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Time-Dependent Changes in the Activity of the Mitochondrial ATP-Dependent Potassium Channel of Rat Liver in CCl_4-Induced Toxic Hepatitis and Their Correction by Polyphenols

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Abstract Mitochondrial ATP-dependent potassium channels (mitoKATP channels) take part in maintaining matrix volume and membrane potential, but data on the activity of this channel in toxic hepatitis and on the effect of biologically active substances on it are scarce. The aim of this study was to determine the activity of the mitoKATP channel of rat liver mitochondria in toxic hepatitis induced by carbon tetrachloride (CCl4), its time-dependent changes, and the effect of the polyphenols gossitan and getasan on it, in comparison with the flavonoid quercetin. Toxic hepatitis was induced in outbred white male rats by injection of 50% CCl4 (0.5 ml/kg) every 3 days for 3 weeks. Channel activity was assessed by the change in optical density of the mitochondrial suspension at 540 nm in the presence of 200 μM ATP; the activity of the control group was taken as 100%. In toxic hepatitis the activity of the mitoKATP channel was inhibited by 29.5±2.6% relative to the control. After 20 days of treatment, quercetin, gossitan and getasan (50 mg/kg) increased the channel activity by 13.0±1.1%, 19.3±1.6% and 14.0±1.2% relative to the hepatitis group. In the 6-week dynamic study the activity of the channel in toxic hepatitis decreased by 34.5±2.1% relative to the control by week 6; when the compounds were administered from week 3, the inhibition at week 6 was 18.5±2.0%, 9.4±1.8% and 14.6±1.5%, respectively, and the activity was higher than at week 3. The results show that gossitan and getasan, like quercetin, may activate K+ flux through the mitochondrial membrane in toxic hepatitis.

Keywords Toxic hepatitis, Carbon tetrachloride, Rat liver mitochondria, Mitochondrial ATP-dependent potassium channel, Gossitan, Getasan, Quercetin

1. Introduction

Hepatocytes are the specific functional cells of the liver and make up 70–85% of its mass. They perform metabolic, secretory and storage functions, and the liver carries out the metabolism of lipids and carbohydrates and regulates the level of proteins and amino acids in the blood [1]. Liver cells are targets of various toxic substances, in which their mitochondria are damaged. Under the action of various xenobiotics the amount of free radicals in liver mitochondria may increase, the antioxidant defense system may change and membrane disturbances may occur. Mitochondria occupy about 20–25% of the cytoplasm of liver cells, and each cell contains 1000–2000 mitochondria [2]. The ion-transport systems located in the liver mitochondrial membrane, the passive ion permeability, ATP synthesis and the antioxidant defense systems determine the physiological regulation of hepatocytes, and in various pathological processes these systems become dysfunctional.

Carbon tetrachloride (CCl_4) is a hepatotoxic substance that is well soluble in fats. The oxidative stress caused by CCl_4 leads to cell damage through the oxidation of cell components such as lipids, proteins and DNA [3], and the binding of CCl_4 to fats and proteins causes peroxidative degeneration of many tissues [4]. Various pharmacological agents can trigger the opening of the mitochondrial permeability transition pore (mPTP) in liver mitochondria [5,6]. The opening of the mPTP is defined as a sudden increase in the permeability of the inner mitochondrial membrane to low-molecular-weight solutes (up to 1.5 kDa) in response to stimuli including high Ca^{2+} and oxidative stress [7]. In toxic hepatitis a sharp increase in the permeability of liver mitochondria to water and water-soluble molecules has been observed, which may lead to a drop of the membrane potential.

Mitochondrial potassium channels have been described as important factors of cell life and death. The activation

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of mitochondrial K^+ channels, such as the ATP-regulated or Ca^{2+}-activated large-conductance channels, may have a cytoprotective effect in cardiac or neuronal tissue, while the inhibition of the mitochondrial Kv1.3 channel may lead to the death of cancer cells [8]. The field began in the early 1990s with the first publication describing a K^+ channel sensitive to ATP and to the antidiabetic sulfonylurea glibenclamide (the mitoK_{ATP} channel) [9]. The discovery that the activation of a mitochondrial K^+ channel by channel openers triggers protective mechanisms in cardiomyocytes showed that these proteins are important in ischemia–reperfusion [10]. This was the point at which trials of directing drugs that affect cell function to various mitochondrial K^+ channels began.

