The Seybold Report

ISSN 1533-9211

DOI 10.17605/OSF.IO/96BCF

# EFFECTS OF GOSSITAN AND GETASAN ON LIPID PEROXIDATION AND HIGH-PERMEABILITY PORE OF LIVER MITOCHONDRIA IN TOXIC HEPATITIS

**TURDIEVA O.M.**

Department of Biology, Kokand State Pedagogical Institute, Uzbekistan.

**Annotation**

In this study, the effect of gossitan and hetasan polyphenols on lipid peroxidation (LPO) process of rat liver mitochondria and Ca2+ ion-mediated mitochondrial inhibition under conditions of $CCl_4$-induced toxic hepatitis was investigated. To induce experimental toxic hepatitis in experimental rats, animals were injected subcutaneously with a 50% solution of $CCl_4$ (0.5 ml/kg) once every 3 days. Toxic hepatitis induced rats were administered per os from gossitan, getasan polyphenols and quercetin flavonoids once a day for 20 days. Gossitan and getasan inhibited $Fe^{2}$+/citrate-induced LPO in rat liver mitochondrial membrane under toxic hepatitis conditions, reduced malondialdehyde (MDA) content, and exhibited membrane-stabilizing effects. Gossitan and getasan polyphenols inhibit the open conformational state of mPTP (Mitochondrial permeability transition pore) of rat liver mitochondria in $CCl_4$-induced toxic hepatitis.

**Keywords:** Liver, Mitochondria, mPTP, $CCl_4$, $Fe^{2}$+/citrate, Gossitan, Getasan, Quercetin

## INTRODUCTION

In recent years, in CCl4-induced toxic hepatitis, interest in the LPO process, which develops in biological membranes, is increasing day by day. In the LPO process, primary, secondary and final products are formed and collected. Accumulation of primary products of LPO (diene conjugates) in the body occurs at the initial stage of oxidation. The concentration of LPO secondary products (MDA) reflects the activity of lipid peroxidation processes and serves as an indicator of the level of endogenous intoxication. End products of LPO disrupt microcirculation in organs and tissues [Iriskulov et al., 2019]. In pathological processes, the formation of $N_2O_2$ free radicals in the membrane as a result of LPO is of great importance. Increased production of free radicals in the mitochondrial respiratory complex weakens the antioxidant defense system and damages membrane lipoprotein components. In $CCl_4$-induced toxic hepatitis and many other pathologies, the intensity of LPO increases based on the development of oxidative stress. In conditions of $CCl_4$-induced toxic hepatitis, biologically active substances are widely used to reduce membrane LPO [Raj, 2014; Meng et al., 2020].

Bioactive compounds isolated from many plants, including antioxidants, have an inhibitory effect on mitochondrial high permeability pore, i.e. mPTP activity, in vitro experiments. Concentration-dependent blocking of the mPTP, which is brought to the open conformational state under the influence of various inducers, is studied by antioxidants that exhibit inhibitory properties. However, the results obtained in vivo are not always the same as those obtained in vitro. We have not investigated the inhibitory effects of these compounds on mitochondrial suppression in in vitro experiments. In various pathological processes, the number of liver

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/96BCF

mitochondria is increased compared to the control [Mihajlovic M., 2022]. There are synthetic drugs that, when administered to animals, destroy mitochondria located in various tissues. Among such drugs, alloxan, streptozotocin, $CCl_4$ and pesticides sharply increase the amount of free radicals in mitochondria, causing a decrease in the potential of the mitochondrial membrane and an open conformation of the highly permeable pore. In the conditions of toxic hepatitis caused by $CCl_4$, sharp disturbances in liver cells and their organoids are observed. Such membrane disorders can be corrected pharmacologically on the basis of plant substances. Among the biologically active substances, gossitan and getasan are important.

