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Research Article

The Influence of Monoammonium Salt of Glycyrrhizinic Acid and Acetylsalicylic Acid on Cardiac and Hematological Processes in Rats with Experimental Myocarditis

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ABSTRACT

This study investigated the effects of GLAS, a supramolecular complex composed of the monoammonium salt of glycyrrhizic acid and aspirin, on vascular-platelet hemostasis, lipid peroxidation (LPO), mitochondrial function, respiratory chain enzyme activity, and the NO-ergic system in an experimental model of adrenaline-induced myocarditis. In vitro, GLAS and aspirin at concentrations of 10^{-2} and 10^{-3} mg/mL significantly reduced ADP-induced platelet aggregation by 2-3 times for aspirin and 2-5 times for GLAS. In vivo, GLAS transiently reduced platelet count and decreased adhesion by 42% and aggregation by 46%. Myocarditis led to a 98% increase in malondialdehyde (MDA), a key marker of LPO, while GLAS reduced MDA levels by 68% in myocardial cell homogenates and by 62.2% in cardiac mitochondria. Additionally, myocarditis impaired oxidative phosphorylation, reduced mitochondrial respiration, and inhibited succinate dehydrogenase and NAD dehydrogenase by 47.6% and 40.7%, respectively. GLAS and aspirin improved mitochondrial function, with GLAS showing greater efficacy in enhancing oxidative phosphorylation and stimulating enzyme activity. Moreover, myocarditis increased nitric oxide metabolite levels due to elevated inducible nitric oxide synthase (iNOS) activity, and GLAS more effectively reduced iNOS expression than aspirin, although it did not fully normalize the parameters. These findings suggest that GLAS, through its pronounced antioxidant and anti-inflammatory effects, offers protective benefits to cardiac muscle cells in myocarditis and may serve as a promising therapeutic agent for cardiovascular complications associated with this condition.

Keywords: experimental myocarditis, monoammonium glycyrrhizinate (MASGA), acetylsalicylic acid (ASA), oxidative stress, myocardial injury, heart inflammation

1. INTRODUCTION

Despite advancements in various treatment methods, cardiovascular diseases remain the leading cause of disability and mortality worldwide. Among these, myocarditis represents a significant challenge in cardiology (Eichhorn et al., 2020). Myocarditis is an inflammatory disease of the heart muscle caused by direct or immune-mediated effects of infections, as well as by chemical and physical factors (Tymińska et al., 2022). It is well established that myocarditis can lead to severe complications, such as chronic heart failure. In recent years, many countries have reported a high incidence of myocarditis, which is partly attributed to the introduction of new, more informative diagnostic methods (Akhmerov and Marban, 2020; Inciardi et al., 2019). According to some researchers, myocarditis accounts for up to 20% of all non-coronary heart diseases and 4-11% of all cardiovascular conditions (Kochi et al., 2020; Long et al., 2020). Due to the significant variability in its clinical manifestations, precise epidemiological data on myocarditis remain unknown. The most representative data are often obtained from autopsy materials (Falleti et al., 2024). It has been shown that myocarditis is present in 8.6-12% of autopsies of young patients who died from sudden cardiac death (Lynge et al., 2019).

Myocarditis is closely associated with lipid peroxidation, a process in which reactive oxygen species (ROS) degrade lipids, particularly those in cell membranes. The inflammatory response in myocarditis leads to the release of mediators that generate oxidative stress, which in turn triggers lipid peroxidation. This process results in the formation of reactive aldehydes and other byproducts that further damage cardiac cells and exacerbate inflammation. Increased ROS production in myocardial tissue contributes to mitochondrial dysfunction, endothelial injury, and impaired heart muscle function (D'Oria et al., 2020). Thus, targeting lipid peroxidation and oxidative stress represents a promising therapeutic approach to mitigate the adverse effects of myocarditis (Li et al., 2024). It is well known that most drugs used to treat cardiovascular diseases have various undesirable side effects on multiple organs and physiological systems (Mladenka et al., 2018). Therefore, the development of low-toxicity, highly effective medications for treating myocarditis remains a critical issue in medicine and pharmacology (Brociek et al., 2023).

Currently, advances in drug development are linked not only to the synthesis of new chemical compounds but also, to a large extent, to the enhancement of existing drugs. This includes creating novel formulations designed for targeted delivery to specific organs (Yadav et al., 2024). One promising strategy involves forming molecular complexes between active pharmaceutical ingredients and plant-derived, carbohydrate-containing metabolites. These metabolites protect the active drugs from rapid metabolism and enhance their transport across biological membranes (Tolstikova et al., 2009). Moreover, such complexation can prolong the drug's action by increasing its affinity for target organ receptors. Among these metabolites, glycyrrhizic acid derived from licorice root is the most studied. Complexation with glycyrrhizic acid has been shown to reduce the required drug dose and associated toxicity while preserving high pharmacological activity (Wahab et al., 2021). These complexes can improve the solubility of hydrophobic compounds in lipids, facilitate cellular penetration, increase bioavailability, enhance therapeutic efficacy, and reduce toxicity. This makes glycyrrhizic acid and its derivatives promising candidates for supramolecular drug delivery systems.