Various K^+ channels are present in the inner mitochondrial membrane [11]: the ATP-regulated channel (mitoK_{ATP}), which was the first K^+ channel described in the inner mitochondrial membrane, the large-, intermediate- and small-conductance Ca^{2+}-activated channels (mitoBK_{Ca}, mitoIK_{Ca}, mitoSK_{Ca}), the voltage-gated channels mitoKv1.3, mitoKv1.5 and mitoKv7.4, the two-pore-domain mitoTASK3 channel, the Na^+-activated mitoSLO2 channel and the mitoHCN channel. Most probably, the mitoK_{ATP} channel is formed by ROMK-type channels [12] or by the recently described CCDC51 protein, and the ABCB8 protein (the mitochondrial sulfonylurea receptor) is a part of the channel [13].

Different tissues contain different sets of mitochondrial K^+ channels [14]. For example, five types of K^+ channels were described in cardiomyocyte mitochondria, but only two types in the mitochondria of skin fibroblasts [15]; the mitoK_{ATP}, mitoBK_{Ca} and mitoKv1.3 channels are the most widespread in mammalian mitochondria [8]. The number of mitoK_{ATP} channels is higher in the brain than in heart mitochondria [16]. Therefore, before targeting specific mitochondrial K^+ channels with drugs, the type of the channel, its presence in the particular tissue and its abundance in the target tissue should be considered.

The following mitochondrial K^+ channels have been described [11]: mitoK_{ATP} (the first channel described in the inner mitochondrial membrane); mitoBK_{Ca} (large-conductance, encoded by the KCNMA1 gene, a splice isoform of the BK_{Ca} channel of plasma membranes); mitoIK_{Ca} (intermediate-conductance, KCNN4); mitoSK_{Ca} (small-conductance, KCNN1–KCNN3); the voltage-gated mitoKv1.3, mitoKv1.5 and mitoKv7.4 (KCNQ4); the acid-sensitive two-pore-domain mitoTASK3 (KCNK9); the Na^+-activated mitoSLO2 (KCNT2 in mammals); and the hypopolarization-activated cyclic-nucleotide-gated mitoHCN channel (HCN1–HCN4). Thus, three parameters should be defined before mitochondrial K^+ channels are considered as pharmacological targets: the molecular identity of the channel, its tissue profile, and its level in the particular tissue (the so-called "3D mitoK channel mapping").

Another aspect of this problem is the presence of the channels in different cell compartments. The BK_{Ca} channels, which are abundant in the membranes of many cells, are present in cardiomyocytes only in the inner mitochondrial membrane and not in the plasma membrane [17]; this is unique for mitochondrial K^+ channels and supports the view that these channels and cardiac tissue are targets for pharmacological agents. The functional consequences of the uneven distribution of the channels and of the intracellular heterogeneity of mitochondria with respect to K^+ channels have not yet been determined.

Before the identification of mitochondrial K^+ channels, the electrogenic flow of K^+ ions into the mitochondrial matrix was described as "potassium uniport" [18]. The mitoK_{ATP} channel takes part in maintaining the matrix volume and in the formation of the membrane potential, and it has a great significance in the formation of adaptation processes of cells in many pathological conditions [19]. The proteins forming this channel are inhibited by physiological concentrations of ATP and take part in the K^+ cycle [20]; the regulation of the channel plays an important role in cell adaptation to hypoxia [21, 22], and the molecular structures of mitochondria may be specific targets for pharmacological agents with antihypoxant properties [23].

In Uzbekistan, the dysfunction of liver mitochondria in CCl_4-induced toxic hepatitis and the effect of bioactive substances on it have been studied by Asrarov and Abdullaeva [24]. In a complex study of the dynamics of mitochondrial functional indices in CCl_4-induced toxic hepatitis it was found that the damaging effect is associated with the transition of the cyclosporin A-sensitive pore into the open conformation and with the activation of endogenous phospholipase A_2, and that these changes intensify as the pathological process develops [25]. The corrective effect of the alkaloid protopine on the functional indices of mitochondria in toxic hepatitis has been found [26]; the effect of flavonoid compounds on mitochondrial enzymes in heliotrine-induced hepatitis [27] and the effect of polyphenol extracts of *Helichrysum maracandicum* on mitochondrial antioxidant enzymes in toxic hepatitis [28] have also been studied.

The search for new agents that prevent liver damage in hepatitis, the identification of bioactive substances in local raw materials with hepatoprotective properties, their isolation from plants in pure form and the study of the mechanisms of their corrective action at the molecular, cellular and tissue levels are among the topical tasks of modern physiology, pharmacology and medicine. Hepatoprotective compounds isolated from plants differ from chemical synthetic preparations in the absence of toxic properties and in having a physiological mechanism of action and pharmacological properties. Therefore, in this work the membrane-active properties of the polyphenols gossitan and getasan were evaluated with the aim of determining membrane disturbances of mitochondria in toxic hepatitis and correcting them with natural substances.

