EFFECT OF HYDROLYZABLE TANNINS OF *EUPHORBIA* PLANTS ON THE RAT ANTIOXIDANT SYSTEM OF UNDER OXIDATIVE STRESS

Authors' contributions

This work was carried out in collaboration between all authors.

Abstract.

Aim: Study of the effect of hydrolyzable tannins isolated from Euphorbia plants on alloxan-induced OS under in vivo conditions. *Methodology:* Polyphenols were isolated from Euphorbia plants using an extraction method, after which the effect of these polyphenols on malondialdehyde (MDA) and glutathione reductase (GR) activity under oxidative stress in rats was determined *in vivo*. *Results:* The data obtained show that the studied hydrolyzable tannins reduce the amount of MDA in organs and tissues to the control level under alloxan-induced OS conditions. It was also found that polyphenols 3GHG, 1GHG and geraniin restore GR activity, and 2DGG had virtually no effect on the restoration of enzyme activity in the blood plasma and pancreas under OS conditions. However, the studied hydrolyzable tannins provide feebly expressed influence on the restoration of GR activity in the liver of rats under OS conditions. *Conclusions:* Thus, these hydrolyzed tannins exhibit strong antioxidant properties when acting on the amount of MDA, but when the GR activity is restored, their activity is weakly expressed.

Keywords.*Oxidative stress, blood plasma, pancreas, liver,hydrolyzable tannins.*

INTRODUCTION

Oxidative stress (OS) develops as a result of a shift in the balance of the antioxidant-prooxidant system in cells to the pro-oxidant side, and it can be systemic in nature or belong to a specific organ.An imbalance in this system causes DNA hydroxylation, protein denaturation, lipid peroxidation (LPO), and apoptosis, which ultimately leads to cell death [1]. OS is associated with many diseases, in particular cardiovascular, neurodegenerative, oncological, and others [2, 3, 4].Free radicals generated as a result of OS are involved in the transmission of electrophilic signals, act as second messengers, and indirectly influence the modulation of basic cellular processes such as autophagy, proliferation, and apoptosis [5]. In addition, the development of an imbalance between reactive oxygen species (ROS) and glutathione causes cell cycle and proliferation disorders [6]. It is worth noting that OS plays an important role in the physiological and pathological processes of the cell [7];in this case, the formation of a large

number of LPO products leads to the development of the OS process and a decrease in the antioxidant defense system.

The purpose of this work was to study the effect of hydrolyzable tannins (polyphenols) isolated from *Euphorbia franchetii*– 3-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose (3GHG), 2,3-di-O-galloyl-β-D-glucose (2DGG) and geraniin, as well as *Euphorbia canescens (L.)*– 1-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose (1GHG) on the production of malondialdehyde (MDA) and glutathione reductase (GR) activity (EC 1.6.4.2) in alloxan-induced OS under *in vivo* conditions.

MATERIALS AND METHODS

Isolation of Polyphenols

The total polyphenols isolated from the aerial part of *E. franchetii* contained 11 compounds. Column chromatography over silica gel with elution by $CHCl_3$:MeOH (methanol) (17:3, 17:4, and 17:5, successively) separated the total polyphenols into three fractions. Rechromatography over a column of silica gel from the third fraction with elution by MeOH solvents (MeOH 60% → MeOH 70%), MeOH: acetone:H_2O (7:2:1 → 6:2:2 → 5:3:2) are isolated the following polyphenols: 3GHG, geraniin, and 2DGG.

The extraction method was used to isolate polyphenols from the aerial parts of *E. canescens* using chloroform ($CHCl_3$) and aqueous acetone. The latter extract was concentrated in vacuo. The aqueous residue was treated with ethyl acetate (EtOAc). The condensed EtOAc extract was treated with $CHCl_3$. The resulting precipitates were filtered off to afford total phenolic compounds in 6.3% yield of the air-dried raw material. Total polyphenols were separated preliminarily by chromatography over a column of hide powder with elution by diethyl ether (Et_2O), water, and aqueous acetone into two fractions. Chromatography from the aqueous acetone fraction over silica gel with elution by Et_2O: EtOAc and pure EtOAc isolated from their physicochemical properties 1GHG and other polyphenols [8]. The purity of these polyphenols is 90-95%.

