

An Experimental Assessment Of The Influence Of Ganoderma Lucidum On The State Of Oxidative Stress

Iriskulov B.U., Saydalikhodjaeva O.Z., Abilov P.M., Seytkarimova G.S., Norboeva S.A., Musaev Kh.A.

Abstract: This article describes the capabilities of Ganoderma Lucidum in the correction of oxidative stress. It has been proven that Ganoderma Lucidum removes free radicals, acts as antioxidants and enhances innate immunity. Materials and research methods: in this article data of histological, biochemical and ultramicroscopic research methods are given. Results of the study: according to the results of a histological study in the 3 main group, in which Ganoderma Lucidum was used, active regeneration of the liver was observed, as evidenced by the frequent mitosis of hepatocytes, fatty degeneration is less pronounced than in 1 and 2 main groups. in which olive oil and 5% -2.0 ml of ascorbic acid were used. In the 3 main group after exposure to Ganoderma Lutsidum, the proportion of intact hepatocytes was $68 \pm 2.0\%$ ($p \leq 0.05$) compared with 2 and 3 main groups ($32.0 \pm 3.5\%$, $p \leq 0.05$). The study showed that the activity of cytochrome c in 2 and 3 main groups was significantly higher than in 1 main group. In particular, this is due to the effect of triterpene fractions and β - δ -polyglucans to increase liver cytochrome oxidase due to an increase in the nonspecific resistance of the organism, as a result of which the optical density of the solution decreases. Moreover, in the 3rd main group, the activity of cytochrome oxidase was higher by 37.8% ($p \leq 0.05$) than in the 1st and 2nd main groups and amounted to 59.0 ± 1.05 nmol of succinate / mg protein per min (at a rate of $61,09 \pm 1.09$ nmol of succinate / mg protein per minute). In groups 1 and 2, the activity of cytochrome c was reduced by 38% ($p \leq 0.01$) and 44% ($p \leq 0.01$), respectively. As a result, after an increase in the activity of cytochrome c, endogenous redox reactions and metabolic processes in tissues accelerated, oxygen utilization improved, and hypoxia in tissues decreased during CCl₄ poisoning.

Index Terms: oxidative stress; Ganoderma Lucidum; triterpenes and polyglucans; mitochondria

INTRODUCTION.

As you know, the basis of many pathological processes is lipid peroxidation (LP) [1-4]. The basis of lipid peroxidation is active oxygen species (AOS). AOS are directly involved in oxidative phosphorylation. It is hypoxia that occurs during lipid peroxidation is that part of oxidative stress, which is very difficult to control. And AOS correction is for the search for new drugs with a prolonged effect. In this regard, I want to look at a natural adaptogen - Ganoderma Lutsidum. Studying the data of literary sources - SCOPUS, Web of Science, we came to the conclusion that at the moment the problem of lipid peroxidation has not been fully studied. Also in the SCOPUS abstract database, studying the data on the Ganoderma, Lutsidum came to the conclusion that there are not enough data in the studies on this fungus [5,6]. So, in the work [7,8] were found triterpenes of Ganoderma Lucidum, which remove free radicals, act as antioxidants and enhance innate immunity. Whereas the polysaccharides of Ganoderma Lucidum act to reduce the oxidative damage caused by ROS and to prevent DNA strand breaks. In studies by Yu Xu (2019), GLPUD activity decreased by 8.96% ($p \leq 0.01$), and glutathione reductase activity (GSH-PX) increased by 12.26% ($p \leq 0.01$). Superoxide dismutase (SOD) activity also increased by 14.90% ($p \leq 0.05$). Low molecular weight polysaccharides significantly counteract increased oxidative stress by lowering lipid peroxidation products and increasing the activity of enzymatic antioxidants.