However, the impact of complex formation on the pharmacological properties of cardiovascular drugs, particularly in the context of myocarditis, remains insufficiently studied. Likewise, the potential of other plant-derived carbohydrate-containing metabolites in modifying the efficacy and safety of such drugs is largely unexplored. In our previous studies using experimental models of venous and arterial thrombosis, we demonstrated that the

supramolecular complex GLAS exhibited greater antithrombotic activity than Cardiomagnyl, a pharmaceutical preparation based on acetylsalicylic acid (ASA), which is widely used in antiplatelet therapy (Khamdamova et al., 2024).

Given that complications of myocarditis are often associated with pulmonary and venous thrombosis, this study aimed to investigate the effects of GLAS a supramolecular complex of monoammonium glycyrrhizinate (MASGA) and ASA on vascular-platelet hemostasis and selected biochemical processes in rat cardiomyocytes using an adrenaline-induced myocarditis model.

2. MATERIALS AND METHODS

2.1. Animals

Experiments were conducted on white outbred rats weighing 140 ± 10 g and Chinchilla-breed rabbits weighing 3.0-3.5 kg. Prior to the experiments, all animals were acclimatized for one week under standard laboratory conditions, including a 12-hour light/dark cycle, a constant temperature of 20 ± 2°C, and unrestricted access to pellet food, vegetables, and water. All experimental procedures complied with the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* and were approved by the Institutional Ethics Committee in accordance with the legislation of the Republic of Uzbekistan. The GLAS drug is a supramolecular complex of monoammonium glycyrrhizinate (MASGA) and acetylsalicylic acid (ASA). Blood samples from rabbits were collected at 30, 60, 120, and 180 minutes after the administration of GLAS and the comparator drug, aspirin (Brand, USA). GLAS and aspirin were administered intravenously at single doses of 5 mg/kg and 50 mg, respectively. In vitro experiments employed GLAS and aspirin solutions at concentrations of 10^{-2} and 10^{-3} mg/mL. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood in a 1:9 anticoagulant-to-blood ratio.

2.2. Experimental Myocarditis Model

Experimental myocarditis was induced in rats by subcutaneous administration of 0.1% adrenaline hydrochloride at a dose of 0.3 mg/100 g body weight for three consecutive days, following the method described by Pearce (1906). The animals were divided into four groups: (1) intact control (C), (2) rats with myocarditis (untreated), (3) myocarditis-induced rats treated with a low therapeutic dose of aspirin, and (4) myocarditis-induced rats treated with GLAS at a dose of 5 mg/kg. The treatments were administered daily for seven days, while the control group received an equivalent volume of saline solution. The effects of the drugs on vascular-platelet hemostasis were assessed by evaluating platelet count, adhesion, aggregation, and clot retraction in peripheral blood. Platelet counts were performed using a Goryaev chamber and expressed in 10^9/L, with Peters's B fluid used as the diluent. Platelet adhesive activity was determined by measuring the percentage of platelets adhered after stirring stabilized blood on a magnetic stirrer, as described by Baluda et al. (1980). Platelet aggregation was assessed using the Born method (Born, 1962), with measurements conducted on platelet-rich plasma obtained from rabbits. The aggregation studies were performed using a FEK-54 photoelectrocalorimeter connected to an electric stirrer. In each 3 mL cuvette, 0.8 mL of plasma, 0.1 mL of distilled water, and 0.1 mL of ADP solution (1 mg of ADP dissolved in 5 mL of Tris buffer, pH 7.4) were used. To evaluate the effects of the test substances, different concentrations of GLAS and aspirin were added to the cuvettes in place of distilled water.

2.3. Isolation of Mitochondria and Biochemical Assays

Mitochondria were extracted from rat heart tissue by differential centrifugation following the method described by Gostimskaya and Galkin (2010). To evaluate the respiratory parameters of myocardial mitochondria and oxidative phosphorylation processes, the polarographic method was employed. The intensity of lipid peroxidation (LPO) in cardiac muscle homogenates was assessed by quantifying the formation of malondialdehyde (MDA). To study LPO within the mitochondrial membrane, a Fe^{2+}/ascorbate-induced system was used, which disrupts the mitochondrial membrane's barrier function, resulting in increased organelle volume and mitochondrial swelling.

