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# Effects of glycyrrhizinic acid, menthol and GA: Mt (2: 1), GA: Mt (4: 1) and GA: Mt (9: 1) supramolecular compounds on mitochondrial functional activity *IN VITRO* experiments.

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**Abstract**

This paper presents the effect of the supramolecular complex of GA (Glycyrrhizic acid) and Mt (menthol) and GA: Mt (4: 1) obtained on their basis can restore functional dysfunction of the liver mitochondria in alloxan diabetes, ie, inhibit lipid peroxidation. The hypoglycemic activity and mitochondrial membrane stabilizing properties of the supramolecular compound GA: Mt (4: 1) in alloxan diabetes were more pronounced than those of menthol, GA and its GK: Mt (2: 1) and GA: Mt (9: 1) compounds. According to the results obtained, the concentration of GA did not affect the peroxidation of lipid membranes of the liver mitochondria. However, a concentration of 15 μM of GA was found to reduce LPO (lipid peroxidation) formed by the effect of $Fe^{2}$+ / ascorbate on the mitochondrial membrane by 58.0 ± 5.0% relative to control. The inhibitory effect of GA and its supramolecular compounds in different proportions with menthol on the peroxidation of lipids in rat heart and brain tissue has been studied

**Keywords:** malon dialdehyde, lipid peroxidation, phospholipid layer, antioxidant activity, liver mitochondria, menthol, glycyrrhizinic acid.

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## Introduction

Menthol ($C_{10}H_{20}O$) is a monoterpenoid that exhibits many biological activities in experiments. Their biological activity is currently being studied in many scientific laboratories. Menthol monoterpenoid has been shown to exhibit gastric ulcer repair using ethyl alcohol [1]. The amount of menthol 50 mg / kg has a gastroprotective effect and exhibits apoptosis, anti-inflammatory and antioxidant activity in cells of the gastric mucosa [1]. Menthol monoterpenoid also affects the physiological processes associated with $Ca^{2}$ + ions present in the cell. Menthol exhibits relaxant activity by inhibiting $Ca^{2}$ + channels in smooth muscle cells of the rat aorta and coronary blood vessels [2]. Based on the literature data, it can be said that the relaxant effect of menthol may lie in the blockade of potential-dependent $Ca^{2}$ + -channels. The effect of menthol on the membrane is due to

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its hydrophobic properties. The breakdown of menthol and other monoterpenes that are part of the biological composition affects membranes and leads to a number of changes. The effect of hydrophobic compounds on membranes is manifested by altering the physicochemical properties of integral proteins (e.g. ion channels, carriers) and the phospholipid layer and by indirectly affecting channel function [3]. In vitro studies have shown that menthol and other hydrophobic monoterpenes affect membrane ion channels in concentrations in the range of 10 μM to 10 mM [4].

Currently, antioxidant and mitoprotective properties of megaferon and GA (glyceric acid) + quercetin supramolecular complexes in rat liver and brain mitochondria have been identified, which are mainly manifested in young animals. In animals, aging has been shown to have a corrective effect of megaferon and glycyrrhizinic acid + quercetin complexes, a decrease in the activity of respiratory chain enzymes in the liver and brain mitochondria, a decrease in ATP (adenosine triphosphate) synthesis and a decrease in protein biosynthesis [5,6]. This will allow in the future to create drugs with geroprotective activity on the basis of supramolecular complexes megaferon and GA + quercetin. However, the biological activity of supramolecular compounds based on menthol and GA (glycyrrhizinic acid) is currently poorly understood, and their effect on the functional parameters of rat liver mitochondria is almost unknown. For this purpose, in our experiments, the effect of menthol, GA (glycyrrhizinic acid) and their supramolecular complexes obtained in different proportions on in vitro and in vivo studies was studied in rat liver mitochondria.