However, data on the activity of the rat liver mitoK_{ATP} channel in toxic hepatitis and on the effect of biologically active substances on it are scarce. In toxic hepatitis the volume of the mitochondrial matrix changes and ion homeostasis may be disturbed, and the K^+-dependent

permeability of the inner membrane is regulated by the mitoK_{ATP} channel. The aim of this work was to determine the activity of the mitoK_{ATP} channel of rat liver mitochondria in CCl_4-induced toxic hepatitis, to study its time-dependent changes, and to determine the effect of the polyphenols gossitan and getasan on it, in comparison with the flavonoid quercetin.

2. Materials and Methods

2.1. Animals

The study was carried out on outbred white male rats weighing 180–200 g, kept on a standard ration in vivarium conditions, in the Laboratory of Molecular Biophysics of the Institute of Biophysics and Biochemistry at the National University of Uzbekistan. The research on experimental animals was carried out in accordance with the Declaration of Helsinki, the guidelines of the Council for International Organizations of Medical Sciences (CIOMS, 1985) and the bioethical regulation of the Institute on the use of laboratory animals in scientific research (2019).

2.2. Toxic Hepatitis Model and Experimental Groups

Toxic hepatitis was induced by injection of 50% CCl_4 dissolved in vegetable oil (0.5 ml/kg) under the abdominal skin once every 3 days [29]. Blood was taken from the animals every 3 days and the activities of ALT and AST were determined; 21 days after the beginning of CCl_4 administration the plasma ALT (normal value 70 ±1 U/l) and AST (normal value 58 ±3 U/l) were increased. The animals were divided into five groups (n=5 in each), as shown in Table 1.

Table 1. Experimental Groups

Group	Treatment	Compound, dose	n
I	Control (healthy)	–	5
II	CCl_4 (toxic hepatitis)	vegetable oil, 0.5 ml/kg	5
III	CCl_4 + quercetin	50 mg/kg	5
IV	CCl_4 + gossitan	50 mg/kg	5
V	CCl_4 + getasan	50 mg/kg	5

The study was carried out in two stages that complemented each other. In the first stage, the changes in the body and liver weights of the rats in toxic hepatitis and the effect of the compounds on them were studied, together with the content of transferases and total bilirubin in the blood plasma and liver. In the second stage, the dysfunction of rat liver mitochondria was corrected with gossitan, getasan and quercetin, and the activity of the mitoK_{ATP} channel was determined. At the end of the experiments the initial and final body weights, the percentage change in body weight, the absolute liver weight and the relative liver weight index were determined as: body weight change (%) = (final body weight – initial body weight) / initial body weight × 100; relative liver weight index (%) = (absolute liver weight, g / final body weight, g) × 100.

In the first experiment, after the increase of ALT and AST (21 days after the beginning of CCl_4 administration), group II received purified vegetable oil (0.5 ml/kg), and groups III, IV and V received quercetin, gossitan and getasan, respectively, orally once daily for 20 days. In the second (dynamic) experiment, CCl_4 was injected for 3 weeks and the compounds were administered orally once daily for 3 weeks, starting from week 3. Mitochondria were isolated from the liver of healthy, hepatitis and treated animals during 6 weeks; in groups III–V this was done at weeks 4, 5 and 6.

