EFFECT OF RASPBERRY SHOOT EXTRACTS ON ECG AND ANTIOXIDANT SYSTEM IN DOXORUBICIN CARDIOMYOPATHY

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Introduction. According to information from World Health Organization (WHO) experts, published in the materials of the "WHO Traditional Medicine Strategy 2014-2023," nearly 80% of the world's population uses plant-based medicines [37]. Many herbal medicines have a long history of use in traditional medicine. Phytomedicines are widely used in the complex therapy of inflammatory diseases, gastrointestinal tract disorders, bronchopulmonary system conditions, cardiovascular diseases, neurological disorders, and more [25].

Most herbal medicines have a wide range of pharmacological properties and are characterized by high safety with sufficient efficacy [25, 37]. Many herbal preparations can be used for a long time both for the purpose of prevention and in the complex treatment of many human diseases.

One of the plants that is widely used in both folk and official medicine and contains a significant amount of biologically active substances (BAS) is raspberry (*Rubus idaeus* L.) [9, 11, 14].

In folk medicine, raspberry (flowers, leaves) is used very widely. It is used as an anti-inflammatory, antipyretic agent [28, 30,25]. Raspberry decoction is used for gargling in throat diseases. Raspberry fruits are used as a high-vitamin remedy, to improve appetite after illnesses [30]. Raspberry fruits and raspberry leaves have a weak urinary and biliary effect in cardiovascular diseases, liver and kidney diseases [30,25].

However, most studies on the pharmacological and clinical activity of raspberries concern leaves or fruits. And only a small number of publications contain data on raspberry shoots, which are usually cut and discarded. It should be noted that some industrial raspberry harvesting technologies include harvesting raspberries in the second year with subsequent mowing of these plantations immediately after harvesting [24].

Considering that Ukraine ranks 4th in raspberry production in the world, raspberry shoots are a huge resource of BAS, which is not used anywhere, which makes them a promising object for further research [21],

Our previous studies have established the antioxidant and anti-inflammatory properties of raspberry shoot extracts and their harmlessness [21, 22].

So, the aim of the study was study of the effect of raspberry shoot extracts on the state of free radical oxidation processes and ECG parameters in rats with experimental doxorubicin cardiomyopathy.

As is known, the anthracycline antitumor antibiotic doxorubicin, despite its clinical effectiveness in the treatment of breast and lung carcinoma, acute leukemia, lymphoma, etc., has a number of side effects, among which cardiotoxicity is particularly acute [34, 27].

Usually, doxorubicin cardiotoxicity is dose-dependent, cumulative and progressive and occurs in approximately 10% of patients who received the drug. Doxorubicin cardiotoxicity can be accompanied by both latent changes in myocardial structure and function, and manifestations of left ventricular dysfunction, a decrease in left ventricular ejection fraction from 10% to 50%, the development of severe cardiomyopathy and congestive heart failure, which can lead to fatal outcomes [34, 27].

One of the leading mechanisms of doxorubicin cardiotoxicity is the activation of free radical oxidation (FRO) processes, both through reactive oxygen species (superoxide anion radical, hydroxyl radical, etc.), and through the NO synthase (NOS) and NADPH oxidase (NOX) systems [17]. With the help of NOX and/or NOS, doxorubicin can be reduced to semiquinone, which also triggers a cascade of free radical destruction of cellular and subcellular structures [30] with the formation of peroxynitrite, which is a highly active oxidant of DNA, proteins and lipids.

Thus, doxorubicin triggers a chain cascade of both oxidative and nitrosative stress, which, against the background of inhibition of the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, quinone reductase, etc.), a decrease in cardiolipin, accumulation of p53, and DNA damage in mitochondria with the occurrence of mitochondrial dysfunction [11]. This leads to the uncoupling of oxidative phosphorylation and tissue respiration, a decrease in ATP production and the development of a number of dysmetabolic, which leads to the death of myocardial cells [35, 33, 27].

