PROSPECTS FOR USE OF SUPRAMOLECULAR COMPLEXES OF MONOAMMONIUM GLYCYRRHIZINATE WITH POLYPHENOLS AND AMINO ACIDS AS THE HEPATOPROTECTORS

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Abstract*. Supramolecular complexes of the monoammonium glycyrrhizinate (MG) with amino acids, to name methionine (Met) and DL-carnitine-HCL (Car) and phenolic compounds, to name quercetin (Que) and methoxycinnamic acid (MethA) in molar ratio 4:1, were generated as promising low-dose cytoprotectors/hepatoprotectors. After induction of acetaminophen-induced hepatitis (1500 mg/kg for 2 days intragastrically) and the 7-day administration of complexes, MG/Met and MG/MethA caused statistically significant shortening of duration of hexobarbital-induced sleep at the dose of 2,5 mg/kg (160±14,0 min in control vs 36,1±2,3 and 33,4±2,2 min, respectively, p<0,05 both) and duration of thiopental-induced sleep at the dose of 5 mg/kg (63,5±4,2 min in control vs 20,7±1,6 and 17,7±1,2 min, respectively, p<0,05 both); MG/Car and MG/Que at the dose 5 mg/kg caused the most significant reduction in activity of the enzyme alkaline phosphatase (245,2±16,4 U/l in control vs 88,33±4,67 and 96,13±4,27 U/l, respectively, p<0,05 both). According to histological studies MG/Met and MG/MethA at the dose of 5 mg/kg caused more significant reduction in the dyscirculatory and edematous events; signs of activation of lymphoid and macrophagal cells in the liver tissue appeared. Under normal physiological conditions, the complexes under study were not found to produce any effects on the bile secreting function of the liver. These findings demonstrate necessity to generate a complex medication combining various effects indispensable for recovery of various functions of the liver after acute injuries, toxic effects of xenobiotics, as well as for prevention of grave complications of hepatitis.*

Keywords*: hepatoprotectors, choleretic activity supramolecular complex, glycyrrhizic acid, amino acids, phenolic compounds.*

INTRODUCTION

To therapeutically correct chronic pathologies of various tissues, antioxidants, as cytoprotectors, are widely used. Known for their low toxicity and multiple favorable effects including those manifesting in the prophylactic medication for cancers, as well as for inflammatory, metabolic and other disorders, they help the innate enzymatic antioxidant system of a human organism combat oxidative stress. Consequently, antioxidants of various natures are the active ingredients in currently used hepatoprotectors and nootropics, as well as immunotropic pharmaceuticals; polyphenols make up the majority of them [1]. However, in addition to high efficacy, polyphenols were demonstrated to have low solubility requiring supramolecular complexes and nanomaterials as the drug delivery systems to improve their pharmacological and

therapeutic properties, as well as their bioavailability. Currently, there are a lot of choices for the nanoparticles; each of them demonstrates both advantages and disadvantages [2].

Glycyrrhizic acid (GA), the major active ingredient of the licorice root with various pharmacological properties, to name anti-inflammatory, anti-bacterial, anti-viral, anti-protozoal, antioxidant, anti-hyperglycemic, anti-hyperlipidemic, as well as hepato- and neuroprotective effects due to modulation of various signaling pathways, such as IKK, ERK1/2, p38, NF-κB and others, is thought to be an effective drug delivery system [3]. All the properties above make GA and its derivatives effective components of various medications for treatment of liver injuries [4] among other things due to their anti-fibrotic features preventing cirrhotic changes in the liver tissues [5].

At the same time, due to a hydrophilic digluguronic unit and a hydrophobic residue in its structure, GA demonstrates amphiphilic nature enabling its molecule to form micelles and aggregates both in the hydrophilic and hydrophobic media as a “host-guest” complex acting as the delivery system of the slightly soluble bioactive agents [6-8].

To obtain an effective hepatoprotector, we generated supramolecular complexes of the monoammonium glycyrrhizinate (MG) with amino acids, to name arginine (Arg), methionine (Met), tryptophan (Trp), cysteine (Cys) and DL-carnitine-HCL (Car) and phenolic compounds, to name flavonoids, such as quercetin (Que) and rutin (Rut), acids, such as cinnamic acid (CinA), methoxycinnamic acid (MethA) and ferulic acid (FA) in molar ratio 4:1, and studied their anti-radical features *in vitro* and hepatoprotective features *in vivo*. Formation of the complexes above was demonstrated to result in the increase of the direct anti-radical activity ($p<0.005$) by 1.3-6.5 times when assessed by phosphomolybdenum and ferricyanide methods; the MG complexes with MethA, Car and Met were found the most active ones. After per oral injection to mice at the dose of 10 mg/kg, the MG complexes with MethA, Met and Que demonstrated high hepatoprotective activity *in vivo*, those with Car and Cys showed anti-inflammatory effects [9]. To proceed, we selected the MG complexes with MethA, Met, Car and Que to study their antioxidant [10], hepatoprotecive [11] and immunotropic [12] properties.