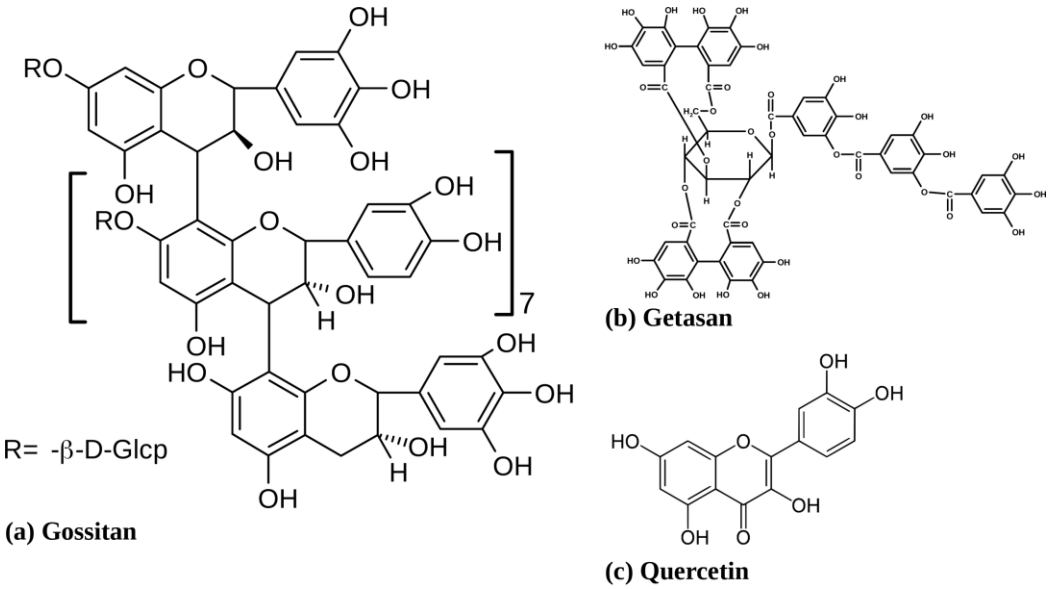


Figure 1. Structural formulas of the compounds used: (a) gossitan ($C_{177}H_{154}O_{85}$) isolated from *Gossypium hirsutum*; (b) getasan isolated from *Geranium sanguineum*; (c) quercetin ($C_{15}H_{10}O_7$) isolated from *Plantago major* L.

2.3. Test Compounds and Reagents

Gossitan ($C_{177}H_{154}O_{85}$) isolated from *Gossypium hirsutum* [30] and getasan ($C_{55}H_{36}O_{34}$) isolated from *Geranium sanguineum* were provided by scientists of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. Gossitan and getasan were compared with the existing hepatoprotective flavonoid quercetin ($C_{15}H_{10}O_7$, M=302 g/mol, isolated from *Plantago major* L.), which was used as a standard prototype [31]. Quercetin is widely found in the vegetative and generative organs of tea (*Camellia sinensis*), apple (*Malus domestica*), onion (*Allium*), grape (*Vitis vinifera*), citrus fruits and tomato (*Solanum lycopersicum*), and anti-inflammatory, antiviral, antiallergic, anticarcinogenic and hypotensive effects of quercetin have been found in *in vitro* experiments. Among the plant substances tested (the diterpenoid alkaloids zongorine and zeravshanizine, the flavonoids isorhamnetin and norwogonin, and the polyphenols gossitan and getasan), only those with hepatoprotective activity in toxic hepatitis were selected by screening; the corrective effect of gossitan and getasan on the disturbances of liver cell and mitochondrial functions was more pronounced than that of the other plant substances. The molar mass of getasan is 1240 g/mol. The structural formulas of the compounds are shown in Figure 1. The following reagents were used: tetrachloromethane (Reakhim, Russia), Tris-HCl, EDTA, EGTA, KH_2PO_4, HEPES, KCl, succinate (Sandoz, Switzerland), oligomycin, rotenone, ATP (Serva, Germany) and sucrose. All reagents had the chemical purity required for the experiments.

2.4. Isolation of Liver Mitochondria

Rat liver mitochondria were isolated by the differential centrifugation method of Schneider [32]. After decapitation the liver was placed in a semi-ice isolation medium (250 mM sucrose, 10 mM Tris-HCl, 1 mM EGTA, pH 7.4), minced, and homogenized in a ratio of 1:6 (liver weight in g to ml of medium) with a Teflon pestle at 6000–8000 rpm. Centrifugation was carried out at 0–2 ℃ in two steps: first at 1500 rpm for 7–8 min to sediment heavy cell aggregates, then the supernatant was centrifuged at 6000 rpm (6000 g) for 17 min. The mitochondrial pellet was washed in an EDTA-free medium (1:5 by volume) and stored in a vessel with ice. Mitochondrial protein was determined by the biuret method.

2.5. Determination of mitoK_{ATP} Channel Activity

The permeability of the mitoK_{ATP} channel was determined by the change in the optical density of the mitochondrial suspension (0.3–0.4 mg/ml) at 540 nm in an open 3-ml cell at 26 ℃ with constant stirring. The incubation medium contained 125 mM KCl, 10 mM HEPES, 5 mM succinate, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 0.005 mM rotenone and 0.001 mM oligomycin, pH 7.4 [33]. The physiological concentration of ATP (200 μM) was present in the incubation medium; the channel activity of the control group under these conditions was taken as 100%.

2.6. Statistical Analysis

Statistical processing and plotting were performed with OriginPro 8.6 (USA). The values were calculated as the arithmetic mean of 4–7 independent experiments. The differences between the control, experimental and experimental + test compound values were calculated by the t-test; $P<0.05$, $P<0.01$ and $P<0.001$ were taken as statistically significant. The results are presented as M ±m (n=5).

3. Results

3.1. Body and Liver Weights

In the hepatitis group (II) the change in the body weight of the rats between the beginning and the end of the experiment was lower (5.6 ±0.4%) than in the control (group I, 11.3 ±1.2%). The absolute liver weight of the rats with toxic hepatitis was 10.6 ±0.9 g, which was 41.3 ±3.4% higher than in the control, and the relative liver weight index was 5.12 ± 0.6%, compared with 3.45 ±0.2% in the control (Table 2).

