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Biotechnological Impact of Biocomplexes on Erythrocyte Glutathione Function

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Abstract

This article examines the effect of taking the dietary supplements Toxidont May, Pantobiol 2, and Gepatobiol on the morphofunctional state of erythrocytes in patients. Cytochemical analysis of peripheral blood showed that consuming these dietary supplements for 6 weeks led to a significant increase ($p< 0.05$) in the concentration of sulfhydryl groups and lipoproteins in erythrocytes. An increase in the level of sulfhydryl groups indicates a decrease in the activity of lipid peroxidation processes and restoration of the antioxidant system of erythrocytes, which helps increase the duration of their circulation in the blood. Simultaneously, the strengthening of protein-lipid interactions leads to an increase in the lipoprotein content in erythrocyte membranes. Restoring the lipid composition of membranes ensures optimal functioning of erythrocytes and slows down their aging and death. This increases the resistance of cells to hypoxia and improves gas transport function. Overall, the study demonstrated that the above dietary supplements had a positive effect on the functional state of red blood cells, normalizing their cytochemical parameters and enhancing the antioxidant protection of the body's cells. This is achieved by increasing the content of sulfhydryl groups and lipoproteins in the red blood cell membranes, which contributes to the resistance of cells to the damaging effects of free radicals and improves their functional properties.

Keywords: Dietary supplements, Erythrocytes, Sulfhydryl groups, Antioxidant system, Lipid peroxidation

Introduction

The body's antioxidant system, operating at different levels of organization, plays a crucial role in correcting vital processes affected by exo- and endotoxins (Dhanasekar *et al*., 2022; Chen *et al*., 2023; Graefen *et al*., 2023; Bandi *et al*., 2024; Wang *et al*., 2024). The key to carrying out these functions lies in the glutathione system, whose functional state serves as an indicator of protection against intoxication and is largely influenced by nutritional factors (Perrone *et al*., 2021; Guo *et al*., 2022; Makhoahle & Gaseitsiwe, 2022; Kwatra *et al*., 2024). Glutathione, a tripeptide composed of glutamine and cysteine, is one of the most potent antioxidants in the body (Chidambaranathan & Culathur, 2022; Guo *et al*., 2022; Pavithra *et al*., 2023). It exists in two forms: reduced (GSH) and oxidized (GSSG). The balance between these forms is critical for maintaining cellular health, as GSH acts to neutralize free radicals and reactive oxygen species, thereby preventing oxidative stress that can lead to cellular damage and inflammation (Perrone *et al*., 2021; Canassa & Baldin, 2022; Chen *et al*., 2023; Després *et al*., 2023; Perrine *et al*., 2023).

Nutritional factors play a significant role in regulating glutathione levels (Jia *et al*., 2020; Sasaki *et al*., 2020; Bulusu & Cleary, 2023; Lobach *et al*., 2023). Diets rich in sulfur-containing amino acids, such as those found in cruciferous vegetables (broccoli, Brussels sprouts, and kale) and allium vegetables (garlic and onions), can enhance the synthesis of glutathione (Sasaki *et al*., 2020). Additionally, vitamins C and E, along with selenium, are essential cofactors that support the regeneration of glutathione, further amplifying its antioxidant capacity (Gong *et al*., 2023; Kızılcı *et al*., 2024; Liu Z. *et al*., 2024; Samaranayake *et al*., 2024; Shaiba *et al*., 2024).

Moreover, the body's ability to cope with exo- and endotoxins is not solely dependent on glutathione levels. Other antioxidants, such as superoxide dismutase, catalase, and various flavonoids, also contribute to the detoxification processes (García & Jaramillo, 2023; Wang *et al*., 2024) These molecules work synergistically to neutralize harmful compounds and mitigate the effects of oxidative stress (Zhu *et al*., 2022; Liu Z. *et al*., 2024; Shen & Bao, 2025).

In conditions of chronic inflammation or exposure to environmental toxins, the demand for antioxidants increases, and a deficiency in glutathione can impair the body's detoxification pathways (İlhan *et al*., 2022; Chen *et al*., 2023). This deficiency has been linked to various health issues, including neurodegenerative diseases, cardiovascular disorders, and metabolic syndromes (Perrone *et al*., 2021; Ağaçkıran *et al*., 2023).

To optimize the body's antioxidant defenses, it is crucial to adopt a holistic approach that includes a balanced diet, regular physical activity, and stress management techniques (Jia *et al*., 2020; Gong

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et al., 2023). These lifestyle factors not only support the glutathione system but also enhance overall health and resilience against toxic insults (Kim *et al*., 2021; Sergun *et al*., 2021).