The work was initiated to proceed with the study on the MG supramolecular complexes selected to assess their effects on the restoration of anti-toxic function of the liver and its tissues in the setting of toxic hepatitis, and to study presence of the choleretic effect.

MATERIALS AND METHODS

Model of acute toxic acetaminophen-induced hepatitis in rats

The study was conducted on 55 Wistar Hannover rats (both sexes, aged 12 weeks old; weighing 180-200 g). Manipulations with the animals were performed in compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. [13]. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. The animals were randomized by the body mass into 11 groups with 5 animals per a group. Acute toxic drug-induced liver injury was induced by the intragastric 2-day administration of acetaminophen at the dose of 1,500 mg/kg [14]. After induction of hepatitis, starting from the 3rd day of experiment MG complexes with MethA, Met, Car and Que (in the molar ratio 4:1) at the doses of 2.5 and 5 mg/kg were administered intragastrically for 7 days. The the analog of Stronger Neo-Minophagen C, Minophagen Pharmaceutical Co., Tokyo, *Japan*)hepatoprotective, immune-modulating and anti-oxidant drug Hepa-NOVO containing glycyrrhizin (0.2%), glycine (2.0%) and L-cystein (0.1%) in physiologocal saline (Stellar Zeneka,

Uzbekistan, was used as the drug of comparison: 10 ml of the combined drug for injections contains Glycyrrhizic acid 20 mg, Glycine 200 mg, L-Cysteine 10 mg. Average daily dose of Hepa-NOVO for a human is 70 ml (40-100ml). The interspecific "animal-human" dose translation was performed by means of a coefficient considering the difference between the body surface areas of a laboratory animal and a human being in compliance with the US FDA Guidance [15]. So, Hepa-NOVO was administered to rats intragastrically at the dose of 6.5 ml/kg (as according to preliminary data, there were no significant differences in results following the intravenous and intragastric administration of Hepa-NOVO). Control animals with and without hepatitis received water in the equivalent volumes for 7 days.

Hexobarbital sleep test was used to assess duration of sleep (in minutes) induced in rats by hexobarbital or thiopental sodium both administered intragastrically at the doses of 60 mg/kg as the intensity of metabolism of the drugs above under effect of the cytochrome P-450-dependent monooxygenase system of hepatocytes. Concentrations of alkaline phosphatase and glucose were measured in the rat blood as the part of a biochemical test. On the 8^{th} day after slaughter, the histology was conducted; the liver was extracted for its pieces to be fixed in the 10%-solution of formalin, dehydrated in the spirits with the increasing concentrations and in chloroform, and finely, embedded in paraffin with wax. 5-8 μm thick histological sections were stained with hematoxylin and eosin to be analyzed microscopically.

Study on choleretic activity of complexes under the normal physiological conditions

Rats with body mass of 180-200g were used in the experiments under the normal physiological conditions. The animals were randomized into groups with 5 animals per a group. The MG complexes were administered intragastrically at the doses of 2.5 and 5.0 mg/kg for 7 days; the control animals received water. Hepa- NOVO administered at the dose of 6.5 ml/kg was used as a drug of comparison. After administration of the drug, the rats were narcotized by the intra-abdominal injection of ethaminal sodium at the dose of 40 mg/kg. The bile was collected by means of catheter introduced into the common bile duct of the animal to determine total amount of the bile within 4 hours of observation; the intensity of bile secretion was measured by the rate of bile excretion in μl in 100 animals per 1 minute.

The data were statistically processed by a computer program for Microsoft Excel 2007. The parameters were presented as the sample arithmetic mean in the group of 5 animals (M) and as the standard error of the mean (m). Difference significance was determined by Student t-criterion with the significance level at $p<0.05$.