When the animals with toxic hepatitis were treated with quercetin (group III) for 20 days, the change in body weight and the relative liver weight index were 11.3 ±0.9% and 3.61 ±0.2%, i.e. the body and liver weights were restored to the control level in comparison with group II. After the administration of gossitan (group IV) and getasan (group V), the change in body weight was 10.2 ±0.8% and 9.2 ±0.7%, and the relative liver weight index decreased relative to the pathological group and was slightly higher than the control (3.81 ±0.4% and 4.06 ±0.3%, respectively; Table 2). Thus, the selected compounds effectively restore the body and liver weights of rats in toxic hepatitis.

Table 2. Effect of Quercetin, Gossitan and Getasan on the Body and Liver Weights of Rats in CCl_4-Induced Acute Toxic Hepatitis (M ±m)

Group	Final weight, g	Weight change, %	Absolute liver weight, g	Relative liver weight index, %
I (control)	217 ±1.5	11.3 ±1.2	7.5 ±0.6	3.45 ±0.2
II (CCl_4)	207 ±1.4*	5.6 ±0.4**	10.6 ±0.9*	5.12 ±0.6*
III (+ quercetin)	216 ±1.6**	11.3 ±0.9*	7.8 ±0.5*	3.61 ±0.2*
IV (+ gossitan)	215 ±1.4*	10.2 ±0.8*	8.2 ±0.6*	3.81 ±0.4*
V (+ getasan)	214 ±1.7*	9.2 ±0.7*	8.7 ±0.5*	4.06 ±0.3*

Group II vs. control; groups III–V vs. group II. *P<0.05;* *P<0.01; n=5.

The decrease in the increase of body weight relative to the control indicates disturbances of metabolic processes and lipid metabolism. In acute toxic hepatitis caused by CCl_4, hepatocytes are damaged and lipid peroxidation is intensified through the metabolic activation of reactive oxygen species. However, the effect of the polyphenols on the morphological indices alone is not sufficient to call them hepatoprotectors; for this the effect on some enzymes and on the total bilirubin content in the blood plasma and liver should be determined.

3.2. Transaminases and Bilirubin in Toxic Hepatitis

After 21 days of CCl_4 administration, the plasma ALT, AST and total bilirubin of the hepatitis group (II) were 203.4±20.5 U/l, 178.6±15.5 U/l and 18.9±1.6 μmol/l, i.e. 2.6, 3.3 and 1.7 times higher than in the control, which confirmed the development of toxic hepatitis (Table 3). In group III, treated with quercetin for 20 days, the plasma ALT (86.6±9.4), AST (68.2±6.6) and bilirubin (12.5±1.4) were lower than in group II by 2.3, 2.61 and 1.5 times, respectively. After 20 days of treatment with gossitan (group IV), ALT, AST and bilirubin were 97.8±10.6 U/l, 82.6±7.5 U/l and 13.4±1.2 μmol/l; with getasan (group V) they were 122.2±11.3 U/l, 94.3±6.2 U/l and 14.8±1.3 μmol/l, i.e. lower than in the hepatitis group by 2.08, 2.16 and 1.41 times for gossitan and by 1.66, 1.90 and 1.27 times for getasan. The effect of gossitan on the increased enzymes and bilirubin was more pronounced than that of getasan.

Table 3. Effect of Quercetin, Gossitan and Getasan on Plasma ALT, AST and Total Bilirubin in Toxic Hepatitis (M±m)

Group	ALT, U/l	AST, U/l	Total bilirubin, μmol/l
I (control)	77.4±6.8	54.1±4.7	10.7
II (CCl_4)	203.4±20.5	178.6±15.5	18.9±1.6
III (+ quercetin)	86.6±9.4	68.2±6.6	12.5±1.4
IV (+ gossitan)	97.8±10.6	82.6±7.5	13.4±1.2
V (+ getasan)	122.2±11.3	94.3±6.2	14.8±1.3

The activities of ALT and AST were also determined in the liver. In the hepatitis group the liver ALT (75.5±5.7) and AST (54.7±4.4) were increased by 211.9% and 154.4% relative to the control. Quercetin significantly decreased the liver ALT (34.6±3.5) and AST (29.8±2.2) relative to group II by 54.2% and 45.5%. In the gossitan group (IV) the liver ALT (39.2±3.7) and AST (33.6±2.2) decreased relative to group II by 48% and 38.5%, and in the getasan group (V) ALT (43.4±3.8) and AST (38.4±3.5) decreased by 42.5% and 29.7% (Figure 2; $P<0.05$; n=5). Thus, gossitan and getasan reduce the activities of ALT and AST in the plasma and liver and the content of total bilirubin, like the existing hepatoprotective compound quercetin.

