

The Effect Of Extracts From The Mushroom Of The Ganoderma Lucidum On The State Of Oxidative Stress

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Abstract: *This article presents data on such a well-known fungus as Ganoderma Lucidum and its effect on the state of oxidative stress. As is known, under oxidative stress, altered Toll receptors cannot independently recognize microbial agents due to the altered chemical formula of L-galactosamine and thus mediators are not activated. It is also known that Ganoderma Lucidum due to β -polyglucans, which by chemical formula are close to this glycoprotein, can interact with leukocyte receptors and increase the adhesion molecules and leukocyte migration. Modern histological, ultrastructural and biochemical research methods are used. The results are presented and the corresponding conclusions are drawn.*

Keywords: *oxidative stress; free radicals; Ganoderma Lucidum; experimental animals*

1. INTRODUCTION:

As you know, the basis of many pathological processes is based on the lipid peroxidation (LP). At the beginning the hypoxia can cause LP through hypoxia with the inducible factor 1α (HIF- 1α), which is produced by the cells during oxygen deprivation and activates the vascular endothelial growth factor, which increases the vascular permeability along with the histamine, bradykin, leukotrienes [1, 12, 21, 26]. At the same time, the important place in occurrence of the oxidative stress is given to the intercellular adhesion molecules iCAM-1, which is essentially the integrin ligand acting as the sialylated oligosaccharide. The initial rolling interactions begin with the selectins [7, 11, 15, 20, 24, 30]. Thus, VCAM-1 is essentially represented by the vascular endothelial adhesion molecule for E-selectin and its integrin VLA-4. At first, they are not active, that is, they have low affinity, but acquiring the higher affinity after the chemokines are attached to them [2, 3, 6, 8, 10, 14, 19, 23, 29]. However, the altered Toll receptors cannot independently recognize the microbial agents under the oxidative stress due to the altered chemical formula of the L-glucosamine and, thus, the mediators are not activated. Currently, the scientific debates are ongoing regarding the HMGB1 protein, as long as the recent studies [4, 5, 9, 13, 16] have proved that this protein regulates the activity of the Toll-like receptors via the signaling pathways and, by the feedback principle, activates and modifies the Toll-receptors. However, the HMGB 1 protein release requires conditions under which this protein binds to the Tig-like receptors via the ligands [17]. These ligands never occurs in the hypoxia. However, there are triterpenes which due to the unique sequence can form the pathway for the ligand formation to the Toll-like receptors and cause activation of such and even modifying such. There are many research works relating the triterpenes, however, the role of such in occurrence of the oxidation-reduction is not fully understood [18, 28]. Thus, Ganoderma Lucidum is one of the well-

known (natural) sources of the triterpenes. So, it is known that the *Ganoderma Lucidum* contains the triterpenes and polyglucans. At the same time, many works [11, 22] show that the *Ganoderma Lucidum* activates the leukocytes by impacting the modified glycoprotein Sialyl-Lewis X causing the stable adhesion to the endothelium via the selectins (E-selectin) [25]. It is known that the *Ganoderma Lucidum* increases expression of the TNF and IL-1, while, at the same time, these cytokines induce expression of the ligands by the endothelial cells to the integrins for VCAM 1 and ICAM 1. It is known that such combination of the cytokine-induced expression of the integrin ligands on the endothelium and activation of the leukocyte integrins leads to the tight integrin-mediated adhesion of the leukocytes to the endothelium in the focus of inflammation. At the same time, LFA 1 and Mac 1 integrins may contain a smaller degree of the Sialyl-Lewis X glycoprotein fucose-containing ligand and, therefore, the leukocyte migration does not occur to a proper degree. So, the *Ganoderma Lucidum* can interact with the leukocyte receptors due to the β -polyclucans, which are close to this glycoprotein by the chemical formula and increase the adhesion molecules and leukocyte migration. At the same time, the IL-8 and C5a complement do not bind to the specific 7-transmembrane G-protein bound by the receptors on the surface of the leukocytes under the oxidative stress [27]. Now then the *Ganoderma Lucidum*, being the intermediate element between these cytokines and the receptor, integrates into the IL-8 cytokine chain using its amino-acid residues, improves its susceptibility and activity and helps to integrate into this receptor. At the same time, it is included into the C5a complement due to the triterpenes, which increase recognition of this complement by the 7-transmembrane G-protein bound receptors.