RESULTS

In vivo study on effects of the complexes on the course of acute acetaminophen-induced hepatitis

First, effects of complexes on the enzymatic apparatus of the liver involved in the biotransformation/ detoxification of drugs were studied. The sleep duration of animals with acetaminophen-induced hepatitis administered with two different sleeping drugs was studied (Table 1). Mean sleep duration in the hepatitis-free controls administered with thiopental sodium and hexobarbital was 26.4±1.3 and 26.4±1.8 min, respectively. The liver injury under acute toxic hepatitis causes suppression of its anti-toxic function; thus, significant sleep duration by 2.4 times (up to 63.5 ±4.2. min, $p<0.05$) could be seen after administration of thiopental sodium and by 6 times (up to 160±14.0 min, $p<0.05$) after administration of hexobarbital. This can be the evidence for significant decline in activity of the enzymatic system of hepatocytes under acetaminophen-

induced hepatitis resulting in its depletion. Various enzymes of cytochrome P-450 were found to differently respond to the effects; thus, thiopental sodium is the substrate of CYP2C19 with a narrow therapeutic index (https://go.drugbank.com/drugs/DB00599), while hexobarbital is the substrate of CYP2C19 and CYP2C9 (https://go.drugbank.com/drugs/DB01355).

Table 1. Effects of complexes on the sleep duration in the acetaminophen-induced hepatitis in rats (M±m, n=5)

Drugs	Dose	Sleep duration, min		Alkaline phosphatase, U/l	Glucose, mmol/l
		thiopental sodium	hexobarbital		
Hepatitis-free controls		26.4±1.3	26.4±1.8	40.0±2.0	4.2±0.2
Acute toxic hepatitis		63.5±4.2 [b]	160±14.0 [b]	245.2±16.4 [b]	10.6±0.9 [b]
Hepa-NOVO	(Glycyrrhizic acid 13 mg + Glycine 130 mg + L-Cysteine 6,5 mg)/ kg (6.5 ml/kg)	35.5±2.4 [a,b]	50.0±4.6 [a,b]	135.3±12.6 [a,b]	11.8±0.9 [b]
MG/Car	2.5 mg/kg	42.5±3.3 [a,b]	43.9±4.4 [a,b]	106.0±15.7 [a,b]	9.7±0.6 [b]
	5 mg/kg	27.5±1.4 [a,c]	40,8±2.2 [a,b]	88.3±4.7 [a,b,c]	8.7±0.5 [b]
MG/ Met	2.5 mg/kg	25.5±1.8 [a,c]	36.1±2.3 [a,b,c]	143.0±9.0 [a,b]	9.4±0.6 [b]
	5 mg/kg	20.7±1.6 [a,b,c]	47.2±4.8 [a,b]	150.4±10.2 [a,b]	9.6±0.6 [b]
MG/ MethA	2.5 mg/kg	23.3±1.5 [a,c]	33.4±2.2 [a,b,c]	213.8±4.3 [b]	11.6±0.8 [b]
	5 mg/kg	17.7±1.2 [a,b,c]	44.2±4.0 [a,b]	206.8±6.7 [b]	11.6±0.8 [b]
MG/Que	2.5 mg/kg	48.3±3.8 [a,b]	46.8±5.4 [a,b]	107.2±4.7 [a,b]	9.4±0.5 [b]
	5 mg/kg	58.3±4.7 [b]	51.7±4.4 [a,b]	96.1±4.3 [a,b,c]	9.4±0.7 [b]

Note: [a] - $p<0.05$ in relation to acute toxic hepatitis; [b] - $p<0.05$ in relation to hepatitis-free controls; [c] - $p<0.05$ in relation to Hepa-NOVO

The 7-day administration of the complexes under study at the doses of 2.5 and 5 mg/kg, as well as injection of HEPA-NOVO after induction of inflammation was found to cause statistically significant shortening of duration of hexobarbital-induced sleep in all cases. However, the values remained significantly higher than the hepatitis-free controls ones to be the evidence for incomplete recovery of the enzymatic system involved in the biotransformation of hexabarbital. It should be noted, that the MG/Met and MG/MethA complexes at the dose of 2.5 mg/kg demonstrated the highest efficacy; thus, the sleep duration reduced approximately by 4.5 times (down to 36.1±1.2 and 33.4±2.2 min, respectively, $p<0.05$ both).