## Research materials and methods.

Preparation of the root extract of the native licorice (Glycyrrhiza glabra L.) and separation of GA from its composition, chemical identification was carried out using standard methods (Figure-1). For the production of supramolecular complex of GA with L - (-) - menthol (Sheme-1), acid solutions of freshly pumped organic solvents (ethyl alcohol, benzene, acetone), as well as alkaline solution and a number of soluble salt solutions were used in the production of L - (-) - menthol.

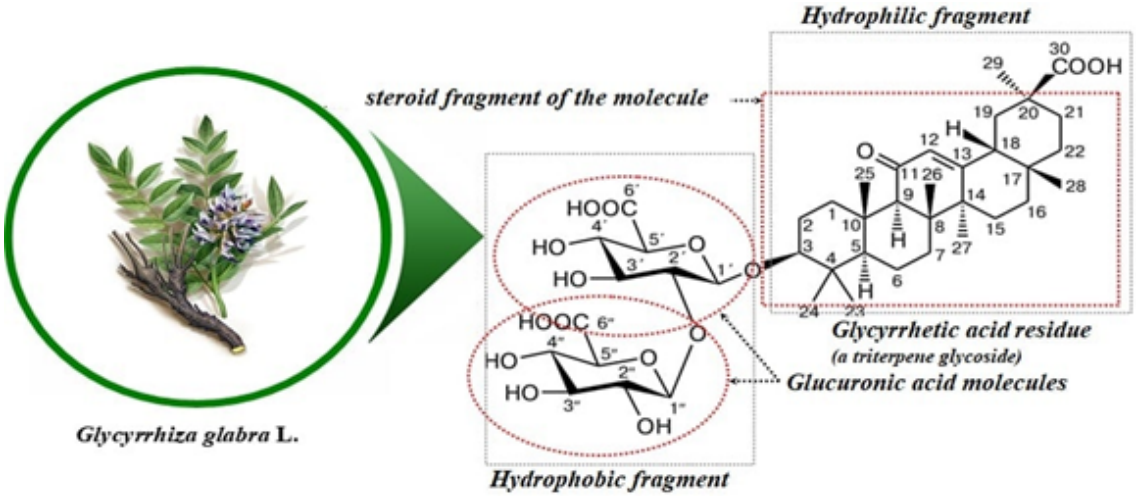
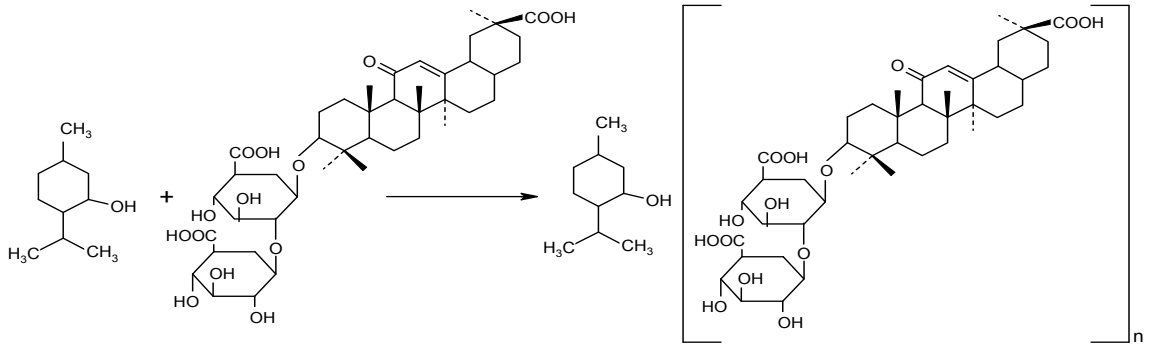



Figure 1. Molecular structures of glycyrrhizic acid (GA) and Menthol.
(A) Glycyrrhizic acid (Empiricalformula - C42H62O16;20β -carboxy-11-oxo-30-norolean-12-en-3β-il-2-O-β-D-glucopyranuronosyl-α-D-glucopyranose-duronic acid) [2]; (B) Menthol (C10H20O)




There n= 2, 4, 9.
Scheme 1. Reaction of the supramolecular complex between GA and Menthol

The feeding and keeping of the animals was carried out in vivary conditions and it was kept at a normal level. The experiments were performed on male white rats without offspring weighing 180-200 g.