Activation of BPO also causes the release of $Ca2+$ from the sarcoplasmic reticulum of the cardiac muscle and vascular endothelium and an increase in its content in the cytosol, which also contributes to myocardial damage [4].

A significant role in the development of cardiac lesions by doxorubicin is also played by its inhibition of topoisomerases - both topoisomerase IIa (which is the main molecular target of the antitumor activity of anthracycline antibiotics) and topoisomerase IIb, which is localized mainly in cardiomyocytes and cells that are in the resting stage [4, 38].

This mechanism causes significant DNA damage and triggers apoptosis [1,10].

Early manifestations of anthracycline-induced cardiomyopathies occur within 1 year, and late manifestations can occur even 7 years after the use of these antitumor antibiotics (irreversible type 1 cardiotoxicity) [7, 17]. In addition to cardiotoxicity, doxorubicin causes damage to the liver, kidneys, etc. [23].

Over the past decades, the literature has seen an exponential increase in publications on reducing the toxic effects of anthracyclines on the myocardium, which indicates that the creation of new effective and safe agents for pharmacocorrection of the cardiotoxic effects of doxorubicin is a very relevant task of modern pharmacy [31].

To reduce the cardiotoxicity of doxorubicin, antioxidant drugs (lipoflavone, corvitin, mexicor, etc.), amino acid agents, etc. are often used [35, 10, 29, 26, 33].

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Material and methods

The object of our research was the native thick extract of raspberry shoots (ERS) and the modified extract of raspberry shoots with L-arginine ("*RubusArg"*). These extracts were obtained and standardized at the Department of General Chemistry of the National University of Pharmacy by PhD, Assoc. Prof. Maslov O. Yu. and PhD, Assoc. Prof. of the Department of Pharmacognosy and Nutritionology Komizarenko M. A., under the supervision of the Head of the Department of General Chemistry, Doctor of Pharmaceutical Sciences, Prof. Kolisnyk S. V., and Doctor of Pharmaceutical Sciences, Prof. Komizarenko A. M. The raspberry shoots were collected during the ripening period in the Kharkiv region in 2021-2022.

The ERS extract was obtained using 60% ethanol from two-year-old shoots of common raspberry according to the technology described in the Ukrainian utility model patent (No. a20201604) *"Method for obtaining a remedy with antioxidant and anti-inflammatory activity from common raspberry shoots"* [19].

A modified extract was also obtained from raspberry shoots, which was extracted using 60% ethanol. Unlike EPM, this extract did not contain derivatives of organic and hydroxycinnamic acids. Additionally, this extract (provisionally named *"RubusArg"*) contained L-arginine in a molar ratio of 3:1 to the total catechin content. L-arginine contains an amino group that fully ionizes the catechin derivatives present in the extract.

The obtained extracts were standardized according to the total catechin content, recalculated as epigallocatechin-3-O-gallate, using a spectrophotometric method [19].

All pharmacological studies were conducted on outbred white rats housed in the vivarium of the Scientific Research and Training Laboratory of the Educational and Scientific Institute of Applied Pharmacy at the National University of Pharmacy. The experiments were carried out in the Educational and Scientific Training Laboratory of Biomedical Research at National University of Pharmacy (NUPh) (certificate No. 01-0084 from DP *Kharkivstandartmetrology*, issued on 06.08.2021).

The experimental animals were kept in a well-ventilated room at an ambient temperature of 20-22 °C, with a standard 12-hour light-dark cycle typical for rodents, a standard diet, and a feeding schedule, with free access to food and water. All experiments were approved by the Bioethics Committee of NUPh (protocol No. 7, dated 21.11.2021).

Animal handling was conducted in accordance with the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* [32, 6, 8].

Doxorubicin (*Doxorubicin-Medac*, Medac Gesellschaft für klinische Spezialpräparate m.b.H., Germany) was administered to the experimental animals intraperitoneally at a dose of 5 mg/kg of body weight once a week for four weeks, with a cumulative dose of 20 mg/kg of body weight [29,34].