Also, these researchers have proved that an increase in sulfate groups in the chains of polysaccharides increases the biological activity of the Lucidum Ganoderma. After analyzing the liver biopsy, these researchers noticed that after exposure to the Ganoderma Lucidum, the liver cells had a clear texture with normal morphology. However, there were a lot of fat droplets in the liver cells of the control group. Low molecular weight polysaccharides can significantly reduce the accumulation of lipid droplets in liver tissue cells. [9-12] Studies have proven that Ganoderma Lucidum can act as donors of electron atoms, turning them into more stable products. Since the hydroxyl radical formed during lipid peroxidation has destructive effects and causes cell damage, studies have shown that Ganoderma Lutsidum has the ability to absorb the hydroxyl radical. The mechanism of this influence is the ability of Ganoderma Lucidum to act as a donor of electrons or hydrogen to remove hydroxyl radicals. However, to date, there is insufficient data on the effect of Ganoderma Lucidum on the state of oxidative stress, and therefore it seemed relevant to us to conduct such a study. Purpose of the study. To conduct a comparative assessment of the influence of Ganoderma Lutsidum on the state of oxidative stress in experimental animal models.

Materials and research methods. To solve this problem, we studied white outbred mice of both sexes in the amount of 100 pcs weighing 15-20 g. The experiment lasted 30 days and was carried out in compliance with the rules provided by the European Commission for the supervision of laboratory and other experiments with the participation of experimental animals of different species. All mice were in standard conditions with a natural change of lighting and observance of a general diet. All animals had free access to food and water.

Materials research. All experimental animals were divided into 4 groups:

- 1 group - 10 mice - control, absolutely healthy mice
- 2 group - 1 main group (20 mice), in which acute liver failure

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was caused by a single intraperitoneal injection of 50% CCl₄ 0.06 ml of an oil solution (olive oil) - at the rate of 0.3 ml per 100 g of the body

- 3 group 2 main (20 mice), in which acute liver failure was caused by a single intraperitoneal injection of 50% CCl₄ 0.06 ml of an oily solution (olive oil) - at the rate of 0.3 ml per 100 g of the body, but correction of metabolic disorders was performed intact drug (5% -2.0 ml of ascorbic acid).
- 4 group 3 main (40 mice), in which acute liver failure was caused by a single intraperitoneal injection of 50% CCl₄ 0.06 ml of an oily solution (olive oil) at the rate of 0.3 ml per 100 g of the body, but the correction of metabolic disorders was performed biologically active substances based on Ganoderma Lucidum (at a dosage of 100 mg / kg body weight).

Euthanasia of animals was carried out under ether anesthesia, after which they were decapitated on 3, 10, 20, and 30 days and the biochemical indices of lipid peroxidation and the activity of antioxidant enzymes were determined in blood serum. An electron microscope FE-TEM HF 5000 from Hitachi HT was used in this work. Resolution is 0.1 nm.

2 RESEARCH METHODS.

1. Determination of the activity of enzymes of the mitochondrial respiratory chain by measuring the activity of cytochrome C, A3, oxidative phosphorylation and conjugacy of oxidative stress on the background of the introduction of Ganoderma Lucidum
2. Determination of the activity of free radicals by determining diene, triene conjugates on the background of the introduction of Ganoderma Lucidum
3. Definition of the architectonics of the structural forms of hepatocytes against the background of the introduction of Ganoderma Lucidum.
4. Statistical (Mann-Winnie test, Wilconson).

Measuring the activity of the enzymes of the mitochondrial respiratory chain by measuring the activity of cytochrome C was determined by a spectrophotometric method, which is based on measuring the optical density of a solution of reduced cytochrome c having an absorption maximum of 550 nm (restoration of cytochrome with a color change from reddish orange to bright pink) serves as the drop in the optical density of the solution over a certain period of time. Measurement of cytochrome A3 activity was determined in a similar way, since cytochrome a3 catalyzes the oxidation of cytochrome c. Determination of oxidative phosphorylation and the conjugacy of oxidative stress was carried out according to the Chance-Williams method, which is based on the fact that phosphorylation does not proceed in the absence of ADP. Oxidation has a certain value. When ADP is added to the incubation medium, phosphorylation begins to occur and the oxidative activity of mitochondria is stimulated. Determination of the activity of free radicals by determining diene, triene conjugates is based on the principle of rearrangement of double bonds and the emergence of a system of conjugated diene structures having an absorption maximum at 232-234 nm with a shoulder in the 260-280 nm region corresponding to conjugated ketodienes. The determination of cytoarchitecture of hepatocytes was carried out according to a standard method. Mice liver pieces were fixed in 10% neutral formalin, embedded in paraffin blocks, 5 μm thick sections were