Moreover, impact of the *Ganoderma Lucidum* on the physiological state of the body, as well as possibility of using of free radical processes to correct changes was not fully studied, which served as the rationale for the study conducted.

Research Goal. To study the effect of the *Ganoderma Lucidum* on free radical processes under conditions of the oxidative stress using the experimental animal models.

Materials and Research Methods. For the purposes of this goal, 100 outbred mice of both sexes weighing 15-20 gr. each were examined. The experiment lasted for 30 days and was performed in compliance with the rules stipulated by the European Commission for Supervision of Laboratory and Other Experiments with Participation of Experimental Animals of Different Species. All mice were put under the standard conditions with the natural change of lighting and observance of the general diet. All animals had free access to the food and water.

Research Materials. All the experimental animals were divided into 4 groups:

Group 1 - 10 mice - control, absolutely healthy mice;

Group 2 - main group 1 (20 mice) where the acute liver failure was caused by a single intraperitoneal injection of 0.06 ml 50% CCl₄ of the oil solution (olive oil) - at the rate of 0.3 ml per 100 g of the body weight;

Group 3 - main group 2 (20 mice) where the acute liver failure was caused by a single intraperitoneal injection of 0.06 ml 50% CCl₄ of the oil solution (olive oil) - at the rate of 0.3 ml per 100 g of the body weight, but the metabolic disorder was corrected using the intact drug (5% -2.0 ml of the ascorbic acid).

Group 4 - main group 3 (40 mice) where the acute liver failure was caused by a single intraperitoneal injection of 0.06 ml 50% CCl₄ of the oil solution (olive oil) - at the rate of 0.3 ml per 100 g of the body weight, but the metabolic disorder was corrected using the biologically active substances based on the *Ganoderma Lucidum* (at the dosage of 100 mg/kg of the body weight).

Animal euthanasia was applied under the ether anesthesia, after which decapitation was applied on 3, 10, 20, and 30 days and the biochemical parameters of the lipid peroxidation and activity of the antioxidant defense enzymes were determined in the blood serum.

The FE-TEM HF 5000 electron microscope from Hitachi HT was used in the work. Resolution is 0.1 nm.

Research Methods.

% 1. Determination of activity of the mitochondrial respiratory chain enzymes by measuring of activity of the Cytochrome C, A3, oxidative phosphorylation and conjugation of the oxidative stress affected by introduction of the *Ganoderma Lucidum*.

% 1. Determination of activity of the free radicals by detecting the diene, triene conjugants affected by introduction of the *Ganoderma Lucidum*.

% 1. Determination of architectonics of the structural forms of the hepatocytes affected by introduction of the *Ganoderma Lucidum*.

% 1. Statistical (Mann-Winnie, Wilconson test).

Activity of the mitochondrial respiratory chain enzymes by measuring of activity of the Cytochrome C was measured using the spectrophotometrical method, which is based on measuring of the optical density of solution of the recovered Cytochrome C having the absorption maximum of 550 nm (Cytochrome C recovery is accompanied with change in color of the solution from reddish-orange to bright pink). The magnitude of drop in the optical density of the solution over a certain period of time serves as the indicator of activity. Activity of the Cytochrome A3 was measured in a similar way, since the Cytochrome A3 catalyzes the Cytochrome C oxidation. The oxidative phosphorylation and conjugation of the oxidative stress were determined using the Chance-Williams method, which is based on the fact that phosphorylation cannot occur in absence of the ADP. The oxidation has a certain value. When ADP is added to the incubation medium, the phosphorylation comennces and the oxidative activity of the mitochondria is stimulated.

Determination of activity of the free radicals by detecting of the diene, triene conjugants is based on the principle of rearrangement of the double bonds and emergence of the system of the conjugated diene structures having the absorption maximum at 232-234 nm with the baseline in the area of 260-280 nm corresponding to the conjugated ketodienes.

Cytoarchitectonics of the hepatocytes was determined using the standard methods. Pieces of the mice liver were fixed in 10% neutral formalin, embedded in the paraffin blocks, from which sections with thickness of 5 μ m were prepared using the microtome. Sections were stained using the hematoxylin-eosin.

The study was performed immediately after the treatment, after 3, 7, 10 and 30 days.

Research Results.

The Results of Histological Studies.

