Int. J. Morphol.,
41(1):231-236, 2023.

Can Pumpkin Save us of Doxorubicin Induced Cardiotoxicity?

¿Puede la Calabaza Salvarnos de la Cardiotoxicidad Inducida por Doxorrubicina?

Milana Bosanac[1]; Jelena Amidzic[1,2]; Maja Stefanovic[3,4]; Jelena Radic[5,6]; Ivana Kolarov-Bjelobrk[5,6]; Stefan Janicic[7]; Zdenka Gojkovic[8]; Bojana Lazic[8]; Dejan Djokanovic[8]; Aleksandra Misan[9]; Biljana Cvetkovic[9]; Alena Stupar[9]; Nikola Martic[10] & Bojana Andrejic Visnjic[1]

BOSANAC, M.; AMIDZIC, J.; STEFANOVIC, M.; RADIC, J.; KOLAROV-BJELOBRK, I.; JANICIC, S.; GOJKOVIC, Z.; LAZIC, B.; DJOKANOVIC, D.; MISAN, A.; CVETKOVIC, B.; STUPAR, A.; MARTIC, N. & ANDREJIC, V. B. Can pumpkin save us of doxorubicin induced cardiotoxicity? *Int. J. Morphol., 41(1)*:231-236, 2023.

SUMMARY: Doxorubicin (DOX) is one of the drugs necessary for the treatment of the 10 most common types of cancer. The leading adverse effect limiting clinical use of DOX is cardiotoxicity. Given that literature data indicate a protective role of carotenoids in doxorubicin-induced toxicity, in our study we compared the cardioprotective effect of a mixture of pumpkin carotenoids and a commercially available antioxidant preparation. Animals were distributed in 8 groups (Control – S; NADES – N; Doxorubicin – Dox; Carotenoids – Car; CardiofortIN – CF; NADES-Doxorubicin – N-Dox; Carotenoids-Doxorubicin – Car-Dox; CardiofortIN-Doxorubicin – CF-Dox). Histological sections were stained with the hematoxylin-eosin (HE) and analyzed for the presence of myocardial damage by doxorubicin damage score (DDS). From the heart tissue homogenate were determined the intensity of lipid peroxidation and specific antioxidative enzyme activity (superoxide dismutase; catalase; glutathione S-transferase; glutathione peroxidase). In Car-DOX and CF-DOX groups, lipid peroxidation is significantly reduced compared to DOX group. Pretreatment of animals with carotenoids and in lesser extent with CardiofortIN led to higher antioxidative enzymes activity, compared to DOX group. Pretreated with carotenoids, only 50 % of animals had some degree of myocardial damage, and no animals had extensive damage. CardiofortIN pretreatment showed less protective effect. Pretreatment with carotenoid extract, reduced DDS significantly, so Car-DOX group has changes equivalent to mild myocardial damage. Although CardiofortIN pretreatment lowered DDS score values, animals still had moderate level of myocardium damage. This in vivo study and its findings indicate that carotenoids extracted from pumpkin may be a promising cardioprotective agent against doxorubicin induced cardiotoxicity, at least in part mediated through inhibition of DOX-induced oxidative stress.

KEY WORDS: Cardiotoxicity; Doxorubicin; Carotenoids; CardiofortIN.

INTRODUCTION

Doxorubicin (DOX) is an antibiotic from the anthracycline group. Data from the World Health Organization (WHO) classify it as one of the drugs necessary for the treatment of the 10 most common types of cancer. The leading adverse effect limiting clinical use of DOX is cardiotoxicity (Ojha *et al*., 2016; Koleini & Kardami, 2017). The mechanism of side effects is still unclear and considered to be multifactorial. In the first place, doxorubicin can increase the production of reactive oxygen species (ROS) that can be neutralized to a certain level, after which oxidative stress (OS) occurs, followed by DNA damage, mitochondrial and later cell membrane rupture, ultimately resulting in cardiomyocyte apoptosis. Another mechanism is that DOX significantly reduces the levels of endogenous antioxidants,