Preparation of the Tissue

The experiments were carried out on albino male rats weighing 180-200 g. All animals were kept in standard vivarium conditions on a standard diet with free access to water and food. The experiments were carried out in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in Experimental Research (1998), and the Rules of Laboratory Practice for Conducting Laboratory Research at the Institute. The animals were divided into 6 groups: control and 5 experimental. Each group used 5 rats. Intact rats were used as control (group I). Alloxan hydrate was administered intraperitoneally at a dose of 150 mg/kg to induce OS in animals [9]. Ramkumar and colleagues

(2014) [9] showed that alloxanhydrate induces OS, causing LPO in other organs and tissues, along with the pancreatic β-cell. Based on this, alloxanhydrate was used to induce OS.Groups of experimental animals: Group II - group with OS; after exposure of animals to alloxanhydrate, from the first day of OS, polyphenols were given *per os* at a dose of 50 mg/kg for 10 days: group III - group receiving3GHG, group IV - group receiving2DGG, group V - group receiving1GHG and group VI - group receiving geraniin. On the 11th day of the experiment, the animals were decapitated, blood and internal organs - pancreas and liver - were taken. Blood was taken from animals with citrate buffer (1:9 ratio), centrifuged at 3000 rpm for 15 min, and blood plasma was separated. The homogenate was prepared from pancreatic and liver tissue using 0.9% physiological NaCl solution in a ratio of 1:10.

Analytical techniques

Determination of MDA in blood plasma and tissue homogenates [10]. To 1.0 ml of blood plasma or 4-four times diluted tissue homogenate, 0.1 M phosphate buffer (pH-7.6), 0.5 ml of 30% TCA, and 2.0 ml of 0.8% TBA. The samples were placed in a boiling water bath for 15 min; the tubes were covered with foil to prevent evaporation. After this, the precipitate that formed was separated by centrifugation for 10 minutes at 3000 rpm. The resulting supernatant was spectrophotometrically at λ=532 nm against a mixture of reagents (2.0 ml of phosphate buffer (pH-7.6), 0.5 ml of 30% TCA and 2.0 ml of 0.8% TBA). We calculated the content of TBA-active products based on the optical density using the formula.

$$C=Ak/\varepsilon l$$

where A – optical density at λ=532 nm; k – dilution factor; ε– molar extinction coefficient for MDA-TBA complex at 532 nm to 1.56×10^5 M^{-1} cm^{-1}; l – optical path length 1 cm.

GR activity was determined by the rate of glutathione reduction in a reaction medium containing 2.0 ml of 50 mM phosphate buffer (pH-7.4), 200 μl of 1.0 mM EDTA, 500 μl of 7.5 mM oxidized glutathione, 200 μl of blood plasma or tissue homogenate, 100 μl NADPH [11]. Enzyme activity was determined by the loss of NADPH at 25°C for 10 min at λ=340 nm. Enzyme activity was expressed as a percentage.

Statistics

Statistical analysis was performed using Student's *t*-test. Data are expressed as means ± SE. Values of P<0.05wereconsidered significantly different.

RESULTS AND DISCUSSION

The experiments studied the effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on the amount of MDA formed as a result of LPO in the blood plasma of rats under conditions of OS induced by alloxan hydrate (Fig. 1, A). According to the results obtained, the amount of MDA in the blood plasma of animals in the control group (group I) was 3.24 ± 0.14 nmol/mg

tissue, while the amount of MDA in animals of group II was 15.2 ± 0.21 nmol/mg tissue. The amount of MDA in the blood plasma of animals of group III, receivingpolyphenol3GHG, was 5.6 ± 0.4 nmol/mg tissue; in group IV, receiving2DGG, the amount of MDA was 3.7 ± 0.14 nmol/mg tissue, and the amount of MDA in group V was 6.2 ± 0.77 nmol/mg tissue and in animals of group VI in the blood plasma the amount of MDA was 7.3 ± 0.21 nmol/mg tissue, which receiving the polyphenol geraniin. As can be seen from the results obtained, it was noted that correction of OS with polyphenols reduces the amount of MDA, a product of LPO, in the blood plasma of experimental animals at a statistically significant level.