The purpose of this experiment was to study the effects of gossitan and getasan polyphenols on the LPO process of rat liver mitochondria and $Ca^{2}$+ ion-mediated mitochondrial contraction under conditions of $CCl_4$-induced toxic hepatitis.

## RESEARCH MATERIALS AND METHODS

The studies were carried out on purebred white male rats weighing 180-200 g. Conducting scientific research on experimental animals was carried out based on the rules of bioethics developed in 1985 by the international Declaration of Helsinki, the council for international medical scientific societies (CIOMS; the council for international organizations of medical sciences).

The rats isolated for the experiment were divided into groups.

Group I control (healthy); Group II experiment ($CCl_4$ 0.5 ml/kg); Group III $CCl_4$ + quercetin (50 mg/kg); IV group CCl4 + gossitan (50 mg/kg); Group V $CCl_4$ + getasan (50 mg/kg). In order to induce experimental toxic hepatitis in II, III, IV and V rats, animals were injected subcutaneously with 50% $CCl_4$ (0.5 ml/kg) once every 3 days. 21 days after the administration of $CCl_4$ to rats, after the increase of ALT (60 Ud/l) and AST (120 Ud/l) enzymes in the blood, purified vegetable oil (0.5 ml/kg) was given once a day to animals of group II, quercetin to group III of the experiment. flavonoid, gossitan to group IV and polyphenol getasan to group V once a day for 20 days per os.

Blood was collected from animals with experimental toxic hepatitis every 3 days, and the amount of ALT, AST enzymes was determined.

Gossitan ($C_{177}H_{154}O_{85}$) extracted from the plant Gossypium hirsutuim and getasan polyphenol ($C_{55}N_{36}O_{34}$) extracted from the plant Geranium sanguineum provided by the scientists of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan were used in this research [Абдулладжанова и др., 2011]. The flavonoid quercetin ($C_{15}H_{10}O_7$) of Plantago major L. (zubturum) plant extract was used as a standard prototype in the experiment. [Махмудов и др., 2010].

Isolation of LPO products was carried out in the presence of thiobarbituric acid (TBK). The reaction was stopped by adding 0.220 mL of 70% trichloroacetic acid to the incubation medium. After this step, the mitochondrial suspension was centrifuged at 4000 rpm for 15 min. Then 2 ml of supernatant was taken and 1 ml of 75% TBK was added. 2 mL of $N_2O$ and 1 mL

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/96BCF

of TBK were added to the control tube. The mixture was incubated in a water bath for 30 min. After cooling, the change in optical density at a wavelength of 540 nm was determined.

When determining the amount of MDA, the molar extinction coefficient (e=1.56×105 M-1 cm-1) in the formula was used: nmol MDA/mg protein=D/1.56x30.

Also, the $Fe^{2}$+/citrate system was used to study the process of LPO in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its functional state, and as a result of membrane LPO, the size of the organelle increased and the mitochondria collapsed. This volume change was determined photometrically at 25°C by constant mixing with the following incubation medium. Incubation medium (mM): KCI – 125, KCl –65, HEPES –10, pH 7.2; The amount of mitochondria is 0.5 mg/ml; Mitochondria were incubated for 2 min in medium containing 2 mM citrate before addition of 50 μM $Fe^{2}$+ [Almeida A.M., 2006].

Kinetics of mitochondrial swelling (0.3-0.4 mg/ml protein) was determined by spectrophotometer (spectrophotometer V-5000) at 540 nm in an open cell (volume 3 ml) with constant stirring of the mitochondrial suspension at 26°C. was determined. The following incubation medium was used to determine mitochondrial PTP permeability: 200 mM sucrose, 20 μM EGTA, 5 mM succinate, 2 μM rotenone, 1 μg/ml oligomycin, 20 mM Tris, 20 mM HEPES, and 1 mM $KH_2PO_4$, pH 7.4. [He L., 2003].

