International Journal of
Inclusive and Sustainable Education
ISSN: 2833-5414
Volume 1 | No 5 | Nov-2022

Effects of Gossitan and Getasan on Lipid Peroxidation and Antioxidant Enzyme Activity in Rat Liver Homogenate in Toxic Hepatitis

Turdieva Odinahon Mamirovna [1], Pozilov Mamurjon Komilovich [2]

[1] Quqon State Pedagogical Institute, Kokand city, A.Temur Street 26
[2] National University of Uzbekistan named after Mirzo Ulugbek, Tashkent

Abstract: In toxic hepatitis caused by carbon tetrachloride (CCl4), the amount of malondialdehyde (MDA) in the liver homogenate of quercetin, gossitan and getasan and the activity of antioxidant enzymes (superoxide dismutase-SOD, catalase, glutathione peroxide GPx) were determined. SOD activity by Matyushin method. Catalase activity was determined by the Karolyuk method.

The experimental group identified ALT, AST enzymes in the blood plasma of animals, and the amount of MDA in the liver was determined when their levels approached control. It was found that the amount of MDA in the liver homogenate of experimental group rats treated with pharmacotherapy with quercetin, gossitane and getasan polyphenol compounds decreased compared to group II indicators.

The liver is one of the most important organs of the body. It plays a key role in the management of various physiological processes and its activity is related to various vital functions such as metabolism, secretion and maintenance of homeostasis. The properties of detoxification of endogenous (metabolites) and exogenous (toxic compounds) substances of the liver, as well as the synthesis of beneficial substances are analyzed by many researchers [Subramoniam, 1999; Adewusi, 2010].

The liver is also involved in growth, nutrient delivery, energy supply, and biochemical processes. In addition, the liver helps in the metabolism of carbohydrates and fats, bile secretion and storage of vitamins [Ahsan et al., 2009]. Despite many studies of all of these functions, liver disease remains one of the major threats to public health, and they are a problem worldwide. Despite the tremendous advances in modern medicine, there are no effective tools that can stimulate liver function, fully protect the organ, or help complete recovery of liver cells [Madrigal-Santillán et al., 2014]. In addition, some medications can cause side effects. Thus, the identification of effective and less toxic alternative pharmaceuticals for the treatment of liver disease is a pressing issue.

The purpose of the work. To determine the effect of quercetin, gossitan and getasan on the amount of MDA in the liver homogenate and the activity of antioxidant enzymes (SOD, catalase, GPx) in toxic hepatitis caused by CCl_4.

Research methods and materials. Studies have been performed on white male rats weighing 180–200 g. There are currently many types of toxic hepatitis modeling in animals. One such classical method is a model of toxic hepatitis induced using tetrachloromethane (SSl4). Animals were divided into groups: group I control (healthy), group II experiment (CCl_4 0.5 ml / kg), group III (CCl_4 + quercetin (10 mg/kg), group IV (CCl_4 + gossitan (20 mg/kg), group V (CCl_4 + getasan (20 mg/kg). To induce experimental toxic hepatitis in group II, III, IV, and V rats obtained for the experiment, animals were injected subcutaneously with 50% (CCl_4 (0.5 ml/kg) dissolved in pure vegetable oil

once every 3 days. 21 days after the administration of (CCl_4 to rats, after an increase in the enzymes ALT (60 Ed/l) and AST (120 Ed/l) in the blood, purified coconut oil (0.5 ml/kg) once a day in group II animals, quercetin in group III of the experiment. flavonoids, group IV gossitane and group V getasan polyphenols were administered once daily for 20 days per os.

Determination of antioxidant enzyme activity in the liver. The activity of SOD, catalase and GPx in liver homogenate was determined. Superoxide dismutase activity (SOD) was determined based on the Matyushin method. Catalase activity was determined by the Karolyuk method [Матюшин. 1991];[Каролюк. 1988]

For the preparation of liver tissue homogenate, experimental groups isolated animal liver. Homogenized in a Potter homogenizer at 120 mM KCI and 30 mM phosphate buffer (pH 7.4) at 0–4 ºC. The nucleus and cell were centrifuged at 600 g for 10 min to remove fractions. The resulting supernatant was used to determine the activity of antioxidant enzymes as liver tissue homogenate. The protein concentration in liver homogenate was determined by the Lowry method [Lowry et al., 1951].