3.3. mitoK$_{ATP}$ Channel Activity After 20 Days of Treatment

In the hepatitis group (II) the permeability of the mitoK$_{ATP}$ channel in the presence of 200 μM ATP in the incubation medium was inhibited by 29.5±2.6% relative to the control (Figure 3). In the hepatitis group treated with quercetin (III) the channel permeability was increased by 13.0±1.1% relative to group II. In groups IV and V, treated with gossitan and getasan for 20 days, the channel permeability was activated by 19.3±1.6% and 14.0±1.2% relative to group II, respectively (Figure 3, Table 4).

Table 4. Effect of Polyphenols on the mitoK$_{ATP}$ Channel Activity After 20 Days of Treatment

Group	Change in channel activity relative to group II, % (M±m)	Direction
III (+ quercetin)	13.0±1.1	Increase
IV (+ gossitan)	19.3±1.6	Increase
V (+ getasan)	14.0±1.2	Increase

Thus, in CCl_4-induced toxic hepatitis the permeability of the rat liver mitoK$_{ATP}$ channel is inhibited, and the polyphenols gossitan and getasan, like the existing hepatoprotective compound quercetin, increase it.

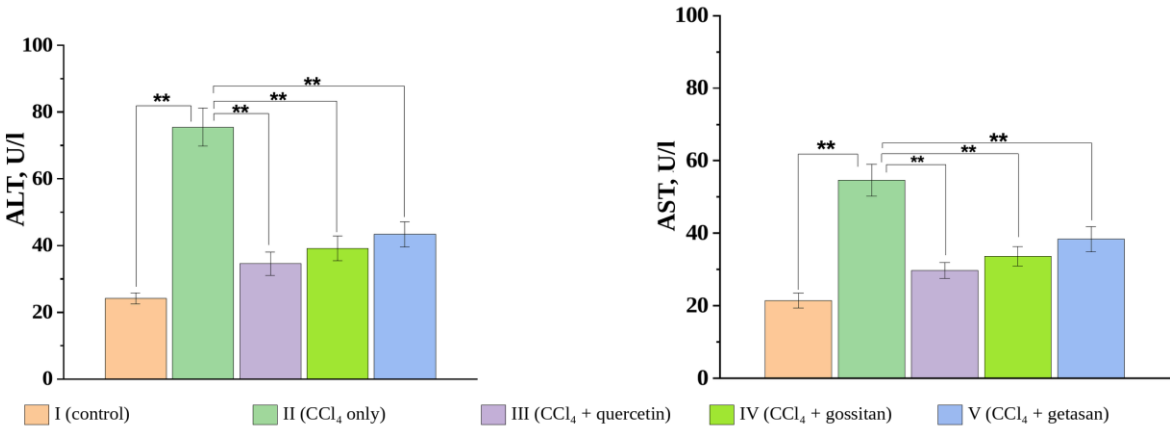


Figure 2. Effect of quercetin, gossitan and getasan on the activity of ALT (left) and AST (right) in the liver of rats with CCl_4-induced toxic hepatitis (**$P<0.05$; group II relative to the control, groups III–V relative to group II; n=5)

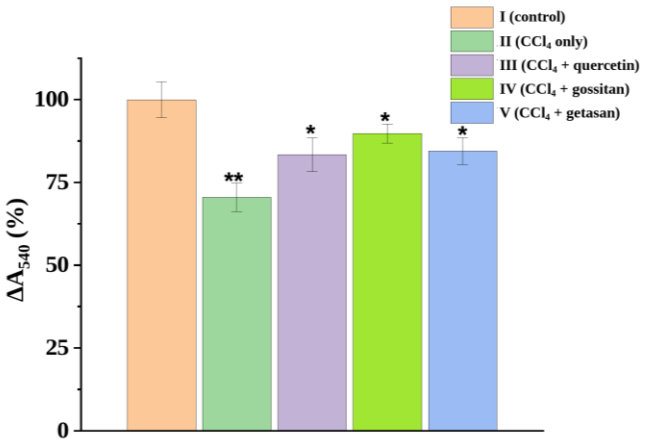


Figure 3. The effect of quercetin, gossitan and getasan on the activity of the mitoK$_{ATP}$ channel of rat liver mitochondria in CCl_4-induced toxic hepatitis after 20 days of treatment (activity of the control group in the presence of 200 μM ATP is taken as 100%; *$P<0.05$ relative to the control, P<0.05 relative to group II; n=5)

3.4. Time-Dependent Changes in mitoK$_{ATP}$ Channel Activity

To determine how the activity of the channel changes with the development of the pathological process and how it depends on the administration of the compounds, the activity of the mitoK$_{ATP}$ channel of the liver mitochondria was followed for 6 weeks (Figure 4). In the presence of 200 μM ATP the activity of the channel in the control (group I) was taken as 100%. In the hepatitis group (II) the activity of the channel decreased with time relative to the control, and by week 6 the decrease was 34.5±2.1%.

