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ORIGINAL ARTICLE

Effects of Heart Cryoextract on Myocardial Antioxidant Capacity in Rats With Adrenaline-Induced Acute Myocardial Dystrophy

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Abstract

Background/Aim: In a healthy heart reactive oxygen species (ROS) are by-products of normal metabolism and perform important signalling functions (sometimes referred to as “redox signalling” or oxidative eustress). Aim of this study was to investigate the cardioprotective mechanisms of porcine heart cryoextract, specifically its effect on the regulation of antioxidant defence in cardiomyocytes in an adrenaline-induced myocardial dystrophy model.

Methods: Eighty-four male rats were divided into four groups: intact control (I), myocardial dystrophy without treatment (II), dystrophy + cryoextract (III) (50 μg peptides per 100 g body weight daily for 14 days) and dystrophy + amiodarone (IV) (10 mg/kg daily for 14 days). Myocardial dystrophy was induced by subcutaneous injection of 0.18 % adrenaline solution (5 mg/kg). On days 2, 7 and 14, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) activities and reduced glutathione (G-SH) content were measured in plasma and cardiac tissue using spectrophotometric and fluorometric assays.

Results: On day 2, myocardial dystrophy caused significant decreases in SOD activity (–69.2 %), catalase (–54.4 %), GPx (–40.1 %) and G-SH content (–46.7 %) compared to intact controls ($p < 0.001$). Cryoextract treatment promoted early restoration of SOD activity (up to 54.6 %), catalase (up to +30.8 %), GPx (+6.7 %) and G-SH (+37.5 %) by day 2 ($p < 0.01$). By day 14, the cryoextract group’s enzyme activities and G-SH levels approached those of intact animals and exceeded the effect of amiodarone on GPx and G-SH ($p < 0.05$).

Conclusions: Porcine heart cryoextract exerts a pronounced cardioprotective effect in an adrenaline-induced myocardial dystrophy model by restoring the activity of key endogenous antioxidant enzymes and reduced glutathione levels. It surpasses amiodarone in the speed and extent of GPx and G-SH normalisation, supporting its further investigation as a biotherapeutic agent for correcting oxidative stress in cardiomyopathies.

Key words: Oxidative stress; Antioxidants; Defence mechanisms; Cardiomyopathies; Cryopreservation; Superoxide dismutase; Catalase; Glutathione peroxidase; Glutathione; Myocardium.

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Introduction

In a healthy heart reactive oxygen species (ROS) are by-products of normal metabolism and perform important signalling functions (sometimes referred to as “redox signalling” or oxidative

eustress). Cardiomyopathies, including adrenaline-induced myocardial dystrophy (AMD), account for a substantial proportion of hospitalisations and mortality, as uncontrolled oxidative stress (OS) impairs the recovery of myocardial contractile function and initiates progressive cardiac remodelling. Meta-analyses of clinical trials have confirmed not only elevated levels of lipid peroxidation markers in patients with cardiovascular diseases (CVDs), but also the limitations of standard antioxidant (AO) pharmacotherapy in reducing overall mortality.[1, 2]

The pathogenesis of OS-mediated cardiac dysfunction is multimodal: mitochondrial dysfunction, excessive activity of NOX2/NOX4 enzymes and the accumulation of reactive aldehydes with insufficient ALDH2 activity constitute a "red" cycle of ROS generation. Selective inhibition of NOX2 or pharmacological activation of ALDH2 has shown promising cardioprotective effects in preclinical models, but should be complemented by strategies that restore overall redox balance.[3]

The AMD model induced by acute or repeated administration of adrenaline/isoproterenol is widely used to reproduce catecholamine-mediated toxicity. Under these conditions, an explosive increase in MDA, a sharp decline in SOD and GPx activity, as well as transcriptional suppression of Nrf2 are observed, correlating with left ventricular dilatation and increased animal mortality. Thus, AMD represents a relevant model for studying approaches to the correction of OS in cardiomyocytes.[4]

The need for comprehensive, multistage solutions stimulates the search for bioactive agents capable of simultaneously modulating multiple pathways of the pathogenesis. Organ cryobiotechnology opens a new niche in cardioprotection: under ultra-deep cooling tissues preserve cardiospecific peptides, exosomes and microRNAs that can potentially activate endogenous repair mechanisms. Experimental data indicate that cryoextracts of various origins retain enzymatic antioxidant potential and reduce free radical–induced damage under reoxygenation conditions.[5, 6]

Modern experimental and clinical cardiology investigates oxidative stress as a key factor in cardiomyopathies of diverse aetiologies. Cell-free agents (cryoextracts, MSC-derived exosomes) enhance antioxidant defence, stabilise cellular membranes and protect organs under inflammatory conditions, including sepsis.[7] In turn, the MSC-conditioned medium exerts a positive effect on myocardial contractile function, as confirmed in an autoimmune myocarditis model through ultrasound assessment.[8] The efficacy of cryopreserved cell-free agents in protecting the heart during autoimmune injury has been confirmed by morphofunctional tissue assessment in preclinical models.[9]

Of particular interest are the results of using placental cryoextract, which reduces lipid peroxidation and normalises energy metabolism in cardiomyocytes in doxorubicin-induced cardiomyopathy,[10] as well as xenogeneic heart cryoextracts, which promote the recovery of myocardial functional parameters after epinephrine-induced injury.[11] Moreover, similar bioagents have been shown to modulate lipid peroxidation and energy metabolism not only in the heart but also in the gastric mucosa, indicating a systemic action under conditions of stress-induced injury.[12]

Thus, further studies may lay the groundwork for the development of personalised biotherapeutic approaches to the treatment of cardiomyopathies, in which the restoration of the endogenous antioxidant system serves as a central component of both pharmacological and biotherapeutic strategies.

Aim of this study was to investigate the mechanisms of cardioprotective activity of porcine heart cryoextract, specifically its effect on the regulation of antioxidant defence in cardiomyocytes in an adrenaline-induced myocardial dystrophy model.

Methods

Experiments were conducted on 84 outbred white male Wistar rats (250–300 g), maintained under standard vivarium conditions (ambient temperature 21 ± 2.4 °C, humidity 55 ± 4 %, with a 12-hour light/dark cycle).