β-cells of the pancreas are a target for alloxan. In the presence of intracellular thiols, especially glutathione, alloxan generates ROS, which ultimately leads to β-cell death and reduces the pancreas antioxidant capacity [12]. Therefore, in subsequent experiments, the amount of MDA was determined in the pancreas tissue of experimental animals (Fig. 1, B). In the pancreas tissue, the amount of MDA in control animals was 11.8 ± 0.8 nmol/mg tissue, while the amount of MDA in group IIanimals induced with OS alloxan hydrate, was 23.4 ± 1.3 nmol/mg tissue.In experimental groups III-IV-V-VI with OS corrected by polyphenols, the following results were recorded: in group III, with the receiving of 3GHG, the amount of MDA was 12.7±1.1 nmol/mg tissue, in group IV, with the receiving of 2DGG, the amount MDA was 15.2±0.8 nmol/mg tissue, the MDA content in group V with the receiving of 1GHG was 12.6±1.2 nmol/mg tissue, and the MDA content in group VI, corrected with geraniin, was 13.4 ±0.7 nmol/mg tissue. The results obtained show that correction of OS induced by alloxan hydrate with polyphenols leads to a decrease in the amount of MDA in pancreatic tissue to the control level in experimental animals.

In the organism, the liver is an important detoxifying organ, and therefore the amount of MDA formed as a result of LPO in alloxan hydrate-induced OS was studied in the liver tissue(Fig. 1, C). From the results obtained, it can be seen that the amount of MDA in control animals was 7.4 ± 0.98 nmol/mg tissue, while the amount of MDA in alloxan hydrate-induced OS was 23.2 ± 2.1 nmol/mg tissue. In animals of experimental groups III-IV-V-VI, the OS state of which was corrected with polyphenols, the following results were recorded. Under OS conditions, the amount of MDA in animals of group III was 7.6±0.7 nmol/mg tissue, corrected with 3GHG, and in group IV, corrected with 2DGG, the amount of MDA was 7.8±0.15 nmol/mg tissue, while the amount of MDA in group V, corrected with 1GHG, was 11.6 ± 0.7 nmol/mg tissue, and in animals of group VI, corrected with geraniin, the amount of MDA was 7.9 ± 0.6 nmol/mg tissue. It can be seen that correction of the OS state with polyphenols reduced the amount of MDA in the liver tissue almost to the control level.

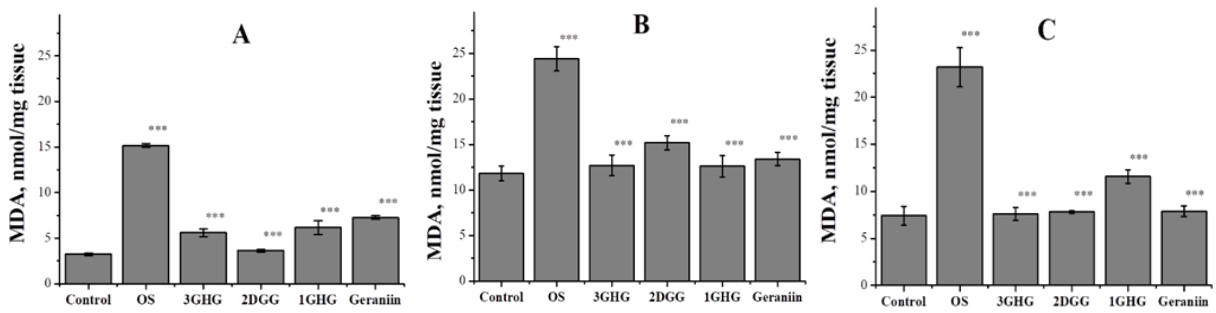

Figure 1. Effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on MDA formation in plasma (A), pancreas (B), and liver (C) under alloxan hydrate-induced OS. Polyphenols were given orally at 50 mg/kg.*** - P<0,001, n=5.