To determine the activities of succinate dehydrogenase and rotenone-sensitive NADH dehydrogenase in cardiac mitochondria, preparations subjected to a single freeze–thaw cycle were utilized. Enzymatic activity was measured using the polarographic method. Cardiomyocyte respiration was also measured under standard conditions using a rotating platinum electrode via the same technique. The composition of the measurement solution in the polarograph cell was as follows (in mM): sucrose 150, KCl 50, KH_2PO_4 5 (pH 7.4), succinate 5, and glutamate 5. The reaction was initiated by adding the mitochondrial suspension to the cell. Mitochondrial respiratory activity was assessed using key parameters. The respiratory control ratio (V_3/V_4) was determined using the method of Chance, and the ADP/O (P/O) ratio was used to evaluate mitochondrial ATP synthesis efficiency. This ratio reflects the amount of oxygen consumed during the complete conversion of added ADP into ATP. The basal respiration rate (V_4) was determined using an incubation medium containing 5 mM glutamate and 5 mM malate substrates of complex I without the addition of ADP. Subsequently, active respiration (V_3) was initiated by the addition of 200 units of ADP. The difference (V_3-V_4) reflects the rate of oxidative phosphorylation, with the respiratory chain serving as the primary determinant of respiration rate.

In the experiments, it was considered that 1 mL of incubation medium at 26 °C contained 500 ng-atoms of oxygen. The mitochondrial respiration rate in various metabolic states was expressed as the amount of oxygen consumed (in ng-atoms) per 1 mg of mitochondrial protein per minute (Chance and Williams, 1955). In both serum and cardiac muscle homogenates from control and experimental rats, the activities of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) were determined using an enzyme-linked immunosorbent assay (ELISA Kit for Nitric Oxide Synthase 2, Inducible (NOS2), Cloud-Clone Corp.). Total protein content was quantified using the Lowry method with Peterson's modification (Peterson, 1977). All data were statistically analyzed using the OriginPro 8.6 software.

3. RESULTS AND DISCUSSION

Preliminary test tube experiments demonstrated that GLAS exhibits a pronounced anticoagulant effect on both whole blood and plasma of rabbits. Given that platelets and their functional activity play a critical role in thrombus formation within microvessels, the subsequent phase of the research aimed to investigate the effect of GLAS on platelet aggregation in *in vitro* conditions. Specifically, the anti-aggregant activity of GLAS was evaluated by examining its influence on ADP-induced platelet aggregation. The experiments were conducted using platelet-rich plasma (PRP) isolated from rabbits, with GLAS and aspirin added at concentrations of 10^{-2} and 10^{-3} mg/mL. The results, summarized in Table 1, show that both concentrations of aspirin significantly reduced the rate of platelet aggregation, by approximately two- and three-fold, respectively. In contrast, GLAS produced an even more

pronounced inhibitory effect, ADP-induced platelet aggregation was reduced by 2-2.5 times relative to aspirin, and by 5-6 times compared to the control group. These findings suggest that GLAS possesses strong anti-aggregant properties under *in vitro* conditions. However, a review of the literature on compounds with anti-aggregant activity reveals that many agents considered "promising" during preclinical *in vitro* evaluations often fail to demonstrate sufficient anticoagulant efficacy *in vivo* (Jannati et al., 2024). Therefore, it became essential to assess whether the anti-aggregant effect of GLAS persists following intravenous administration in experimental animals.

Table 1. Effect of GLAS and aspirin in *in vitro* experiments on ADP-induced platelet aggregation (n=6; M±m)

Drugs	**10^{-2} mg/mL**		**10^{-3} mg/mL**	
	Aggregation, sec	**%**	**Aggregation, sec**	**%**
Control	15 ± 1.2	100	15 ± 1.2	100
Aspirin	30 ± 2.5*	200	45 ± 3.6*	300
GLAS	75 ± 6.8*	500	90 ±8.7*	600

*p<0.01 compared to the control

In the subsequent series of experiments on rabbits, the effect of GLAS on platelet count and functional activity was investigated. Table 2 presents the results of GLAS and a low therapeutic dose of aspirin on both the number and functional status of rabbit platelets. Measurements were recorded at baseline and at 30, 60, 120, 180, and 240 minutes following drug administration to assess the time-dependent effects. As shown in Table 2, GLAS administration caused minimal changes in platelet count during the first 30-60 minutes. A significant reduction approximately 29% was observed at 120 minutes post-administration. This decline was transient, as platelet counts began to recover and showed a slight increase at subsequent time points. In parallel, the effect of GLAS on platelet adhesion was assessed, revealing a 33-46% reduction between 30 and 120 minutes after administration. To further evaluate the effect of GLAS on vascular-platelet hemostasis, clot retraction time was measured. The results indicated a notable increase in clot retraction time, ranging from 25% to 100%, between 60 and 180 minutes following administration. These findings support the role of GLAS in modulating platelet function and hemostatic parameters in vivo.