In the first in vitro studies of the effect of supramolecular complexes obtained on the basis of menthol, GA and their different ratios on the functional parameters of rat liver mitochondria, the biological activity of the supramolecular complex GA: Mt (4: 1) was more pronounced than other supramolecular compounds. Therefore, in the later stages of our study, it was selected to study the hypoglycemic property in IN VIVO experiments.

## Separation of mitochondria.

Mitochondria were isolated from rat liver using the Schneider [7,8] differential centrifugation method. By decapitating the animal, the liver was removed from the abdominal cavity and placed in a frozen separation medium in a beaker. Separation medium composition: 250 mM sucrose, 10 mM tris-chloride, 1 mM EDTA, pH 7.4. After the liver mass was measured, it was homogenized in a Teflon homogenizer in a 6-fold separation medium by mechanical pressing. For the first time, a 600 g rotation was centrifuged at 0-1 oC for 7 min at an

angle-type rotor TsLR-1, and the nucleus and cell fragments were separated. The supernatant was centrifuged at 6000 g for 15 min at the above temperature for 15 min. The precipitate was suspended in a separation medium in a ratio of 10: 1 (the amount of the separation medium in ml per gram of liver mass). During the experiment, mitochondria were kept in an ice bath.

## Determination of protein content.

We identified mitochondrial protein by biuret reaction [9]. To prepare the biuret reagent, 750 mg of copper sulphate (CuSO4 x 5H2O) and 3 g of sodium potassium hydroxide (NaKC4H4O6 x 4H2O) were dissolved in 500 ml of H2O. To stop oxidation to the prepared solution, 150 ml of 10% NaOH and 1 g of KJ were added and stored in a polyethylene container to a volume of 1 l.

## Determination of mitochondrial PTP (permeability transition pore) permeability.

Mitochondrial swelling (swelling) kinetics (0.3-0.4 mg / ml) was determined by varying the optical density of the mitochondrial suspension in an open cell (volume 3 ml) at 540 nm while constantly stirring at 26 ° C.

## Results and discussion

### Determination of lipid peroxidation (LPO) products in mitochondria.

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 IM. After this step, the mitochondrial suspension was centrifuged at 15,000 rpm for 15 min. Then 2 ml of supernatant was obtained and 1 ml of 75% TBK was infused. 2 ml of H2O 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 (malondialdehyde), the molar coefficient extinction (e = 1.56 × 105 M-1 cm-1) was used in the formula: MDA / mg protein = D / 1.56x30.

$Fe^{2}$ + / ascorbate system was also used to study the LPO process in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its barrier function, resulting in an increase in organelle size and the suppression of mitochondria. This volume change was detected photometrically. IM: KCl - 125 mM, tris-HCl - 10mM, pH 7.4; Concentrations: FeSO4 - 10 μM, ascorbate - 600 μM; mitochondrial volume 0.5 mg / ml;

It is known that many inhibitors of mitochondrial mPTP have antioxidant properties, which in turn effectively affect the process of ATP synthesis. In this regard, in order to determine the antioxidant properties of menthol, GK and their supramolecular complexes in rat liver mitochondria, the effect of lipid peroxidation on mitochondria caused by $Fe^{2}$ + / ascorbate was studied. Initially, the effect of menthol monoterpenoid and GK triterpenoid on liver mitochondrial LPO was determined. After $Fe^{2}$ + / ascorbate was added to the incubation medium, the induced LPO process, i.e., mitochondrial tumor rate, was assumed to be 100%. In this experiment, the

products resulting from the peroxidation of lipids disrupt the barrier function of the mitochondrial membrane, resulting in an increase in its swelling rate relative to control. In experiments, the effect of menthol monoterpenoid on LPO in the mitochondrial membrane at a concentration of 10 μM was not significant. However, concentrations of menthol 20, 30, and 40 μM were 76.5 ± 4.5%, respectively, relative to the control of peroxidation of hepatic mitochondria under $Fe^{2}$ + / ascorbate; Decreases were found to be 89.4 ± 3.0% and 93.5 ± 1.7%, respectively (Fig. 2, A). This result indicates that menthol concentrations of 20, 30 and 40 μM have antioxidant properties. The antioxidant properties of menthol are also consistent with the literature [10] but it was first discovered that it exhibits this activity in the hepatic mitochondria.

