVA, OVA+ та V3 на культивовані Т-лімфоцити клітинної лінії МТ-4 та первинні культури Т-лімфоцитів щурів підтвердив безпеку цих біопрепаратів у досліджуваному діапазоні концентрацій (0,031–1,0 мг/мл). OVA+ значно підвищив активність мітохондріальної дегідрогенази (МТТ-аналіз), що свідчить про підвищену життєздатність клітин, порівняно з контролем, тоді як OVA демонстрував незначне пригнічення. Рівень глюкози в культуральному середовищі залишався незмінним під час обробки OVA та V3. Однак обробка OVA+ призвела до 1,5-кратного ($p < 0,05$) та 2-кратного ($p < 0,05$) збільшення рівня глюкози в первинних культурах Т-лімфоцитів щурів і клітинах МТ-4 відповідно, порівняно з контролем. Крім того, рівні загальних і зв'язаних з білком SH-груп у клітинах МТ-4 були значно підвищені під час обробки OVA+, тоді як під час обробки OVA або V3 жодних змін не спостерігали.

**Висновки.** Препарати V3, OVA та OVA+ не виявляють цитотоксичної або цитостатичної дії на Т-лімфоцити. Найвираженіший ефект має препарат OVA+, який викликає підвищення рівня глюкози в культуральному середовищі й активності дегідрогеназ, а також збільшення вмісту SH-груп у клітинах, що свідчить про його потенціал у підтриманні клітинного метаболізму та захисту. У той самий час V3 та OVA дають у заданих умовах нейтральний ефект.

***Ключові слова:*** Т-лімфоцити, біологічні препарати, рівень глюкози, SH-групи

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