[1] Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 2100, Novi Sad, Serbia.
[2] Department of Pathology and Cytology, General Hospital Vrbas, Vrbas, Serbia.
[3] Department of Emergency Medicine, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia.
[4] Institute of Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia.
[5] Department of Internal Medicine, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia.
[6] Oncology Institute of Vojvodina, Sremska Kamenica, Serbia.
[7] Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 2100, Novi Sad, Serbia.
[8] Faculty of Medicine Banja Luka, University of Banja Luka, Banja Luka, Bosnia and Herzegovina.
[9] Institute of Food Technology in Novi Sad, University of Novi Sad, Serbia.
[10] Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia, 21000 Novi Sad, Serbia.

Received: 2022-10-29 Accepted: 2022-12-10

which affects the capacity of anti-oxidative defense of the organism/cell (Sangomla *et al*., 2018). In order to reduce the effect of doxorubicin-induced cardiotoxicity, the use of many drugs as well as various herbal substances have been studied.

Carotenoids are liposoluble plant pigments. The most common are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zexanthin (Ciccone *et al*., 2013). There are several factors, which determine their bioavailability, and in the first place it is the medium in which they are dissolved (solvent). Unlike conventional solvents, the latest generation of «green» solvents, the so-called NADES (natural deep eutectic solvents), are not toxic (Liu *et al*., 2018). Since NADES are specific solvents, it is possible to adjust their properties in order to achieve high solubility capacity, high extraction power and high stabilization ability of some natural products (Dai *et al*., 2015). Carotenoids are powerful antioxidants which makes them point of interest in DOX-induced cardiotoxicity prevention. Certain carotenoids can be converted to vitamin A, important in the regulation of vision (Bungau *et al*., 2019). Also, carotenoids are associated with potential influences on reproduction, embryogenesis, immune system, intracellular connections as well as metabolic pathways (Indu *et al*., 2014).

Given that literature data indicate a protective role of carotenoids in doxorubicin-induced toxicity, in our study we compared the cardioprotective effect of a mixture of pumpkin carotenoids and a commercially available antioxidant preparation

MATERIAL AND METHOD

Applied chemical substances, experimental groups and protocol. Healthy male white laboratory Wistar rats were used. During one week of acclimation and during the experiment, animals were placed in standard laboratory conditions in the vivarium of the Department of Pharmacology, Toxicology and Clinical Pharmacology at Faculty of Medicine Novi Sad. They had free access to water and identical standard food. The animal study was approved by the Ethics Committee for the protection of the welfare of laboratory animals by the University of Novi Sad and the approval of the Ministry of Agriculture, Forestry and Water Management of Republic of Serbia was obtained (Belgrade, Serbia; No. 323-07-10388/2021-05).

Animals were distributed in 8 groups of 10 animals. Control (S group) received only 1ml of saline, orally during 9 days. In order to induce cardiotoxicity (DOX group) we applied doxorubicin (Doxorubicin Ebewe® 50 mg/25ml, Ebewe Pharma GmbH, Unterach, Austria). Doxorubicin was applied on 8th experimental day (single i.p. dose, 15 mg/kg) in DOX group and in groups CF-DOX, Car-DOX and N-DOX groups. Animals of CF group received (perorally) solution of CardiofortIN for 9 days, and in Car-DOX group, mixture of carotenoids extracted from pumpkin pulp (Cucurbita pepo., 900 μg/kg). Extract was prepared at the Institute of Food Technology in Novi Sad. The doses of doxorubicin, CF and carotenoids used in animals were converted from the usual therapeutic dose for humans using formula (Food and Drug Administration formula – FDA) for conversion between human and animal doses. In order to exclude any cardiotoxic impact of carotenoid solvent on myocardium, we treated N group with 1 ml of NADES solvent. N-DOX group received both doxorubicin and NADES, in order to exclude cardioprotective features of NADES. Car group received only extract for 9 days, and CF group received only CardiofortIN solution. On 9th day, general urethane anesthesia (0.75 g/kg, i.p.) was administered, and after that animals were exsanguinated by cardiopunction. An autopsy was performed on each animal, the weight of the heart was measured, and then two pieces of myocardial tissue were taken - one for histological processing and one for and determination of lipid peroxidation and antioxidant enzymes.