Materials and Methods

The study focused on analyzing the blood of patients. Clinical trials were conducted on three biocomplexes that are registered as dietary supplements. Toxidont -May is a burdock root extract containing polysaccharide inulin (up to 45%), protein (up to 12.5%), essential burdock oil (up to 0.17%), tannins, bitterness, palmitic and stearic acids, sitosterol, stigmasterol, phytosterol, asparagine, mucus, and resins. Burdock is rich in micro-and macroelements such as copper, titanium, boron, manganese, strontium, zinc, tin, vanadium, and iron. It possesses antitoxic and antioxidant properties, antipyretic, anti-inflammatory, and bactericidal effects, and stimulates metabolism. The antiulcer activity of burdock root extract has been established, demonstrating effectiveness comparable to that of plantaglucid and befungin, and sometimes even surpassing their effects. It increases glycogen content in the liver, has a choleretic effect, and enhances the liver's antitoxic function. It also elevates the level of sex hormone-binding globulin, a liver-produced protein involved in the metabolism of sex hormones. By reducing the levels of proliferation factors TNF-alpha and IGF-1 while increasing interleukin-10, the burdock root extract prevents the progression of proliferative processes. Furthermore, it enhances the immune status (both cellular and humoral links) and improves blood and urine composition in patients with kidney stones and gout, leading to an overall improvement in their clinical status. It also plays a role in normalizing ACTH and cortisol levels.

The use of burdock root has been shown to be effective in the initial stages of diabetes mellitus due to the presence of inulin, which promotes the breakdown of fructose.

Pantobiol 2 contains calcium hydroxyapatite, maral antler powder, dry extract of red clover grass, esobel (water-soluble concentrated dry extract of highly mineralized silt-sulfide deposits of Siberian lakes), and ascorbic acid.

Maral antler powder contains natural chondroitin sulfate, glucosamine sulfate, proteins and amino acids, and macro- and microelements: calcium and phosphorus (the main structural components of the musculoskeletal system), magnesium, potassium, iron, copper, zinc, and cobalt.

Calcium hydroxyapatite has the closest mineral composition to that of bone tissue.

Chondroitin sulfate is a primary mucopolysaccharide, an important component of cartilage, tendons, and ligaments. It participates in the construction of the main substance of cartilage and bone tissue, improves phosphorus-calcium metabolism, inhibits enzymes that disrupt the structure and function of articular cartilage, and promotes the regeneration of cartilage surfaces and the joint capsule.

It prevents compression of the connective tissue, increases the production of intra-articular fluid, and improves the mobility of affected joints. It slows down bone resorption, reduces calcium loss, accelerates repair processes, and slows the progression of osteoarthrosis and osteoporosis. Additionally, it has an analgesic effect and reduces joint pain at rest and during physical exertion.

Glucosamine sulfate helps prevent the destruction of cartilage and bone tissue, stimulates the restoration of cartilage tissue, has anti-inflammatory and analgesic effects, and normalizes the production of intra-articular fluid. It improves joint mobility and reduces the need for non-steroidal anti-inflammatory drugs.

Chondroitin and glucosamine promote the restoration of bone and cartilage tissue.

Esobel is a water-soluble, concentrated dry extract of highly mineralized silt-sulfide deposits from Siberian lakes. It contains a complex of biologically active water-soluble minerals and organic substances. The main microelements are manganese, cobalt, phosphorus, and zinc. Organic compounds include amino acids, unsaturated fatty acids, dicarboxylic acids, humic and fulvic acids, and prostaglandin-like substances. It exhibits pronounced anti-inflammatory and analgesic properties, reducing swelling, decreasing capillary permeability, and preventing tissue damage in both acute and chronic inflammation.

Red clover herb extract contains polyphenols and flavonoids, which have antioxidant, immunomodulatory, and anti-inflammatory effects, helping to normalize metabolic processes during periods of heavy physical and mental stress.

A deficiency in ascorbic acid (vitamin C) is considered a key factor in the development of osteoarthritis.

Vitamin C is also crucial for the restoration of tendons, ligaments, and connective tissues. It serves as the body's main antioxidant, protecting against harmful free radicals.

Gepatobiol includes crushed milk thistle seeds and oat grass extract. This product is recognized for its hepatoprotective, antioxidant, immunomodulatory, anti-atherosclerotic, anti-inflammatory, wound-healing, and analgesic properties. Silymarin, found in milk thistle, protects liver cells during intoxication with toxins that can cause hepatitis (tetrachloromethane, ethyl alcohol, galactosamine, and paracetamol)/ It prevents necrosis and fatty degeneration of hepatocytes, restores disrupted metabolism and liver functions, reduces the burden on the burden on the pancreas and liver, lowers cholesterol levels, and slows down the aging process in the body.