Quercetin, gossitan and getasan were administered to the animals with toxic hepatitis (groups III, IV and V) from week 3, and mitochondria were isolated at weeks 4, 5 and 6. In group III the activity of the channel was inhibited up to week 3; after the administration of quercetin, from week 4 the permeability was higher than at week 3, and at week 6 the activity of the mitoK$_{ATP}$ channel was inhibited by 18.5±2.0% relative to the control, i.e. it was activated under the action of quercetin compared with week 3.

In groups IV and V the activity of the mitoK$_{ATP}$ channel was inhibited at week 3 by 25.0±3.0% and 24.0±2.0% relative to the control, respectively. When gossitan and getasan were administered from week 3, at week 6 the inhibition relative to the control was 9.4±1.8% and 14.6±1.5%, respectively. When these week 6 values were compared with the values at week 3, the activity of the mitoK$_{ATP}$ channel was found to be increased (Figure 4, Table 5).

Table 5. Inhibition of the Activity of the mitoK$_{ATP}$ Channel of Rat Liver Mitochondria at Week 6

Group	Inhibition at week 6 relative to control, % (M±m)	Activity compared with week 3
II (CCl_4)	34.5±2.1	–
III (+ quercetin)	18.5±2.0	Increased
IV (+ gossitan)	9.4±1.8	Increased
V (+ getasan)	14.6±1.5	Increased

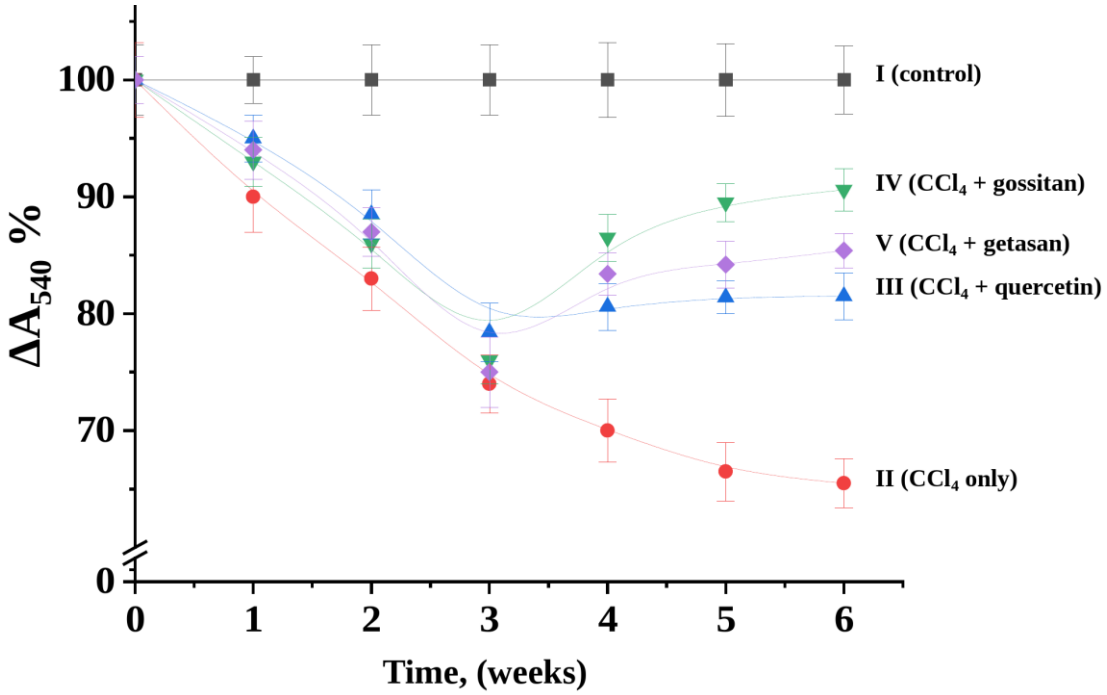


Figure 4. Time-dependent changes in the activity of the mitoK$_{ATP}$ channel of rat liver mitochondria in CCl_4-induced toxic hepatitis and the effect of quercetin, gossitan and getasan (all points P<0.05; n=5). The activity of the control group in the presence of 200 μM ATP was taken as 100%; treatment of groups III–V was started at week 3

4. Discussion

The results show that in CCl_4-induced toxic hepatitis the activity of the mitoK_{ATP} channel of rat liver mitochondria is inhibited (by 29.5±2.6% in the first experiment) and that the inhibition increases with the development of the pathological process (up to 34.5±2.1% by week 6). The mitoK_{ATP} channel takes part in maintaining the matrix volume and in the formation of the membrane potential, and the K^+-dependent permeability of the inner membrane is regulated by this channel. In toxic hepatitis the matrix volume changes and ion homeostasis may be disturbed. Because of the inhibition of the channel, the flow of K^+ ions into the matrix sharply decreases and the volume of the mitochondria changes; as a result, the K^+ cycle in the mitochondria may be disturbed and the membrane potential may decrease.