Toxic liver damage by exposure to CCl_4 leads to occurrence of signs of inflammation, fatty degeneration, fibrosis in the liver of mice. Further exposure to the carbon tetrachloride leads to occurrence of the connective tissue. The hepatocytes along the connective tissue cords are vacuolated and contain a large number of the lipid drops in the main groups 1, 2 (Fig. 1).

Fig. 1. Signs of Inflammation in Hepatocytes after Exposure to CCl_4 . View under the electron microscope (zoom. X10000000). A- on the 1st day of the experiment. B- on the 2nd day of the experiment.

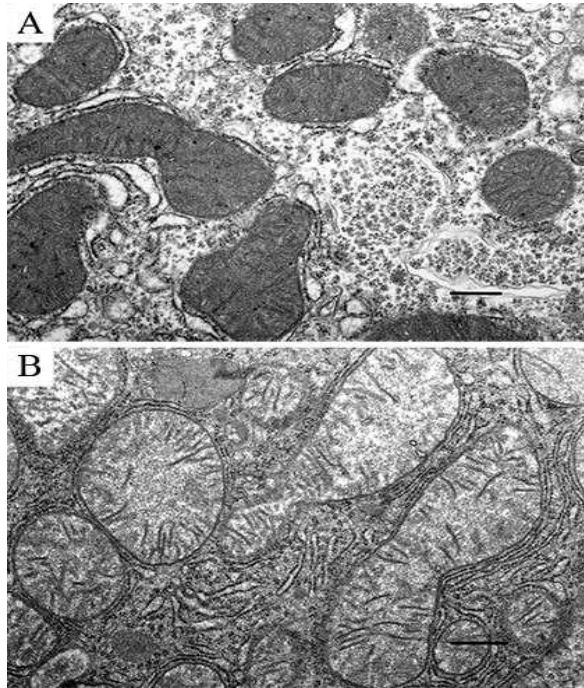


Fig. 2. Pit-cell after Exposure to the Ganoderma Lucidum on the 14th Day of the Experiment. View under the Electron Microscope (zoom.x1000000)

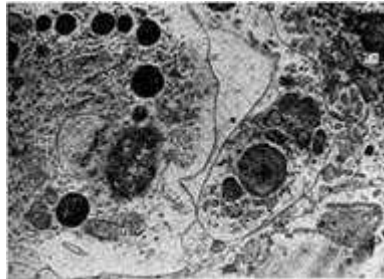


Fig. 3. Mitochondria after Exposure to the Ganoderma Lucidum on the 14th Day of the Experiment. View under the Electron Microscope (zoom.x1000000)

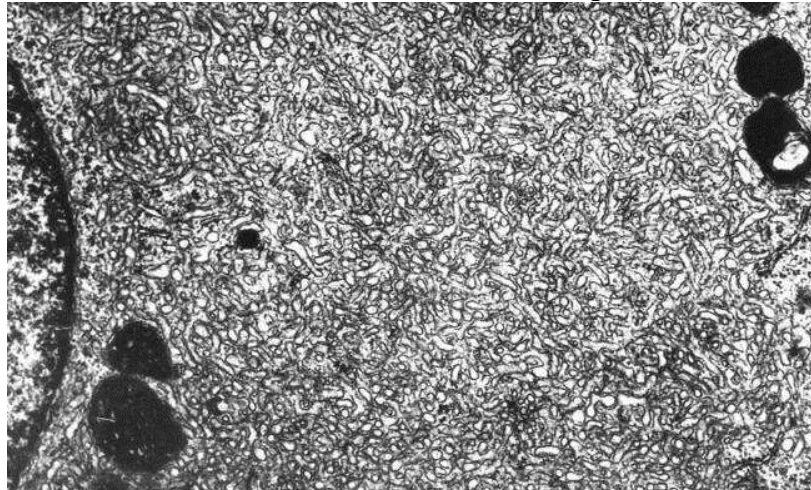
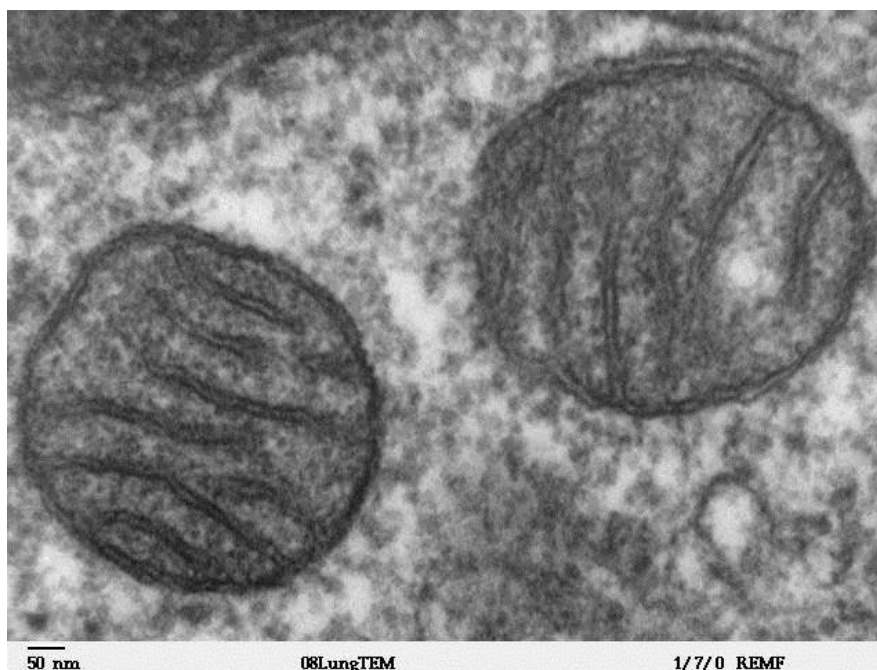