After administration of thiopental sodium, improvement in antitoxic function of hepatocytes could be seen for all complexes under study excluding the MG/Que complex administered at the dose of 5 mg/kg, and for the drug of comparison; thus, the sleep duration was significantly shorter than in the rats with the untreated hepatitis ($p<0.05$). The MG/Met and MG/MethA complexes demonstrated the highest activity, but the effective dose was 5 mg/kg causing the reduction in the sleep duration by 3 times; down to 20.7±1.6 and 17.7±1.2 min for the MG/Met and MG/MethA complexes, respectively. These values turned out significantly lower

than those in the hepatitis-free control rats to be the evidence for recovery of the enzymatic system of hepatocytes and increase in detoxification of thiopental sodium. The MG/Car complex administered at the dose of 5 mg/kg demonstrated high activity, too; the sleep duration reached the one of the physiological norms (27.5 ±1.4 min). The MG/Que complex turned out effective at the dose of 2.5 mg/kg only.

Next, serum concentrations of alkaline phosphatase (ALP) were measured. Alkaline phosphatase is basically present in the liver cells and bile ducts, participating in the metabolism of phosphoric acid. Damages to the liver cells and cholestasis significantly increase its serum concentrations [16].

As it can be seen, there was a 6-fold increase in the alkaline phosphatase activity in animals with untreated hepatitis, from 40.0±2.0 up to 245.2±16.4 U/l ($p<0.05$) (Table). The 7-day administration of complexes under study was found to cause significant reduction in the alkaline phosphatase activity in all groups, excluding those administered with the MG/MethA complex. The drug of comparison was found to produce an intermediate effect on the serum ALP concentrations (135.30±12.6 U/l). Administration of the MG/Car and MG/Que complexes caused the most significant reduction in activity of the enzyme (88.33±4.67 U/l and 96.13±4.27 U/l, respectively, $p<0.05$ both), as compared to the one in the untreated animals ($p<0.05$) and to the one found in animals administered with the drug of comparison ($p<0.05$). But the values did not reach those of hepatitis-free controls ($p<0.05$).

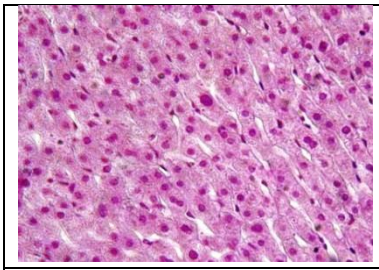
Next, concentrations of glucose in blood of animals with acute toxic hepatitis were measured, for the enzymatic system of hepatocytes participates in the processes of blood glucose regulation, while the dysfunctions of hepatocytes cause hyperglycemia [17]. In our experiments, blood glucose level under acute toxic hepatitis was 10.6±0.9 mmol/l, to be significantly higher than the physiologically normal one (4.2 ±0.21 mmol/l $p<0.05$). Administration of the complexes caused slight but statistically insignificant reduction in blood glucose in all groups of animals with acute hepatitis; the most pronounced reduction could be seen after administration of the MG/Car complex at the dose of 5 mg/kg (8.7±0.5 mmol/l).

The following are histological studies of normal liver tissue (Fig.1A), tissue under acute toxic hepatitis (Fig.1B) and tissues under acute toxic hepatitis treated with complexes under study (Fig.1C-F). Under acetaminophen-induced acute toxic hepatitis, fatty dystrophy in the cytoplasm of hepatocytes could be seen. Fatty degeneration engulfed the central and intermediate zones of the liver lobule; there was hypertrophy of Kupffer cells with brown pigment of both lipidogenic and bilirubinogenic origin. Accumulation of large amounts of macrophages could be seen in the focus of the liver injury around necrotically changed hepatocytes; hyperplasia of lipocytes or Ito cells with high concentrations of lipidogenic inclusions in the cytoplasm was found in the perisinusoidal spaces. The nuclei of hepatocytes were in the state of shrinkage and vacuolization as the ones of the “empty liver cell nuclei”; the foci of the centrilobular lipogenic necrosis could be seen.

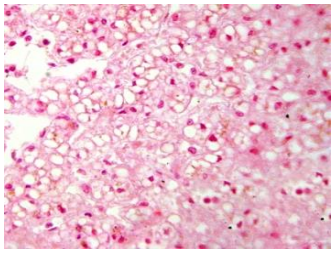
After treatment of acute toxic hepatitis with the MG/Car complex at the dose of 5 mg/kg, in most cases dystrophic changes of mixed character could be seen to preserve. In the third functional zone of the hepatic lobules, signs of protein and vacuole dystrophy, as well as of colliquative necrosis could be seen. Both sinusoidal lumina and the space of Disse with the damage to the plate-like position of hepatocytes were found significantly widened in the focus of necrosis.