Determination of *in vivo* antioxidant activity. Liver samples were mixed with a physiological solution in a ratio of 1:4 w:v and homogenized at a temperature of 3 °C using an electric homogenizer type B, Braun, Potter S (Melsungen, Germany). Subsequently, the intensity of lipid peroxidasis (LPx) was estimated by measuring the amount of malonil-aldehide (MDA). Activity of antioxidative enzymes (catalase-CAT, superoxide dismutase-SOD, glutathione reductase-GR, glutathione peroxidase-GPx, glutathione S-transferase-GSH), were determined according to previously described methods (Teofilovic *et al*., 2021).

Histological tissue preparation. A heart tissue sample from the area of the left ventricle and/or heart septum was processed using standard histological techniques, embedded in paraffin, after which 5 mm thick tissue sections were cut on a rotary microtome. In order to assess the histological manifestations of cardiotoxicity and to assess the potential protective effect of the tested substances, the obtained histological sections were stained with the hematoxylin and eosin (HE). Stained tissue sections were analyzed qualitatively under a Leica DMLB microscope, at magnifications of 20x, 40x and 63x. The heart tissue of each animal was analyzed in 20 fields of view (VF). Photographs for semi quantitative analysis were made using a Leica MC190 HD camera, at 20x magnification.

Semi quantitative assessment of histological changes and determination of the doxorubicin damage score (DDS). Semi quantitative evaluation of myocardial damage was performed according to the modified method of Saad *et al*. (Saad *et al*., 2004). We analyzed 20 (not 10) visual fields and added analysis and grading of 4 more histological parameters in relation to their work (Fig. 1). Parameters interstitial edema, cardiomyocyte edema, cardiomyocyte disorganization, disorganization of myofilaments, nucleus morphology were scored as 0 (present changes in less than 5 VF) or 1 (pervasive changes in more than 5 VF). Parameters necrosis and the presence of neutrophils were scored as 0 (not observed) and 1 (present in at least 1 VF). Vacuoles in cardiomycytes were scored as 0 (sum of all vacuoles in 20 VF is less than 10) and 1 (the sum of all vacuoles in 20 VF is greater than 10).

The sum of grades of all analyzed parameters of myocardial damage for sample (one animal) gives a total score, in our research we named it "doxorubicin damage score" (DDS).

Based on the score value, it is interpreted as:

- Negative DDS score (score 0 or 1), the absence of myocardial damage or
- Positive DDS score (score 2 - 7), the existence of damage
  - Mild myocardial damage (score 2 - 3),
  - Moderate myocardial damage (score 4 - 5) or
  - Extensive myocardial damage (score 6 - 7).

Statistical data processing: Student's t-test and the Kruskal-Wallis were applied for statistical data processing, and the processing was performed in the computer program Microsoft Office Excel 2007. Statistically significant values at the level of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were noted.

RESULTS

Lipid peroxidation (LPx). Groups DOX and N-DOX have statistically significant rise in lipid peroxidation, compared to control group (S) and solvent (N) (?<0.01). In Car-DOX and CF-DOX groups, MDA and LPx are significantly reduced compared to DOX group ($p<0.05$) (Fig. 2).

Activity of antioxidative enzymes. DOX lead to decrease in activity of all analyzed antioxydative enzymes (CAT, SOD, GR, GPx, GSH), compared to saline administration in control group (S). Activity of antioxidative enzymes in N, Car and CF groups is in line with S group. Pretreatment of animals with carotenoids and in lesser extent with CardiofortIN led to higher antioxidative enzymes activity, compared to DOX group ($p<0.05$) (Table I).