prepared using a microtome. Sections were stained with hematoxylin-eosin. The study was carried out immediately after treatment, after 3.7, 10 and 30 days.

3 THE RESULTS OF THE STUDY.

The results of histological studies.

Toxic damage to the liver by exposure to CCl₄ leads to the appearance in the liver of mice of signs of inflammation, fatty rebirth, fibrosis. Further exposure to carbon tetrachloride leads to the appearance of connective tissue. In 1, 2 main groups, hepatocytes along the connective tissue strands are vacuolized and contain a large number of lipid droplets (Fig. 1).

Fig.1. Signs of inflammation in hepatocytes after exposure to CCl₄. View under an electron microscope (magnification X1000000). And on the 1st day of the experiment. On the 14th day of the experiment.

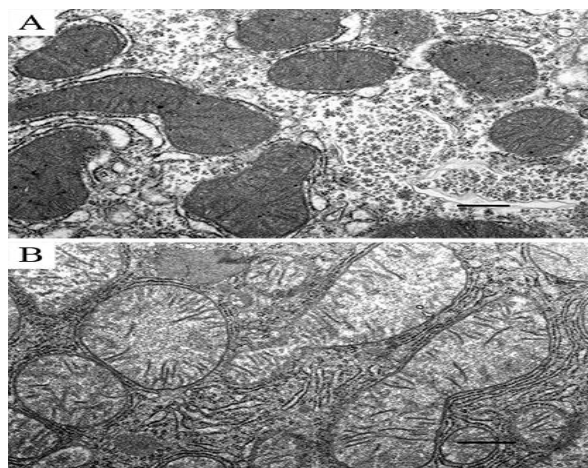


Fig.2. Pit-cell after exposure to Ganoderma Lucidum on the 14th day of the experiment. View under the electron microscope (magnification x1000000)

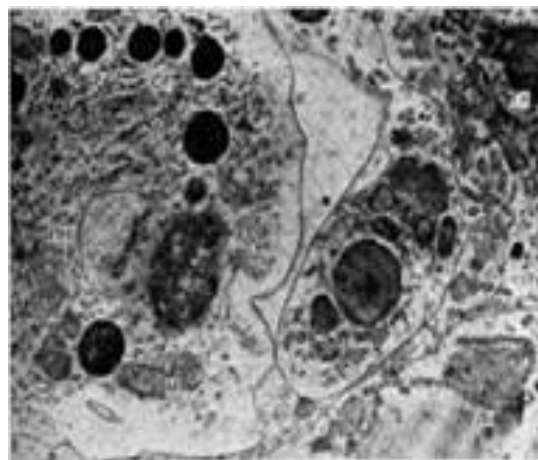


Fig. 3. Mitochondria after exposure to *Ganoderma Lucidum* on the 14th day of the experiment. View under an electron microscope (magnification x10000000)

