

Effect of Derivatives of Glycyrrhetic Acid on The Intensity of Free Radical Processes During Immobilization Stress

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Abstract. The effect of glycyrrhizic acid derivatives (18-dehydroglyceric acid - 18 DGA, monoammonium salt of glycyrrhizic acid - MSGA) on the intensity of free radical processes during immobilization stress was studied. The experiments were performed on mature male rats with an initial weight of 165–185 g. Experimental groups of animals were formed taking into account body weight. The animals were divided into 4 groups of 6 each. The first group consisted of healthy animals, while the rats of the remaining groups were subjected to immobilization stress. In contrast, in animals treated with a monoammonium salt of glycerol acid, the number of rats with mucosal injury was reduced by 2 times, and the number of linear and fine-dot wounds by 15.3 and 57.7%, respectively. The number of wounded rats was 67% and the Pauls index decreased to 64.1%. We noted high gastro protective activity in a group of animals treated with 18-dehydroglyceric acid. Thus, the number of mucosal injuries in this group of animals decreased by 65.3%, and the number of spot injuries decreased by 71.8%. It is noteworthy that the number of linear erosions decreased by 36.6%. From the data in Table 2 in these groups, it can be seen that the number of rats with ulcers decreased by 45%.

Keywords: Peptic ulcer, 18-dehydroglyceric acid, monoammonium salt of glyceric acid, immobilization stress, Sel'e triad.

Introduction. In the mechanism of the development and progression of the pathology of organs and the systems, as it is known, an increase in the processes of lipid peroxidation is assigned. Proceeding from this, many scientists consider the use of substances that suppress the intensity of free radical processes to be pathogenetically justified [1,2,3].

In this regard, it seems relevant to study the significance of inhibition of lipid peroxidation (LPO) processes in the implementation of the pharmacological effect of anti-inflammatory drugs. Glycyrrhetic acid derivatives have distinct anti-inflammatory activity [4,5]. However, their activity as a means of inhibiting the development of LPO remains insufficiently studied.

Purpose of the study: To study the effect of derivatives of glycyrrhetic acid (18-dehydroglycyrrhetic acid - 18 DHA, monoammonium salt of glycyrrhetic acid - MASGA) on the intensity of free radical processes during immobilization stress.

Material and research methods: The experiments were carried out on sexually mature male rats with an initial weight of 165-185 g. The experimental animals were kept in a vivarium (with natural lighting, at a temperature of 22-24°C and a relative humidity of 40-50%) using a standard diet. The animals were quarantined and acclimatized in a vivarium for 14 days. Experimental studies were carried out in accordance with the rules of a quality laboratory (GLP) for preclinical studies, as well as the rules and International Recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Research Purposes (ETS No. 123) (Strasbourg, 03/18/1986).

The experimental groups of animals were formed taking into account the body weight. The animals were divided into 4 groups of 6 animals each. The first group consisted of intact animals, and the rats of the other groups were subjected to immobilization stress.

Immobilization stress was simulated by fixing laboratory animals in the supine position for 18 hours. Experienced rats were injected orally with 18-DHA and MASGA at the same dose of 50 mg / kg one hour before stress exposure. Control rats received an equivalent volume of distilled water. Animals of the control and experimental groups, at the end of the stress exposure, were subjected to one-stage decapitation under

general ether anesthesia and the severity of the Selye triad was determined: the mass of the adrenal glands, spleen and thymus gland (thymus), the state of the gastric mucosa(GM), blood was collected in which the concentration of products was determined LPO and the activity of enzymes of the antioxidant system. The stomachs of all rats were removed and washed with cold saline. In the gastric mucosa (GM), the total, as well as the number of stripe-like and punctate lesions were calculated separately, and the Pauls index was calculated.