Evaluation of myocardial damage according to doxorubicin damage score (DDS). Negative DDS score (absence of histological changes of myocardium) was detected in C, N, Car and CF groups. Highest DDS score was registered in DOX group, and based on DDS values, together with N-DOX group, has moderate histological damage of myocardium.

Pretreatment with carotenoid extract, reduced DDS significantly, so Car-DOX group has changes equivalent to mild myocardial damage. Although CardiofortIN pretreatment lowered DDS score values, still they are in range of moderate myocardial damage, same as DOX and N-DOX groups. Kruskal-Wallis test confirmed statistical significance of differences in DDS score among experimental groups ($H(3) = 8.17$, $P = .013$) (Table II).

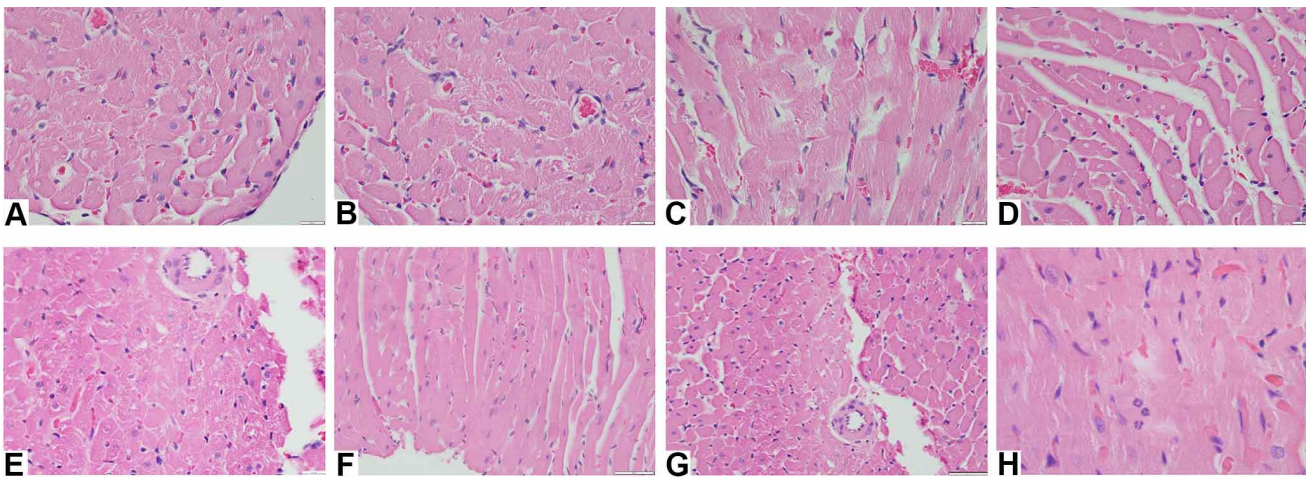

Fig. 1. Histological changes in the heart tissue evaluated in order to form the DDS score: (A) – Interstitial edema (HE, 63x); (B) - cardiomyocyte edema (HE, 63x); (C) - Cardiomyocyte disorganization (HE, 63x); (D) - Vacuoles (HE, 63x); (E) - Necrosis (HE, 40x) ; (F) - Disorganization of myofilaments (HE, 40x); (G) - Nucleus appearance changes (HE, 63x); (H) - The presence of neutrophils (HE, 63x).

