Comparation of animal models of acute toxicity of doxorubicin

Milana Bosanac [1], Bojana Andrejić Višnjić [1], Golub Samardžija [1, 2], Marko Marjanović [1], Jelena Amidžić [1, 3], Nemanja Martić [1], Maja Stefanović [1, 2]

SUMMARY

Background: *Animal models are essential for research in biomedicine. The cardiotoxicity of doxorubicin is the most common and severe side effect of this potent chemotherapeutic agent, and in order to investigate its prevention, a large number of studies have been performed on animal models. Unfortunately, the models are not uniform and the applied doses as well as the effectiveness vary significantly, often with great animal suffering.*

Methods: *Male Wistar rats were divided into three groups of 10 animals each and treated with saline solution intraperitoneally (group C), or with doxorubicin intraperitoneally in a single dose of 15 mg/kg (group G15) or 20 mg/kg (group G20). Body weight, mortality, the condition of animals, intensity of lipid peroxidation, activity of antioxidant enzymes and myocardial tissue damage (using doxorubicin damage score, DDS) were analyzed.*

Results: *Group 20, in comparison with groups G15 and C, exhibited the worse general condition, higher mortality and significantly higher intensity of lipid peroxidation. Both doses of doxorubicin induced a statistically significant decrease of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) activity compared to the control group. Among the doxorubicin-treated groups, GR is significantly more reduced in G15. GST is significantly more reduced in G20 while SOD and GSH-Px do not differ. The DDS score indicates myocardial tissue injury in both G15 and G20.*

Conclusion: *Both applied doses induce animal models of acute doxorubicin induced oxidative stress and cardiotoxicity. A higher dose is more suitable for studies of oxidative stress, but should be applied with caution due to higher mortality. A lower dose is more suitable for studies focused on tissue morphology.*

Keywords: *Animal models, doxorubicin, cardiotoxicity, myocardium, oxidative stress*

Arch Oncol 2025;31(2):3-8

https://doi.org/10.2298/AOO250224008B

1 Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
2 Institute for Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia
3 General Hospital Vrbas,Vrbas, Serbia

Correspondence to:
Milana Bosanac
email: milana.bosanac@mf.uns.ac.rs

Received: **2025-02-24**
Received in revised form: **2025-07-03**
Accepted: **2025-07-04**
Published online: **2025-08-12**

INTRODUCTION

Animal models play an important role in experimental research understanding various aspects of disease pathogenesis and the discovery of new therapies (1).

Depending on the goal of the study, it is necessary to choose the most adequate model as well as the species that has the most similarities with humans in that aspect. Over time, the field of research in which animals are used has been regulated both on a scientific and ethical-legal basis. One of the principles by which the Ethics Committee members are guided when deciding on granting consent is the so-called "3R rule" which implies: Replacement (replace experimental animals from the vertebrate subtype with alternative animal of a lower level of evolutionary development); Reduction (reduction of the number of animals); Refinement (refinement of the experimental procedure to a degree where it is possible to completely avoid or reduce unpleasant physical and emotional experiences of experimental animals) (2).

Very important characteristics that an ideal model should possess are that: it depicts the condition under investigation as faithfully as possible and corresponds to the accommodation and experimental capacities, it is easily accessible, it is a large enough sample and easy to handle. Also, it is particularly important that the animals live long enough to obtain valid results. Therefore, it is important to pay attention to their mental and physical health throughout the experiment. As ethics committees approve a minimum number of animals for the research, the dose used must at the same time induce changes specific to the disease under investigation without endangering the survival of the animals or their general condition to the extent that they must be euthanized or excluded (2).

Doxorubicin (DOX) is an anthracycline antibiotic which is used as a cytotoxic agent. Drugs from this group are used for the treatment of various neoplasias, among which the most common are breast cancer, lung cancer, Hodgkin's lymphoma and leukemia (3).

The clinical use of anthracyclines is limited by their adverse effects (AE). The most common AE of doxorubicin is cardiotoxicity, but, the mechanism of the side effects is still unclear and is thought to be multifactorial. Just some of them are: the increased production of reactive oxygen species (ROS), reduction of antioxidant mechanisms, incorporation into host DNA and binding to topoisomerase II, as well as drug accumulation in heart tissue, calcium overload, and impairment of autoimmune regulation of heart function (4-6). The incidence of doxorubicin induced cardiotoxicity is dose-dependent, which sharply increases when the cumulative dose of DOX reaches 550 mg/m^2 (4,7).