The animals were divided into four separate groups. Animals were randomly assigned to experimental groups using a random number generator. Group I (n = 21) consisted of intact control rats. Group II (n = 21) comprised rats with the AMD model receiving no treatment (control

group); these animals were administered an equivalent volume of 0.9 % NaCl solution. Group III (n = 21) included rats with AMD, which received intraperitoneal administration of cryopreserved porcine heart fragment extract (CHFE) at a dose of 50 μg of peptides per 100 g body weight daily for 14 consecutive days. Group IV (n = 21) comprised AMD rats receiving intramuscular administration of amiodarone hydrochloride at 10 mg/kg daily for the same period.

Amiodarone (10 mg/kg/day, im) was selected as a reference comparator due to its established clinical and experimental efficacy in catecholamine-mediated cardiotoxicity, integrating anti-adrenergic, membrane-stabilising and antioxidant effects. Despite its broad cardioprotective profile, amiodarone is associated with important off-target toxicities, including pulmonary fibrosis, hepatotoxicity, thyroid dysfunction and renal oxidative stress. These detrimental effects highlight the need for alternative or adjunctive approaches that can provide antioxidant protection without systemic organ toxicity.

AMD was reproduced according to the protocol of Markova (1998): animals were given a single subcutaneous injection of 0.18 % adrenaline tartrate solution (5 mg/kg; PJSC "Pharmaceutical Company "Darnitsa"", Ukraine).[13] Cryopreserved porcine heart fragments were obtained according to the method of Halchenko (2005).[14] Peptide concentrations in the extracts were quantified spectrophotometrically at a wavelength of 280 nm.

Animals were withdrawn from the experiment on days 2, 7 and 14. Rat heart tissue homogenates and mixed (venous-arterial) blood, obtained following animal decapitation, were used for the analyses. For plasma preparation, whole blood was collected into EDTA-containing tubes and centrifuged for 15 minutes at 3000 g. Serum was obtained from blood samples without anticoagulant: blood was allowed to clot at 20–26 °C, after which the liquid phase was separated and centrifuged for 15–20 minutes at 3000 g.

For the preparation of heart tissue homogenate, the organ was washed with cold (+4 °C) 1.15 % KCl solution and minced (Teflon–glass, 3000 rpm) in the same buffer at a 1:10 ratio (250 mg tissue + 2.25 mL solution), resulting in 10 % suspension. Post-nuclear supernatants were prepared by centrifuging the homogenates at 600 g for 30 minutes and aliquots were subsequently collected into Eppendorf microtubes. To prepare a deproteinised extract, 0.6 M trichloroacetic acid was added to the homogenate, followed by neutralisation with 5 M potassium carbonate.

Serum superoxide dismutase (SOD) activity was measured according to a modified method of Chevari et al. The method is based on the competition between SOD and nitroblue tetrazolium (NBT) for superoxide anions generated during the aerobic interaction of reduced NADH with phenazinemethosulfate. In the control system, NBT is reduced to hydrazine-formazan, whereas in the presence of SOD, the extent of this reduction is decreased. A 1 mL serum sample was used; one arbitrary unit of enzymatic activity was defined as the amount of SOD that inhibits NBT reduction by 50 %.

Serum catalase activity was measured spectrophotometrically according to the method of Korolyuk et al.[16]

Glutathione peroxidase (GPx) activity in the myocardium was assessed spectrophotometrically by measuring the rate of reduced glutathione (G-SH) depletion before and after incubation with tert-butyl hydroperoxide (t-BuOOH).[17] The remaining G-SH was quantified based on the intensity of the coloured product formed with 5,5'-dithiobis(2-nitrobenzoic acid), which has an absorption maximum at 540 nm; the signal intensity correlated linearly with the number of reactive SH groups. Enzyme activity was expressed as μmol of reduced glutathione equivalents per mg of protein per minute.

The content of reduced glutathione (G-SH) in cardiac tissue was quantified fluorometrically using a method based on the reaction of o-phthalic anhydride with reduced glutathione resulting in a fluorescent complex detected at Ex/Em = 340/420 nm. Glutathione levels were calculated using a calibration curve.[18] G-SH content was expressed in mmol/kg.

Data processing was performed using Microsoft Excel 2010. The Shapiro–Wilk test (n < 50) was used to evaluate the normality of data distribution in each group. Homogeneity of variances was assessed using Levene's test. Comparisons between groups were conducted based on the distribution characteristics: for normally distributed data, Student's t-test was applied, while the Mann–Whitney U test was applied for non-nor-

mally distributed variables. Results following a normal distribution are presented as M ± m or M (95 % CI), whereas non-normally distributed data are reported as Me [LQ; UQ].[19]

Results

Analysis of serum superoxide dismutase (SOD) activity in rats demonstrated a distinct time-dependent dynamic. On day 2 after induction of myocardial dystrophy, superoxide dismutase (SOD) activity in the untreated AMD control group decreased to 0.14 ± 0.006 AU/L, representing a 69.2 % reduction compared to intact rats (0.45 ± 0.013 AU/L; p < 0.001). SOD activity in the CHFE-treated group SOD activity was 0.21 ± 0.012 AU/L (54.6 % of the intact level; p < 0.01), while in the amiodarone-treated group it was 0.24 ± 0.012 AU/L (47.0 %; p < 0.001).

By day 7, an increase in SOD activity was observed in all groups. In the control group, SOD activity reached 0.38 ± 0.026 AU/L, representing a 172.5 % increase compared to day 2 (p = 0.009). SOD activity in the CHFE-treated group reached 0.41 ± 0.009 AU/L (+93.6 %; p = 0.009), while in the amiodarone group it was 0.40 ± 0.008 AU/L (+67.9 %; p = 0.009). Compared to intact animals, statistically significant differences persisted in all groups (p = 0.01–0.2).

By day 14, SOD activity in all experimental groups nearly recovered to the levels observed in intact controls. In untreated rats, SOD activity was 0.45 ± 0.026 AU/L (+222.7 % vs day 2, p = 0.01; +18.6 % vs day 7, p = 0.05). In the CHFE-treated group, SOD activity was 0.44 ± 0.012 AU/L (+104 %, p = 0.01 and +5.5 %, p = 0.03, respectively), whereas in the amiodarone-treated group it was 0.46 ± 0.028 AU/L (+93.9 %, p = 0.01 and +15.5 %, p = 0.05, respectively). All groups exhibited a moderate increase in enzyme activity compared to day 7, consistent with the data shown in Figure 1.