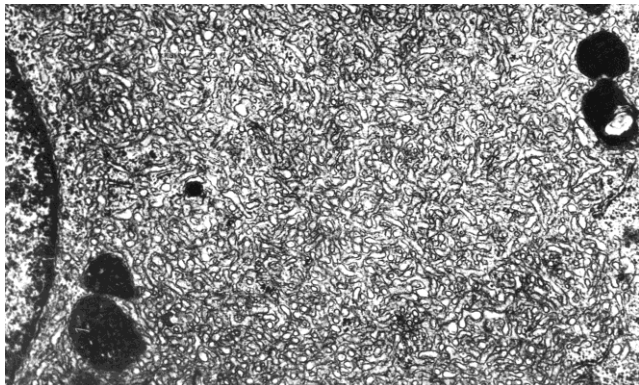
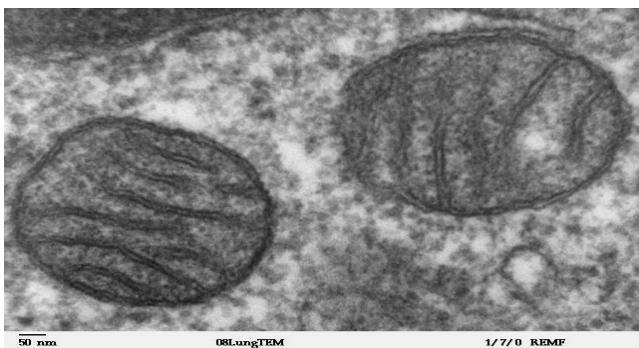


Fig. 4. Mitochondria after exposure to *Ganoderma Lucidum* on the 14th day of the experiment. View under an electron microscope (magnification x10000000)



The walls of blood vessels are thickened and fibrosed. In the liver parenchyma, especially near the vessels, activated Kupffer cells and leukocyte infiltrates are visible. In 1 and 2 main groups, the liver parenchyma regenerates very weakly, which is associated with the almost complete absence of mitoses in hepatocytes. In the main group 3, the liver parenchyma actively regenerates, as evidenced by frequently occurring hepatocyte mitoses, at the same time in this group in which mice received *Ganoderma Lucidum*, fatty dystrophy was much weaker, and the effect of *Ganoderma Lucidum* was evaluated on the ability of biologically active substances increase the degree of fibrosis processes and reduce inflammatory processes. Isolated hepatocytes in the control group (normal mice) had a regular round shape with a centrally located nucleus, a clearly defined plasma membrane, and a large amount of cytoplasm. In the 1 and 2 main groups, the hepatocytes were significantly more diverse in size; very large cells with large polyploid nuclei were found. In the cytoplasm vacuoles were often present, the plasma membrane had many protrusions. In group 3, after exposure to *Ganoderma Lucidum*, the proportion of intact hepatocytes was $68 \pm 2.0\%$ ($p \leq 0.05$) compared with 2 and 3 main groups ($32.0 \pm 3.5\%$, $p \leq 0.05$).

4 THE RESULTS OF BIOCHEMICAL STUDIES.

Hepatitis toxic nature are becoming increasingly common in the world, which determines the great interest of researchers