This pathological model involved 57 male white rats weighing 220–310 g. Given the high mortality rate, each group (except for the intact control) included 10 animals:

- Group 1 – Intact control (7 animals);
- Group 2 – Control pathology group;
- Group 3 – Animals that received daily administration of raspberry shoot extract (ERS) at a dose of 2 ml/kg for four weeks, equivalent to 26.4 mg/kg of catechins recalculated as epigallocatechin-3-O-gallate;
- Group 4 – Animals that received *RubusArg* under the same regimen at a dose of 2 ml/kg (equivalent to 6.5 mg/kg catechin content per body weight);
- Group 5 – Animals that received catechin (*Sigma Aldrich*, Lublin, Poland) at a dose of 2 ml/kg (6.5 mg/ml);
- Group 6 – Animals that received quercetin at a dose of 20.5 mg/kg (comparison drug manufactured by *Borshchahivskiy Chemical-Pharmaceutical Plant*, Ukraine).

During the experiment, animal survival was assessed. At the end of the experiment, ECG parameters were recorded in groups of animals using an EKIT 03 M2 electrocardiograph. The ECG parameter was determined in the second standard lead. Subsequently, the animals were anesthetized under light chloroform anesthesia and biochemical studies of blood serum and myocardial homogenate were performed to determine the state of free radical oxidation processes and antioxidant systems [32].

In blood serum, the content of isoprostane-8 (an indicator of oxidative stress) was determined by the enzyme-linked immunosorbent assay using a set of reagents from IBL (Hamburg, Germany).

In the heart homogenate, the content of TBA reactants was determined by reaction with thiobarbituric acid by the method of Asakawa T. et al. by optical density at a wavelength of $\lambda = 535$ nm, and expressed in μmol/g of tissue [2].

To assess antioxidant protection in myocardial homogenate and blood serum, the content of reduced glutathione (G-SH) and the activity of SOD and catalase were determined by standard methods [32].

Catalase activity was determined spectrophotometrically at a wavelength of $\lambda = 410$ nm by the reaction of ammonium molybdate formation [9].

Antioxidant-prooxidant index (API) was calculated by the formula: API = (catalase activity/TBC-RP content) × 100 [12]

SOD activity was determined spectrophotometrically by the reaction of nitrotetrazolium blue reduction at a wavelength of $\lambda = 540$ nm [9]. The content of reduced glutathione (VG, G-SH) was determined spectrophotometrically by the method of Beutler E.D. et al. [24] by reaction with 5,5-dithio-bis-2-nitrobenzoic acid.

To obtain statistical conclusions, the standard software package "Statistica 10.0" was used, the significance level was assumed to be $p<0.05$. The normality of the distribution was checked using the W-Shapiro-Williams criterion. [16].

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Results and discussion

The results of the experiment are given in Tables 1-2 and Fig. 1.

Doxorubicin cardiomyopathy in rats was characterized by a rather high mortality, which at the end of the experiment was 40% (Table 1).

Against the background of experimental cardiomyopathy, activation of FRO processes was observed in the myocardium: the level of TBA reactants in the homogenate increased by 1.6 times, and in the blood serum the content of 8-isoprostane increased by 4.24 times ($p<0.001$).

All these changes are inherent in this pathology [1], and contribute to the intensification of heart damage.

Against the background of anthracycline-induced heart damage, there was a decrease in antioxidant capacity activity: the level of reduced glutathione in myocardial homogenate decreased by 1.55 times, in blood serum - by 1.9 times, which indicates the depletion of the reduced glutathione reserve during the neutralization of FRO products.

Doxorubicin cardiomyopathy was also characterized by a decrease in the activity of the endogenous enzymatic system: SOD activity in myocardial homogenate decreased by 55.5%, in blood serum - by 40%; catalase activity in myocardial homogenate decreased by 31.3% and 32.14% in blood serum (Table 1).