SOD superoxidation-radicals (O^{-2}) catalyze the dismutation reaction, converting them into less harmful molecules (N_2O_2 and O_2) and thus protecting cell function. Superoxide radicals are formed in the presence of dehydrogenases, amino oxidases, cytochromoxidase enzymes. Increasing the concentration of superoxide radicals in the cell stimulates membrane lipid peroxidation (LPO) and triggers inflammatory processes. Depending on what metal (Cu, Zn, Fe) is present in the active center of the enzyme, three types of SOD have been identified. Enzyme activity was expressed in units of Ed/mg protein.

Catalase activity was determined using a method based on the formation of a yellow compound of hydrogen peroxide with molybdenum salts. [Каролюк 1988].

Separation of LPO products was performed in the presence of thiobarbituric acid (TBK). The reaction was stopped by adding 0.220 ml of 70% trichloroacetic acid to the IM. After this stage, the mitochondrial suspension was centrifuged at 15,000 rpm for 15 min. Then 2 ml of sedimentary liquid was obtained and 1 ml of 75% TBK was poured. 2 ml of N_2O and 1 ml of TBK were added to the control solution. The mixture was incubated for 30 min in a water bath. After cooling, a change in optical density at a wavelength of 540 nm was detected.

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

Statistical processing of the obtained results was performed using the computer program OriginPro 7.5 (Microsoft, USA). The difference between the values obtained from the control, experiment, and experiment + research material was calculated on the t-test. In this case, the values of $P < 0.05$ and $P < 0.01$ represent statistical reliability.

Results obtained and their analysis. In experimental hepatitis, the toxic effect of metabolites in the liver is associated with the acceleration of the process of lipoperoxidation, which leads to structural-functional disorders of the cell membrane. In toxic hepatitis, liver tissue can reduce the intensity of the LPO process on the basis of plant substances. To investigate this, we determined the amount of MDA in liver tissue, the end product of the LPO process.

In our experiment, an increase in the amount of MDA in the liver homogenate of group II animals of the hepatitis model caused by CCl4 - was observed in comparison with the indicators of control rats (group I) (Fig. 1). In particular, the increase in the amount of MDA (3.2 ± 0.1 nmol/mg protein) in the liver homogenate of animals with group II hepatitis increased by 109.4% compared to control indicators and amounted to 6.7 ± 0.45 nmol/mg protein. The literature shows that an increase in the amount of MDA in the liver under the influence of LPO process activation in the conditions of experimental toxic hepatitis has been detected [Ritesh et al., 2015]. Our experience also confirms the above points.

Quercetin (50 mg/kg), gossitan (50 mg/kg), and getasan (50 mg/kg) polyphenol compounds were administered orally for 20 days, respectively, in group III, IV, and V rats called toxic hepatitis.

The experimental group identified ALT, AST enzymes in the blood plasma of animals, and the amount of MDA in the liver was determined when their levels approached control.

It was found that the amount of MDA in the liver homogenate of experimental group rats treated with pharmacotherapy with quercetin, gossitan and getasan polyphenol compounds decreased compared to group II (Fig. 1).

The amount of MDA in the liver homogenate of rats in group III rats delivered quercetin obtained as a standard prototype was 4.0 ± 0.2 nmol / mg of protein, in group IV sent 4.9 ± 0.21 nmol / mg of protein, and in group V sent to getasan 5.4 ± 0 , 24 nmol / mg protein, 84.4%, respectively, compared to group II indicators; MDA levels were found to decrease by 56.3% and 40.7%, respectively (Figure 1).

Many polyphenol compounds have been shown to exhibit hepatoprotective and antioxidant activity in experimental toxic hepatitis conditions.