Mitochondrial protein was determined using the Biuret method. Statistical processing of the obtained results and drawing of pictures were carried out using the computer program OriginPro 7.5 (Microsoft, USA). In the experiments, the kinetics of mitochondrial decay was calculated as a percentage of the maximum, as the arithmetic mean value of 4-7 different experiments was calculated. The difference between the values obtained from control, experiment and experiment+study material was calculated by t-test. In this case, $R<0.05$ and $R<0.01$ values represent statistical reliability.

**The obtained results and their analysis**

In our next experiment, we investigated the effects of gossitan and getasan on LPO of liver mitochondria in $CCl_4$-induced toxic hepatitis. The effects of gossitan and getasan on liver mitochondrial LPO in $CCl_4$-induced toxic hepatitis are shown in Figure 1 below. According to the obtained results, MDA formation in mitochondria isolated from the liver of rats with toxic hepatitis (group II) was found to increase by 97.9±5.4% in toxic hepatitis compared to the control group (group I) (Fig. 1). Therefore, MDA is formed as a result of increased LPO process in the liver mitochondria membrane of CCl4 injected rats. An increase in the amount of MDA causes pathophysiological changes in physiological (ion homeostasis, matrix volume changes) and biochemical (respiratory chain enzyme activity decreases, ion channel protein conformation changes) processes in mitochondria. However, LPO damage in the liver mitochondrial membrane as a result of toxic hepatitis can be corrected with herbal substances. The effect of gossitan and hetasan polyphenols, which have shown hepatoprotective activity in experiments, on the amount of MDA, a product of rat liver mitochondrial membrane LPO, in a model of toxic hepatitis, was studied in vivo. Group III rats with $CCl_4$-induced toxic hepatitis

ISSN 1533-9211

DOI 10.17605/OSF.IO/96BCF

were administered per os at a dose of 50 mg/kg once a day for 20 days for comparative analysis, after 21 days after CCl4 injection and the increase of indicator enzymes. Mitochondria were isolated from the liver of rats treated with quecetin and their LPO was determined. It was found that the amount of MDA in liver mitochondria of animals with hepatitis decreased by 64.6±4.4% under the influence of quercetin compared to group II.

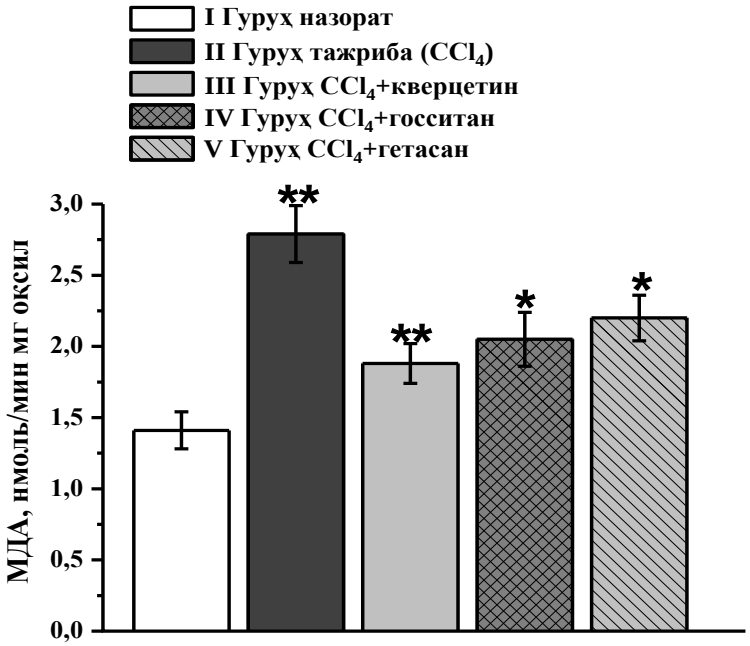


**Figure 1: Effects of quercetin, gossitan and getasan on rat liver mitochondrial MDA content in $CCl_4$-induced toxic hepatitis. (*R<0.05; **R<0.01; n=6)**