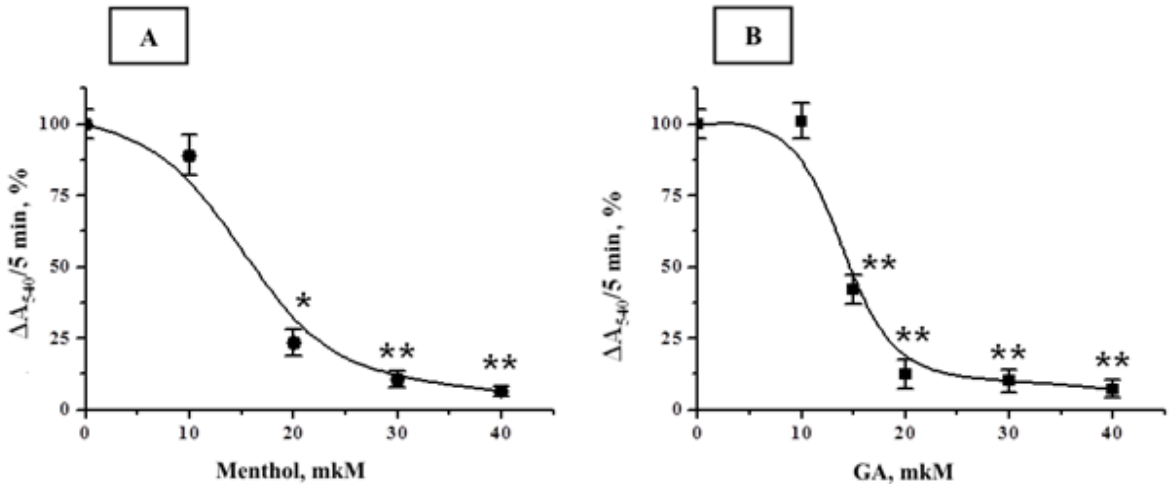



Figure 2. Effect of menthol (A) and GA (B) on hepatic mitochondria to Fe2++ascorbate-inducedLPOprocess (control reliability * R 〈0.05; ** R 〈0.01; n = 5).

In our next experiment, the effect of GA concentrations in the range of 10-40 μM on the peroxidation of lipids induced by $Fe^{2}$ + / ascorbate in rat liver mitochondria was studied (Fig. 2, B). According to the results obtained, the concentration of GA 10 μM did not affect the peroxidation of lipid membranes of the liver mitochondria. However, a concentration of 15 μM of GA was found to reduce LPO formed by the effect of $Fe^{2}$ + / ascorbate on the mitochondrial membrane by 58.0 ± 5.0% relative to control. Concentrations of GA 20, 30, and 40 μM were 87.5 ± 5.0%, respectively, relative to LPO rate control in hepatic mitochondria; Further reductions of 89.1 ± 4.0% and 92.8 ± 3.0% were found (Fig. 2, B). Thus, a concentration of 10 μM of GA does not affect the LPO induced by the $Fe^{2}$ + / ascorbate inducer of the mitochondrial membrane, but it has been noted that its high 15, 20, 30, and 40 μM levels may have a reliable inhibitory effect on LPO intensity[11]. The inhibitory effect of GA and its supramolecular compounds synthesized with various flavonoids on lipid peroxidation in rat heart and brain tissue is consistent with the literature data [11]. However, in our experiments, it was first identified that GA exhibited antioxidant

properties in the hepatic mitochondria under the influence of the $Fe^{2}$ + / ascorbate inducer.