Table 1. Effect of native and modified raspberry shoot extract on animal survival and indicators of FRO processes in myocardial homogenate and blood serum in doxorubicin-induced cardiomyopathy in rats (M±m)

Parameter	Group of rats					
	Intact control	Control patology	ESR	RubusArg	Catechin	Quercetin
Survived animals, %	100	60	80	90	70	80
Myocardial homogenate						
TBA-reactants, µmol/g	59.21± 2.57	95.5±6.95*	68.8± 2.1**/#	65.8± 2.9**/#/##	79.38± 2.11**	74.2± 2.74**
G-SH, µmol/g	1.68±0.08	1.083±0.059*	1.41± 0.034**	1.47± 0.034**	1,32± 0.072	1.108± 0.06
SOD, mmcat/g	0.90±0.01	0.54±0.01*	0.82± 0.01**	0.85± 0.02**	0.65± 0.02**	0.70± 0.01**
Catalase, µcat/g	1.02±0.04	0.70±0.02*	0.83± 0.02**	0.90±0.02**	0.80± 0.02**	0.75±0.02**
Blood serum						
8-isoprostan, ng/mL	5.3 ± 0.32	22.49 ± 1.53*	13.16 ±0.71**/##	10.56± 0.45**/#/##	17.8± 0.77**	14.16 ±0.94**/#
G-SH, µmol/L	2.39±0.02	1.20±0.05*	2.00± 0.01**	2.35± 0.05**	1.55± 0.02**	1.80± 0.03**
SOD, µcat/L	0.95±0.01	0.50±0.01*	0.80± 0.01**/#	0.84± 0.02**/#	0.68± 0.02	0.76± 0.01**
Catalase, µcat/g	1.40±0.05	0.95±0.03*	1.2±0.02**	1.23± 0.02**	0.75±0.02	1.08±0.05**

Notes: 1. *the deviation is significant relative to the intact control group ($p \leq 0.05$);
2.** - the deviation is significant relative to the control pathology group ($p \leq 0.05$);
3. # - the deviation is significant relative to the catechin group ($p \leq 0.05$);
4. ## - the deviation is significant relative to the quercetin group ($p \leq 0.05$).

Therapeutic and prophylactic administration of the studied drugs and the comparison drug quercetin increased the survival of animals, which was 90% when using RubusArg, 80% - ERS and quercetin, 70% - catechin.

The inhibitory effect of the studied drugs on the intensity of FRO processes and activation of the EEA is reflected in changes in the indicators of TBA reactants, 8-isoprostane, reduced glutathione, SOD enzymes, catalase. The results of the analysis of the studied drugs for these indicators are given in Table 1.

During the experiment, it was found that the studied drugs inhibited the accumulation of primary lipoperoxidation products in both blood serum and myocardial homogenate in relation to the control pathology. By conducting a comparative analysis among the studied drugs, it was proven that the most powerful

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suppression of the intensity of FRO was carried out under the influence of RubusArg, which was confirmed by a significant decrease in the level of 8-isoprostane in blood serum by 2.1 times (p≤0.01), in myocardial homogenate the indicator of TBA-reactants decreased by 1.45 times compared to the untreated control.

When using the studied drugs ERS and quercetin, there was also a significant decrease in TBA-reactants in myocardial homogenate by 1.38 times and 1.28 times and a decrease in 8-isoprostane in blood serum by 1.7 times and 1.58 times, respectively (p≤0.05).

Catechin reduced the level of TBA-reactive substances in myocardial homogenate by 1.2 times and decreased the level of 8-isoprostane in blood serum by 1.26 times ($p \leq 0.05$).

As is known, 8-isoprostane is a metabolic product of arachidonic acid peroxidation (an isomer of prostaglandin F2, and its quantity is directly proportional to the level of free radicals formed [5].

Thus, the results of our study convincingly demonstrate the powerful antioxidant effect of both raspberry shoot preparations and quercetin. Considering literature data indicating that isoprostanes act as vasoconstrictive agents (through activation of the TxA2 prostanoid receptor) and are modulators of various cardiovascular diseases, contributing to increased cardiovascular risk [5], the significant reduction in 8-isoprostane levels following the administration of ERS and RubusArg is not only evidence of their antioxidant action but also a predictor of improved functional status of the cardiovascular system.