Table 2. Effect of GLAS on platelet count and functional activity (n=8; M±m)

Indicators	**Time after drug administration (min.)**				
	Amount	**30**	**60**	**120**	**180**
Platelets, 109/l	240 ± 14	220* ± 10	215*±12	175*±15	266 ±10
Clot retraction, min	16 ± 1.0	20* ± 1.4	20* ±1.4	30* ±1.6	32* ±1.5
Platelet adhesion, %	29 ± 1.0	15,5*±1.2	19,0*±2.0	16,3*±1.4	17* ±1.2
Spontaneous aggregation, %	33,4 ± 2.4	10,0*±1.1	16,3*±1.4	18* ±1.4	19* ±2.0

*p<0.05 compared to baseline

Similar experiments were carried out using a low therapeutic dose of aspirin (50 mg/kg). Aspirin administration led to a reduction in platelet adhesion, aggregation, clot formation, and platelet count. Although both aspirin and GLAS produced comparable effects on platelet functional activity, a significant difference was observed in the effective doses of the two drugs. GLAS was effective at a dose of 5.0 mg/kg, which is ten times lower than that of aspirin. The results from both *in vitro* and *in vivo* experiments demonstrated that GLAS and aspirin significantly inhibited platelet aggregation and functional activity. These findings align with existing literature on the antiplatelet effects of aspirin, which remains widely used due to its

ability to inhibit the cyclooxygenase pathway involved in platelet activation. However, our results revealed that GLAS exhibited a stronger effect on the studied parameters despite being administered at a substantially lower dose. This enhanced efficacy is attributed to the presence of monoammonium glycyrrhizinate (MASGA), which possesses broad pharmacological activity. MASGA appears to enhance the antiplatelet effect of acetylsalicylic acid (ASA), allowing for a lower effective dose and a more pronounced therapeutic outcome.

According to literature, the myocardium primarily derives its energy from the oxidation of free fatty acids and glucose, contributing approximately 70% and 30%, respectively, to total oxygen consumption (Maack et al., 2018). Under hypoxic conditions, the accumulation of unoxidized fatty acid intermediates serves as a major substrate for free radical reactions, which contribute to membrane damage and cardiomyocyte injury. Moreover, oxygen deprivation disrupts mitochondrial respiratory chain function, leading to the formation of superoxide radicals. Given this background, the next phase of our study focused on evaluating lipid peroxidation (LPO) in rat cardiac muscle homogenates using an adrenaline-induced myocarditis model. LPO intensity was assessed by measuring the levels of malondialdehyde (MDA), a secondary product of lipid peroxidation. The findings are presented in Figure 1.

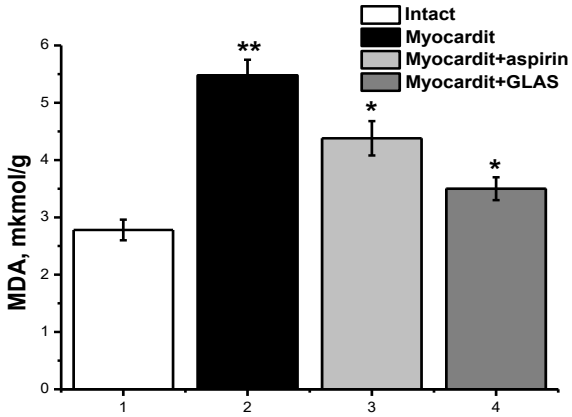

Figure 1. Effect of GLAS and aspirin on MDA levels, a product of lipid peroxidation (LPO), in rat heart homogenate during experimental myocarditis (*p<0.05; **p<0.01; n=8)

As shown in Figure 1, adrenaline-induced myocarditis resulted in a 95% increase in malondialdehyde (MDA) levels in the cardiac muscle cell homogenates compared to intact animals, indicating a substantial intensification of lipid peroxidation (LPO) due to adrenaline exposure. Treatment of myocarditic animals with GLAS and aspirin over a 7-day period led to a significant reduction in MDA levels by 68% and 43%, respectively, with GLAS demonstrating superior efficacy in suppressing MDA formation. To further validate these findings, additional experiments were conducted to assess LPO in mitochondrial membranes using a Fe^{2+}/ascorbate-induced system. The results, presented in Figure 2, demonstrate that the presence of Fe^{2+} and ascorbate in the incubation medium markedly intensified lipid peroxidation, resulting in a disruption of mitochondrial membrane integrity and a substantial increase in mitochondrial volume compared to the control. Specifically, mitochondrial swelling in the cardiac tissue of myocarditic rats increased 7.2-fold relative to intact animals, reflecting enhanced oxidative damage within mitochondria.

These findings suggest that myocarditis is associated with pronounced mitochondrial dysfunction, where increased free radical formation contributes to the deterioration of critical organelle functions. Notably, treatment with GLAS and aspirin effectively attenuated these pathological changes, reducing mitochondrial swelling by 62.6% and 42.6%, respectively, compared to untreated myocarditic rats. These results indicate that both GLAS and aspirin

possess antioxidant properties that confer protective effects on the mitochondria of cardiac muscle cells. Their administration led to the stabilization and partial restoration of the lipoprotein structure of mitochondrial membranes in animals with myocarditis.