The polyphenol compounds we selected also correspond to the literature data on the reduction of LPO in liver tissue under conditions of toxic hepatitis [Shehab et al., 2015].

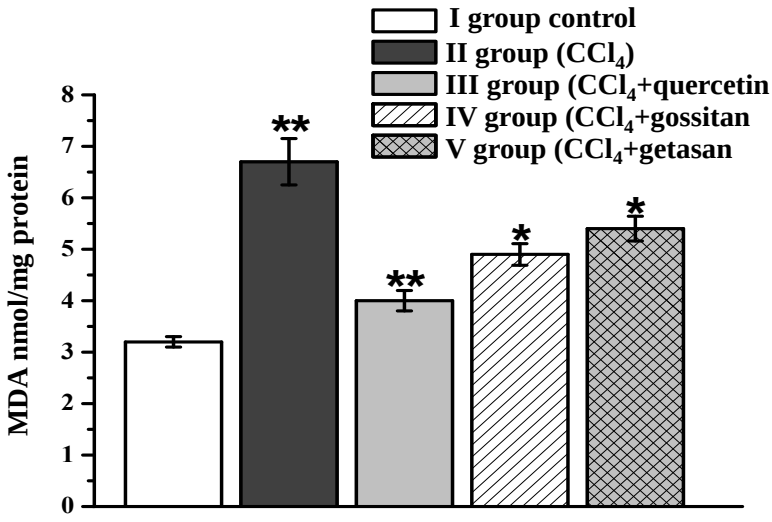

Figure 1. The impact of quercetin, gossitane and getasan in the convenient toxic hepatitis with CCl_4.

Reliability levels Experience Group II was determined against the control and the I II, IV, V Groups II * r <0.05; ** r <0.01; N = 6.1.

Thus, gossitane and getasan polyphenols had an inhibitory effect on the increase in the amount of MDA in liver homogenate under conditions of toxic hepatitis. However, their activity had a slightly weaker effect on the existing hepatoprotective compound quercetin. With respect to getasan, it was found that gossitan significantly reduced the intensity of cell membrane LPO by reliably reducing the formation of MDA in the liver in toxic hepatitis.

Effect of gossitane and getasan on SOD and catalase activity in rat liver homogenate in toxic hepatitis.The role of oxidizing substances in cells is complex, and their activity depends on the balance between oxidizing and antioxidant biomolecules.

Antioxidant enzymes (SOD, catalase and GPx) and non-enzyme compounds (tocopherol, vitamin E, beta carotene, ascorbate and glutathione) perform protective functions against ROS resulting from LPO in tissue cells [Abou Seif 2016].

In conditions of toxic hepatitis, free radicals accelerate cell membrane damage as a result of antioxidant imbalance in liver tissue.

One of the antioxidant enzymes, SOD superoxidanion radicals (O^{-2}) catalyzes the dismutation reaction and breaks them down into less harmful molecules (N_2O_2 and O_2), thereby protecting cell function.

In our next experiment, changes in SOD activity in liver tissue homogenates under the conditions of toxic hepatitis caused by CCL_4 and the effect of polyphenol compounds on them were studied (Fig. 2).

According to the results, in the conditions of toxic hepatitis caused by CCl_4, the SOD content in the liver homogenate of group II rats was 55.2 ± 3.5 Ed/mg protein compared with control (group I) (22.4 ± 1.8 Ed/mg protein). Decreased by 59.6%.

Superoxide radicals are formed in the presence of dehydrogenases, amino oxidases, cytochrome oxidase enzymes. Increasing the concentration of superoxide

The group of toxic hepatitis caused by CCl_4 indicates that the active forms of oxygen in the liver tissue of rats, increased intensity of formation of superoxide radicals - N_2O_2, damage membranes and reduce the activity of antioxidant enzymes.

Continuing the experiments, rats of groups III, IV, and V caused by toxic hepatitis were treated with pharmacotherapy for 20 days with polyphenol compounds.