When we administered pharmacotherapy to group IV animals with toxic hepatitis with gossitan once a day for 20 days, it was noted that the amount of MDA in the mitochondria isolated from the liver was inhibited by 52.5±4.3% compared to group II. As a result of administration of getasan to group V animals, which was called a toxic hepatitis model, it was found that the MDA content of liver mitochondria decreased by 41.9±3.5% compared to group II (Fig. 1). Thus, in $CCl_4$-induced toxic hepatitis, gossitan and getasan affected the intensity of LPO in rat liver mitochondria, reduced the concentration of MDA and enhanced the antioxidant defense system. In this case, it was found that the antioxidant effect of gossitan polyphenol on liver mitochondria is more active than getasan in conditions of toxic hepatitis. So far, the effects of the polyphenolic compounds gossitan and getasan on $Fe^{2}$+/citrate-induced LPO of liver mitochondria in toxic hepatitis have not been studied in vivo. Membrane LPO can be determined in experiments by staining mitochondria. For this, $Fe^{2}$+/ascorbate and Fe2+/citrate are often used as inducers in experiments. In the presence of 50 μM $Fe^{2}$+ and 2 mM citrate in the incubation medium, the LPO process is dramatically enhanced, resulting in a disruption of

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/96BCF

the barrier function of mitochondria and an increase in their size compared to the control [Almeida et al., 2006]. Membrane LPO was studied using $Fe^{2}$+/citrate in $CCl_4$-induced toxic hepatitis and liver mitochondria of corrected rats.

According to the obtained results, in the presence of 125 mM sucrose, 65 mM KCl, 10 mM HEPES, 1 mM EGTA and pH 7.2 in the standard incubation medium, no swelling was observed in the liver mitochondria of intact animals (Fig. 2). In order to determine the inhibition of intact mitochondria, acute inhibition occurred when we added 2 mM citrate to the incubation medium and exposed to 50 μM $Fe^{2}$+ 2 min after the initiation of LPO, and this was recorded as a control. Under the same conditions, it was found that mitochondria isolated from the liver of group II rats with toxic hepatitis were increased by 45.4±3.6% under the influence of $Fe^{2}$+/citrate compared to the control group (group I) (Fig. 2). Therefore, in the presence of $Fe^{2}$+/citrate in the incubation medium, the mitochondria swelling increases, and this indicates that the membrane LPO process has been completed. Compared to healthy liver mitochondria, liver mitochondria of animals with hepatitis were significantly increased by $Fe^{2}$+/citrate. Rats with toxic hepatitis showed increased $Fe^{2}$+/citrate-induced suppression of liver mitochondria, indicating that membrane LPO was accelerated compared to controls. Continuing our experiment, when pharmacotherapy of group III animals with toxic hepatitis with quercetin taken as a standard, it was found that the liver mitochondria with $Fe^{2}$+/citrate was inhibited by 33.0±2.6% compared to the indicators of group II, and the intensity of LPO decreased. The antioxidant activity of the flavonoid quercetin is more effective than other bioactive compounds and neutralizes the formation of free radicals. [Xu D., Hu M.J. 2019]. In addition, quercetin increases the activity of antioxidant enzymes in toxic hepatitis, reduces the amount of LPO product MDA [Zhang et al., 2014]. In our experience, quercetin may have antiradical activity by inhibiting ROS generation in liver mitochondria in $CCl_4$-induced hepatitis.

Continuing our experiment, IV and V groups of rats were injected with 50% tetrachloromethane dissolved in vegetable oil (0.5 ml/kg) once every 3 days. 21 days after challenge of hepatitis model, group IV and group V rats were pharmacotreated by oral administration of gossitan (50 mg/kg) and getasan (50 mg/kg) for 20 days, respectively. When the liver mitochondria of IV and V rats with pharmacotreated toxic hepatitis were studied by $Fe^{2}$+/citrate-induced membrane LPO, their mitochondrial function was inhibited by 25.9±1.8% and 19.5±1.6%, respectively, compared to pathological group II values. was determined (Fig. 2).