On day 2 after AMD induction, serum catalase activity in the untreated control group was 0.13 μkat/g protein (95 % CI: [0.12; 0.13]), representing a 54.4 % decrease compared to intact animals (0.29 μkat/g protein; 95 % CI: [0.25; 0.29]; p = 0.001). This marked decrease in enzyme activity indicates severe oxidative

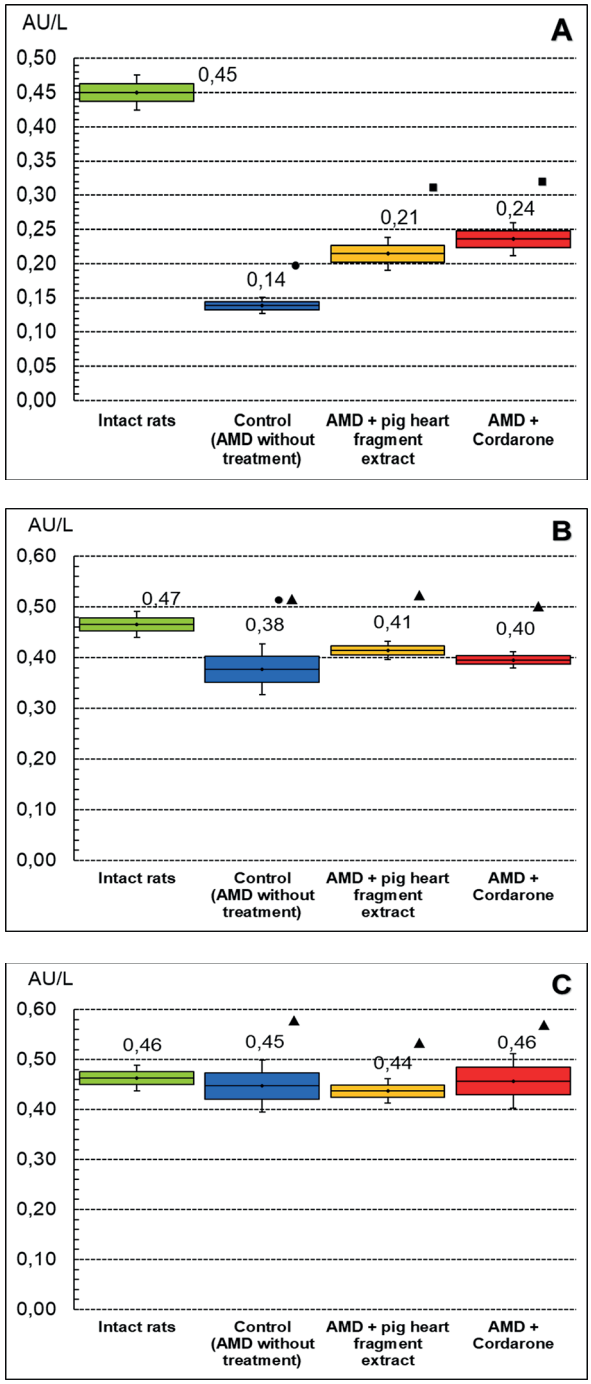

Figure 1: Serum superoxide dismutase (SOD) activity in the adrenaline-induced myocardial dystrophy model under the influence of cryopreserved porcine heart fragment extract, AU/L (M ± m, 95 % CI, n = 84; A – experimental day 2; B – experimental day 7; C – experimental day 14)

Values in each experimental group follow a normal distribution. Boxes represent the standard error of the mean; vertical lines outside the boxes indicate the 95 % confidence interval. The horizontal line within each box indicates the mean. ● – p < 0.05 compared with intact rats; ■ – p < 0.05 compared with untreated AMD rats (control); ▲ – p < 0.05 compared with day 2 of the experiment.