When rats with toxic hepatitis III were pharmacotherapy with quercetin for 20 days, the SOD content in their liver homogenate was 45.5 ± 3.1 Ed/mg protein.

It was found that the flavonoid quercetin, obtained as a hepatoprotector, activated the amount of SOD in the liver homogenate by 41.8% compared to group II rats (Fig. 2).

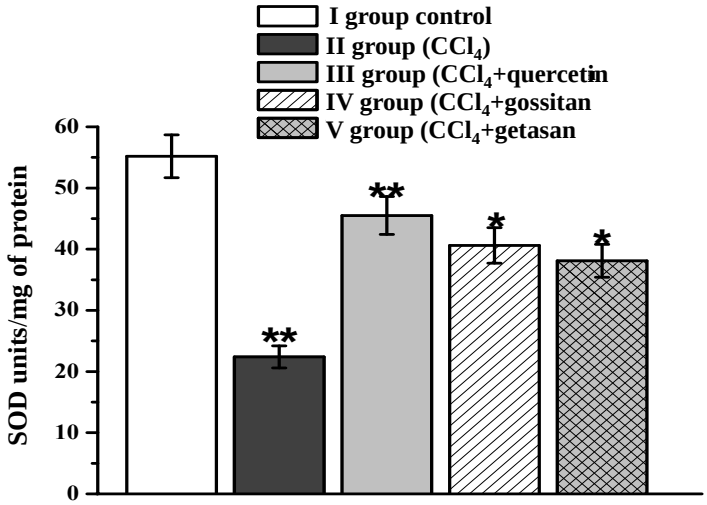

Figure 2. Effect of quercetin, gossitan, and getasan on the amount of SOD in liver homogenate in toxic hepatitis caused by CCl4. * R <0.05; ** R <0.01; n = 6.

The gossitane and getasan polyphenols obtained for the study were 40.6 ± 2.9 and 38.1 ± 2.7 Ed/mg protein, respectively, in the liver homogenate of animals with group IV and V toxic hepatitis induced by CCl_4.

It was found that gossitane and getasan polyphenols increased the SOD activity in liver homogenates of group IV and V rats with toxic hepatitis by 32.9% and 28.4%, respectively, compared with group II rats (Fig. 2).

Gossitane and getasan polyphenols have been found to reliably increase the amount of decreased enzyme SOD enzyme in liver tissue in rats caused by toxic hepatitis.

The antioxidant activity of gossitane and getasan polyphenols in toxic hepatitis conditions was noted to have a similar effect to the quercetin flavonoid obtained for the comparative standard. This suggests that the results obtained are consistent with the literature [Elsawy et al., 2019].

In our next experiment, changes in the activity of another important antioxidant enzyme catalase in toxic hepatitis conditions were studied. Catalase is an enzyme of the oxyreductase class that enters the antioxidant system of the cell and performs a protective function against peroxide [Bezruchko i dr., 2012].

Catalase is located in the body mainly in liver and erythrocyte cells. Catalase is a toxic product of respiration and prevents the accumulation of metabolites formed in metabolism in cell tissues and separates them into water and oxygen [Gabitova i dr., 2006].

Catalase is a very active enzyme and no energy is required to activate it. Decreased catalase activity occurs with an increase in methionine, cystine, copper, spirit [Chesnokova et al., 2006]. Чеснокова Н.П

In the CCl_4 poisoning model, changes in the activity of catalase, one of the antioxidant system enzymes in liver homogenate, were noted (Fig. 3).

According to the results obtained, the development of an imbalance in the activity of the antioxidant system of liver cells in the conditions of toxic hepatitis has been proven in our experience.

In particular, catalase activity in liver tissue in hepatitis II caused by CCl_4 was found to be 17.9 ± 1.4 mkM / min / mg protein in the liver homogenate of group II rats, a decrease of 49.7% compared to controls.

The catalase content in the liver homogenate of group III hepatitis rats treated with quercetin for 20 days was 30.9 ± 2.0 mkM/min/mg protein, which was 36.4% more active than in group II rats.