In the space of Disse, macrophages, single brown cells containing pigments of both lipidogenic and bilirubinogenic origin in the cytoplasm could be seen (Fig.1C).

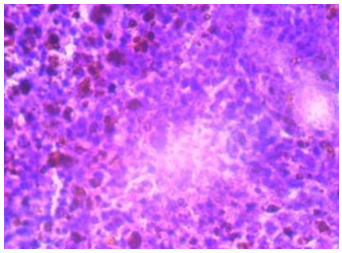
Findings from the morphological study of the liver after treatment with the MG/Que complex at the dose of 5 mg/kg in the second functional zone of hepatic lobules demonstrated paralytic widening of the sinusoidal lumina, damages of the wall with the marked edema of perisinusoidal space and single neutrophilic and eosinophilic leukocytes in them. Hepatocytes in this zone are compressed by the edematous sinusoids from one side being subjected to the vacuole and focal fatty dystrophy from the other. Nuclei of the hepatocytes are in the state of kariolysis and kariopyknosis. In this zone, hepatocytes are restored almost completely with their plate-like position preserved. Their nuclear structures have uniform structure and position; cytoplasm is stained with eosin uniformly to be the evidence for restoration of structural-functional elements of hepatocytes under effect of the MG/Que complex. There is cell infiltration consisting of lymphoid and granulocytic cells in some portal tracts. These cells diffusely infiltrate periportal conjunctive tissue penetrating the perisinusoidal space in some places (Fig.1D).



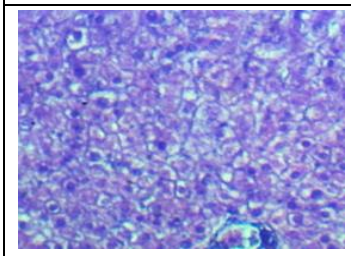
A. Physiological norm (hepatitis-free controls)



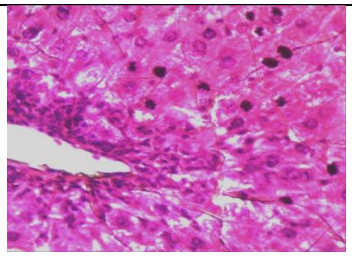
B. Acute toxic hepatitis



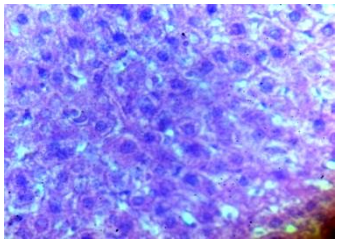
C. Acute toxic hepatitis. Treatment with MG/Car, 5 mg/kg



D. Acute toxic hepatitis.. Treatment with MG/Que, 5 mg/kg



E. Acute toxic hepatitis. Treatment with MG/Met, 5 mg/kg



F. Acute toxic hepatitis.. Treatment with MG/MethA, 5 mg/kg

Figure 1. Tissues of normal liver, under toxic hepatitis and treatment with MG-complexes Staining with hematoxylin and eosin.

After treatment of acute toxic hepatitis with the MG/Met complex at the dose of 5 mg/kg, restoration of structural-functional components of hepatocytes could be seen almost in all zones of the hepatic lobules. Only local small drop fatty dystrophy could be seen to preserve in the periportal zone. The edema of vascular-stromal tissue was found to disappear, while some disorganized events with restoration of basal membrane and fibrous structures of the hepatic framework could be observed (Fig.1E).

After treatment of acute toxic hepatitis with the MG/MethA complex at the dose of 5 m/kg slight periportal infiltration consisting of lymphoid and granulocytic cells was demonstrated. In macrophages and the Kupffer cells, accumulation of hemosiderin was observed. Among cell elements of periportal conjunctive tissue, there were lymphoid cells and single granular leukocytes

found. In some portal tracts, cell infiltration consisting of lymphoid and granulocytic cells was found (Fig.1F).

Thus, in treatment of acute toxic hepatitis with the complexes under study, the dyscirculatory and edematous events were found to decline; signs of activation of lymphoid and macrophagal cells, particularly, in treatment with the MG/Met and MG/MethA complexes could be seen.