Quercetin, gossitan and getasan increased the activity of the channel in toxic hepatitis. After 20 days of treatment the increase relative to the hepatitis group was 13.0±1.1%, 19.3±1.6% and 14.0±1.2%, respectively. In the dynamic experiment, when the compounds were administered from week 3, the activity of the channel at week 6 was higher than at week 3, although the inhibition relative to the control remained (18.5±2.0%, 9.4±1.8% and 14.6±1.5% for quercetin, gossitan and getasan, respectively); the smallest inhibition at week 6 was observed in the gossitan group. Thus, the polyphenols gossitan and getasan may activate the flow of K^+ ions through the mitochondrial membrane in toxic hepatitis induced by CCl_4.

The development of toxic hepatitis in this model was confirmed by the decrease in the increase of body weight, the increase in the absolute liver weight and the relative liver weight index, and by the increase of ALT, AST and total bilirubin. Aminotransferases catalyze the transfer of amino groups from amino acids to keto acids, and pyridoxal phosphate, the active form of vitamin B_6, serves as their coenzyme. Their activity in the blood plasma is very low, and it increases considerably when the membranes of liver cells are damaged and their integrity is disturbed; this is why the determination of the activities of both ALT and AST is important in toxic hepatitis. Gossitan and getasan decreased the activities of these enzymes in the plasma and liver and reduced the increased bilirubin, and the effect of gossitan was more pronounced than that of getasan, which indicates their hepatoprotective properties.

The corrective effect of the polyphenols on the mitoK_{ATP} channel is consistent with the other findings obtained in the same model: in toxic hepatitis the permeability of liver mitochondria for K^+, H^+, Ca^{2+} and Mg^{2+} ions and the opening of the mPTP increased, and gossitan, getasan and quercetin inhibited the permeabilization of the membrane; they also reduced the content of the lipid peroxidation product MDA and restored the activity of the antioxidant enzymes SOD, catalase and glutathione peroxidase, which indicates their antioxidant properties. In addition, the polyphenols decreased the activity of ALT and AST and the content of bilirubin, i.e. they showed a hepatoprotective effect.

The experiments were carried out in two complementary stages. In the first stage the polyphenols corrected the changes of body and liver weights, the plasma and liver transferases and bilirubin; in the second stage they corrected the dysfunction of liver mitochondria, including the activity of the mitoK_{ATP} channel. The mechanisms of the corrective action of the compounds on the dysfunction of mitochondria in toxic hepatitis were determined at the mitochondrial level, which may serve as a theoretical and practical basis for the creation of new preparations against liver diseases on the basis of the revealed properties of these compounds.

Therefore, the study of disturbances of organ, tissue and cell functions in toxic hepatitis at the mitochondrial level, of the corrective role of the K_{ATP} channel, and the identification of new promising hepatoprotective substances and their mechanisms of action remain relevant problems. Biologically active substances that correct the dysfunction of liver homogenate and mitochondria in hepatitis may serve as a theoretical and practical basis for the development of new hepatoprotective drugs.

5. Conclusions

(1) In CCl_4-induced toxic hepatitis the plasma ALT, AST and total bilirubin increased by 2.6, 3.3 and 1.7 times, and gossitan and getasan decreased them, like quercetin; the body and liver weight indices were also restored. The permeability of the mitoK_{ATP} channel of rat liver mitochondria in the presence of 200 μM ATP is inhibited by 29.5±2.6% relative to the control.

(2) After 20 days of treatment, quercetin, gossitan and getasan (50 mg/kg) increased the activity of the channel by 13.0±1.1%, 19.3±1.6% and 14.0±1.2% relative to the hepatitis group.

(3) With the development of toxic hepatitis the activity of the mitoK_{ATP} channel decreases with time and by week 6 is 34.5±2.1% lower than in the control. When quercetin, gossitan and getasan were administered from week 3, the activity of the channel at week 6 was higher than at week 3; the inhibition relative to the control was 18.5±2.0%, 9.4±1.8% and 14.6±1.5%, respectively.

(4) The polyphenols gossitan and getasan may activate K^+ flux through the mitochondrial membrane in toxic hepatitis, and their effect is comparable to that of quercetin.

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