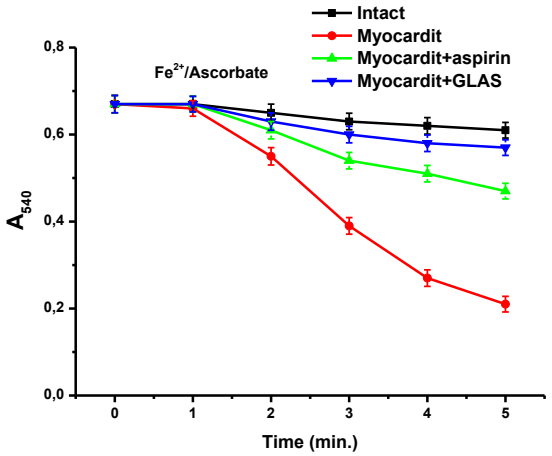

Figure 2. Effect of GLAS and aspirin on Fe^{2+}-ascorbate-induced changes in mitochondrial volume in rat hearts during experimental myocarditis induced by adrenaline ($p<0.05$ in all cases; n=6)

In the next phase of our research, we evaluated the functional state of mitochondria in cardiomyocytes isolated from both myocarditic and intact animals, with the findings presented in Figure 3. Succinate and malate were employed as substrates for mitochondrial oxidation in these experiments. Mitochondria isolated from the cardiac tissue of intact animals exhibited high coupling efficiency and well-preserved oxidation and phosphorylation processes during the oxidation of both substrates. In contrast, myocarditis induced significant mitochondrial dysfunction, evidenced by reduced respiration rates, impaired phosphorylation efficiency, and diminished respiratory control. Specifically, oxygen consumption in state V_3 (active respiration during the ADP phosphorylation phase) decreased by 44% when succinate was used as the substrate (Figure 3A). Additionally, alterations in state V_4 (resting respiration following ADP consumption) contributed to a marked decline in both the respiratory control ratio (RC) and the ADP/O (P/O) ratio (Figure 3B), indicating impaired mitochondrial bioenergetic performance in myocarditic animals.

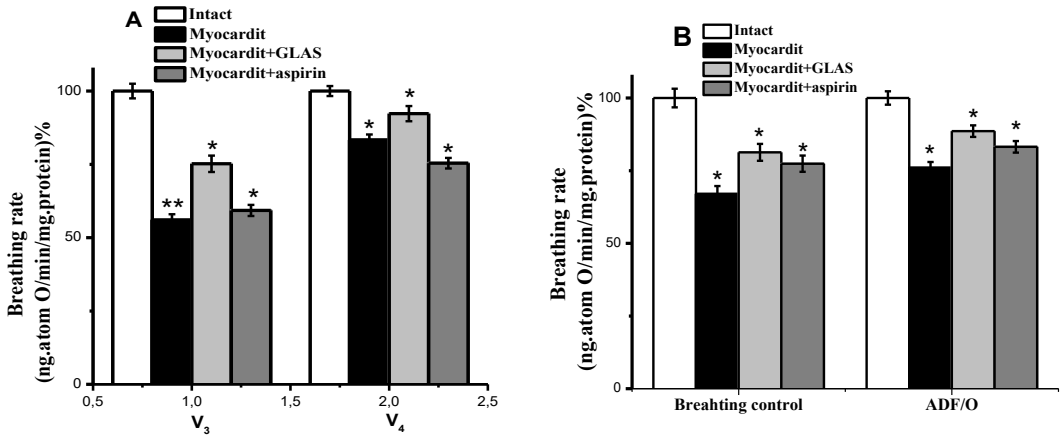

Figure 3. Effect of GLAS and the drug aspirin on the respiration and oxidative phosphorylation of rat heart mitochondria in experimental myocarditis induced by adrenaline; V_3 - the rate of oxygen consumption during the ADP phosphorylation cycle; V_4 - the rate of oxygen consumption after the ADP phosphorylation cycle; (*$p<0.05$; **$p<0.01$; n=6)

Administration of the GLAS complex at a dose of 5.0 mg/kg to rats with experimental myocarditis led to an increase in mitochondrial oxygen consumption rates by 23% in the active state (V_3) and 19% in the resting state (V_4), compared to untreated myocarditic animals. Similar improvements were observed when malate was used as the oxidation substrate, suggesting a broad enhancement of mitochondrial respiratory activity. Aspirin treatment also produced some degree of functional recovery in cardiomyocyte mitochondria; however, its effect was significantly less pronounced than that of GLAS.