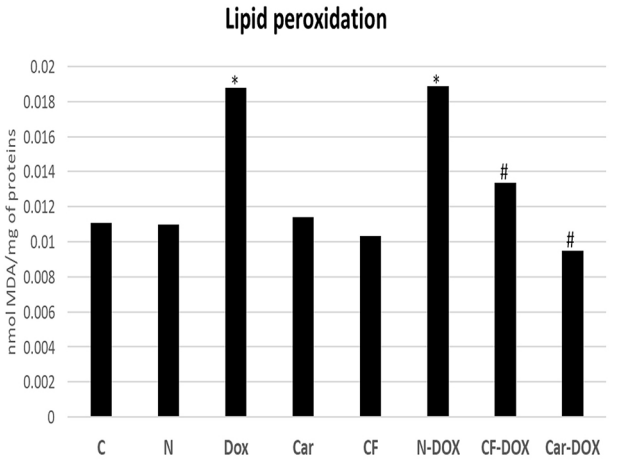

Fig. 2. Lipid peroxidation intensity. * - statistically significant compared to control group; # - statistically significant compared to doxorubicine (DOX) group.

Range (grade) of myocardial damage, according to DDS score, among experimental groups is given in Figure 3. Myocardial damage was not detected in C, N and Car groups, while one animal in CF groups had mild damage. All animals in DOX group had damage, mostly of moderate and extensive grade. When pretreated with carotenoids, only 50 % of animals had some degree of myocardial damage, and no animals had extensive damage. CardiofortIN pretreatment showed less protective effect.

Table I. Activity of antioxidative enzymes.

Group	SOD (X±SD)	CAT (X±SD)	GPx (X±SD)	GR (X±SD)	GST (X±SD)
S	30.1±34.74	43.1±8.39	65.66±20.39	38.04±20.8	60.81±21.09
N	52.84±9.65	42.54±5.67	53.99±8.61	29.71±17.53	53.2±26.08
DOX	26.37±15.24	22.17±8.11	31.51±10.28	21.88±9.63	41.17±6.98
Car	59.14±26.26	39.9±6.79	50.99±7.81	28.39±5.41	50.97±18.05
CF	54.21±36.38	31.62±3.94	72.85±64.4	28.79±12.03	51.68±26.65
N-DOX	35.53±17.07	26.38±9.5	37.25±6.09	23.37±10.52	41.51±12.92
CF-DOX #	52.02±21.16	52.93±13.3	56.17±13.08	32.61±10.52	45.18±13.16
Car-DOX #	66.71±16.47	51±10.84	47.87±10.49	43.92±34.97	65.73±19.82

S – saline, N – NADES solvent, DOX – doxorubicin, Car – carotenoids, CF – CardiofortlN, N-DOX – NADES and doxorubicin, CF-DOX – CardiofortIN and doxorubicin, Car-DOX – carotenoids and doxorubicin; SOD – superoxide dismutase; CAT – catalase ; GPx – glutathione peroxidase ; GR – glutathione reductasa; GST – glutathione S-transferase; # - statistically significant compared to DOX group.

Table II. Average values of doxorubicin damage score (DDS) in experimental groups.

Group	C	N	DOX	N-DOX	Car	Car-DOX	CF	CF-DOX
DDS	0.17	0.63	5	3.29	0.5	1.75	0.5	3

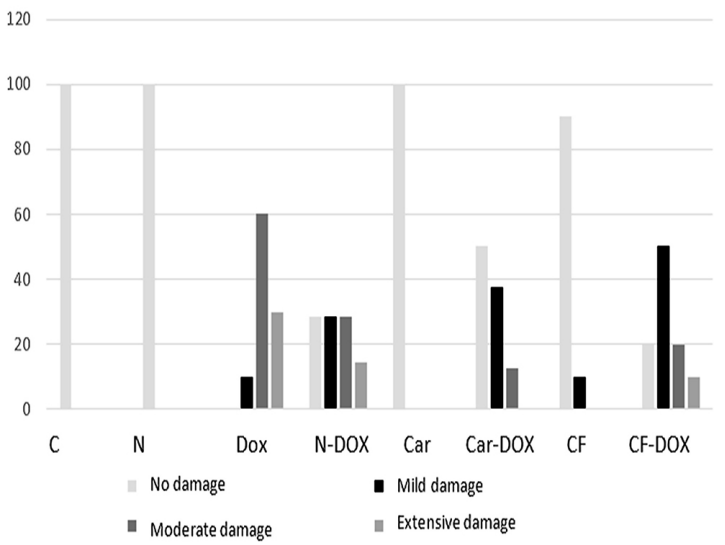

Fig. 3. Degree of myocardial damage according to DSS values.