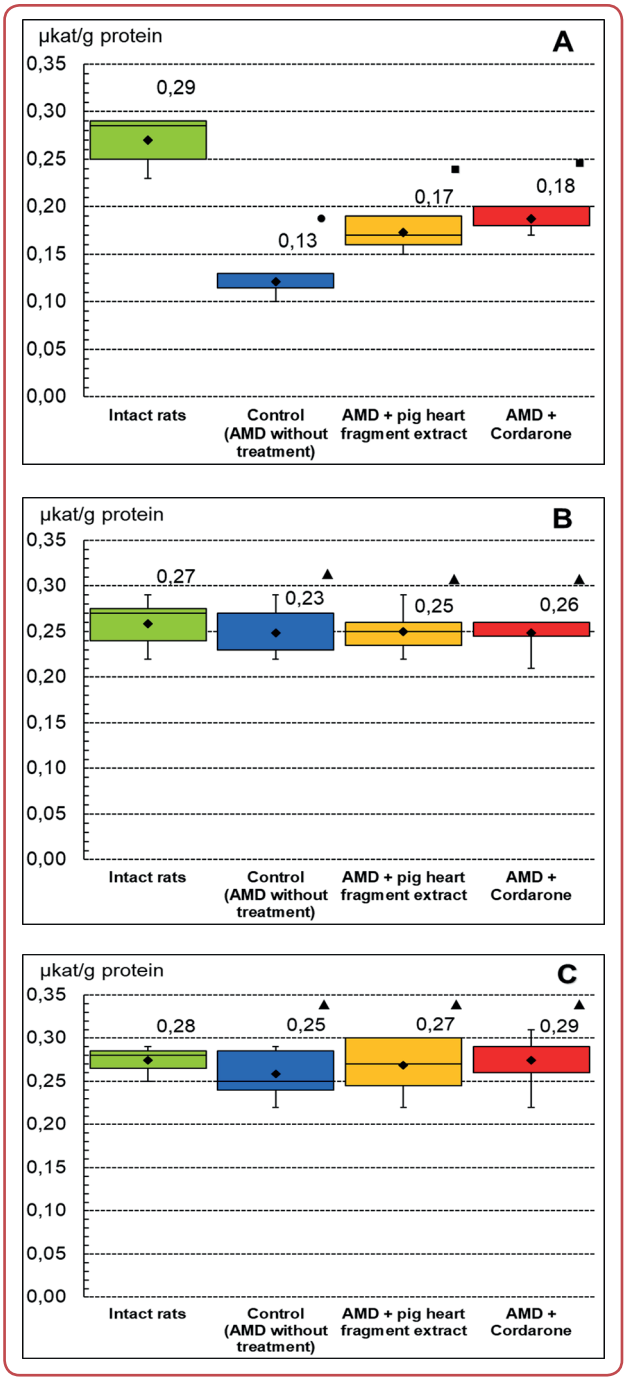

Figure 2: Serum catalase activity in the adrenaline-induced myocardial dystrophy model under the influence of cryopreserved porcine heart fragment extract, µkat/g protein (Me [LQ; UQ], n = 84; A – experimental day 2; B – experimental day 7; C – experimental day 14)

Values follow a non-normal distribution. Boxes show the interquartile range (25th–75th percentile). Vertical lines outside the boxes indicate the minimum and maximum values. The horizontal line within each box represents the median; ♦ inside the box – mean value; ● – $p < 0.05$ compared with intact rats; ■ – $p < 0.05$ compared with untreated AMD rats (control); ▲ – $p < 0.05$ compared with day 2 of the experiment.

stress. Catalase activity in the CHFE group was 0.17 µkat/g protein (95 % CI: [0.16; 0.19]), while in the amiodarone-treated group it was 0.18 µkat/g protein (95 % CI: [0.18; 0.20]), corresponding to increases of 30.8 % and 38.5 % relative to the control ($p < 0.001$ and $p < 0.01$, respectively). Despite the positive trend, catalase levels in these groups remained statistically lower than those in intact animals (Figure 2).

By day 7, enzymatic activity had increased across all experimental groups. Catalase activity in the control group reached 0.23 µkat/g protein (95 % CI: [0.23; 0.27]), representing a 76.9 % increase compared to day 2 ($p = 0.009$).Catalase activity in the CHFE and amiodarone-treated groups was 0.25 µkat/g protein (95 % CI: [0.24; 0.26]) and 0.26 µkat/g protein (95 % CI: [0.25; 0.26]), respectively, corresponding to increases of 47.1 % and 44.4 % relative to baseline values ($p = 0.009$ for both). However, even under these conditions, levels remained lower than in intact animals, indicating only partial compensation of antioxidant deficiency.

On day 14, catalase activity in the control group was 0.25 µkat/g protein (95 % CI: [0.24; 0.29]), representing a 92.3 % increase compared to day 2 ($p = 0.01$), but not significantly different from day 7 values ($p = 0.3$). Catalase activity in the CHFE group reached 0.27 µkat/g protein (95 % CI: [0.25; 0.30]), while in the amiodarone-treated group it was 0.29 µkat/g protein (95 % CI: [0.26; 0.29]). The increases relative to day 2 were 58.8 % and 61.1 %, respectively ($p = 0.01$ for both groups); however, a statistically significant difference compared to intact values persisted.

Experimental AMD progression was associated with significant decrease in GPx activity in the myocardium. On day 2 post-induction, GPx activity in the control group (untreated AMD) was 48.9 ± 0.83 µmol reduced glutathione/mg protein/min, nearly half of the values observed in intact animals (81.6 ± 1.84 µmol; $p < 0.001$). This reduction reflects activation of oxidative stress and impairment of antioxidant enzyme function. CHFE treatment led to a moderate increase in GPx activity to 52.1 ± 0.80 µmol ($p = 0.02$; +6.7 % relative to control), while amiodarone administration resulted in a more substantial increase to 57.1 ± 0.59 µmol ($p < 0.001$; +17.0 %).

On day 7, a tendency for continued restoration of enzyme activity was observed. In the CHFE group, GPx activity reached 70.0 ± 1.31 µmol ($p < 0.009$; +34.2 %), whereas in the amiodarone group it was 66.3 ± 2.23 µmol ($p = 0.01$; +16.0 %).

However, even under these conditions, values remained lower than those of intact animals. In the untreated control group, GPx values remained reduced (61.9 ± 0.77 µmol; $p < 0.009$; –26.6 % compared to intact animals).

By day 14, GPx activity in treated animals had nearly fully recovered. In the CHFE group, GPx activity reached 80.0 ± 1.43 µmol ($p = 0.01$; +53.4 % relative to control), approaching intact values. GPx activity in the amiodarone-treated group reached 74.7 ± 2.79 µmol ($p = 0.01$; +30.7 %), showing significant improvement, although the recovery rate was slightly slower compared to CHFE treatment.

Both therapeutic strategies produced a statistically significant increase in GPx activity in the cardiac tissue of rats with AMD, thereby reducing manifestations of oxidative stress. Although a more pronounced effect was observed with amiodarone at early stages, by the end of the experiment the CHFE group approached the values of intact animals, suggesting a potentially more sustained impact of this approach (Figure 3).

The study demonstrated that reduced glutathione (G-SH) levels in the cardiac tissue of rats with AMD varied dynamically, depending on the experimental group and the time point of observation.

On day 2 after AMD induction, G-SH level in the control group (untreated AMD rats) was 0.80 mmol/kg, significantly lower than in intact rats (1.50 mmol/kg; $p < 0.001$, a 46.7 % difference). In the AMD rats treated with CHFE, G-SH level reached 1.10 mmol/kg, representing a 37.5 % increase compared to the control group ($p < 0.001$), yet remaining below the levels of intact animals. In amiodarone-treated rats, G-SH levels were 1.20 mmol/kg, also indicating a significant increase compared to controls ($p = 0.004$; 50.0 % difference), although values did not reach those of intact animals (Figure 4).

By day 7, G-SH levels in rats treated with CHFE increased to 1.30 mmol/kg, representing an 18.2 % rise compared to day 2 ($p = 0.047$). In the control group, G-SH levels reached 1.20 mmol/kg, representing a 50 % increase compared to day 2 ($p = 0.009$). Amiodarone-treated rats showed less pronounced changes: G-SH levels were 1.40 mmol/kg, a 16.7 % increase compared to day 2, although this difference was not statistically significant ($p = 0.08$).