In our next study, the effect of GA and menthol supramolecular complexes on the LPO process induced using the $Fe^{2}$ + / ascorbate system of the rat liver mitochondrial membrane was studied. According to the results, the supramolecular compound of GA and menthol in the ratio GA: Mt (2: 1) to LPO induced by Fe2 + / ascorbate of rat liver mitochondria in the amounts of 20 and 50 μg / ml, respectively, 46.6 ± 5.5% and 57.7 ± A 2.8% inhibitory effect was found (Fig. 3, A).

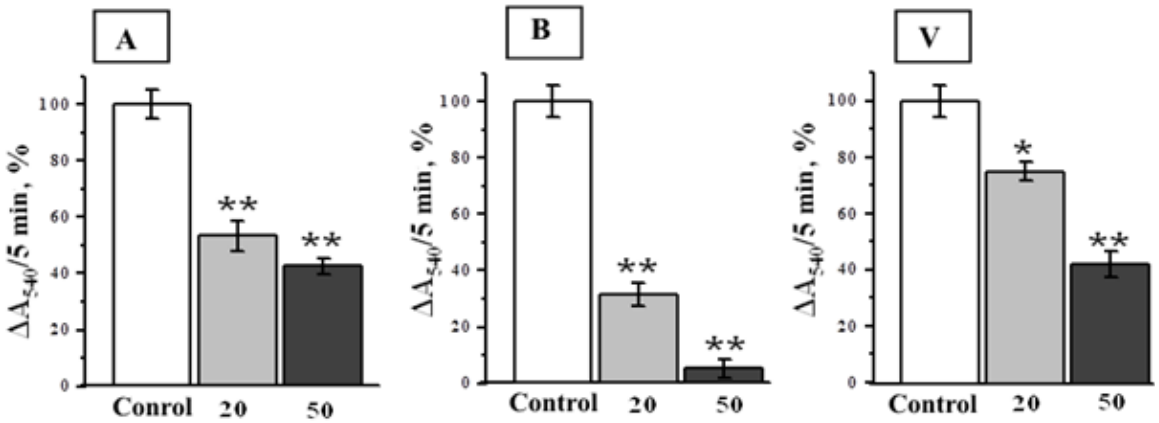



Figure 3. Supramolecular compounds of GA and menthol in ratios GA: Mt (2: 1) (A), GA: Mt (4: 1) (B) and GA: Mt (9: 1) (V) were induced by Fe2++ascorbateofratlivermitochondriaEffectonLPOprocess (reliability with control * R ⟨0.05; ** R ⟨0.01; n = 5).

In a subsequent experiment, the effect of GA and menthol on the rate of lipid peroxidation of the mitochondrial membrane of the rat liver supramolecular complex in the ratio GA: Mt (4: 1) was studied. According to the experimental results, the rates of GA: Mt (4: 1) supramolecular complex of 20 and 50 μg / ml of the lipid peroxidation rate of hepatic mitochondria caused by $Fe^{2}$+ / ascorbate were 68.6 ± 4.4% and9 4.6 ± 3, respectively. 2% inhibitory effect was noted (Fig. 3, B).

The GA: Mt (9: 1) supramolecular complex of GA and menthol also had an inhibitory effect on the peroxidation of lipids located in the hepatic mitochondrial membrane. Their doses of 20 and 50 μg / ml were found to inhibit mitochondrial LPO by 25.0 ± 3.4% and 58.6 ± 4.5%, respectively (Fig. 3, V). The inhibitory effect of the GA: Mt (2: 1) supramolecular complex on the LPO process was found to be effective against the GK: Mt (4: 1) and (9: 1) compounds. GA and menthol and their derived GA: Mt (2: 1), GA: Mt (4: 1) and GA: Mt (9: 1) supramolecular compounds to further demonstrate antioxidant activity in rat liver mitochondria the effect on maleindialdehyde (MDA) levels was also studied.

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