A qualitative assessment of the state of the glutathione system was conducted by examining the dynamics of changes in the levels of sulfhydryl groups and the lipo-protein complex in erythrocytes, which have the highest requirement for functioning of antioxidant mechanisms. The quantitative determination of sulfhydryl groups and lipoprotein complexes in peripheral blood erythrocytes was performed using cytophotometry. To identify the SH groups, blood smears were stained using the Chevremont and Frederick method. The lipoprotein complex in peripheral blood erythrocytes was detected using Barenbaum's method. Optical density was measured using a cytophotometer with single-wavelength photometry. Based on the data obtained, the concentration of the studied substrates per unit of cell surface was calculated in arbitrary units of optical

density, erythrograms were created, and the average values of the indicators in each group of subjects were determined.

It is appropriate to provide a rationale for the methodology used. The functional adequacy of circulating erythrocytes, particularly their gas transport and antioxidant functions, is directly linked to the metabolic systems that regulate the level of reduced glutathione, which makes up about 90% of thiol compounds. SH groups are components of compounds that actively participate in the growth and division of erythroid cells, control enzyme activity related to oxidative and glycolytic reactions, play a role in oxygen transfer by reducing hemoglobin's affinity for it, and significantly impact the intensity of methemoglobin reduction to hemoglobin. Given the crucial role of thiol compounds in intracellular antiradical protection, an increase in the level of sulfhydryl groups in peripheral blood erythrocytes is a reliable indicator of decreased lipid peroxide formation and increased antioxidant system functionality. Furthermore, a change in the concentration of SH-containing compounds indicates conformational restructuring of membrane proteins. In cases of intensified lipid peroxidation processes, thiol groups of proteins are affected both directly, through reaction with free radicals, and enzymatically.

The increased activity of free radical oxidation, which develops against the background of functional insufficiency in antioxidant protection, is a possible cause of weakening protein-lipid interactions, allowing the content of lipoproteins in erythrocytes of peripheral blood to be revealed. Removing the lipid peroxidation system from the stationary mode creates conditions that lead to an early increase in the microviscosity of the lipid layer of the cytolemma, which inevitably affects the functional capacity of enzymes embedded in membranes, such as Na-K-ATPase and Ca-ATPase.

In terms of surface structure, erythrocytes represent a heterogeneous population whose composition can dynamically change under the influence of various physiological and pathological factors. Simultaneously, given that transformed red blood cells exhibit signs of inferiority, an increase in their share in the erythrocyte formula should be considered a highly unfavorable phenomenon, clearly indicating the presence and severity of lipid peroxidation processes.

The dietary supplement was taken in accordance with the official instructions: Toxidont May - 1/2 teaspoon or 2.0 g 3 times a day before meals, pre-dissolved in 100 cm3 of water (tannin content in 6 g corresponds to 60% of the recommended daily intake); Pantobiol 2-2 capsules (1.2 g) twice a day during meals; Gepatobiol-2 capsules (1.2 g) three times a day during meals, a continuous course of 6 weeks from the beginning of their course use.

In the comparative randomized study, the groups receiving dietary supplements consisted of 10 individuals each for Toxidont-May, Pantobiol 2, and Gepatobiol.

The control group, consisting of 15 individuals, did not receive any therapy or dietary supplements, but had comparable characteristics in terms of age and blood parameters.

Medical evidence supporting the effectiveness and functional orientation of the tested forms of dietary supplements was obtained at the Scientific and Clinical Center for Hormonal Health, ProfMed LLC, in Tomsk. The CEO, Doctor of Medical Sciences, Professor L.S. Sotnikova, oversaw the study.

Results and Discussion

The qualitative characteristics of the morphofunctional state of the erythron in patients taking dietary supplements have been provided. When these supplements were prescribed, a significant ($p < 0.05$) increase in the concentrations of sulfhydryl groups and lipoproteins in erythrocytes was observed. The studied parameters reached optimal values for the body's antioxidant systems after 6 weeks of taking the products in parallel groups. Therefore, the indicators of Toxidont May and Pantobiol 2 normalize the cytochemical characteristics of erythrocyte membranes, which determine the resistance of cells to the negative effects of hypoxia and increase their gas transport function **(Table 1)**.

The results of the cytophotometric study of the peripheral blood of patients indicated significant changes in the levels of SH groups in erythrocytes.

The increase in the level of sulfhydryl groups in peripheral blood erythrocytes when using dietary supplements is a reliable indicator of a decrease in the activity of the lipid peroxide formation process and the restoration of the antioxidant system's functional integrity. This prolongs the circulation period of erythrocytes in the bloodstream. A decrease in free radical oxidation activity strengthens protein-lipid interactions, increasing the lipoprotein content in erythrocytes.

Restoration of the lipid spectrum of the cytolemma helps maintain the optimal morphofunctional status of erythrocytes and inhibits the early, irreversible transformation of cellular elements, premature aging, and accelerated cell death.