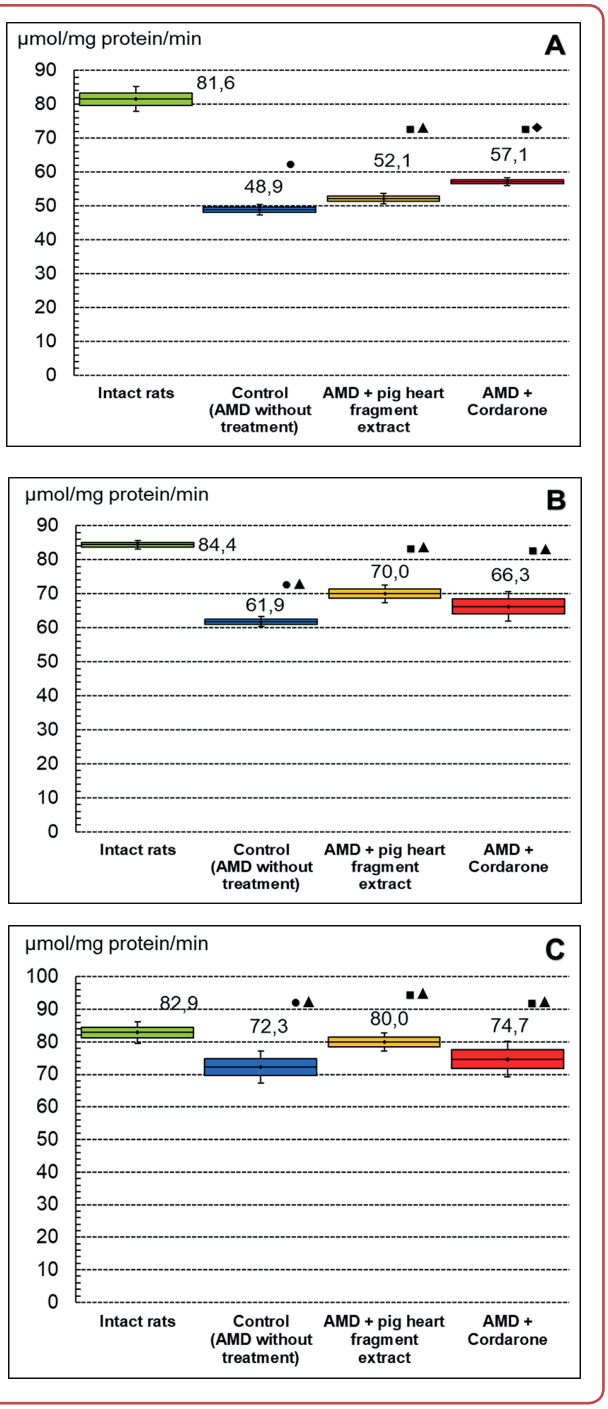

Figure 3: Glutathione peroxidase (GPx) activity in the cardiac tissue of rats with adrenaline-induced myocardial dystrophy under the influence of the extract from cryopreserved porcine heart fragments, µmol reduced glutathione/mg protein/min (M ± m, 95 % CI, n = 84; A – experimental day 2; B – experimental day 7; C – experimental day 14)

Values follow a non-normal distribution. Boxes show the interquartile range (25th–75th percentile). Vertical lines outside the boxes indicate the minimum and maximum values. The horizontal line within each box represents the median; ♦ inside the box – mean value; ● – $p < 0.05$ compared with intact rats; ■ – $p < 0.05$ compared with untreated AMD rats (control); ▲ – $p < 0.05$ compared with day 2 of the experiment.

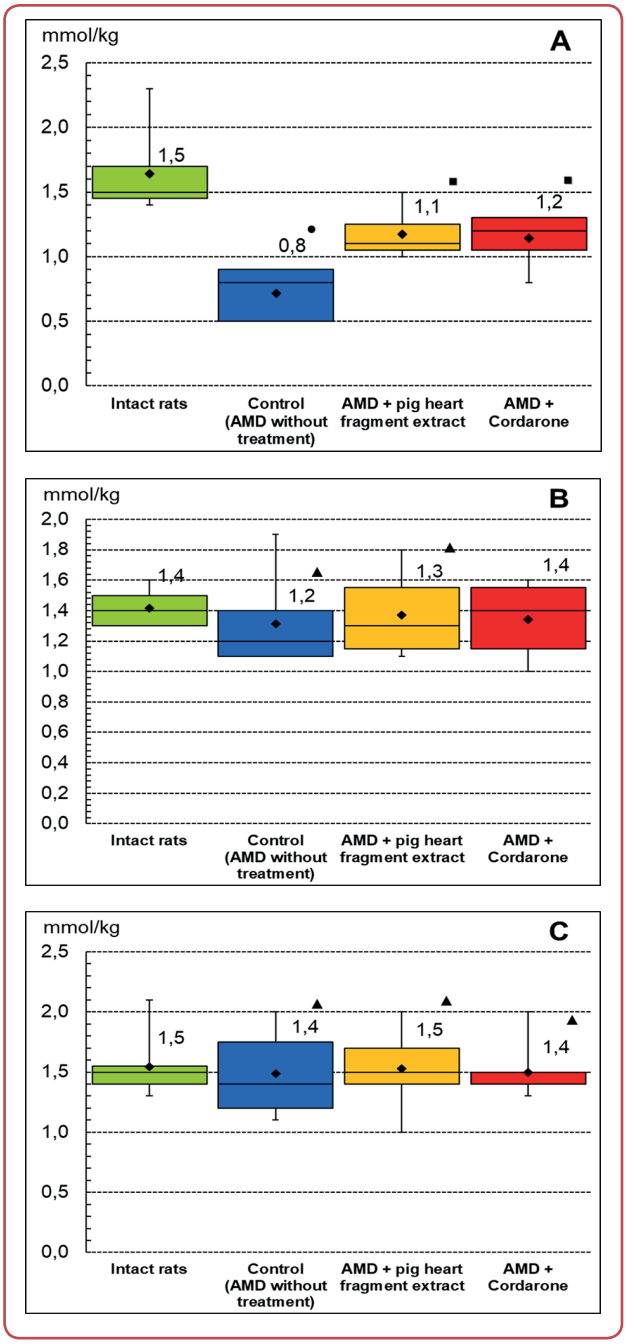

Figure 4: Reduced glutathione (G-SH) levels in the cardiac tissue of rats with adrenaline-induced myocardial dystrophy under the influence of the extract from cryopreserved porcine heart fragments, mmol/kg (Me [LQ; UQ], n = 84; A – experimental day 2; B – experimental day 7; C – experimental day 14)