Fig. 4. Mitochondria after Exposure to the Ganoderma Lucidum on the 14th Day of the Experiment. View under the Electron Microscope (zoom.x1000000)



The walls of the vessels are thickened and fibrosed. The activated Kupffer cells and leukocyte infiltrates are visible in the liver parenchyma, especially near the vessels. The liver parenchyma regenerates very weakly in the 1st and 2nd main groups, which is associated with the almost complete absence of mitoses in the hepatocytes.

The liver parenchyma actively regenerates in the main group 3 as evidenced by frequent mitosis of the hepatocytes; at the same time the fatty degeneration was much weaker in this group, where the mice received the *Ganoderma Lucidum*, while the effect of the *Ganoderma Lucidum* was evaluated based on the ability of the biologically active substances increase degree of the fibrosis of processes and reduce the inflammatory processes.

The isolated hepatocytes in the control group (normal mice) had a regular round shape with the centrally located nucleus, clearly defined plasma membrane, and a large amount of cytoplasm. In groups 1 and 2, the hepatocytes were significantly more diverse in size; very large cells with large polyploid nuclei were found. The vacuoles were often present in the cytoplasm; the plasma membrane had many protrusions. In the main group 3 after exposure to the *Ganoderma Lucidum*, proportion of the intact hepatocytes was $68 \pm 2.0\%$ ($p \leq 0.05$) compared with the main groups 2 and 3 ($32.0 \pm 3.5\%$, $p \leq 0.05$).

The Results of Biochemical Studies.

The hepatitis of toxic nature becomes more widespread in the world, which determines the great interest of the researchers in this problem (Ryabinin, 2002). According to the general opinion of the researchers, the key chain in the pathological process is disorder of the respiratory chain of the hepatocyte mitochondria, which is based on the increased production of ROS - oxidative stress (Avasarala et al., 2016; Wang, Cederbaum, 2016). At the same time, the literature data on the state of the mitochondrial respiratory chain in toxic hepatitis are incomplete and very contradictory.

The study proved that activity of the Cytochrome C in main groups 2 and 3 was significantly higher than in the main group 1. In particular, this is associated with influence of the triterpene fractions and β - δ -polyglucans to increase of the liver cytochrome oxidase due to increase in the nonspecific resistance of the organism, leading to decrease of the optical density of the solution. Moreover, in the 3 main group, activity of the cytochrome oxidase was higher by 37.8% ($p \leq 0.05$) than in the main groups 1 and 2 and amounted to 59.0 ± 1.05 nmol of the succinate/mg protein per min (at the rate of $61,09 \pm 1.09$ nmol of the succinate/mg

protein per minute). In the groups 1 and 2, activity of the Cytochrome C was reduced by 38% ($p \leq 0.01$) and 44% ($p \leq 0.01$), respectively. As a result, endogenous redox reactions and metabolic processes in the tissues accelerated after increase in activity of the Cytochrome C, the oxygen utilization improved, and hypoxia in the tissues decreased in CCl_4 poisoning.