Administration of these substances to animals with doxorubicin-induced cardiomyopathy normalized antioxidant homeostasis. Specifically, under the influence of RubusArg, the level of G-SH in myocardial homogenate significantly increased by 1.35 times, while ERS and the reference drug quercetin increased it by 1.3 times. In contrast, catechin administration led to a 1.2-fold decrease in this parameter ($p \leq 0.05$).

A similar trend in G-SH normalization was observed in blood serum. Specifically, administration of RubusArg significantly increased this parameter by 1.35 times compared to the pathological control, while ERS and quercetin increased it by 1.3 times ($p \leq 0.05$). However, the increase in G-SH levels following catechin administration was only a trend and was not statistically significant.

Comparing the degree of antioxidant activity of the studied preparations, it should be noted that RubusArg and ERS surpassed both catechin and the reference drug quercetin in their effect on SOD. They significantly increased SOD levels in myocardial homogenate by 1.57 and 1.5 times, respectively, and in blood serum by 1.6 and 1.68 times, respectively (Table 1). In contrast, quercetin increased SOD levels by 1.3 times, while catechin increased them by 1.2 times.

A similar trend was observed in the effect of RubusArg on catalase activity, with a significant increase in enzyme activity by 1.28 times in myocardial homogenate and by 1.3 times in blood serum. ERS also increased catalase activity by 1.2 times in both myocardial homogenate and blood serum.

Thus, analysis of the experimental results confirmed that the most pronounced antioxidant effect was observed with the administration of RubusArg and ERS, as evidenced by the antioxidant-prooxidant index (Fig.1).

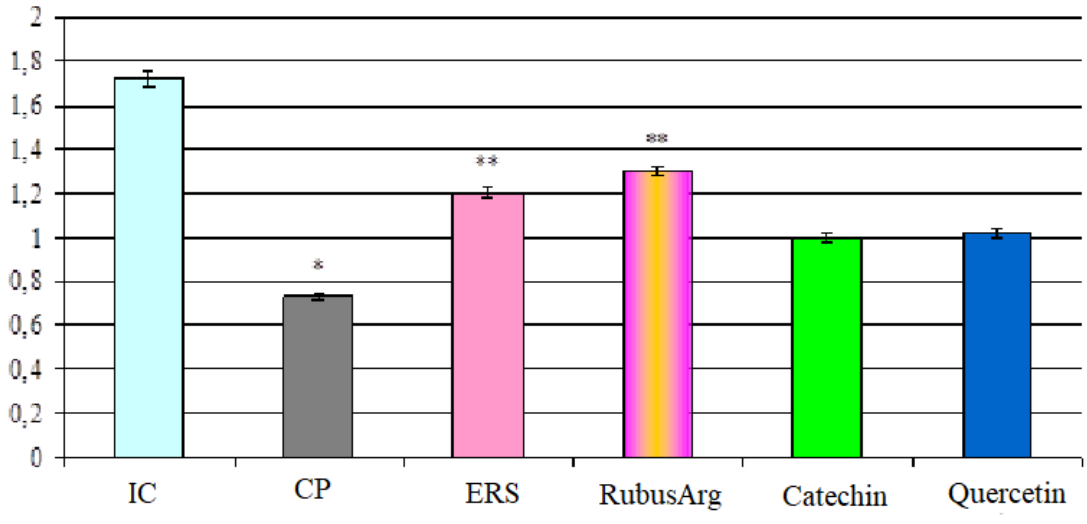

Figure 1 Changes in the antioxidant-prooxidant index following the administration of ERS and RubusArg. Notes: 1. * - Significant difference ($p \leq 0.05$) compared to the intact control group (IC); 2. ** - Significant difference ($p \leq 0.05$) compared to the pathological control group (PC).