Regarding the doses used to induce the desired animal model of doxorubicin induced cardiotoxicity, there are very varied data in the literature - from 5 mg/kg to 40 mg/kg (4). The wide range of literature values imposes the need to compare the effects of certain doses, which is the goal of this study.

MATERIALS AND METHODS

Experimental animals

Healthy male white laboratory rats (Wistar strain, 2 months old, with a body weight of 250-300 g) were used in the experiment. During the experiment, the animals were kept under standard laboratory conditions in the vivarium of the Department of Pharmacology, Toxicology and Clinical Pharmacology of the Faculty of Medicine in Novi Sad. The temperature ranged between 20 to 25°C, air humidity 55±1.5%, with a light-dark cycle that alternated at intervals of 12 hours. Animals had free access to food and water at all times.

The Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia issued Decision No. 323-07-10388/2021-05 on the approval of the implementation of the planned experiment on animals.

Experimental design

A total of 30 animals were used, which were classified into 3 groups by the method of random selection.

Group C: 10 animals received a single intraperitoneal dose of 1 mL of saline (Natrii chloridi infundibile 0.9%, Hemofarm, Vršac, Serbia) (day 1).

Group G15: 10 animals received a single intraperitoneal dose of 15 mg/kg doxorubicin (Doxorubicin Ebewe® 50 mg/25 mL, Ebewe Pharma GmbH, Unterach, Austria (day 1).

Group G20: 10 animals received a single intraperitoneal dose of 20 mg/kg doxorubicin (Doxorubicin Ebewe® 50 mg/25 mL, Ebewe Pharma GmbH, Unterach, Austria (day 1).

The doses of the tested substances applied to animals were recalculated from the usual therapeutic dose for humans using the formula for conversion between human and animal doses, for a human weighing 70 kg:

$$\text{Rat dose}\left(\frac{\text{mg}}{\text{kg}}\right)=\frac{\text{human equivalent dose}\left(\frac{\text{mg}}{\text{kg}}\right)}{\frac{\text{the mass of the animal (kg)}}{\text{mass of man (kg)}}*0{,}33} \quad (8).$$

During the experiment, the indicators of the general condition of the animals were monitored (fur quality, blood quantity, serum quantity and quality, and mortality). The body weight (BW) of the animals was measured at the beginning of the experiment (day 1) and on the day of sacrifice (24 hours after the given dose of doxorubicin–day 2). Animals were anesthetized (urethane, intraperitoneally at a dose of 0.75 mg/kg) and sacrificed by cardiopuncture. After necropsy, two pieces of myocardial tissue were taken from each animal–one for homogenization and the other for tissue histological processing.

Determination of antioxidant enzymes

Heart samples (1 g of heart tissue) were mixed with saline at a ratio of 1:4 w:v, whereby a homogenate was made at a temperature of 3°C using an electric homogenizer type B, Braun, Potter S (Melsungen, Germany). The intensity of lipid peroxidation (LPx) was assessed by measuring malondialdehyde (MDA) levels using the TBARS assay according to Ohkawa et al. and expressed as nmol MDA per mg protein (9).Then, the samples were held in an ultrasonic bath for 2 min, then centrifuged for 15 minutes at 1372 RCF (Relative Centrifugal Force), after which the supernatant and cytosol were separated (10,11). The obtained supernatant was used for the assays of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione-S-transferase (GST). The acitivity of SOD was measured at 550 nm according to the method of McCord et al. (11). The activity of glutathione peroxidase (GSH-Px) was determined according to the methods of Chiu et al. (9,12) and the activity of glutathione reductase (GR) was determined according to the methods of Glatzle et al (9,10,13). The glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene as a substrate (9). All the measurements were obtained on the Boeco S-220 UV/Vis spectrophotometer (Hamburg, Germany).

Histological processing of tissue

After sampling, the heart tissue samples intended for histological analysis were fixed in 4% buffered formalin for 24 hours, after which the process of dehydration with isopropanol alcohol of different concentrations (70%, 80%, 96% and absolute) was started. After that, the tissue was placed in paraffin, at a temperature of 56°C, to permeate the tissue at night. On the fifth day, the tissue was cast in paraffin blocks, on a casting console (Sakura, Japan). Then, the tissue was cut into 5 μm thick slices on a microtome (Sakura, Japan), transferred to a water bath (DiaPath, Italy), after which the slices were transferred to a glass slide and a thermostat (Memmert, Germany), and then stained with standard histological staining, hematoxylin and eosin (HE).