In the subsequent series of experiments, changes in the activity of succinate dehydrogenase and rotenone-sensitive NADH dehydrogenase were assessed in the cardiac mitochondria of myocarditic rats. The influence of GLAS and aspirin on these mitochondrial enzymes under pathological conditions was also evaluated (Figure 4). The data showed that myocarditis reduced the activity of succinate dehydrogenase and NADH dehydrogenase by 47.6% and 40.7%, respectively, compared to intact animals. Treatment with GLAS partially restored succinate dehydrogenase activity by 35%, whereas aspirin achieved only a 17.7% improvement (Figure 4A). Additionally, GLAS demonstrated a stabilizing effect on NADH dehydrogenase activity (Figure 4B), although neither treatment fully restored enzyme levels to baseline. These results are consistent with previous studies (Dalimova et al., 2020) and indicate that experimental myocarditis is associated with impaired mitochondrial respiration and oxidative phosphorylation, along with inhibition of key respiratory chain enzymes. This enzymatic inhibition likely results not only from direct mitochondrial dysfunction but also from increased free radical generation in the respiratory chain, triggered by toxic doses of adrenaline. Both GLAS and aspirin exhibited mitochondrial stabilizing effects, with GLAS demonstrating superior efficacy in preserving mitochondrial enzyme activity.

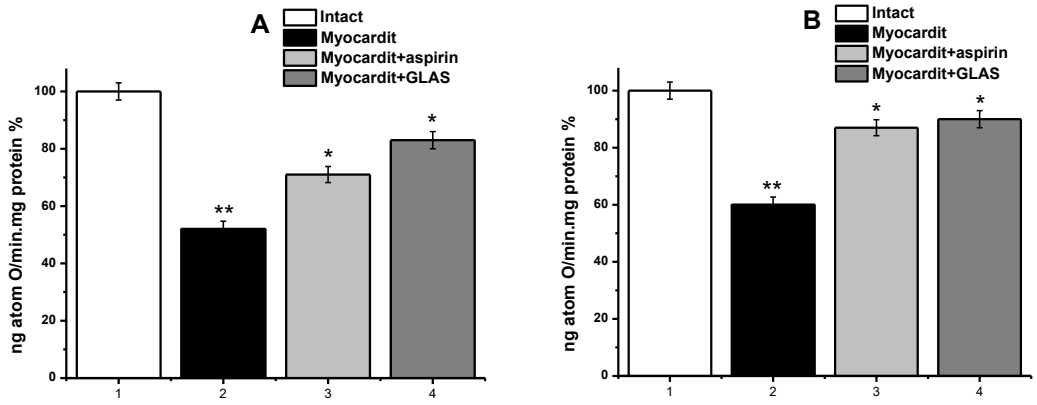

Figure 4. Effect of GLAS and aspirin on the activity of mitochondrial respiratory chain enzymes: succinate dehydrogenase (A) and rotenone-sensitive NADH dehydrogenase (B) in rat heart mitochondria during experimental myocarditis induced by adrenaline (*$p<0.05$; **$p<0.01$; n=6)

It is well established that glycyrrhizic acid and its derivatives inhibit the activation and nuclear translocation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that regulates antioxidant defenses as well as proinflammatory, proapoptotic, and oncogenic signaling pathways under conditions of cellular stress. Suppression of NF-κB activity is associated with reduced expression of proinflammatory cytokines (IL-1α, IL-1β, IL-6), chemokines (CCL5, MCP-1), adhesion molecules (VCAM-1, NCAM), and oxidative stress markers (MnSOD, GST, HO-1, GPx, NOX2), along with decreased production of reactive oxygen species (ROS) and inhibition of caspase-3 (Li et al., 2014; Abo et al., 2018; Lingappan, 2018). Furthermore, GC has been shown to prevent mitochondrial dysfunction, support mitochondrial energy metabolism, increase mitochondrial

mass and ATP production, stimulate mitochondrial biogenesis, and normalize gene expression profiles (Rashedinia et al., 2019). In experimental models of ischemia reperfusion injury, GC reduced coronary artery endothelial cell apoptosis by inhibiting autophagy/mitophagy, decreasing mitochondrial ROS levels, and preserving mitochondrial membrane potential (Tang et al., 2020).

Numerous studies have emphasized the role of nitric oxide (NO) in regulating vascular tone (Treuer and Gonzalez, 2015). NO is a key signaling molecule involved in the control of basal arterial pressure and blood flow. Alterations in NO production either deficiency or excess are commonly observed in various cardiovascular disorders, including myocarditis (Schror and Rauch, 2015). Since myocarditis is frequently accompanied by acute hypoxia, the ability of cardiotonic agents to modulate nitric oxide metabolism is a critical factor in therapeutic development. Three isoforms of nitric oxide synthase (NOS) are responsible for NO synthesis: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). While eNOS and nNOS are membrane-bound and regulate physiological NO production in cardiomyocytes, iNOS is a soluble enzyme that is minimally expressed under normal conditions but upregulated during inflammation and oxidative stress (Hennekens et al., 2010). With this background, the next phase of our study investigated the activities of nitric oxide synthase isoforms in rats with adrenaline-induced myocarditis. The results are presented in Figure 5.