Anthracycline-induced myocardial damage is accompanied not only by changes in the antioxidant-prooxidant index, but also by disorders in other systems. Against the background of the use of doxorubicin in the control pathology group, functional myocardial disorders were noted, which was confirmed by significant changes in ECG parameters (Table 2).

During the development of the pathology, a significant decrease in the indicators characterizing the contractile function of the myocardium was observed (a decrease in the systolic index (SI) by 53%, a decrease in

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the amplitude of the R waves by 60% and the P waves by 49%), a decrease in the heart rate by 46% (indicating the development of bradycardia), an increase in the time of ventricular excitation at the moment of systole (an increase in the duration of the QRS interval by 30%). On the ECG, depression of the ST segment from the isoline was observed, which indicated the development of ischemia in the myocardium (Table 2). Electrocardiographic indicators in doxorubicin cardiomyopathy reflect the development of disorders of the contractile function of the myocardium, which is primarily due to damage to the membrane apparatus of cardiomyocytes [1].

Table 2. Effect of native and modified raspberry shoot extract on electrocardiogram parameters in doxorubicin-induced cardiomyopathy in rats

Parameter	Group of animals					
	Intact control	Control patology	ESR	RubusArg	Catechin	Quercetin
HR, bpm	460.70±8.5	260.23± 9.5*	360.0± 25.0**	380.50± 15.5**	270.30± 20.0	370.00± 15.50**
PQ,s	0.036± 0.002	0.050± 0.01*	0.040± 0.01	0.040± 0.01	0.045± 0.001	0.045± 0.001
QRS,s	0.015± 0.002	0.016± 0.002	0.020± 0.003	0.020± 0.002	0.015± 0.002	0.017± 0.002
QT,s	0.056± 0.002	0.075± 0.04	0.06± 0.01	0.05± 0.01	0.06± 0.003	0.07± 0.003
R,mV	0.455± 0.021	0.175± 0.035*	0.35± 0.002**	0.40± 0.002**	0.155± 0.002	0.335± 0.033**
P,mV	0.095± 0.007	0.05± 0.02*	0.10± 0.02	0.05± 0.02	0.05± 0.02	0.06± 0.01
T, mV	0.16± 0.01	0.145± 0.02	0.140± 0.05	0.150± 0.03	0.140± 0.03	0.090± 0.02
SP,%	45.43± 0.95	21.45± 1.65*	40.35± 3.25**	41.35± 3.25**	25.50± 1.70	42.50± 1.80**
ST offset, mm from the isoline	0.00 (0÷1)	-4.35 (-10÷0)	-3.20 (-3÷0)	-2.05 (-3÷0.5)	-4.00 (-10÷0)	-2.05 (-3÷0.5)

Notes:
1) * - deviation is significant in relation to the intact control group ($p \leq 0.05$);
2) ** - deviation is significant in relation to the control pathology group ($p \leq 0.05$);
3) ST - deviation of ST from the isoline.

Under the influence of the RubusArg, ERS, and comparison drug quercetin, normalization of heart rate was statistically significant; for the catechin drug, there was only a slight trend toward normalization of this parameter.

The use of RubusArg, ERS, and the comparison drug quercetin eliminated signs of hypoxia and myocardial ischemia, as evidenced by an increase in the ST segment to the baseline; no significant changes in this parameter were observed after catechin administration.

Thus, the comparative analysis of biochemical and electrocardiographic research methods allows us to conclude that the introduction of these drugs improves the course of doxorubicin-induced cardiomyopathy. However, the most pronounced cardioprotective activity was exhibited by the investigated drug RubusArg and ERS, which were more effective for this pathology compared to catechin and quercetin.

In our opinion, the greater effectiveness of RubusArg and ERS in this pathology can be explained by the composition of these agents. According to the studies of O. Maslov and M. Komisarenko, raspberry shoots contain a significant amount of phenolic compounds, primarily flavan-3-ols, with epicatechin and (+)-catechin as the main components [23]. In addition to flavan-3-ols, raspberry shoots are rich in derivatives of ellagitannins, primarily sangvinin N-6, N-10, and lambertiannins C [23].