Effects of supramolecular complexes on the bile secretion function of the liver under physiologically normal conditions

Amounts of bile and intensity of its secretion is thought to be a major parameter of normal function of the liver. In addition, absence or presence of choleretic effect produced by medications is important; accordingly, in some pathologies, such as the viral injuries of the liver, cholestasis, chronic pancreatitis and others, the choleretics are contraindicated [18]. Under normal physiological conditions, after the 7-day administration of water, mean bile secretion rate within 4 hours of observation was 2.38±0.18 μl/100g/min. The intragastric administration of the complexes under study at the doses of 2.5 and 5 mg/kg and Hepa-NOVO at the dose of 6.5 ml/kg did not result in any changes in the bile secretion; the values measured during the whole period of observation did not significantly differ from those under physiologically normal conditions. This can be the evidence for lack of the choleretic effect in the complexes under study, and medications with the complexes can be recommended for therapy of viral hepatides and cholestasis of various etiologies.

DISCUSSION

Acetaminophen-induced death of hepatocytes resulting in acute toxic hepatitis is a generally recognized experimental model. Damages to cells are triggered by depletion of glutathione (GSH) and accumulation of N-acetyl-p-benzoquinone imine (NAPQI) binding with the proteins (metabolic phase, 0-3 hours), including the mitochondrial ones, to start early phase of damage resulting in apoptosis/necrosis (2-6 hours). In the final phase of damage and early phase of restoration, the activation of the innate immune response (12-24 hours) deepens the processes; within the next 24-96 hours, the regenerative phase with the activation of the cell division starts [19].

The phase includes complex time- and dose-dependent interaction of some signal mediators including factors of growth, cytokines, angiogenic factors and other was of mitosis induction [20].

Acetylcysteine, also known as N-acetylcysteine (NAC), is the only FDA-approved medication that is used to treat acetaminophen overdose. Standard per oral or intramuscular administration for NAC is the most effective in patients with the signs of moderate overdose manifesting within 8 hours after the medication has been taken [21].

Similar to NAC in anti-oxidant properties, but differing in structures hypotaurine (HYTAU) and taurine (TAU) demonstrated equivalent patterns of protection and, to a certain extent, equipotent protective actions against acetaminophen in the liver when tested in equimolar doses [22]. But in patients with late referral or great overdose, the efficacy of NAC was found to decline [21] to demonstrate high efficacy of anti-oxidant medications administered on the stage of oxidative damage to cells.

However, considering the processes taking place in the liver in acute course of disease, as well as in chronization of inflammation, an up-to-date hepatoprotector should combine antioxidant

effects with those providing restoration of the hepatic functions and prevention of severe complications in septic inflammation including fibrosis and malignant transformation. Consequently, to select low-molecular bioactive agents as constituents for a medication based on the MG supramolecular complex the medications were administered on the next day after onset of acute hepatitis, that is, at the regenerative stage with activation of cell division. In the study, the MG/Met and MG/MethA complexes at the doses of 2.5 and 5 mg/kg turned out the most effective in restoration of detoxification activity of hepatocytes; reduction of dyscirculatory and edematous events and appearance of signs for activation of lymphoid and microphagal cells in the liver tissues was observed. The MG/Car and MG/Que complexes at the dose of 5 mg/kg were found more effective in restoration of hepatocyte integrity. At the same time, the MG/Car complex caused no significant reduction in the serum ALP concentrations; the MG/Que complex produced no effect on the sleep duration.

In addition, under acetaminophen-induced acute toxic hepatitis the MG/Que complex at the doses of 2.5 and 5 mg/kg and HEPA-NOVO in the therapeutic doses was demonstrated to significantly reduce lipid peroxidation in the rat liver mitochondria [10]; the MG/Que complex administered at the dose of 2.5 mg/kg demonstrated more pronounced restoration of biochemical parameters, to name ALT, AST, total blood protein, total cholesterol and urea [11].

Next, the immune-stimulating activity of the complexes under study was assessed since the liver acts as an immune organ where the synthesis of most immune molecules takes place in circulation to name acute phase proteins and complement system, antibodies, cytokines/interleukins and others, and most cells of innate immunity, including macrophages and T-cells, including natural killers, γδ T-cells, mucosa-associated invariant T-cells (MAIT-cells) are localized [23].

Single per oral administration of the MG/Met and MG/MethA complexes at the doses of 2.5 and 5.0 mg/kg, respectively, was demonstrated to cause increase in the antibody-producing cells of the murine spleen under physiologically normal conditions; the stimulation index is similar to the one of the HEPA-NOVO [12]. The data are consistent with the findings from histological studies performed in the frames of the work identifying activation of lymphoid and macrophagal cells in the tissues of the liver with injury.