As shown in Figure 5A, eNOS activity was suppressed by 37.6% in myocarditis animals compared to intact controls. Treatment with GLAS led to a partial restoration of eNOS activity by 24%, while aspirin showed a weaker effect, increasing eNOS activity by only 17%. In contrast, the analysis of iNOS activity (Figure 5B) revealed a substantial increase by 95.5% in myocarditis animals, consistent with inflammatory activation.

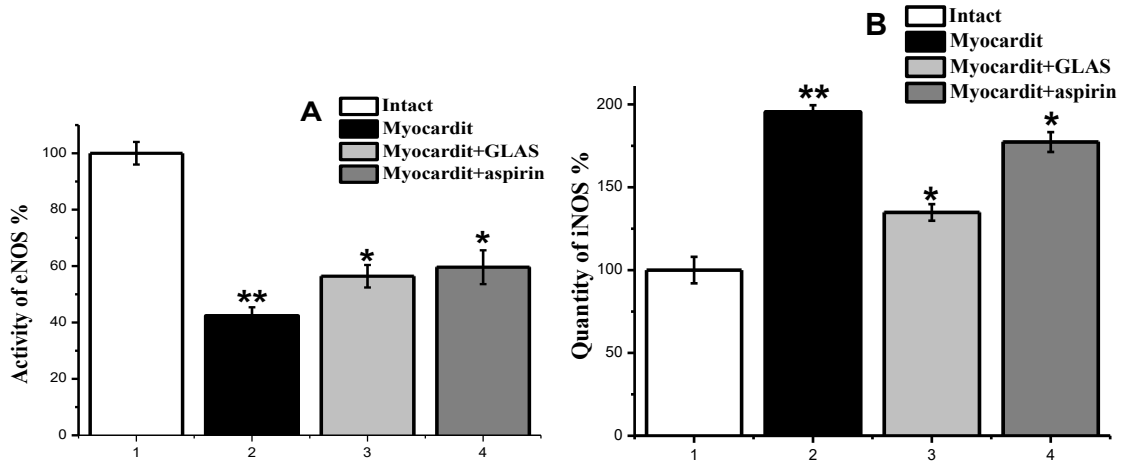

Figure 5. Effect of GLAS and aspirin on the activity of endothelial nitric oxide synthase (eNOS) (A) and the quantity of inducible nitric oxide synthase (iNOS) (B) in the plasma of myocarditis rats (*p<0.05; **p<0.01; n=7)

According to existing literature, the stimulation of inducible nitric oxide synthase (iNOS) is regulated by multiple factors and is typically expressed in endothelial cells and macrophages only under pathological conditions, particularly during inflammatory responses (Bonafede, 2018; Ewid, 2020). iNOS plays a central role in the synthesis of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1β (IL-1β), and is known to be expressed in cardiac tissue during myocardial infarction, myocarditis, and heart failure (Totzeck et al., 2017). In our study, correction of the observed increase in iNOS activity in myocarditis was evaluated following treatment with GLAS and aspirin. The results showed that GLAS reduced

iNOS activity by 60%, while aspirin led to a smaller reduction of 19%, indicating the superior modulatory effect of GLAS on this inflammatory marker.

A similar pattern was observed in heart tissue homogenates from myocarditic rats, as illustrated in Figure 6. In this model, eNOS activity was suppressed by 21%, while iNOS levels nearly doubled compared to intact controls. These findings are consistent with previous reports in the literature (Costa et al., 2017; Baltina, 2003). According to these studies, the modulatory effect of aspirin on eNOS and iNOS activity is likely related to its acetylating properties. The pharmacological activity of low-dose aspirin (100-300 mg/day) in the cardiovascular system is believed to be largely, if not entirely, dependent on the acetylation of specific molecular targets (Hennekens et al., 2010). Furthermore, the enhanced efficacy observed with the GLAS supramolecular complex may be attributed to the synergistic interaction between aspirin and monoammonium glycyrrhizinate (MASGK), which appears to potentiate the anti-inflammatory and nitric oxide-modulating properties of aspirin.