The pharmacological activity of a substance largely depends on its chemical structure and bioavailability, which is influenced by the degree of ionization. It should be noted that ionized compounds, due to their electric charge, can differ significantly in their chemical and biological properties from their uncharged forms [23, 23]. This was confirmed in our studies, where the ionized form of RubusArg exhibited the highest pharmacological activity. Given that the molecular mechanisms of action of quercetin and catechin in cardiovascular diseases are somewhat similar (reduction of malondialdehyde levels; NF-kB, increase in GSH; SOD, reduction of TNF-α levels, etc.) [21], it can be assumed that there are other alternative mechanisms involved in the cardioprotective action of raspberry shoot extract-based drugs (whose major component is catechins). Primarily, the presence of ellagitannins in the extracts, whose cardioprotective properties are well known [35, 27], should be considered. Also, catechins and gallic acid (which are present in the studied raspberry shoot extracts) can chelate metal ions, thereby reducing the intensity of FRO processes, including in doxorubicin-induced

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cardiomyopathy, and protecting mitochondrial DNA from damage [35,18].

The cardioprotective effect of raspberry extracts, primarily the modified L-arginine extract RubusArg, is likely due to its effect on the NO system in the vascular endothelium. As is well known, endothelial NO is formed by the oxidation of L-arginine to L-citrulline in reactions catalyzed by the endothelial Ca2+-dependent constitutive isoform of eNOS [23, 23]. Thus, RubusArg can be considered as a donor of L-arginine. Under the action of a number of toxicants and a significant amount of reactive oxygen species, eNOS starts generating superoxide anion radicals and initiating nitrosative stress development [35,12].

Reduction of the intensity of FRO processes and membrane damage (which was confirmed in our studies by the impact on the levels of 8-isoprostane, TBK-AP, prooxidant-antioxidant index, GSH levels, and SOD and catalase activities) normalizes the eNOS-NO system, which not only limits the manifestations of both oxidative and nitrosative stress but also reduces endothelial dysfunction. It is precisely due to the multimodal impact on key links of the pathogenesis of doxorubicin-induced cardiomyopathy that we can explain the advantages of RubusArg.

Our previous studies on molecular docking and the anti-inflammatory activity of native and ionized raspberry shoot extracts (RubusArg) showed that ionized forms of (+)-catechin and epicatechin can inhibit key inflammatory enzymes (cyclooxygenase-2, myeloperoxidase, 5-lipoxygenase, NF-kB, etc.) up to 10 times more effectively, which likely reduces the manifestations of the inflammatory reaction [19, 22, 20]. Considering the role of these enzymes in the formation of doxorubicin-induced cardiomyopathy, the advantages of RubusArg are evident [20, 36].

Thus, the conducted study indicates the promising application of this extract in the correction of anthracycline-induced myocardial lesions.

Conclusion

1. The administration of doxorubicin at a dose of 5 mg/kg body weight intraperitoneally once a week for 4 weeks leads to the activation of FRO processes, a decrease in the functional activity of the body's antioxidant system, which worsens the functional condition of the heart and increases mortality in the experimental animals.
2. The use of native and modified L-arginine raspberry shoot extracts (RubusArg) contributes to the improvement of the antioxidant-prooxidant index, reduction of FRO intensity, increased activity of SOD, catalase, increased levels of reduced glutathione, improved ECG parameters, and reduced mortality. According to these indicators, both native and modified L-arginine raspberry shoot extracts either exceed or act at the level of the comparison drug quercetin.
3. The study confirmed the hypothesis that ionized forms of (+)-catechin and epicatechin, which are components of RubusArg, are more active antioxidants than their non-ionized forms.
4. The conducted study indicates the promising use of the modified L-arginine raspberry shoot extract, which contains ionized forms of (+)-catechin and epicatechin, for the correction of anthracycline-induced myocardial damage.