to this problem (Ryabinin, 2012). According to the general opinion of researchers, a key link in the pathological process is the disruption of the respiratory chain of hepatocyte mitochondria, which is based on increased ROS production - oxidative stress (Avasarala et al., 2016; Wang, Cederbaum, 2016). At the same time, the literature data on the study of the state of the mitochondrial respiratory chain in toxic hepatitis is not complete and highly controversial. The study showed that cytochrome c activity in 2 and 3 main groups was significantly higher than in 1 main group. In particular, this is due to the influence of triterpene fractions and β - δ -polyguenes to increase liver cytochrome oxidase due to an increase in the nonspecific resistance of the organism, as a result of which the optical density of the solution decreases. Moreover, in the 3 main groups, the cytochrome oxidase activity was higher by 37.8% ($p \leq 0.05$) than in 1 and 2 main groups and was 59.0 ± 1.05 nmol succinate / mg protein per minute (at a rate of $61,09 \pm 1.09$ nmol succinate / mg protein per minute). In 1 and 2 main groups, the activity of cytochrome c was reduced by 38% ($p \leq 0.01$) and 44% ($p \leq 0.01$), respectively. As a result, after an increase in cytochrome c activity, endogenous redox reactions and metabolic processes in tissues accelerated, oxygen utilization improved, and tissue hypoxia decreased due to CCl₄ poisoning. As is known, the mechanism of oxylic phosphorylation and the conjugacy of oxidative stress lies in the fact that the transfer of each pair of electrons from NADH to oxygen is accompanied by the translocation of 6 protons from the inner to the outer side of the mitochondrial membrane. In the conducted study it was studied that in 1 and 2 groups there was no closed membrane system. As a result of poisoning with carbon tetrachloride, ATP in mitochondria cannot be formed as a result of disturbance of the components of the respiratory chain, and the activity of enzymes located on the inner mitochondrial membrane was lower by $45 \pm 2.0\%$ ($p \leq 0.05$), since the phosphorylation subunits and their proteins did not form ATP synthase, which is most likely due to the absence of the so-called stalk, which binds the phosphorylating subunits to the membrane protein subunit and does not produce an ATP molecule. Moreover, in groups 1 and 2, apparently, protons do not attack oxygen atoms, as a result of which ATP is not formed. In group 3, after taking *Ganoderma Lucidum*, due to the constituent organic germanium, new protons are added, which attack the oxygen atoms and as a result ATP is formed. Moreover, *Ganoderma Lucidum* leads to the formation of a closed membrane system, as a result of which the vector ATP synthase functions and the components of the respiratory chain are arranged in the membrane in an orderly manner. In particular, what happens is that the F1 subunit is activated as a result of three loops of the respiratory chain. In 1 and 2 main groups, the content of ADP was reduced by $45 \pm 3.0\%$ ($p \leq 0.01$) and $56 \pm 2.5\%$ ($p \leq 0.01$), respectively. In the 3 main group after taking *Ganoderma Lutsidum* and on the 3rd, 7th, 10th and 30th day there was a significant increase in the content of ADP 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 indications of oxidative phosphorylation and conjugacy of oxidative stress before treatment

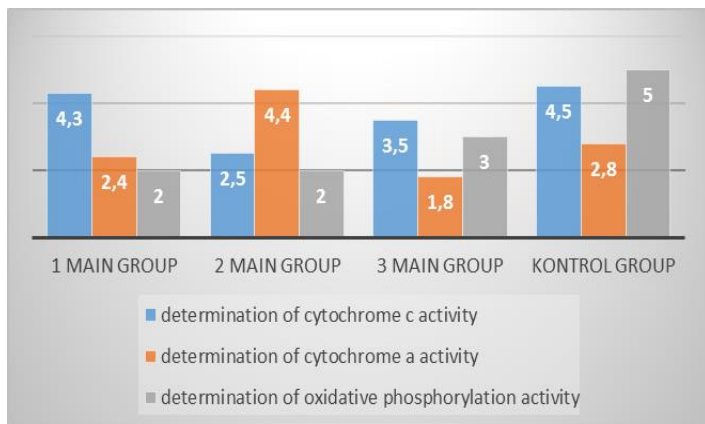
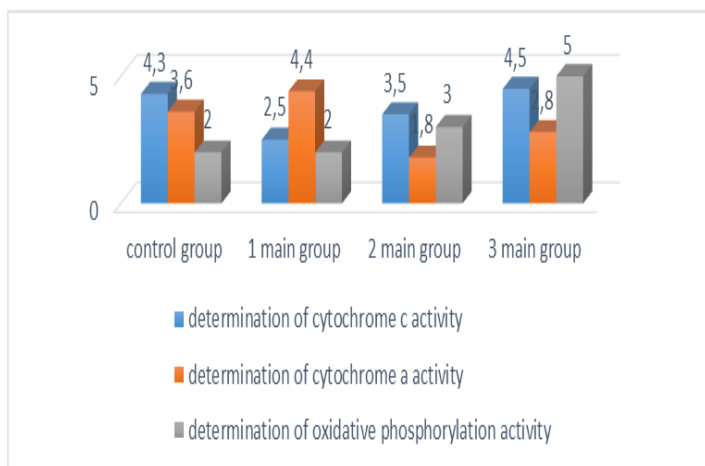


Fig.6. The dynamics of the indications of oxidative phosphorylation and the relationship between oxidative stress after treatment.