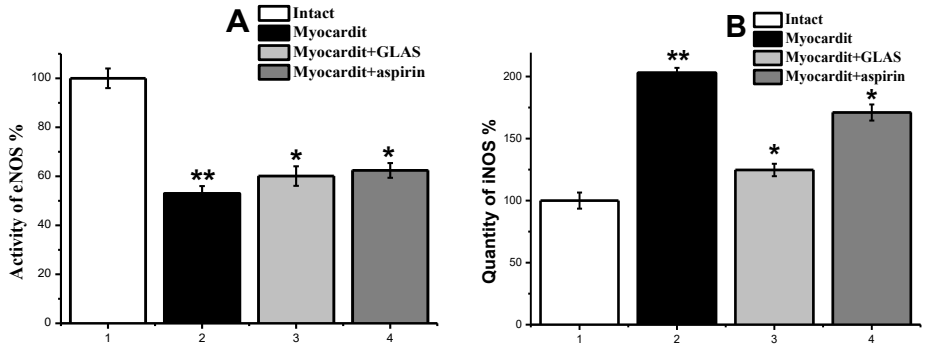

Figure 6. Effect of GLAS and aspirin on the activity of endothelial nitric oxide synthase (eNOS) (A) and the quantity of inducible nitric oxide synthase (iNOS) (B) in the heart homogenate of myocarditis rats (*p<0.05; **p<0.01; n=7)

Currently, various pharmaceutical preparations containing glycyrrhizic acid derivatives extracted from licorice root are used in clinical practice. The formation of complexes between GA derivatives and therapeutic agents has been reported to reduce the toxic side effects of the primary drug and enhance its pharmacological efficacy. Glycyrrhizic acid is known to act as a plant-derived selective thrombin inhibitor. It has been shown to attenuate the elevation of serum marker enzymes such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) in blood, prevent glycogen depletion in the myocardium, increase total lipid content, activate lipid peroxidation, and reduce antioxidant activity in serum. Moreover, GA improves electrocardiographic parameters and shows therapeutic promise in cardiac pathological conditions involving inflammation and necrosis, particularly myocarditis and myocardial infarction (Shi et al., 2021).

According to Kuhl and Schultheiss (2009), cardiovascular pathologies are commonly associated with the suppression of endothelial nitric oxide synthase (eNOS) activity and a concurrent upregulation of inducible nitric oxide synthase (iNOS). In myocarditis, pro-inflammatory cytokines significantly increase iNOS expression, resulting in excessive NO production. This overproduction of NO exerts cytotoxic effects on myocardial cells, amplifies the inflammatory response, promotes cardiac muscle remodeling, and contributes to the progression of heart failure. Biopsy samples from patients with heart failure have confirmed elevated iNOS levels and reduced eNOS activity (Li et al., 2020). Under oxidative stress conditions characteristic of myocarditis, the surplus NO molecules react with other free radicals, producing highly reactive species that damage both cardiac and vascular cells. The

administration of GLAS under these pathological conditions has been shown to induce beneficial changes in the disrupted NO-ergic system of cardiac and vascular tissues in myocarditis-induced rats, highlighting its therapeutic potential as a modulator of nitric oxide metabolism and inflammatory response.

4. CONCLUSION

Thus, as a result of the conducted studies it was established that the model of experimental adrenaline myocarditis created in rats is accompanied by serious disturbances in myocardial cells, expressed by almost 2-fold increase in the LPO process, decrease in the coupling of oxidative phosphorylation and mitochondrial respiration parameters, as well as inhibition of the enzymes of the respiratory chain of mitochondria of cardiomyocytes - succinate dehydrogenase and NAD-dehydrogenase by 47.6% and 40.7%, respectively, compared to intact animals. At the same time, an increase in nitric oxide metabolites was found, due to an increase in the activity of iNOS by 95.5 and 200%, respectively. Treatment of such animals with the GLAS supramolecular complex showed that it has pronounced antioxidant activity, the ability to restore the functional state and activity of the respiratory chain enzymes of the mitochondria of mouse heart cells, as well as the ability to normalize the NO-ergic system of the myocardium and blood vessels. The combined use of cardiotropic drugs is one of the most promising areas of pharmacotherapy for various cardiovascular diseases. This applies to both traditionally used cardioprotectors and new drugs that are only undergoing clinical trials. The prospects and possibilities of the combined use of glycyrrhizic acid and its derivatives with various drugs are determined, on the one hand, by the pharmacotherapeutic properties of the molecule itself, and on the other hand, by its features as a form-forming substance. The ability of glycyrrhizic acid to form self-associates and complexes with drugs leads to an increase in the degree of their absorption, an increase in the therapeutic effect and a decrease in toxicity, including by reducing the dose used, which was shown in our studies.

Conflict of Interest
The authors declare that they have no competing interests.

Author Contribution Statement
Surayyo Dalimova and Muslima Yunusova: Conceptualisation and design; Muslima Yunusova, Sherali Kuziev, Gulbahor Umarova, Nigora Hamdamova, Guzal Mukhammadjonova, Mukhlisa Dadakhonova, Sobir Khamroev: Methodology and analysis; Zaynieva Makhbuba, Gafurov Maxmudjon, Surayyo Dalimova, Farkhod Eshboev, Sherali Kuziev: Writing, review & editing.

Data Availability Statement
The authors confirm that the data supporting the findings of this study are available within the article.

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