DISCUSSION

Doxorubicin is an anthracycline antibiotic, and was first used as a cytotoxic medication in 1969. To this date, it is used in the treatment of leukemia, lymphoma, and several types of carcinomas and sarcomas (Tacar *et al*., 2013). Its most serious side effect is dose dependent cardiotoxicity. This cardiotoxicity can be acute, sub-acute, or chronic. Acute cardiotoxicity occurs in about 11 % of cases and it is manifested within 2–3 days of treatment. It can be reversible, and its onset predicts the risk of heart failure in the future. The incidence of chronic cardiomyopathy is much less than acute toxicity and is estimated to be 1.7 % (Abushouk *et al*., 2017).

Oxidative stress has been shown to be a basic mechanism of Dox-induced cardiotoxicity (Zhang *et al*., 2017). In our research, acute dose of DOX showed cardiotoxic effect on myocardium, manifested trough oxidative stress (increased lipid peroxidation and decrease of antioxidative enzymes activity). This confirms preexisting data on DOX-induced oxidative stress. Application of NADES solvent did not aggravate nor enhance the toxicity of DOX, proving it to be nontoxic and neutral for application.

Doxorubicin is a highly effective drug for the treatment of malignancies; therefore, research efforts must be directed to prevent and treat its associated cardiotoxicity. Therefore, researchers have been trying to use phytochemicals for prevention and treatment of DOX-induced cardiotoxicity. Phytochemicals (plant-derived small molecules) are non-nutritive plant chemicals that have protective or disease preventive properties (Ojha *et al*., 2016). These chemicals can be of higher pharmaceutical values than the current drugs because they are less expensive and easily available in natural food (Kok *et al*., 2000). Preclinical trials identified several mechanisms, underlying the cardioprotective effects of phytochemicals, including antioxidant, anti-inflammatory, and antiapoptotic mechanisms. Understanding these mechanisms at the cellular and molecular levels will help developing new prophylactic and therapeutic agents for DOX cardiotoxicity. That was our goal when we compared protective features of commercial drug, and natural extract. CardiofortIN is made as a combination of several antioxidative compounds (coenzyme Q, folic acid, resveratrol, lycopene, Vit D). The major carotenoid in pumpkin (Cucurbita pepo) is b-carotene (>80 %), with lesser amounts of lutein, lycopene, a-carotene and cis-b-carotene (Seo *et al*., 2005). Regarding the parameters of oxidative stress, carotenoid extract showed somewhat greater protection from lipid peroxidation. In terms of enzyme activity, compared to CF, carotenoids had stronger inductive effect on SOD, GR and GSH, equal on GPx and less to CAT activity. This is in accordance with other studies. Indu *et al*. (2014) established that administration of carotenoids prevented the depletion of antioxidants in the heart and liver, thereby protecting the tissue damage and release of marker enzymes. Carotenoids also prevented DOX-induced variation in tissue architecture in heart and liver tissues (Indu *et al*., 2014). Myocardium in our research showed many histological alterations under the influence of DOX – cardiomyocyte disorganization, vacuolization, necrosis, presence of neutrophils, disorganization of myofilaments, interstitial edema, impairment of fibers and myofibrils, changes in nuclear morphology. Based on grading of all changes, and determination of DDS score, we established that DOX induced moderate myocardial damage. While pretreatment with carotenoids alleviated these changes, application of CF had preventive effect in aspect of some histological parameters, but in spite of CF, by DDS score – myocardium had suffered moderate damage, similar to DOX group.