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/96BCF

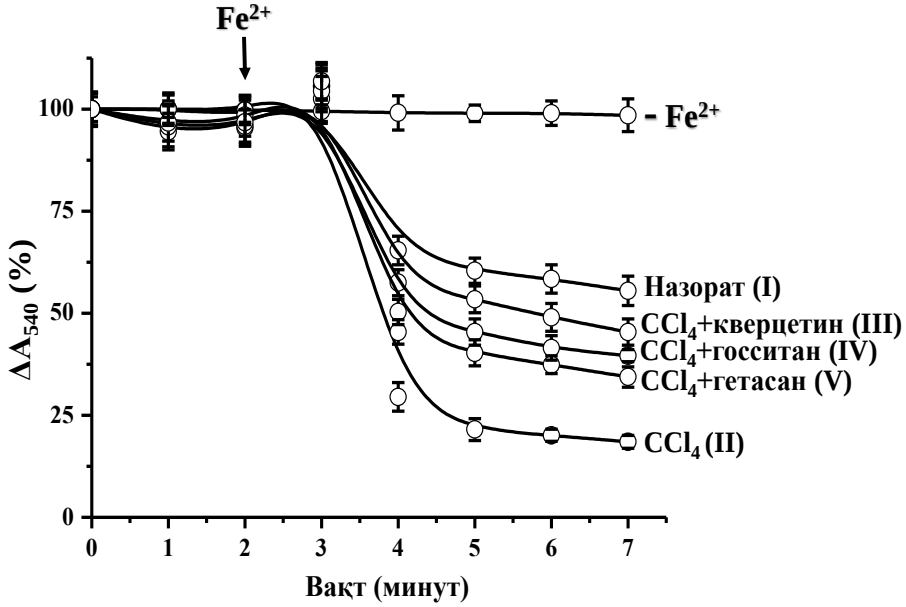


**Figure 2: Effects of quercetin, gossitan and hetasan on $Fe^{2}$+/citrate-dependent mitochondrial membrane LPO in $CCl_4$-induced toxic hepatitis (all cases R<0.05; n=6)**

Iron compounds induce LPO in the mitochondrial membrane. LPO induced in the presence of $Fe^{2}$+/citrate in the mitochondrial membrane causes a sharp increase in permeability dependent on $Ca^{2}$+ ions [Castilho et al., 1999]. Under these conditions, mitochondrial membrane potential decreases, free radicals increase, swelling and loss of matrix components occur. [Castilho et al., 1999; Kowaltowski et al., 2001]. It can be seen that gossitan and getasan inhibit LPO of the rat liver mitochondria membrane under conditions of toxic hepatitis, and the stabilizing effect on the membrane can be explained by their antioxidant properties. Gossitan and hetasan polyphenols may also cause increased ATF synthesis and decreased LPO process by reducing free radical generation from the liver mitochondrial respiratory chain. This, in turn, allows the use of gossitan and getasan in physiological studies as a corrective agent in the development of toxic hepatitis. But their pharmacological effect in toxic hepatitis conditions requires further research.

The classic toxicity of $CCl_4$ is liver injury and fibrosis. Liver fibrosis is the result of chronic liver disease that can progress to cirrhosis or even hepatocarcinoma. However, the toxicological mechanisms of $CCl_4$-induced liver damage are not fully understood. White male rats were challenged with $CCl_4$ every 3 days for 3 weeks in a toxic hepatitis model, and blood ALT and AST enzymes were examined. Toxic hepatitis model animals were injected with polyphenolic compounds once a day for 3 weeks. Enzymes were also determined in the corrected rat groups. The development of toxic hepatitis in rats for 6 weeks and the dynamics of changes in some functional activities of liver mitochondria under the influence of pharmacotherapy were determined.