The content of hydroperoxides was determined by the spectrophotometric method [6], and – MDA (monoammonium dialdehyde) using thiobarbituric acid [7]. Superoxide dismutase (SOD) activity was determined by the ability of the enzyme to inhibit the autoxidation of adrenaline at pH 10.2 [8], catalase activity - by the method based on the ability of hydrogen peroxide to form persistent coloration with molybdenum salts [9]. The obtained digital material was processed statistically using the standard package of the Biostat 2009 program according to the well-known method of variation statistics with an assessment of the significance of indicators (M + m) and differences in the samples under consideration by Student's t-test. Differences in the compared groups were considered significant at a significance level of 95% (p <0.05).

Results and its discussion. At present, pathologies in the pathogenesis of stress are quite common, and the lack of effectiveness of modern medications dictate the need to develop effective and safe drugs that can increase the body's adaptive capabilities and provide an adequate level of physical and cognitive activity in experimental conditions. In this regard, the most promising direction is the development of adaptogens based on medicinal plants, which have a number of advantages over synthetic drugs [1,2,10].

The results of the studies have shown that under immobilization stress there are distinct changes characteristic of the stress reactions of the Selye triad, expressed in the involution of immunocompetent organs and adrenal hypertrophy. Thus, in control rats, compared with healthy rats, the weight of the thymus decreased by 55.4%, and the weight of the spleen by 47.5%; at the same time, an increase in the weight of the adrenal glands by 60.0% was noted. Prophylactic administration of the studied drugs at a dose of 50 mg / kg orally had a pronounced antistress effect, as evidenced by a decrease in the degree of adrenal hypertrophy by 28.4% and 17.9% and a significant increase in the relative mass of the thymus (by 96.3% and 74.8%) and spleen (53.6% and 35.2%, respectively, in the groups receiving 18-DHA and - MASGA, compared with the control). It is noteworthy that such an effect under immobilization stress is observed in cordekaim and eleutherococcus [11,12].

Table №1
 Effect of glycyrrate acid derivatives on the mass of the adrenal, spleen and thymus glands(M ± m)

Group	Thymus gland, mg	Spleen, g	Adrenal gland, mg
Control	202±17.4	0.391±0.031	76.8±7.3
Experienced	91.6±8.8*	0.196±0.038*	123.4±7.1*
Experimental + 18DHA	179.8±14.3 ^α	0.321±0.031 ^α	88.4±8.1 ^α
Experimental + - MASGA	160.1±15.2 ^α	0.265±0.024*	101.3±9.6

Note: * - statistically significant differences compared to control

α - statistically significant differences compared to untreated animals

Stress is an important pathogenetic factor in the development of gastropathy. Therefore, it is believed that one of the manifestations of stress reactions is damage to the coolant. In thermal injuries, myocardial infarction, after surgery, the development of stress leads to damage to the (GM) [13, 14]. The results of studies carried out in this regard have shown that the immobilization of animals leads to severe damage to the coolant. So, in animals of the control group, all rats showed damage to the (GM) to one degree or another, with a total area of damage of 1.48±0.73 mm². In this case, the number of stripe-like injuries was 1.31 ± 0.47, and the number of punctate injuries was 4.61 ± 0.67. Consequently, the development of stress during the

immobilization of animals leads to severe gastropathy. The mechanism of this condition is known to be due to increased activity of the corticoadrenal system [14].

In contrast to this, in animals treated with the monoammonium salt of glycyrrhetic acid, the number of rats with injuries to the GM decreased by 2 times, and the number of stripe and pinpoint injuries by 15.3 and 57.7%, which caused a decrease in the area of damage by 3.4%, although the number of rats with ulcers was 67%, and the Pauls Index decreased by 64.1%. We noted a higher gastroprotective activity in the group of animals treated with 18-dehydroglycyrrhetic acid. Thus, the amount of damage to the coolant in animals of this group decreased by 65.3%, and the number of point injuries by 71.8%. It is noteworthy that the number of strip erosion decreased by 36.6%. As can be seen from the data of table No. 2 in the indicated groups, the number of rats with ulcers decreased to 45%.