The amount of catalase in the liver homogenate of rats of groups IV and V of the experiment corrected with gossitane and getasan polyphenols was 29.2 ± 1.6 and 26.1 ± 1.4 mkM/min/mg protein respectively.

It was found that the activity of catalase increased by 31.7% and 23.0% respectively, compared with the indicators of toxic hepatitis group II (Figure 3). Thus, in toxic hepatitis, a decrease in the activity of SOD and catalase in liver tissue is observed, which indicates the presence of peroxidative oxidation of lipids.

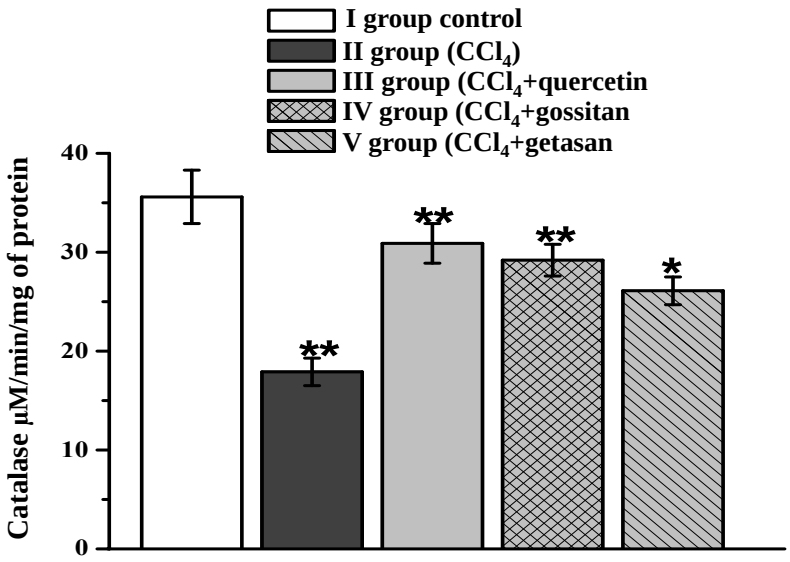

Figure 3. Effect of quercetin, gossitan, and getasan on catalase levels in liver homogenate in CCl_4-induced toxic hepatitis. * R <0.05; ** R <0.01; n = 6.

Catalase is an active enzyme that acts as an antioxidant that plays a key role in the protection system against LPO products. Numerous studies have found a significant decrease in catalase activity in toxic hepatitis caused by CCl_4 [Mahmoodzadeh et al., 2017; Khan et al., 2012].

Changes in the activity of antioxidant system enzymes in the context of toxic hepatitis are the result of an increase in the amount of LPO products and an increase in the generation of active forms of oxygen. It is a potent inhibitor of superoxide radical catalase in particular.

Selected goositan and getasan polyphenols may increase the activity of antioxidant enzymes in toxic hepatitis conditions. The antioxidant activity of polyphenols has been shown to be close to them in comparison with quercetin. This indicates that these compounds exhibit antioxidant activity.

In our next experiment, another antioxidant enzyme glutution peroxidase activity was also detected. In CCl_4 induced toxic hepatitis, the GPO enzyme in group II rat liver homogenate was found to be 46.4 ± 3.3% lower than in the control (Fig. 4).