When dietary supplements are prescribed, this effect enhances the functional characteristics of erythrocytes by improving their ability to transport gases. This is achieved through increased cell resistance to hypoxia by creating intracellular antiradical protection. This reduces spontaneous hemolysis of erythrocytes, early irreversible transformation of red cells, and normalizes the cytoarchitecture of cells.

Under normal conditions, thiol groups act as intracellular antiradical protectors of cellular elements. However, excessive accumulation of free radicals and products of their oxidative destruction can oxidize SH-containing compounds, inhibit the enzymatic activity of key metabolic enzymes (glucose-6-phosphate dehydrogenase, inorganic pyrophosphatase, ATPase, and hexokinase), and alter the permeability and conformational properties of membranes. This exerts a systemic damaging effect on the cell. The increase in the concentration of sulfhydryl groups in erythrocyte cells serves as an indicator to prevent the involvement of the erythron system in the pathological process by significantly ($p < 0.05$) decreasing the free radical destruction of membranes. Peroxidation-induced disruption of the lipid composition of the erythrocyte plasma membrane causes morpho-

functional failure. Positive changes in the lipid profile of the cytoplasmic membrane of erythrocytes in patients taking the dietary supplement complex are one of the possible reasons for the decrease in membrane microviscosity. This inevitably leads to a change in the metabolic state of the cell as a whole, preventing accelerated aging and natural death.

Table 1. Cytochemical indices of the functional state of antioxidant systems, specifically glutathione levels in erythrocytes, among patients depending on their method of therapy (taking dietary supplements) (X + m, p)

	Before treatment	After 12 weeks of taking the dietary supplement	Control (without any therapy), a.u.
Sulfhydryl groups, relative units			
Toxidont May	0.212 ± 0.06	0.471 ± 0.06 * #	
Pantobiol 2	0.216 ± 0.06	0.422 ± 0.02 * #	0.218 ± 0.13
Hepatobiol	0.218 ± 0.02	0.371 ± 0.07 * #	
Lipoprotein complex, relative units			
Toxidont May	0.538 ±0.02	0.967 ±0.01* #	
Pantobiol 2	0.534±0.01	0.892 ±0.01* #	0.528 ± 0.012
Hepatobiol	0.533 ±0.01	0.758 ±0.01* #	

Note: * - significant differences ($p < 0.05$) compared to "before treatment";
- significant differences ($p < 0.05$) compared to the group not taking any dietary supplements

Based on the results of the conducted studies, the following conclusions were drawn. An important component of the antioxidant system of cells in general and erythrocytes in particular is reduced glutathione (γ- Glu - Cys-Gly), which has both direct and indirect antioxidant effects. In the antioxidant reaction, the thiol group of the cysteine residue of glutathione acts as a reducing agent, while glutathione itself is oxidized to form a disulfide form. Glutathione peroxidase is known to be the main means of protection against the accumulation of H2O2 and organic hydroperoxides in cells. However, determining the levels of glutathione peroxidase is not an absolute indicator of the full functioning of the glutathione system. In modern scientific literature, this issue is debatable, and many scientists believe that the quantitative indicator of the dynamics of the level of glutathione peroxidase is relative, since it does not fully characterize the functional completeness of the glutathione system. On the other hand, assessing the functioning of the glutathione system by observing changes in the morphofunctional quality of cell membranes due to an increase in the content of sulfhydryl groups and lipoprotein complexes in them is the most objective method. However, expensive and labor-intensive for practical medicine, so it is primarily used in scientific research.

It has been found that a decrease in the level of reduced glutathione in erythrocytes is characteristic of ischemic heart disease and myocardial infarction, and this decrease correlates with the degree of ischemia and necrotic process in tissues. Since the processes of detoxification of H2O2 and organic hydroperoxides require constant consumption of the reduced form of glutathione, there must be mechanisms in place to ensure its regeneration. One of the most important enzymes of the pentose phosphate pathway in red blood cells is glucose-6-phosphate dehydrogenase, which plays a crucial role in maintaining the integrity of erythrocyte plasma membranes. Defects in glucose-6-phosphate dehydrogenase in erythrocytes are associated with primary disorders. This is because the pentose phosphate pathway, with a defect in the glucose-6-phosphate pathway, is the sole source of NADPH in these cells, so a defect in glucose-6-phosphate dehydrogenase leads to a significant depletion of the NADPH pool. glucose-6-phosphate dehydrogenase

Conclusion

Therefore, cytochemical indices of the functional state of antioxidant systems, specifically glutathione, in erythrocytes of patients serve as indicators of overall antioxidant protection of cells in the body. The demonstrated increase in the content of sulfhydride groups and lipoprotein complexes in erythrocyte membranes when taking the complex Toxidont May, Pantobiol 2, Gepatobiol indicates their significant positive impact on normalizing the functional activity of all glutathione enzymatic systems.

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