Values follow a non-normal distribution. Boxes show the interquartile range (25th–75th percentile). Vertical lines outside the boxes indicate the minimum and maximum values. The horizontal line within each box represents the median; ♦ inside the box – mean value; ● – $p < 0.05$ compared with intact rats; ■ – $p < 0.05$ compared with untreated AMD rats (control); ▲ – $p < 0.05$ compared with day 2 of the experiment.

On day 14, G-SH levels in rats with intact hearts remained stable at 1.50 mmol/kg, with no significant change compared to day 7 (p = 0.09). In the AMD rats treated with CHFE, G-SH levels reached 1.50 mmol/kg, a 36.4 % increase compared to day 2 (p = 0.02); however, no significant changes were observed relative to day 7. The control group remained stable at 1.40 mmol/kg, showing no significant changes throughout the study period.

Discussion

The study of CHFE and amiodarone on serum catalase activity in rats under induced myocardial dystrophy revealed significant fluctuations of this parameter at different stages of the experiment. Catalase, as a key enzyme of the antioxidant system, catalyses the decomposition of hydrogen peroxide into water and molecular oxygen, preventing reactive oxygen species accumulation and the development of oxidative stress. The latter represents a primary pathogenetic mechanism of cardiomyocyte and cardiac tissue structural damage in AMD. Reduced catalase activity promotes persistent lipid peroxidation and progressive cellular damage, highlighting the significance of restoring its activity as part of therapeutic strategies.

Treatment with CHFE and amiodarone led to a significant increase in serum catalase activity in rats with experimental AMD, indicating a reduction in oxidative stress intensity. At the same time, neither therapeutic approach fully restored the parameters to the levels of intact animals even by day 14, highlighting the limited compensatory potential of the applied treatment regimens. The obtained results underscore the necessity for further research aimed at optimising dosing regimens, treatment duration and the potential for combined application of antioxidants to attain more pronounced and sustained recovery of myocardial antioxidant function under AMD conditions.

The dynamics of G-SH levels indicate a positive effect of treatment in the groups receiving CHFE and amiodarone. CHFE administration from day 2 improved the parameters, demonstrating the potential of this extract to reduce oxidative stress and restore cardiac tissue function in AMD. The positive effect was sustained throughout the study period, although it decreased by day 14, which may indicate the need for additional measures to maintain the therapeutic effect.

At the same time, amiodarone showed a less pronounced but still positive effect on G-SH levels, supporting its potential role in the treatment of heart diseases, though with lower efficacy in counteracting oxidative stress. These results emphasise the necessity for further investigation of innovative therapeutic approaches, particularly the use of biological materials such as CHFE, for restoring cardiac function in AMD.

The study also indicated that changes in G-SH levels may serve as an important marker of therapeutic efficacy in AMD, as glutathione plays a critical role in protecting cells from oxidative stress and facilitating detoxification in cardiac tissues. Thus, based on the obtained results, it can be suggested that an increase in reduced glutathione levels may serve as a useful indicator for assessing treatment efficacy in AMD.

The study demonstrated that CHFE administration not only restored G-SH levels but also positively affected other indicators of antioxidant activity, suggesting an overall beneficial effect on cardiac tissue. At the same time, the effect of amiodarone was less pronounced and needs additional studies to explore its mechanisms of action at the cellular level.

The results obtained demonstrate that the administration of CHFE and amiodarone significantly affects key antioxidant defence enzymes, such as SOD, catalase, GPx, as well as G-SH levels under conditions of experimental AMD. According to current data, alterations in the activity of these antioxidant systems represent a critical marker of OS progression and ischemic injury in the myocardium.[20] No deaths or observable adverse effects (lethargy, weight loss, changes in grooming or feeding) were recorded in animals receiving CHFE during the 14-day experiment.

In the AMD model, an acute increase in oxidative stress is observed in the myocardium, characterised by a reduction in the activity of key antioxidant enzymes. Presented findings revealed that by day 2 after AMD induction, the activities of SOD, catalase and GPx were significantly reduced, which corresponds to the phase of ROS accumulation during ischaemia. Similar findings have been reported in other studies, which demonstrated a rapid decline in antioxidant defence during the early stages following reperfusion injury.[21]

By day 7 of observation, a restoration of enzymatic activity was noted, indicating an adaptation to stress induced by the endogenous defence system. For example, combined therapy with diltiazem and exogenous SOD contributed to a reduction in ROS and preservation of mitochondrial structure in a rat model of ischaemia. Presented findings demonstrate that treatment with CHFE and amiodarone significantly accelerated the recovery of SOD activity compared to the control group suggesting a cardioprotective effect of CHFE through the enhancement of SOD-dependent mechanisms of superoxide neutralisation.

Regarding glutathione peroxidase and reduced glutathione, presented results are also in agreement with current literature data. GPx and G-SH are critical components of the cellular antioxidant system, responsible for mitigating damage induced by free radicals.[20] GPx utilises G-SH to detoxify organic peroxides, thereby maintaining the redox balance of the cellular environment. In this model, treatment with CHFE and amiodarone led to an earlier increase in GPx activity and G-SH levels compared to the control group, demonstrating a synergistic effect of the enzymatic antioxidant defence systems.

At the same time, presented results indicate an incomplete restoration of antioxidant homeostasis over the 14-day treatment period. This is consistent with the findings of Tong et al, who reported that prolonged administration of antioxidants or combined approaches (eg, polyphenols together with coenzyme Q10) may provide a more sustained normalisation of myocardial homeostasis.[22]

Thus, based on the analysis of current studies, it can be concluded that cardiac cryoextract possesses significant potential as a cardioprotective agent with antioxidant properties. Considering its biogenic nature, CHFE may offer advantages over conventional pharmacological agents, such as amiodarone, in reducing the risk of side effects and improving biocompatibility. For further clinical application, more detailed studies are required, taking into account optimal dosages, treatment duration and the potential for combined therapeutic regimens.