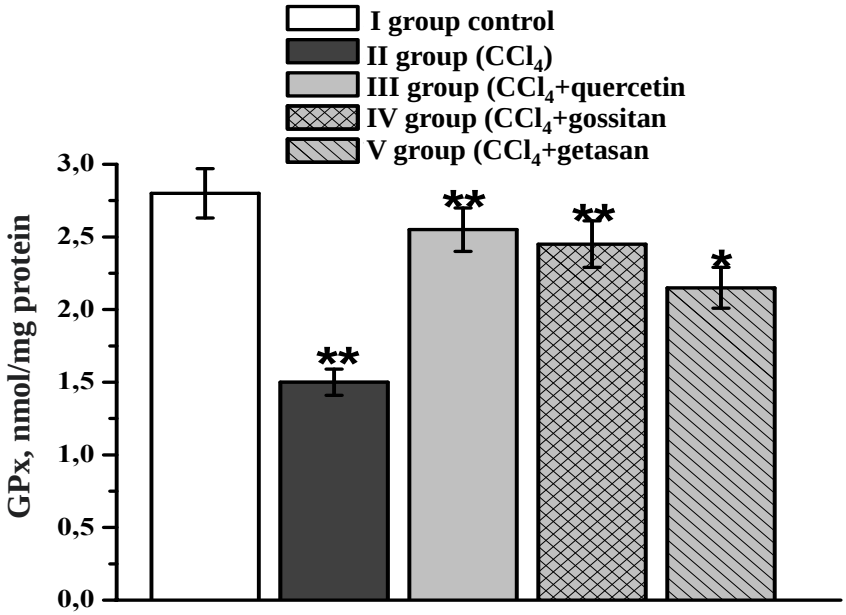

Figure 4. Effect of quercetin, gossitan, and getasan on GPx activity in liver homogenate in toxic hepatitis caused by CCl_4. * R <0.05; ** R <0.01; n = 6.

Accumulation of LPO-derived lipid hydroperoxide in toxic hepatitis conditions inhibits enzyme activity and leads to changes in cell function, forming a covalent bond with ketones, aldehydes and dialdehydes, proteins and other biomolecules.

Oxidation of lipids leads to the development of a free radical reaction, while prooxidants stop antioxidants. The enzyme GPx also breaks down lipid hydroperoxides, protecting cells from their toxic effects.

In conditions of toxic hepatitis, GPO inhibition in liver homogenate may be activated by plant-derived compounds. In order to determine the effect of gositan and getasan polyphenols on GPO in liver homogenate in the conditions of toxic hepatitis in experiments, pharmacotherapy was performed by sending them to animals.

The results showed that quercetin flavonoid, obtained as a standard prototype, activated GPO activity in liver homogenates of group III animals with toxic hepatitis by 37.5 ± 2.7% compared to group II indicators.

In experimental toxic hepatitis group IV, rats were given gossitane and group V were given getasan polyphenols for 20 days. Pharmacotherapeutic group IV and V rats were found to restore GPO

activity in liver homogenate by 33.9 ± 2.5% and 23.2 ± 1.7%, respectively, compared to group II (Figure 4).

Hence, the selected compounds indicated that CCl_4 induced toxic hepatitis rats corrected the disrupted antioxidant imbalance in liver tissue. The results obtained on the reduction of antioxidant enzyme GPO activity in the liver in CCl_4 induced toxic hepatitis and their correction using natural compounds are consistent with the results of the literature [Thanh et al., 2015].

Under conditions of toxic hepatitis, LPO production in liver tissue increases, resulting in a sharp change in membrane lability. Disruption of the membrane system, especially its increased ion transport permeability, is of great importance in injury and disease progression.

Phenol compounds have antioxidant effects by interacting with peroxides and radicals formed during the LPO process. One or more phenol groups of the antioxidant molecule strongly inhibit LPO reactions.

Some antioxidant molecules with a phenolic structure, such as flavonoids, are thought to have the property of chelating metal cations [Shakhmardanova et al., 2016]. The antioxidant properties of quercetin, gossitane and getasane grave compounds belonging to the phenol group may also depend on their antioxidant concentration, the process and time of free radical formation.

It can effectively correct disorders of the enzymatic antioxidant system present in hepatic hepatocytes associated with toxic hepatitis.

Conclusions. An increase in the amount of MDA in liver tissue in toxic hepatitis indicates an acceleration of LPO intensity. This is manifested by a violation of the balance of the antioxidant system in liver cells in hepatitis. Increased levels of LPO product MDA in rat liver tissue homogenate in toxic hepatitis may reduce the confidence of quercetin, gossitane and getasan compounds. It has also been found that gossitan and getasan polyphenols, such as quercetin, reliably increase the activity of antioxidant enzymes such as SOD, catalase, and GPx in the liver relative to control in hepatitis. This may be due to the ability to neutralize the cytotoxic effects of free radicals formed as a result of LPO processes in the liver.