Histological analysis of samples

The preparations were analyzed under a microscope (Leica DMLB 100T) and photographed with a camera (Leica MC 190 HD). For each model, 20 microscopic visual fields (VF) were photographed and analyzed at 200× magnification. The semiquantitative analysis of myocardial tissue damage was performed according to the scoring system (doxorubicin damage score–DDS score) described earlier (Table 1) (14).

Table 1. Semiquantitative scoring system–doxorubicin damage score (DDS score).

PARAMETERS	MARK 0	MARK 1
Interstitial edema and cardiomyocyte edema	<5 VF	≥5 VF
Cardiomyocyte disorganization	<5 VF	≥5 VF
Disorganization of myofilaments	<5 VF	≥5 VF
Nucleus morphology	<5 VF	≥5 VF
Hemorrhage	<5 VF	≥5 VF
Presence of neutrophils	0	≥1
Necrosis	0	≥1
Vacuoles (in 20 VF)	<10	≥10

Based on the score value, it is interpreted as:

- Negative DDS score (score 0 or 1,99), the absence of myocardial damage or
- Positive DDS score (score 2–7), the existence of damage:
  - Mild myocardial damage (score 2–3,99),
  - Moderate myocardial damage (score 4–5,99) or
  - Extensive myocardial damage (score 6–7).

Statistical analysis

Among the methods for testing statistical hypotheses, the parametric ANOVA method of variance analysis was used to prove the difference between three or more groups. As non-parametric statistical methods, the Mann Whitney test was used to determine the difference between two groups, the Kruskal Wallis test to determine the difference between three or more groups, and the Wilcoxon test of equivalent pairs. The hypotheses were tested at the level of statistical significance (α level) of 0.05.

RESULTS

Body weight and the general condition of animals

All the animals of the control group were healthy throughout the duration of the experiment. The animals in the G20 group had thinning fur, with the presence of bleeding from the nose and gastrointestinal tract. Mortality was higher in the G20 group (50%) compared to the G15 group (10%). During the autopsy, the serohemorrhagic liquid content and coagulum in the chest were observed. The animals from both groups lost an average of about 20 g of body mass from the beginning to the end of the experiment and there was no statistically significant difference between these two models, but these changes in body weight were statistically significantly higher in both groups compared to the control group (Table 2).

Table 2. Average body weight (BW) of the animals.

GROUP	C (saline)	G15 (15 mg/kg)	G20 (20 mg/kg)
BW (day 1) (g)	315.9	379.6	389.8
BW (day 2) (g)	323	357.4	368.8
ΔBW	+7.1	-22.2*	-21.0*

* - p<0.001; compared to the control group

Intensity of lipid peroxidation and antioxidant enzyme activity

The intensity of lipid peroxidation (LPx) (Figure 1A, Table 3) was statistically significantly higher in the group G20 compared to the control group, as well as to group G15.

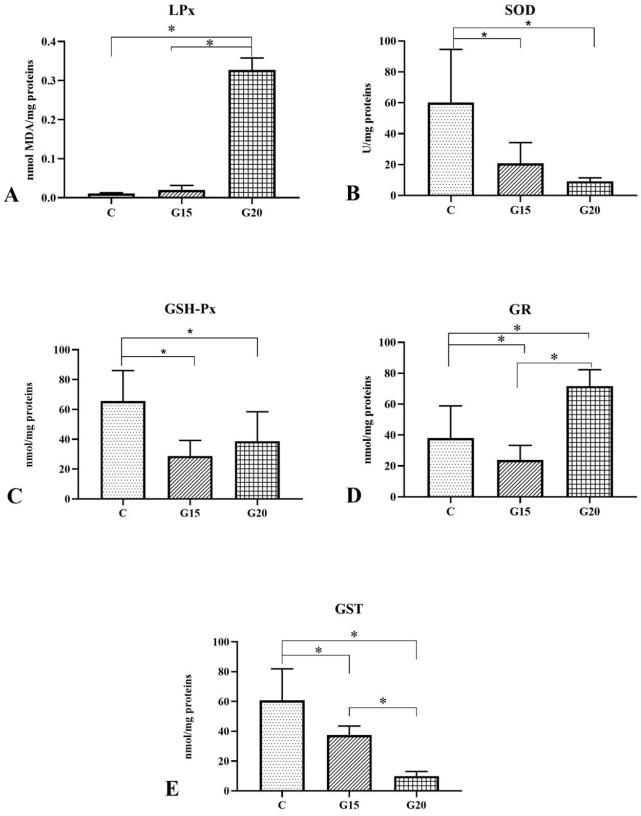

Figure 1. Graphical comparison of experimental groups based on measured oxidative stress parameters.