The results obtained showed that polyphenols isolated from plants*Euphorbia* reduce the amount of MDA in blood plasma, pancreatic and liver tissues, preventing alloxan hydrate-induced OS. It was noted that the amount of MDA in pancreatic and liver tissues is relatively higher than its amount in blood plasma.

Subsequent studies examined GR activity. It has been established that GR plays an important role in the regulation and modulation of cell redox homeostasis. GR is an enzyme that reduces reduced glutathione (GSH), one of cells'most common reduced thiols. It has been shown that GSH is involved in regulating ROS in cells, thereby creating conditions for controlling intracellular redox processes and activating programmed cell death [13]. It has also been shown that the activity of glutathione enzymes and the level of GSH, along with lipoperoxidative enzymes, decreases in the liver under OS conditions [14].

Therefore, in the following experiments, the effect of polyphenols on the activity of the antioxidant enzyme GR in the blood plasma, pancreatic tissue and liver of experimental animals was studied under conditions of OS induced by alloxan hydrate (Fig. 2). Initially, the experiments studied the effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on the GR activity in blood plasma under OS conditions (Fig. 2, A). Based on the results obtained, it was established that GR activity in the blood plasma of rats of group II, which were administered alloxanhydrate, decreased by 34.2% (P<0.05) compared to the control. As a result of the administration of 3GHG to experimental animals of group III under OS conditions, GR activity in the blood plasma increased by 32.0% (P>0.05) compared to group II. In animals of group IV receiving2DGG, virtually no changes in GR activity in the blood plasma were observed compared to group II. At the same time, it was found that GR activity in the blood plasma of experimental animals receiving 1GHG in group V and geraniin in group VI under OS conditions increased by 44.0% (P<0.05) and 48.0% (P <0.05), respectively, compared to group II.

In further experiments, we studied the effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on GR activity in pancreatic tissue under alloxan-dependent OS conditions (Fig. 2, B). Based on the results obtained, it was established that GR activity in the pancreas tissue of experimental animals of group II, which were administered alloxanhydrate, decreased by 53.5% ($P<0.01$) compared to the control. In group III, those receiving 3GHG, GR activity in the pancreas increased by 39.1% ($P>0.05$) compared to animals in group II, but no significant difference was observed. In experimental animals of group IV, which received the 2DGG in the pancreas under OS conditions, the same situation was observed as in the blood plasma, while practically no changes in GR activity were observed compared to that of group II. Under OS conditions, GR activity in the pancreas of experimental animals that received 1GHG in group V and received geraniin in group VI increased by 43.4% ($P>0.05$) and 78.1% ($P<0.01$), respectively, compared to group II. Thus, the studied polyphenols have different effects on GR activity in the pancreas under OS conditions. Polyphenols other than 2DGG showed a tendency to restore enzyme activity, and geraniin was shown to cause a significant change in GR activity.

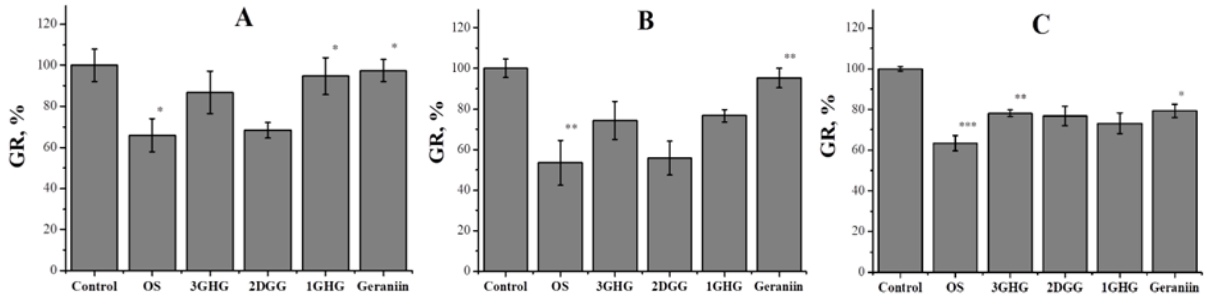

Figure 2. Effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on GR activity in plasma (A), pancreas (B), and liver (C) under alloxan hydrate-induced OS.*- P<0,05;**- P<0,01; ***- P<0,001; n=5.