Findings from the study demonstrate necessity to generate a complex medication combining various effects indispensable for recovery of all functions of the liver after acute injuries, toxic effects of xenobiotics, as well as for prevention of grave complications. In this case, the MG complex could serve a dual function: the ones of a hepatoprotector and of a carrier. In selection of components for the complexes, various models of hepatitis, the chronic one included, should be considered while assessing their effects on the prevention of complications.

CONCLUSIONS

Under normal physiological conditions, the complexes under study were not found to produce any effects on the bile secreting function of the liver.

After 7-day intragastric administration in the setting of acute toxic hepatitis

- MG/Met and MG/MethA at the dose of 2.5 mg/kg caused a reduction of sleep duration after administration of hexobarbital by 4.5 times (from 160±14.0 min in the controls down to 36.1±2.3 and 33.4±2.2 min ($p<0.05$), respectively; the complexes at the dose of 5 mg/kg administered with thiopental sodium caused a reduction of sleep duration by 3 times (from 63.5±4.2 min in the control down to 20.7±1.6 and17.7±1.2 min ($p<0.05$), respectively;

- MG/Met and MG/MethA at the dose of 5 mg/kg caused more significant reduction in the dyscirculatory and edematous events; signs of activation of lymphoid and macrophagal cells in the liver tissue appeared;

- MG/Car и MG/Que at the dose of 5 mg/kg caused a reduction in the activity of alkaline phosphatase by 2.5-2.8 times, from 245.2±16.4 U/l in the control down to 88.33±4.67 and 96.13±4.27 U/l ($p<0.05$), respectively.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

ETHICAL CONSIDERATIONS

The protocol of experimental animal study was approved by Animal ethical committee based on Institute of Bioorganic chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 26, 2019).

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REFERENCES

1. Demirci-Çekiç, S., Özkan, G., Avan, A. N., Uzunboy, S., Çapanoğlu, E., Apak, R. Biomarkers of oxidative stress and antioxidant defense . Journal of pharmaceutical and biomedical analysis. 2022; V.209: 114477 https://doi.org/10.1016/j.jpba.2021.114477
2. Costa, M., Sezgin-Bayindir, Z., Losada-Barreiro, S., Paiva-Martins, F., Saso, L., Bravo-Díaz, C. Polyphenols as antioxidants for extending food shelf-life and in the prevention of health diseases: Encapsulation and interfacial phenomena. Biomedicines.2021;9(12):1909. https://doi.org/10.3390/biomedicines9121909
3. Heidari S, Mehri S, Hosseinzadeh H. The genus Glycyrrhiza (Fabaceae family) and its active constituents as protective agents against natural or chemical toxicities. Phytother Res. 2021;35(12):6552-6571. doi: 10.1002/ptr.7238
4. Li, M., Luo, Q., Tao, Y., Sun, X., & Liu, C. Pharmacotherapies for Drug-Induced Liver Injury: A Current Literature Review. Frontiers in Pharmacology. 2022;12:806249. doi: 10.3389/fphar.2021.806249
5. Abo El-Magd, N. F., El-Karef, A., El-Shishtawy, M. M., & Eissa, L. A. Glycyrrhizin and Omega-3 fatty acids have hepatoprotective roles through toll-like receptor-4. Egyptian Journal of Basic and Applied Sciences. 2019; 6(1):82-98 doi.org/10.1080/2314808X.2019.1669111
6. Hou, Y., Zou, L., Li, Q., Chen, M., Ruan, H., Sun, Z.,Xu X., Yang J., Ma, G. Supramolecular assemblies based on natural small molecules: Union would be effective. Materials Today Bio. 2022:100327. https://doi.org/10.1016/j.mtbio.2022.100327
7. Nascimento M. H. M., de Araújo D. R. Exploring the Pharmacological Potential of Glycyrrhizic Acid: From Therapeutic Applications to Trends in Nanomedicine. Future Pharmacology 2022; 2(1):1-15 doi.org/10.3390/futurepharmacol2010001
8. Selyutina OY, Polyakov NE. Glycyrrhizic acid as a multifunctional drug carrier - From physicochemical properties to biomedical applications: A modern insight on the ancient drug. Int J Pharm. 2019;559:271-279. doi: 10.1016/j.ijpharm.2019.01.047