The use of natural products in combination with chemotherapeutic agents to reduce side effects is a novel approach, and several studies have shown promising results (Motlagh *et al*., 2021). This *in vivo* study and its findings indicate that carotenoids extracted from pumpkin may be a promising cardioprotective agent against doxorubicin induced cardiotoxicity, at least in part mediated through inhibition of DOX-induced oxidative stress.

ACKNOWLEDGMENTS. This research is financially supported by the Autonomous Province of Vojvodina (Project No 142-451-2543/2021-02)

BOSANAC, M.; AMIDZIC, J.; STEFANOVIC, M.; RADIC, J.; KOLAROV-BJELOBRK, I.; JANICIC, S.; GOJKOVIC, Z.; LAZIC, B.; DJOKANOVIC, D.; MISAN, A.; CVETKOVIC, B.; STUPAR, A.; MARTIC, N. & ANDREJIC, V. B. ¿Puede la calabaza salvarnos de la cardiotoxicidad inducida por doxorrubicina? *Int. J. Morphol., 41(1)*:231-236, 2023.

RESUMEN: La doxorrubicina (DOX) es uno de los fármacos necesarios para el tratamiento de los 10 tipos más comunes de cáncer. El principal efecto adverso que limita el uso clínico de DOX es la cardiotoxicidad. Debido a que los datos de la literatura indican un papel protector de los carotenoides en la toxicidad inducida por doxorrubicina, en nuestro estudio comparamos el efecto cardioprotector de una mezcla de carotenoides de calabaza y una preparación antioxidante disponible comercialmente. Los animales se distribuyeron en 8 grupos (Control – S; NADES – N; Doxorrubicina – Dox; Carotenoides – Car; CardiofortIN – CF; NADES-Doxorrubicina – N-Dox; Carotenoides-Doxorrubicina – Car-Dox; CardiofortIN-Doxorrubicina – CF-Dox). Las secciones histológicas se tiñeron con hematoxilina-eosina (HE) y se analizaron para detectar la presencia de daño miocárdico mediante la puntuación de daño por doxorrubicina (DDS). A partir del homogeneizado de tejido cardíaco se determinó la intensidad de la peroxidación lipídica y la actividad enzimática antioxidante específica (superóxido dismutasa, catalasa, glutatión S-transferasa, glutatión peroxidasa). En los grupos Car-DOX y CF-DOX, la peroxidación lipídica se redujo significativamente en comparación con el grupo DOX. El pre tratamiento de los animales con carotenoides y, en menor medida, con CardiofortlN condujo a una mayor actividad de las enzimas antioxidantes, en comparación con el grupo DOX. Al ser pre tratados con carotenoides, solo el 50 % de los animales tenían algún grado de daño miocárdico y ningún animal tenía daño extenso. El pre tratamiento con CardiofortIN mostró un efecto protector menor. El pre trata-

miento con extracto de carotenoides redujo significativamente el DDS, por lo que el grupo Car-DOX mostró cambios equivalentes a un daño miocárdico leve. Aunque el pre tratamiento con CardiofortIN redujo los valores de la puntuación DDS, los animales aún tenían un nivel moderado de daño al miocardio. Este estudio *in vivo* y sus hallazgos indican que los carotenoides extraídos de la calabaza pueden ser un agente cardioprotector prometedor contra la cardiotoxicidad inducida por doxorrubicina, al menos en parte mediada por la inhibición del estrés oxidativo inducido por DOX.

PALABRAS CLAVE: Cardiotoxicidad; Doxorrubicina; Carotenoides; CardiofortIN.

REFERENCES

Abushouk, A. I.; Ismail, A.; Salem, A. M. A.; Afifi, A. M. & Abdel-Daim, M. M. Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. *Biomed. Pharmacother., 90*:935-46, 2017.

Bungau, S.; Abdel-Daim, M. M.; Tit, D. M.; Ghanem, E.; Sato, S.; Maruyama-Inoue, M.; Yamane, S. & Kadonosono, K. Health Benefits of Polyphenols and Carotenoids in Age-Related Eye Diseases. *Oxid. Med. Cell. Longev., 2019*:9783429, 2019.