References

1. Subramoniam A., Pushpangadan P. Development of phytomedicine for liver diseases // Indian J Pharmacol. – 1999. – V.31: – P.166-175.saemlshaet
2. Adewusi E.A., Afolayan A.J. A review of natural products with hepatoprotective activity // J Med Plants Res. – 2010. – V.4: – P.1318-1334.
3. Ahsan M.R., Islam K.M., Bulbul I.J.Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats // Global J Pharmacol –2009. – V.3: – P.116-122.
4. Madrigal-Santillán E., Madrigal-Bujaidar E., Álvarez-González I., Sumaya-Martínez M.T., Gutiérrez-Salinas J., Bautista M., Morales-González Á., García-Luna y González-Rubio M., Aguilar-Faisal J.L., Morales-González J.A. Review of natural products with hepatoprotective effects // World J Gastroenterol – 2014. – V. 20. (40): – P. 14787-14804.
5. Lowry O.H., Rosebrough N.J., Farr A. L., Randall R.J. "Protein measurement with the Folin phenol reagent" // The Journal of Biological Chemistry. – 1951. – V.193(1). – P. 265–275.
6. Ritesh K.R., Suganya A., Dileepkumar H.V., Rajashekar Y., Shivanandappa T. A single acute hepatotoxic dose of CCl4causes oxidative stress in therat brain // Toxicol Rep. – 2015. – V.11(2): – P. 891-895.
7. Shehab N.G., Abu-Gharbieh E., Bayoumi F.A. Impact of phenolic composition on hepatoprotective and antioxidant effects of four desert medicinal plants // BMC Complementary and Alternative Medicine – 2015. – V.15: – P.1-12.

8. Abou Seif H.S. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants // Journal of basic and applied sciences – 2016. – V.5: – P.134-146.
9. Elsawy H., Badr G.M., Sedky A., Abdallah B.M., Alzahrani A.M., Abdel-Moneim A.M. Rutin ameliorates carbon tetrachloride (CCl4)-induced hepatorenal toxicity and hypogonadism in male rats // PeerJ. – 2019. – V.7: – P. 1-20.
10. Mahmoodzadeh Y., Mazani M., Rezagholizadeh L. Hepatoprotective effect of methanolic Tanacetum parthenium extract on CCl4-induced liver damage in rats // Toxicology Reports – 2017. – V.4: – P.455-462.
11. Khan R.A., Khan M.R., Sahreen S. CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat // BMC Complementary and Alternative Medicine – 2012. – V.12 (178). – P. 1-6
12. Thanh T.B., Thanh H.N., Thi Minh H.P., Thi-Thu H.L., Thi Ly H.D., Duc L.V. Protective effect of Tetracera scandens L. leaf extract against CCl4-induced acute liver injury in rats // Asian Pac J Trop Biomed – 2015. – V. 5(3): – P. 221-227.
13. Габитова Д.М., Рыжикова В.О., Рыжикова М.А. Антиоксидантная защитная система организма // Башкирский химический журнал. – 2006. – Т.13. № 2. – С. 94–96.
14. Чеснокова Н.П., Понукалина Е.В., Бизенкова М.Н. Общая характеристика источников образования свободных радикалов и антиоксидантных систем // Успехи современного естествознания. – 2006. – № 7. – С.37–41.
15. Шахмарданова С.А., Гулевская О.Н., Селецкая В.В., Зеленская А.В., Хананашвили Я.А., Нефедов Д.А., Галенко-Ярошевский П.А. Антиоксиданты: классификация, фармакотерапевтические свойства, использование в практической медицине // Журнал фундаментальной медицины и биологии – 2016. – №3. – С.1-12.