In subsequent experiments, we studied the effect of polyphenols3GHG, 2DGG, 1GHG, and geraniin on GR activity in the liver of experimental animals under conditions of alloxan-induced OS (Fig. 2, C). From the results obtained, it was established that GR activity decreased by 36.6% ($P<0.001$) in the liver of experimental animals of group II under OS conditions. It was found that GR activity in the liver of experimental animals receiving 3GHG increased by 23.2% ($P<0.01$) compared to group II. We also studied the GR activity in the liver of experimental animals of group IV who received 2DGG under OS conditions, it increased by 21.1% ($P>0.05$) compared to group II. We also determined the GR activity in the liver of experimental animals

that received the 1GHG in group V and received the geraniin in group VI, it increased by 15.4% ($P>0.05$) and 25.0% ($P<0.05$), respectively, compared with group II.

Consequently, under conditions of alloxan-induced OS, GR activity was reduced compared to the control. In experimental groups corrected with polyphenols, restoration of GR activity did not occur completely. However, polyphenols1GHG and geraniin caused a relative restoration of GR activity in blood plasma, no significant change in GR activity under the influence 3GHG was observed, and 2DGG had virtually no effect on the restoration of GR activity. Although the same situation was observed in the pancreas as in the blood plasma, the restoration of GR activity was more pronounced under the influence of geraniin. However, it has been shown that, under the influence of polyphenols, the restoration of GR activity in the liver occurs very slowly. Thus, although polyphenols belonging to the studied group of hydrolysable tannins inhibited the amount of MDA formed in the processes of LPO under conditions of alloxan-induced OS to the level of control indicators, however, changes in GR activity appeared in a scattered state.

According to the literature, polyphenols are noncompetitive inhibitors of the GR enzyme [15], but they have been shown to increase the sensitization of cancer cells to drugs by inhibiting the binding of drugs to glutathione in cancer cells [16]. However, the literature reports that alloxan, in addition to the β-cells of the pancreas, has a direct effect on other organs, in particular the liver [17], and it is noted that it leads to an increase in the sinusoid in the liver tissue, micro - and macrovascular fatty degeneration in hepatocytes, steatohepatitis, and periportal fibrosis [18]. It can be seen that the slow restoration of GR activity in the liver in relation to the amount of MDA under OS conditions with polyphenols can be explained by destructive changes caused by alloxan. At the same time, it has been shown that in type I diabetes mellitus, the regenerative potential of the liver decreases due to changes in metabolic processes in hepatocytes [19],and under conditions of alloxan-induced OS, specific destructive conditions develop in the liver, which can lead to a slowdown in the restoration of tissues and cells in the process of correction of liver function by polyphenols.

CONCLUSIONS

It can be concluded that the hydrolyzable tannins studied in experiments reduce the amount of MDA in organs and tissues to the control level under alloxan-induced OS conditions. In this case, polyphenols attach to reactive free radicals caused by alloxan, which normalize the antioxidant system, ensuring its removal from the body. It was also found that polyphenols 3GHG, 1GHG, and geraniin restore GR activity in the blood plasma and pancreas under OS conditions. However, the polyphenol 2DGG had virtually no effect on the restoration of GR activity in the blood plasma and pancreas under OS conditions. Among these polyphenols,

1GHG and geraniin have been shown to be superior to other polyphenols in restoring GR activity in plasma. However, it has been established that polyphenols in the liver have little effect on the restoration of GR activity.

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