The importance of assessing oxidative homeostasis in cardiovascular pathology is heightened in the context of emerging clinical challenges. In patients with ischaemic heart disease, a significant correlation has been observed between the

level of oxidative stress and impaired immune function, which complicates disease progression and delays recovery after ischaemia.[23] Simultaneously, the impact of systemic inflammation and infectious factors, particularly COVID-19, on the risk of cardiovascular complications is being investigated, with oxidative distress serving as a key pathophysiological mechanism.[24] Additionally, clinical cases report the occurrence of stress-induced cardiomyopathy (Takotsubo) in the context of sepsis, emphasising the strong link between systemic inflammation, redox imbalance and myocardial functional exhaustion.[25]

In surgical practice, particularly during procedures in patients with reduced cardiac reserve, monitoring redox homeostasis parameters is considered a key tool for personalised fluid management.[26] Equally significant is the impact of oxidative stress in transfusiology – under wartime conditions, shifts in lipid peroxidation are observed even in blood donors, which may potentially affect the quality of erythrocyte products and their efficacy in cardiac surgery.[27]

Thus, the obtained results not only confirm the potential of using CHFE as a cardioprotective agent but also broaden the understanding of the role of combination therapy in the correction of oxidative homeostasis. Current trends in cardiology emphasise the integration of antioxidant approaches with anti-inflammatory and immunomodulatory strategies, which is particularly relevant in comorbid conditions, especially in patients with inflammatory rheumatic diseases, where pharmacological interventions may affect cardiovascular risk.[28] In this context, data on the cardioselectivity of cryobiotechnological agents[29] open up possibilities for their application in both cardiological and rheumatological practice, with the potential adaptation of dosage and administration regimens to the needs of individual patients.

Certain elements of the methodology and conceptual framework were previously reported by presented group;[30] the present study, however, extends these findings by applying them to the adrenaline-induced myocardial dystrophy model and assessing specific antioxidant parameters. Limitations of this study include the absence of a dose–response analysis of cryoextract, the lack of long-term safety follow-up. In addition, no histological or ultrastructural evaluation of myocardial tissue was performed, which limits conclusions regarding the structural correlates of the biochemical findings. The follow-up period was restricted to 14 days, which does not allow evaluation of long-term functional or remodelling outcomes. Finally, possible off-target effects of amiodarone or the cryoextract in other organs was not assess, which may be relevant to systemic oxidative balance. These aspects should be addressed in future studies.

Conclusion

Cryopreserved porcine heart fragment extract (CHFE) effectively restored the activity of key antioxidant defence enzymes (SOD, catalase, GPx) and increased the level of reduced glutathione, thereby contributing to the stabilisation of redox homeostasis and cellular membranes in adrenaline-induced myocardial dystrophy. The biogenic origin of CHFE may provide better biocompatibility and reduced risk of adverse effects, highlighting its potential for clinical application. These findings indicate the feasibility of using CHFE as a pathogenetic agent for cardioprotection in adrenergic myocardial dystrophy. The incomplete restoration of antioxidant parameters over 14 days necessitates further studies to clarify the optimal therapy regimen and the potential for combined treatment.

Ethics

The research protocol was reviewed and approved by the Bioethics Committee of IPC&K, NAS of Ukraine (Protocol No 2), dated 3 January 2022. The study was conducted in accordance with the "General Principles for Animal Experiments" (III National Congress on Bioethics, Kyiv, 2007) and complied with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes and the Law of Ukraine "On the Protection of Animals from Cruelty" No 3447-IV of 21 February 2006.

Acknowledgement

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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References

1. Wafi AM. Nrf2 and autonomic dysregulation in chronic heart failure and hypertension. Front Physiol. 2023;14:1206527. doi:10.3389/fphys.2023.1206527.
2. Chyzh MO, Belochkina IV, Hloba VY, Sleta IV, Mykhailova IP, Hladkykh FV. [Ultrasound examination of rat hearts after experimental epinephrine injury and application of xenocardiac extract]. J V N Karazin Kharkiv Nat Univ Ser Med. 2024;32(2(49)):185-97. doi: 10.26565/2313-6693-2024-49-06. Ukrainian.
3. Dawi J, Affa S, Gonzalez E, Misakyan Y, Nikoghosyan D, Hajjar K, et al. Ferroptosis in cardiovascular disease and cardiomyopathies: therapeutic implications of glutathione and iron chelating agents. Biomedicines. 2024;12(3):558. doi: 10.3390/biomedicines12030558.
4. Qian K, Song L, Guo JM, Fu D, Shi J, Ma Y, et al. Baicalin improves isoproterenol-induced cardiac remodeling by regulating the Nrf2-dependent signaling pathway. BMC Cardiovasc Disord. 2024;24(1):733. doi: 10.1186/s12872-024-04395-9.
5. Chen J, Liu X, Hu Y, Chen X, Tan S. Cryopreservation of tissues and organs: present, bottlenecks, and future. Front Vet Sci. 2023;10:1201794. doi:10.3389/fvets.2023.1201794.
6. Koshurba IV, Hladkykh FV, Chyzh MO, Belochkina IV, Rublova TV. Hepatotropic effects of triple anti-ulcer therapy and placental cryoextract: the role of sex factors in lipid peroxidation. FiziolZh. 2022;68(5):25-32. doi: 10.15407/fz68.05.025.
7. Matvieienko MS, Hladkykh FV, Oleksiuk OB. Therapeutic potential of exosomes from mesenchymal stromal cells in sepsis. Karazin J Immunol. 2024;7(1):84-97. doi: 10.26565/3083-5615-2024-13-09.
8. Hladkykh FV, Liadova TI, Komorovsky RR, Chyzh MO. Ultrasound characterization of functional changes in myocardium following the use of mesenchymal stem cell-conditioned medium in an autoimmune myocarditis model. Ukr J Cardiol. 2024;31(6):35-46. doi: 10.31928/2664-4479-2024.6.3546.