Legend: A) Lipid peroxidation (LPx); Activity of : **B)** Superoxide dismutase (SOD); **C)** Glutathione peroxidase (GSH-Px); **D)** Glutathione reductase (GR); **E)** Glutathione-S-transferase (GST) in animals treated with saline (C), 15 mg/kg (G15) and 20 mg/kg (G20) doxorubicin.* -p<0.001

The activities of the SOD, GSH-Px and GST (Figures 1B, 1C and 1E, Table 3) were statistically significantly lower in the groups G15 and G20 compared to the control group. The activity of the SOD was higher in group G15 compared to group G20. In opposite, the activity of the GSH-Px was lower in group G15 compared to the group G20. However, these differencies were not statistically significant. The activity of the enzyme glutathione reductase (Figure 1D, Table 3) was statistically significantly higher in the group G20 in comparison to group G15 and control group.

Table 3. Quantitative values of oxidative stress parameters (mean±SD).

	LPx (nmol MDA/mg proteins)	SOD (U/mg proteins)	GSH-Px (nmol/mg proteins)	GR (nmol/mg proteins)	GST (nmol/mg proteins)
C (saline)	0.011±0.002	60.09±34.47	65.65±20.39	38.04±20.80	60.81±21.08
G15 (15 mg/kg)	0.020±0.0112	20.77±13.47	28.77±10.44	23.87±9.46	37.42±6.10
G20 (20 mg/kg)	0.328±0.030	9.2±2.2	38.69±19.76	71.71±10.56	9.94±3.12

Legend: **A)** Lipid peroxidation (LPx); Activity of : **B)** Superoxide dismutase (SOD); **C)** Glutathione peroxidase (GSH-Px); **D)** Glutathione reductase (GR); **E)** Glutathione-S-transferase (GST) in animals treated with saline (C), 15 mg/kg (G15) and 20 mg/kg (G20) doxorubicin.* -p<0.001

Semiquantitative analysis of the effect of doxorubicin on myocardial tissue (DDS score)

The myocardial tissue of the control group showed the usual histological structure (elongated cardiomyocytes, transversely striated acidophilic cytoplasm, with a basophilic nucleus with regular borders). The histological structure of the myocardium in both groups shows the disorganization of cardiomyocytes in all animals (impaired shape, arrangement and direction of muscle cells) (Figure 2C). Disorganization is often accompanied by interstitial edema (Figure 2A), which was more pronounced in group G20. Cardiomyocyte edema (Figure 2B) and the absence of cytoplasmic cross striations was observed in all animals of both groups, in the majority of the analyzed tissue, indicating the disorganization and damage of the myofilaments (Figure 2F). These changes were more pronounced in group G15. Also, there are pronounced changes in the appearance of cardiomyocyte nuclei (from mild hyperchromasia to the reduction in nuclear size, irregularities of the nuclear membrane and karyopyknosis), and the presence of perinuclear halo (Figure 2G). The presence of numerous intracytoplasmic vacuoles was noticeable as well in the myocardium of all the animals in both the G15 and G20 model (Figure 2D). In half of the samples of the G15 group, the presence of myocardial infiltration by neutrophils was observed, while this finding was not detected in the tissue of the animals of the G20 group (Figure 2H). The necrosis of the myocardium (seen as the absence of transverse striations, absence or indistinct nuclear membrane, indistinct cell borders between cardiomyocytes) was present in both models, but more pronounced in G20 (Figure 2E).

After evaluating the observed parameters, the DDS score values in the G15 group indicate a moderate degree of damage, while in the G20 group a mild degree of damage was caused. However, there is no statistically significant difference between the DDS score of the G15 and G20 groups (Table 4).

Table 4. Results of the DDS score (doxorubicin damage score) of both experimental groups in comparison to the control group.