Ciccone, M. M.; Cortese, F.; Gesualdo, M.; Carbonara, S.; Zito, A.; Ricci, G.; De Pascalis, F.; Scicchitano, P. & Riccioni, G. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators Inflamm., 2013*:782137, 2013.

Dai, Y.; Witkamp, G. J.; Verpoorte, R. & Choi, Y. H. Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chem., 187*:14-9, 2015.

Indu, R.; Azhar, T. S.; Nair, A. & Nair, C. K. Amelioration of doxorubicin induced cardio-and hepato-toxicity by carotenoids. *J. Cancer Res. Ther., 10(1)*:62-7, 2014.

Kok, L. D.; Wong, Y. P.; Wu, T. W.; Chan, H. C.; Kwok, T. T. & Fung, K. P. Morin hydrate: a potential antioxidant in minimizing the free-radicals-mediated damage to cardiovascular cells by anti-tumor drugs. *Life Sci., 67(1)*:91-9, 2000.

Koleini, N. & Kardami, E. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. *Oncotarget, 8(28)*:46663-80, 2017.

Liu, Y.; Friesen, J. B.; McAlpine, J. B.; Lankin, D. C.; Chen, S. N. & Pauli, G. F. Natural deep eutectic solvents: properties, applications, and perspectives. *J. Nat. Prod., 3(81)*:679-90, 2018.

Motlagh, P. E.; Novin, A. G.; Ghahari, F.; Nikzad, A.; Khoshandam, M.; Mardani, S.; Khanbabaei, H.; Farsinejad, A.; Sathyapalan, T.; Sahebkar, A.; *et al*. Evaluation of the effect of crocin on doxorubicin-induced cardiotoxicity. *Adv. Exp. Med. Biol., 1328*:143-53, 2021.

Ojha, S.; Taee, H. A.; Goyal, S.; Mahajan, U. B.; Patil, C. R.; Arya, D. S. & Rajesh, M. Cardioprotective potentials of plant-derived small molecules against doxorubicin associated cardiotoxicity. *Oxid. Med. Cell. Longev., 2016*:5724973, 2016.

Sangomla, S.; Saifi, M. A.; Khurana, A. & Godugu, C. Nanoceria ameliorates doxorubicin induced cardiotoxicity: Possible mitigation via reduction of oxidative stress and inflammation. *J. Trace Elem. Med. Biol., 47*:53-62, 2018.

Seo, J. S.; Burri, B. J. C.; Quan, Z. & Neidlinger, T. R. Extraction and chromatography of carotenoids from pumpkin. *J. Chromatogr. A., 1073(1-2)*:371-5, 2005.

Tacar, O.; Sriamornsak, P. & Dass, C. R. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J. Pharm. Pharmacol., 65(2)*:157-70, 2013.

Teofilovic, B.; Tomas, A.; Martic, N.; Stilinovic, N.; Popovi´, M.; Capo, I.; Grujic, N.; Ilincic, B. & Raskovic, A. Antioxidant and hepatoprotective potential of sweet basil (*Ocimum basilicum* L.) extract in acetaminophen-induced hepatotoxicity in rats. *J. Funct. Foods, 87*:104783, 2021.

Zhang, J.; Cui, L.; Han, X.; Zhang, Y.; Zhang, X.; Chu, X.; Zhang, F.; Zhang, Y. & Chu, L. Protective effects of tannic acid on acute doxorubicin-induced cardiotoxicity: Involvement of suppression in oxidative stress, inflammation, and apoptosis. *Biomed. Pharmacother., 93*:1253-60, 2017.

Corresponding author:
Andrejic Visnjic Bojana
Department of Histology and Embryology
Faculty of Medicine
University of Novi Sad
Hajduk Veljkova 3, 2100
Novi Sad
SERBIA

E-mail: bojana.andrejic-visnjic@mf.uns.ac.rs