9. Hladkykh FV, Liadova TI, Komorovsky RR. Cell-free cryopreserved biological agents for cardiac protection in autoimmune myocarditis: morphofunctional study in animal model. Scr Med. 2025;6(2):233-43. doi: 10.5937/scriptamed56-56858.
10. Hladkykh FV, Chyzh MO, Koshurba IV, Belochkina IV, Komorovsky RR, Marčenko MM, et al. Anthracycline-induced cardiac injury and the effect of placenta cryoextract on myocardium in doxorubicin cardiomyopathy. Ukrainian J Radiol Oncol. 2023;31(2):190-205. doi: 10.46879/ukroj.2.2023.190-205.
11. Chyzh MO, Hladkykh FV, Liadova TI, Matvieienko MS, Komorovsky RR. Metabolic changes in the myocardium during adrenaline-induced injury and the effect of heart cryoextract on lactate-pyruvate metabolism. Ukr J Cardiovasc Surg. 2025;33(2):53-61. doi: 10.63181/ujcvs.2025.33(2).53-61
12. Koshurba IV, Hladkykh FV, Chyzh MO. Modulation of lipid peroxidation and energy metabolism in the gastric mucosa as a mechanism of the activity of placental cryoextract in healing stress-induced erosive and ulcerative lesions. Gastroenterol (Ukr). 2022;56(3):149-55. doi: 10.22141/2308-2097.56.3.2022.503.
13. Markova OO, Popovich IL, Tserkovniuk AV, [Adrenal myocardial dystrophy and organism reactivity]. Kyiv: Computerpress; 1997. 126 p. Ukrainian.
14. Halchenko SYe. [Extracts of cryopreserved xenogeneic organ fragments: preparation and biological action]. Probl Cryobiol Cryomed. 2005;15(3):403–6. Ukrainian.
15. Engelbrecht I, Horn S, Giesy JP, Pieters R. Determining superoxide dismutase content and catalase activity in mammalian cell lines. MethodsX. 2023;11:102395. doi: 10.1016/j.mex.2023.102395.
16. Hadwan MH, et al. An efficient protocol for quantifying catalase activity in biological samples (CUPRAC-CAT method). Bull Natl Res Centre. 2024;48:34. doi: 10.1186/s42269-024-01189-z.
17. Ali AF, Hadwan MH. Simple kinetic method for assessing catalase activity in biological samples. MethodsX. 2021;8:101434. doi: 10.1016/j.mex.2021.101434.
18. Kamyshnikov VS. [Handbook of clinical-biochemical studies and laboratory diagnostics]. MEDpress-inform; 2009. 896 p. Ukrainian.
19. Zar J H. Biostatistical Analysis. 5th ed. Englewood Cliffs (NJ): Prentice Hall; 2014. 960 p.
20. Zhang H, Hu H, Zhai C, Jing L, Tian H. Cardioprotective strategies after ischemia-reperfusion injury. Am J Cardiovasc Drugs. 2024;24(1):5–18. doi: 10.1007/s40256-023-00614-4.
21. Haugsten Hansen M, Sadredini M, Hasic A, Eriksen M, Stokke MK. Myocardial oxidative stress is increased in early reperfusion, but systemic antioxidative therapy does not prevent ischemia-reperfusion arrhythmias in pigs. Front Cardiovasc Med. 2023;10:1223496. doi: 10.3389/fcvm.2023.1223496.
22. Tong C, Zhou B. Cardioprotective strategies in myocardial ischemia-reperfusion injury: implications for improving clinical translation. J Mol Cell Cardiol Plus. 2024 Dec 16;11:100278. doi: 10.1016/j.jmccpl.2024.100278.
23. Havrylenko TI, Lomakovskyi OM, Pidhaĭna OA, et al. Immunological reactivity and intensity of oxidative stress in stable ischemic heart disease. Ukr J Cardiovasc Surg. 2023;31(3):22-30. doi: 10.30702/ujcvs/23.31(03)/GL008-2230.
24. Voloshyna OB, Kovalchuk LI, Balashova IV, Buheruk VV, Zbitnieva VO. Coronavirus disease: impact on risk and clinical course of cardiovascular diseases. Odesa Med J. 2023;2:98-103. doi: 10.54229/2226-2008-2023-2-18.
25. Yevsieieva VV, Kharchenko KV. The role of the POCUS protocol in the diagnosis of urosepsis-caused Takotsubo syndrome in a patient with cervical cancer. Ukr J RadiolOncol. 2024;32(4):589-96. doi: 10.46879/ukroj.4.2024.589-596.
26. Tsivenko OI, Matvieienko MS, Liashok AL, et al. Monitoring of infusion therapy in patients with reduced cardiac reserve during abdominal operations. J V N KarazinKharkiv Natl UnivSer Med. 2024;32(1):73-85. doi: 10.26565/2313-6693-2024-48-08.
27. Kalynychenko T, Anoshyna M, Malyhon O, Belousov A, Yagovdik M, Parubets L, et al. Levels of lipid peroxidation markers and main laboratory health indicators in blood donors during wartime. East Ukr Med J. 2024;12(4):827-45. doi: 10.21272/eumj.2024;12(4):827-45.
28. Garmish O, Smiyan S, Hladkykh F, Koshak B, Komorovsky R. The effects of disease-modifying antirheumatic drugs on cardiovascular risk in inflammatory joint diseases: current evidence and uncertainties. Vasc Health Risk Manag. 2025;21:593-605. doi: 10.2147/VHRM.S523939.
29. Hladkykh FV, Liadova TI, Chyzh MO, Komorovsky RR, Babaeva HH, Matvieienko MS. Cardioselectivity of cryobiotechnological agents in the treatment of cardiovascular diseases. Vinnytsia: TVORY; 2025. p. 384. doi: 10.46879/2025.4.
30. Chyzh MO, Matvieienko MS, Hladkykh FV, Liadova TI, Komorovsky RR, Kozlova TV. Evaluation of the cardioprotective activity of cardiac cryoextract in an adrenaline-induced myocardiodystrophy model based on oxidative stress markers. J V N Karazin Kharkiv Nat Univ Ser Med. 2025;33(2(53)):178–93. doi: 10.26565/2313-6693-2025-53-02.