Table №2
 The effect of some derivatives of glycyrrhetic acid on
 state of the coolant during immobilization stress (M ± m)

Groups	Number of coolant lesions	Number of stripe erosions	Number of pinpoint erosions	Pauls index	Rats with ulcers%	Area of damage in mm ²
Control	6,140	1,31±0,47	4,61±0,67	3,34	100	1,48±0,18
18-DHA	2,13±0,70*	0,83±0,12	1,30±0,32*	0,49	45	0,91±0,10
MASGA	3,06±0,57*	1,11±0,15	1,95±0,29*	1,20	67	1,43±0,24

Note: * - statically significant differences compared to control

Thus, the studied derivatives of glycyrrhetic acid have a distinct gastroprotective effect under immobilization stress. According to the data of morphological studies by M.A. Mamadzhanova [15], under the influence of 18-dehydroglycyrrhetic acid, a more accelerated and complete epithelialization of the wound surface occurs, the epithelium and connective tissue differentiate earlier, and the muscle layers of the injured stomach wall are restored, which indicates a stimulating and regulating effect drug based on the stages of the regeneration process (rebuilding, proliferation, differentiation).

Therefore, based on the results of experimental studies, it can be stated that glycyrrhetic acid derivatives prevent the development of a stress reaction. This effect of drugs is probably due to the supression of free radical processes that play an important role in the pathogenesis of many diseases. At the same time, it is considered proven that in the development of pathology by the universal reaction of the organism to the action of various exogenous and endogenous damaging factors, an important role is given to oxidative stress. The latter develops as a result of a violation of the pro- and antioxidant balance with the intensification of the processes of lipid peroxidation (LPO). To suppress the latter, it is considered quite justified to use antioxidants - substances with the ability to quench free radicals [1,16,17].

Analysis of the results of experimental studies showed that the immobilization effect in rats causes a significant increase in the free radical process manifested in an increase in lipid peroxidation products against the background of a decrease in the activity of enzymes of the antioxidant system. Thus, in experimental animals, in comparison with the control in blood serum, the concentration of acyl hydroperoxides increases almost threefold, and MDA - 4.5 times. Against this background, the activity of catalase was reduced by 26%, and SOD - by 42.6%. Consequently, under immobilization stress, there is a substantial increase in free radical processes, the probable cause of which, on the one hand, is a decrease in the activity of enzymes of the antioxidant system: SOD and CT(Catalase), and on the other, an increase in the intensity of free radicals. We observed a different picture in animals preemptively receiving glycyrrhetic acid derivatives. Thus, in the group of rats receiving 18-DHA, the ACHP (acyl hydroperoxide) content was 63% low compared to untreated animals, and in the group of rats receiving MASGA - by 57%. It is noteworthy that, along with the initial LPO products, under the influence of 18-DHA and MASGA, the level of the intermediate product MDA decreases by 51% and 45.5%, respectively. Along with this, under the influence of the studied drugs, there is an increase in catalase activity by 85% and 53.9%, and such SOD activities by 112.5 and 100%, respectively.

Table №3

Effect of 18-DHA and MASGA on the content of lipid peroxidation products and the activity of SOD and catalase in the blood during immobilization stress in rats

Groups	Hydroperoxides D233	MDA, nmol / ml	Catalase, nmol / min.mg protein	SOD, conventional units / mg protein
Control	1.29±0.111	0.52±0.036	0.0175±0.0007	0.195±0.021
Experimental + H2O	4.94±0.122*	2.86±0.101*	0.0130±0.0008*	0.112±0.009*
Experimental + 18 DHA	1.84±0.093*. ^α	1.40±0.106*. ^α	0.0241±0.0018 ^α	0.238±0.012 ^α
Experimental + MASGA	2.14±0.106*. ^α	1.56±0.079*. ^α	0.0200±0.0018 ^α	0.224±0.022 ^α

Note: * - statistically significant differences compared to control

α - statistically significant differences compared to untreated animals

Conclusion. Analyzing the results obtained, it can be concluded that the derivatives of glyceric acid 18-DHA and MASGA have a pronounced antioxidant property, which is probably due to their anti-inflammatory effect. Taking into account the low toxicity and high anti-stress and anti-ulcer properties, 18-DHA can be recommended as a means of prevention and treatment of gastropathy of various origins.

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