As it is known, the mechanism of the oxidative phosphorylation and conjugation of the oxidative stress is based on transfer of each pair of electrons from NADH to the oxygen and accompanied with translocation of 6 protons from the inner to the outer side of the mitochondrial membrane.

The study conducted proved that no closed membrane system occurred in the groups 1 and 2. As a result of the carbon tetrachloride poisoning, ATP in mitochondria cannot be formed as a result of disorder of the components of the respiratory chain, while activity of the enzymes located on the inner membrane of the mitochondria was lower by $45 \pm 2.0\%$ ($p \leq 0.05$), since the phosphorylating subunits and their proteins did not form ATP synthase, which is the most likely due to absence of the so-called stem, which binds the phosphorylating subunits to the membrane protein subunit and forming no ATP molecule. Moreover, protons, apparently, do not attack the oxygen atoms in the groups 1 and 2, as a result of which ATP is not formed.

After taking the Ganoderma Lucidum in the group 3, due to the organic germanium that is part of such, new protons are added, which attack the oxygen atoms that is leading to formation of the ATP. Moreover, the Ganoderma Lucidum leads to formation of the closed membrane system resulting in functioning of the vector ATP synthase and systematically arranging of the respiratory chain components in the membrane. In particular, F_1 subunit is activated as a result of three loops of the respiratory chain.

In the main groups 1 and 2, ADP content was reduced by $45 \pm 3.0\%$ ($p \leq 0.01$) and $56 \pm 2.5\%$ ($p \leq 0.01$), respectively. After taking the Ganoderma Lucidum on the 3rd, 7th, 10th and 30th day in the main group 3, significant increase occurred in the ADP content by $34.0 \pm 2.0\%$, $42.5 \pm 2.0\%$, $57.0 \pm 3.0\%$ and $67 \pm 2.5\%$ ($p \leq 0.05$), respectively.

Fig. 5. Dynamics of Oxidative Phosphorylation Indications and Conjugation of Oxidative Stress before Treatment

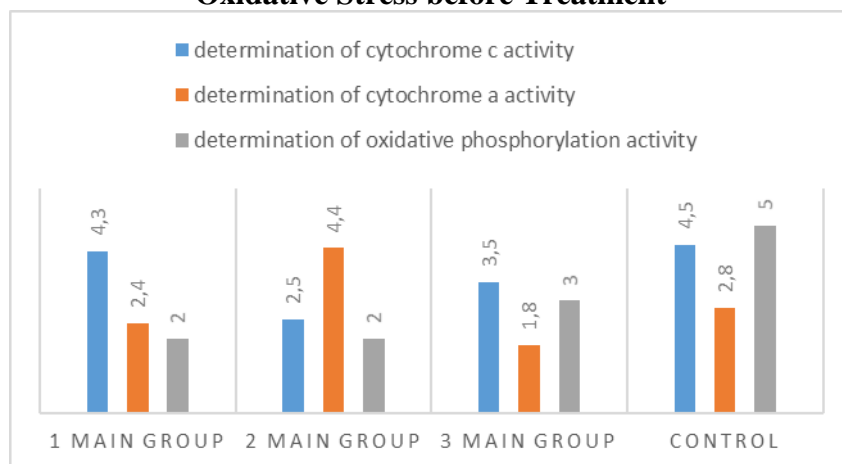
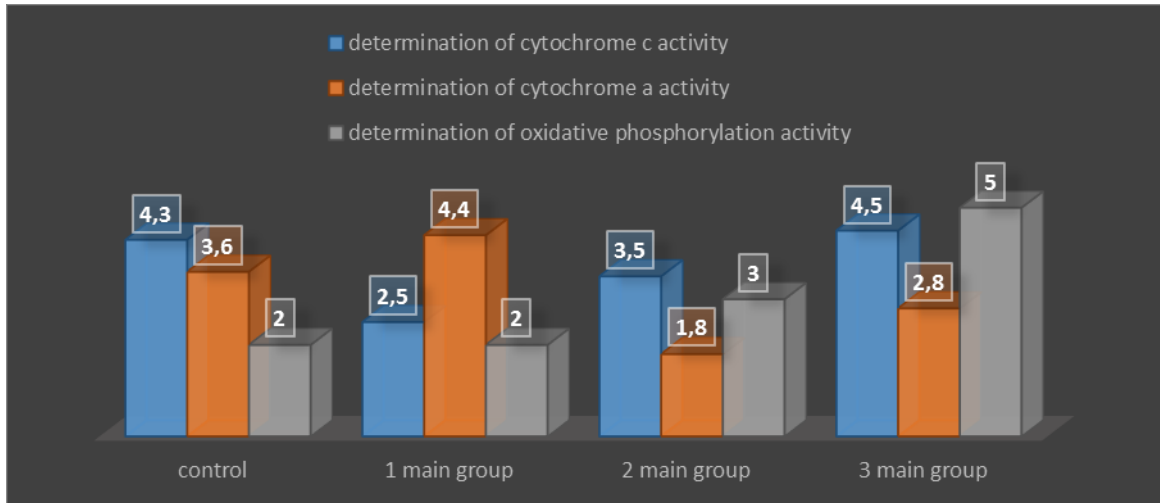