ISSN 1533-9211

DOI 10.17605/OSF.IO/96BCF

**Analysis of the dynamics of changes in mPTP function of rat liver mitochondria in $CCl_4$-induced toxic hepatitis**

In the following experiment, $CCl_4$, a drug with such toxic properties, was administered to rats, and we studied the effect of polyphenolic compounds selected for research on the dynamics of mPTP, i.e., inhibition of liver mitochondria in in vivo experiments. The data is shown in Figure 4.9 below. Rats were challenged with 50% $CCl_4$ dissolved in vegetable oil every 3 days for 3 weeks in a toxic hepatitis model. Toxic hepatitis model animals were injected with polyphenolic compounds once a day for 3 weeks. Mitochondria were isolated from the livers of healthy, toxic hepatitis and polyphenol compound-corrected rats for 6 weeks to determine the dynamics of mitochondrial destruction**.** From the obtained results, it was revealed that no change was observed in the dynamics of liver mitochondria division in rats of the I control group for 6 weeks. However, group II rats treated with $CCl_4$ had an increased number of liver mitochondria compared to controls during the week. In this case, the permeability of the hepatic mPTP of $CCl_4$-treated rats increased sharply by 78±4.5% compared to the control, and after 3 weeks, there was no significant change in the swelling dynamics (Fig. 3). Group III, IV, and V rats, called toxic hepatitis model, were treated with quercetin, gossitan, and getasan polyphenols for 3 weeks, respectively, starting from the 3rd week.

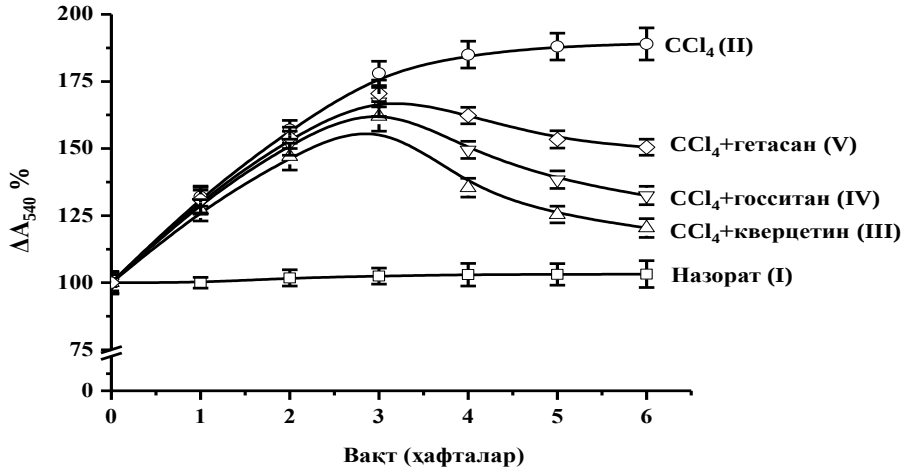


**Figure 3: Analysis of the dynamics of changes in mPTP function of rat liver mitochondria in $CCl_4$-induced toxic hepatitis (all cases R<0.05; n=6)**

In rats with quercetin-treated hepatitis III, liver mitochondria were found to be inhibited by 49.6±3.5%, 62.6±4.7%, and 68.6±4.8% compared to control at 4, 5, and 6 weeks, respectively. In rats with IV toxic hepatitis administered Gossitan, liver mitochondria were inhibited by 35.5±2.6%, 49.6±3.8%, and 56.5±4.3% compared to control at 4, 5, and 6 weeks, respectively. Hepatic mitochondria were inhibited by 22.7 ± 2.2%, 34.6 ± 3.0%, and 38.5 ± 2.5% compared to the control at weeks 4, 5, and 6 in rats with toxic hepatitis V treated with Getasan (Fig. 3 picture). As can be seen from the obtained results, the introduction of polyphenolic compounds

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/96BCF

at a dose of 50 mg/kg body weight of the animal every day for 3 weeks inhibited the conformation of mPTP in the mitochondria of the liver of animals with toxic hepatitis.