After treatment with Ganoderma Lucidum, it is seen that there is an increase in the activity of the entire respiratory chain (cytochrome c, a, oxidative phosphorylation and conjugacy of oxidative stress). The principle of the method for the determination of diene conjugates is based on the determination of the content of primary POL products in the blood according to the absorption of monochromatic light flux in the ultraviolet region of the spectrum by the lipid extract, since molecules with two conjugated bonds (diene conjugates) have an absorption maximum at 233 nm. Moreover, in 1 and 2 main groups, the content of diene conjugates varied at a level of 0.400 units. wholesale PL / mg of lipids ($p \leq 0.001$) and in group 3 after treatment with Ganoderma Lucidum, this figure was 0.300 units. wholesale PL / mg of lipids ($p \leq 0.001$). A gradual decrease in diene and triene conjugates indicates successful treatment and a favorable prognosis.

Fig. 7. Indicators of diene and triene conjugates before and after treatment and 30 days after treatment

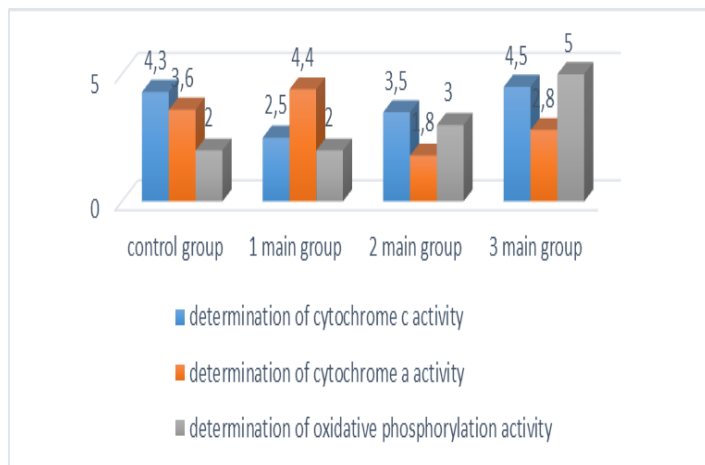
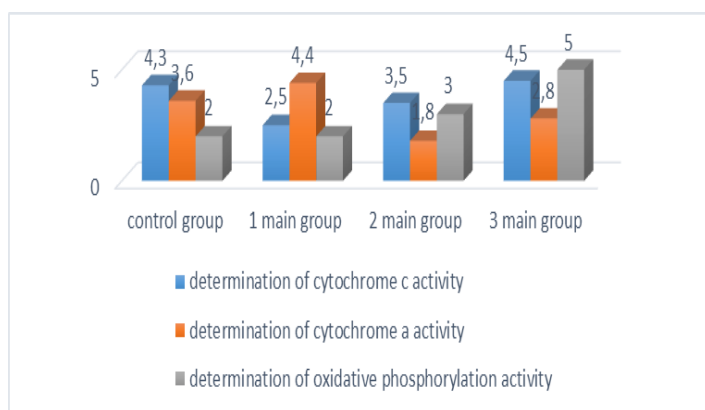


Fig. 8. Dynamics of the content of diene and triene conjugates before treatment, after treatment and 30 days after treatment



5 FINDINGS:

1. A study of the cytoarchitecture of hepatocytes revealed that after exposure to a toxic poison, such as carbon tetrachloride against the background of Ganoderma Lucidum, active regeneration of the liver parenchyma occurred, fatty degeneration was much weaker than in the 1 and 2 main groups.
2. The activity of cytochrome c, and after exposure to Ganoderma, Lutsidum was significantly higher, which is associated with the action of triterpene fractions and β - δ -polyglucans to increase liver cytochrome oxidase by increasing the nonspecific resistance of the organism.
3. A gradual decrease in diene and triene conjugates with Ganoderma Lucidum in use indicates successful treatment and a favorable prognosis.
4. The presented results of the treatment of oxidative stress with biologically active substances based on Ganoderma Lucidum can also be used in clinical practice.

All authors declare no conflict of interest.

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