Fig. 6. Dynamics of Oxidative Phosphorylation Indications and Conjugation of Oxidative Stress after Treatment



After treatment using the Ganoderma Lucidum, increase in activity of the entire respiratory chain (Cytochrome C, A, oxidative phosphorylation and conjugation of the oxidative stress) is obvious.

The principle of the method for determining of the diene conjugants is based on determination of content of the primary lipid peroxidation products in the blood by absorption of the monochromatic light flux by the lipid extract in the ultraviolet area of the spectrum, since the molecules with two conjugated bonds (the diene conjugates) have the maximum absorption at 233 nm.

Moreover, content of the diene conjugants in the main groups 1 and 2 fluctuated at the level of 0.400 units. opt. pl/mg lipids ($p \leq 0.001$), while this indicator was 0.300 units. opt. pl/mg lipids ($p \leq 0.001$) in the group 3 after treatment using the Ganoderma Lucidum.

The gradual decrease in the diene and triene conjugants proves successful treatment and favorable prognosis.

Fig. 7. Indicators of Diene and Triene Conjugants before and after Treatment and 30 days after Treatment.

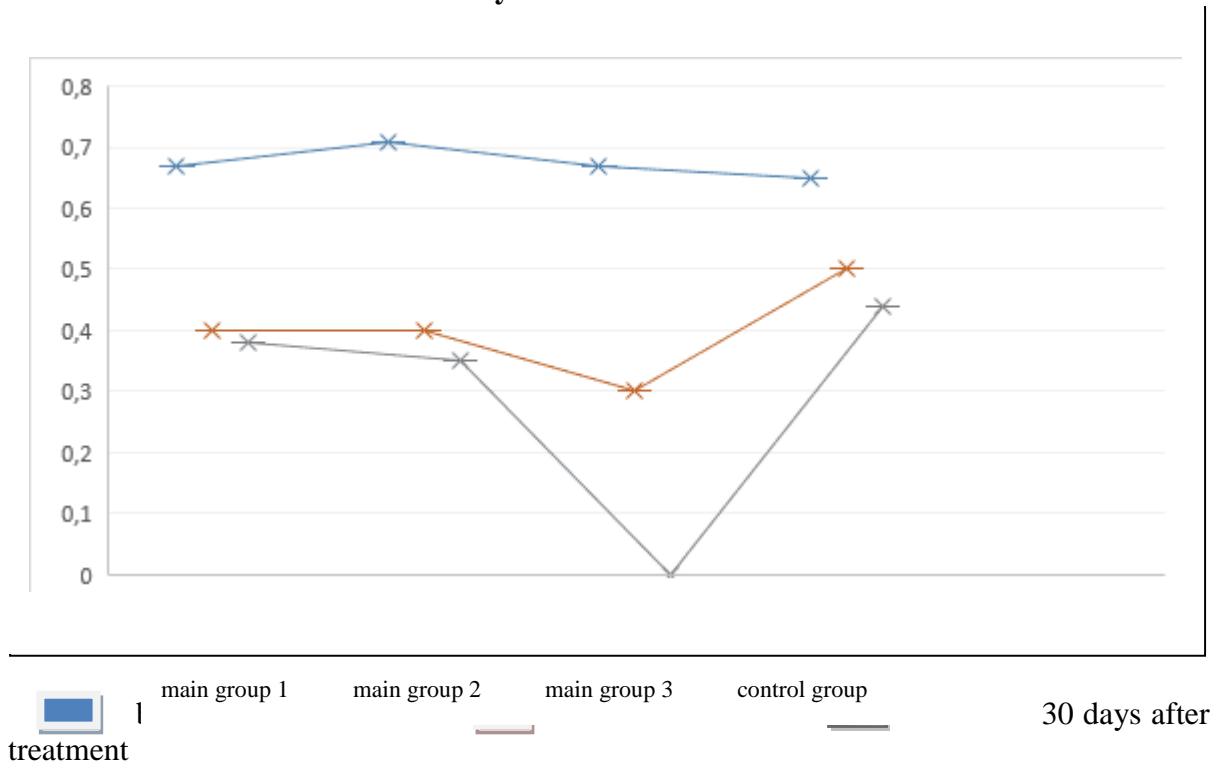
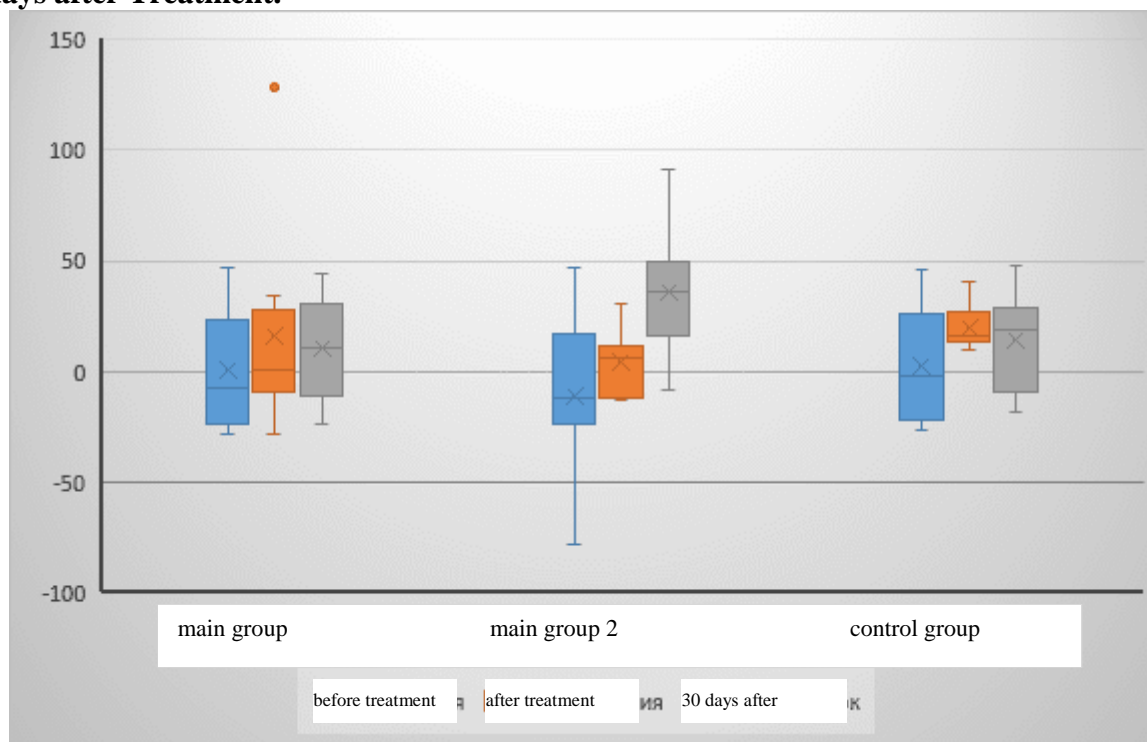


Fig. 8. Dynamics of Diene and Triene Conjugants before and after Treatment and 30 days after Treatment.



2. CONCLUSIONS:

Study of the hepatocytes cytoarchitectonics revealed that exposure to the toxic poison, such as carbon tetrachloride affected by the Ganoderma Lucidum, resulted in the active regeneration of the liver parenchyma, while the fatty degeneration was significantly weaker compared to the main groups 1 and 2.

Activity of the Cytochrome C, A was significantly higher after exposure to the Ganoderma Lucidum, which is associated with ability of the triterpene fractions and β - δ -polyglucans to increase the liver cytochrome oxidase due to the increased nonspecific resistance of the body.

The gradual decrease in the diene and triene conjugants affected by the Ganoderma Lucidum proves successful treatment and favorable prognosis.

The presented results of treatment of the oxidative stress using the biologically active substances based on the Ganoderma Lucidum can be applied in the clinical practice.

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