## CONCLUSIONS

It was found that the antioxidant effect of gossitan polyphenol on liver mitochondria in toxic hepatitis is more active than that of getasan. Gossitan and getasan exhibited membrane-stabilizing effects by inhibiting $Fe^{2}$+/citrate-induced LPO in rat liver mitochondria under toxic hepatitis conditions. In $CCl_4$-induced toxic hepatitis, the mPTP open conformational state of rat liver mitochondria is inhibited by the polyphenols gossitan and getasan. The properties of gossitan and getasan, which correct the dysfunction of liver mitochondria in toxic hepatitis, were found to be close to the flavonoid quercetin.

**Literature**

1. Iriskulov B.U., Abilov P.M., Norboyeva S.A., Musayev Kh.A., Urinov A.M. Modern condition of the problem of lipid peroxide oxidation // Central Asian Journal of Medicine – 2019. – V. (1). – P. 52-58.
2. Raj S., Gothandam K.M. Hepatoprotective effect of polyphenols rich methanolic extract of Amorphophallus commutatus var. wayanadensis against CCl4 induced hepatic injury in swiss albino mice // Food Chem Toxicol – 2014. – V.67: – P. 105-112.
3. Meng X., Tang G-Y., Liu P-H., Zhao C-J., Liu Q., Li H-B. Antioxidant activity and hepatoprotective effect of 10 medicinal herbs on CCl 4 -induced liver injury in mice // World Journal of Gastroenterology – 2020 – V.26 (37): P. 5629-5645.
4. Almeida A.M., Bertoncini C.R., Borecky J., Souza-Pinto N.C., Vercesi A.E. Mitochondrial DNA damage associated with lipid peroxidation of the mitochondrial membrane induced by $Fe^{2+}$-citrat // An. Acad. Bras. Cienc. – 2006. – V. 78(3). – P. 505-514.
5. Абдулладжанова Н.Г., Мавлянов С.М., Салихов Ш.И., Камаев Ф.Г. Новые полифенольние соединение сем. Euphorbiaceae // ДАН РУз. – 2011. – №3. – С. 60-62.
6. Махмудов Р.Р., Абдулладжанова Н.Г., Мавлянов С.М., Камаев Ф.Г. Фенольные сеодинения Plantago major L. // Вестник НУУз. 2010. №3/1. - С.150-151.
7. He L., Lemasters J.J. Heat shock suppresses the permeability transition in rat liver mitochondria // J. Biol. Chem. – 2003. – V. 278(19). – P. 16755-16760.
8. Mihajlovic M., Vinken M. Mitochondria as the target of hepatotoxicity and drug-induced liver injury: molecular mechanisms and detection methods // Int. J. Mol. Sci. – 2022 – V.23 (6) – P.
9. Xu D., Hu M.J.,Wang Y.Q., Cui Y.L. Antioxidant activ ities of quercetin and its complexes for medicinal application // Molecules – 2019 – V.24(6): – P. 1–15.
10. Zhang J.Q., Shi L., Xu X., Huang S.C., Lu B., Ji L.L., Wang Z.T. Therapeutic detoxification of quercetin against carbon tetrachloride-induced acute liver injury in mice and its mechanism // J Zhejiang Univ Sci B. – 2014. – V. 15(12): – P. 1039–1047.
11. Castilho R.F., Meinicke A.R., Vercesi A.E., Hermes-Lima M. The role of Fe(III) in Fe(II)-citrate mediated peroxidation of mitochondrial membrane lipids // Mol Cell Biochem – 1999. – V.196. – P. 163– 168.
12. Kowaltowski A.J., Castilho R.F., Vercesi A.E. Mitochondrial permeability transition and oxidative stress // FEBS Lett – 2001. V.495. – P. 12-15.