9. Tagayalieva N. A., Makhmudov L. U., Aripov T. F., Vypova N. L., Yuldashev Kh. A., Esanov R. S., Gafurov M. B. Antiradical and hepatoprotective properties of supramolecular complexes of monoammonium glycyrrhizinate with amino acids and phenolic compounds. ScienceAsia. 2022: 6(48): 887-893. doi: 10.2307/ scienceasia887-893.2022.SA2165
10. Dalimova S.N., Khamdamova N.A., Levitskaya Yu.V., Kuziev Sh.N., Gafurov M.B., Yuldashev Kh.A., Umarova G.B., Mukhamadjanova G.M., Vypova N.L., Tagayalieva N.A., Makhmudov L.U., Khamraev S.Kh. Study on antioxidant properties of some soluble forms of supramolecular complexes based on the monoammonium salt of glycyrrhizic acid. Universum: chemistry and biology, 2021;1(79):16-22.URL: https://7universum.com/ru/nature/archive/item/11158) (in Russian)
11. Ashurova F.K., Yuldashev V.Sh., Tagayalieva N.A., Khodjanazarova S.J., Azizova Z.Sh., Makhmudov L.U., Ergasheva B.Sh., Avezdurdieva T.A. Study on biochemical activity of the complexes on the model of acute toxic hepatitis. Journal of theoretic and clinical medicine, 2022;3:11-14 (in Russian)
12. Ashurova F.K., Yuldashev V.Sh., Tagayalieva N.A., Khodjanazarova S.J., Azizova Z.Sh., Makhmudov L.U., Avezdurdieva T.A. Study on immunotropic activity of the complexes in the physiological normal conditions. Journal of theoretic and clinical medicine, 2022;1:14-17 (in Russian)
13. European Directive 2010/63/EU on the protection of animals used for scientific purposes. September 22, 2010. Official Journal of the European Union, L 276/33- L276/79 http://data.europa.eu/eli/dir/2010/63/oj
14. Myshkin V.A., Enikeev D.A. Chemical damage of organism (molecular-cellular mechanisms, pathogenesis, clinical picture and treatment. Training manual for students. Ufa, FED Publishing House, 2000. (in Russian)
15. US FDA. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Adult Healthy Volunteer. Rockville, MD: US Food and Drug Administration; 2005 https://www.fda.gov/media/72309/download
16. Kamyshnikov V.S. Clinical-laboratory diagnosis of the liver diseases. Laboratory Diagnostics. Eastern Europe. 2016;5(1):150-163 (in Russian)]. http://recipe.by/wp-content/uploads/woocommerce_uploads/2016/10/1_2016_LD.pdf#page=150
17. Chatterjee S., Majumder B., Basu D. A Review To Explore The Association Between Diabetes Mellitus And Hepatitis . IOSR Journal of Dental and Medical Sciences. 2021;20(9):13-22. doi: 10.9790/0853-2009121322
18. Yakovenko E.P. Choleretics in the clinical practice. Gastroenterology. Attachment to Consilium Medicum. 2003;2:21-27 (in Russian) https://elibrary.ru/item.asp?id=27002063
19. Jaeschke H., Adelusi O. B., Akakpo J. Y., Nguyen N. T., Sanchez-Guerrero G., Umbaugh D. S., Ramachandran A. Recommendations for the use of the acetaminophen hepatotoxicity model for mechanistic studies and how to avoid common pitfalls .Acta Pharmaceutica Sinica B. 2021;11(12):3740-3755. https://doi.org/10.1016/j.apsb.2021.09.023
20. Bhushan B., Apte U. Liver regeneration after acetaminophen hepatotoxicity: mechanisms and therapeutic opportunities .The American journal of pathology. 2019;189(4): 719-729. https://doi.org/10.1016/j.ajpath.2018.12.006

21. Akakpo J. Y., Ramachandran A., Curry S. C., Rumack B. H., Jaeschke H. Comparing N-acetylcysteine and 4-methylpyrazole as antidotes for acetaminophen overdose. Archives of Toxicology. 2022; 96(2):453-465. https://doi.org/10.1007/s00204-021-03211-z
22. Acharya, M., Lau-Cam, C.A. Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. J Biomed Sci 17 (Suppl 1), S35 (2010). https://doi.org/10.1186/1423-0127-17-S1-S35
23. Wang, R., Tang, R., Li, B., Ma, X., Schnabl, B., & Tilg, H.Gut microbiome, liver immunology, and liver diseases./Cellular & molecular immunology. 2021; 18(1): 4-17. https://doi.org/10.1038/s41423-020-00592-6