Effect of raspberry shoot extracts on ecg and antioxidant system in doxorubicin cardiomyopathy

Daryna Horopashna, Lyudmyla Derymedvid

Introduction. In folk medicine, raspberry (fruits, leaves and shoots) is applied in treatment of influenza, sore throat. Despite the extensive research on the pharmacological properties of raspberry leaves and fruits, relatively little attention has been given to raspberry shoots. These shoots, which are typically pruned and discarded, remain largely underexplored. Industrial raspberry farming often follows a two-year cultivation cycle, after which plantations are mowed following the harvest. This practice highlights an untapped potential for utilizing raspberry shoots as a valuable resource for medicinal applications. **Material and methods.** The object of the study was extract of shoot raspberry (ERS) and its modified extract with L-arginine ("RubusArg"); antioxidant effect of extracts was determined by catalase, superoxide dismutase, and reduced glutathione assays; cardiomyopathy was induced by doxorubicin model. **Results and discussion.** The therapeutic and prophylactic administration of the studied extracts, along with the reference drug quercetin, significantly improved animal survival rates. Survival reached 90% with RubusArg, 80% with both ERS and quercetin, and 70% with catechin. RubusArg demonstrated the strongest suppression of free radical oxidation, as evidenced by a significant 2.1-fold reduction in serum 8-isoprostane levels ($p \leq 0.01$). Additionally, myocardial homogenate showed a 1.45-fold decrease in TBA-reactive substances compared to the untreated control. The extract also significantly enhanced antioxidant enzyme activity, increasing catalase levels by 1.28-fold in myocardial homogenate and 1.3-fold in blood serum. Superoxide dismutase activity rose by 1.5-fold in myocardial homogenate and 1.68-fold in blood serum, while myocardial glutathione levels significantly increased by 1.35-fold. The administration of RubusArg, ERS, and the reference drug quercetin resulted in a statistically significant normalization of heart rate, whereas catechin only showed a slight trend toward improvement. RubusArg, ERS, and quercetin also effectively eliminated signs of hypoxia and myocardial ischemia, as indicated by the restoration of the ST segment to baseline levels. In contrast, catechin administration did not produce significant changes in this parameter. A comparative analysis of biochemical and electrocardiographic data confirms that these treatments contribute to the improvement of doxorubicin-induced cardiomyopathy. However, the most pronounced cardioprotective effects were observed with RubusArg and ERS, which demonstrated greater efficacy in managing this pathology compared to catechin and quercetin. The reduction in FRO intensity and membrane damage—confirmed in our

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study by changes in 8-isoprostane levels, TBK-AP, the prooxidant-antioxidant index, GSH levels, and the activities of SOD and catalase—contributes to the normalization of the eNOS-NO system. This, in turn, not only mitigates oxidative and nitrosative stress but also alleviates endothelial dysfunction. The superior efficacy of RubusArg can be attributed to its multimodal action on key mechanisms underlying doxorubicin-induced cardiomyopathy. **Conclusion.** The administration of doxorubicin at a dose of 5 mg/kg body weight intraperitoneally once a week for 4 weeks leads to the activation of FRO processes, a decrease in the functional activity of the body's antioxidant system, which worsens the functional condition of the heart and increases mortality in the experimental animals. The use of native and modified L-arginine raspberry shoot extracts (RubusArg) contributes to the improvement of the antioxidant-prooxidant index, reduction of FRO intensity, increased activity of SOD, catalase, increased levels of reduced glutathione, improved ECG parameters, and reduced mortality. According to these indicators, both native and modified L-arginine raspberry shoot extracts either exceed or act at the level of the comparison drug quercetin. The study confirmed the hypothesis that ionized forms of (+)-catechin and epicatechin, which are components of RubusArg, are more active antioxidants than their non-ionized forms. The conducted study indicates the promising use of the modified L-arginine raspberry shoot extract, which contains ionized forms of (+)-catechin and epicatechin, for the correction of anthracycline-induced myocardial damage.

Keywords: doxorubicin, cardiomyopathy, raspberry shoots, catechins, rats, ECG.

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