	C (saline)	G15 (15 mg/kg)	G20 (20 mg/kg)
DDS score	0	4*	2*

* -p<0.001; compared to the control group

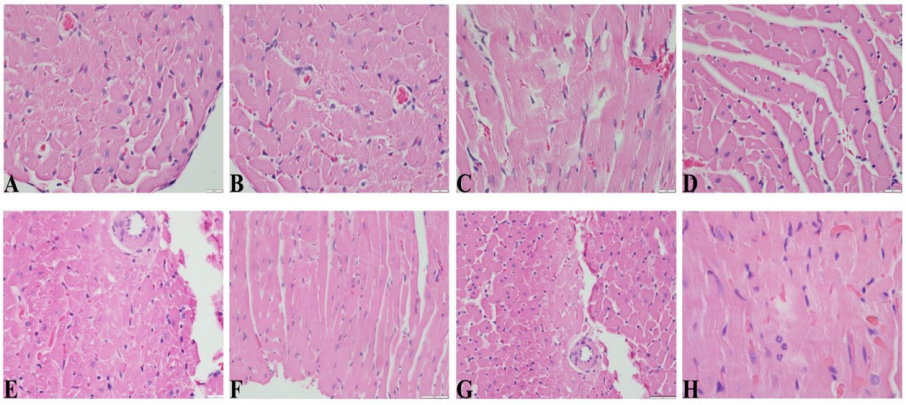

Figure 2. Histological parameters of DDS score evaluated in myocardial tissue.

Legend: A) Interstitial edema, 400×; **B)** Hemorrhage, 200×; **C)** Cardiomyocytes disorganization, 400×; **D)** Intracytoplasmic vacuoles, 400×; **E)** Necrosis, 400×; **F)** Disorganization of myofilaments, 400×; **G)** Perinuclear halo, 400×; **H)** Myocardial infiltration by neutrophils, 400×, HE.

DISCUSSION

At the beginning of the research on animals, the focus was exclusively on the well-being of people, which can be obtained from their results. However, over time, awareness developed and empathy and care for the well-being of the animals included in the research became the focus of the study planning. Simply put, the goal is to get as much (results, data and samples) as possible with as few animals, while minimising the animals suffering, stress and invasive methods (15). The research to date has mainly focused on the cardiotoxicity of doxorubicin, and the administered doses of doxorubicin vary widely. In our research, we wanted to compare the advantages and disadvantages or the shortcomings of the two most commonly used models, with the applied doses of 15 mg/kg and 20 mg/kg. We evaluated the survival, general condition and body weight of the animals, parameters of oxidative stress, as well as the degree of damage caused to the heart by the application of each of the doses.

Some authors indicate a rapid loss of BW in animals that received DOX in a short period of time (3,16,17). Also, according to the data from the same study, BW was unchanged in all rats regardless of whether they received an intravenous (i.v.) or intraperitoneal (i.p.) dose of DOX. The research on pigs showed that two days of consecutive use of this antibiotic is enough for BW to start to visibly decrease (17). In the animals given two consecutive i.v. doses at an interval of 24 hours, after 96 hours a loss of about 16% of body weight compared to the initial one was detected (16). This is, compared to our study, a higher percentage of BW loss considering that both G15 and G20 groups lost about 5%. This BW loss in G15 and G20 groups was statistically significant compared to group C (control), which clearly suggests doxorubin exerted its toxic effects. In our research, similar to other studies (16), the animals from both experimental groups lost an average of about 20 g throughout the experiment, that is, both doses equally affect the loss of body weight, without leading to a statistically significant decrease in BW.

Cardiomyocytes are particularly sensitive to oxidative damage due to the increased mitochondrial volume. Oxidative damage is supported by the increased intensity of lipid peroxidation, expressed indirectly through the concentration of malonylaldehyde, which is produced as a degradation product (9). A dose of 20 mg/kg doxorubicin caused a statistically significant higher intensity of lipid peroxidation compared to the group of animals treated with 15 mg/kg doxorubicin and saline. This suggests that the higher dose of doxorubicin (20 mg/kg) is more effective in inducing the animal model of doxorubicin-induced oxydative injury.

The interaction of doxorubicin with iron contributes to the increased production of oxygen free radicals, and it has been shown that doxorubicin treatment reduces the levels of endogenous antioxidant mechanisms, primarily SOD (18). In our study, the administration of both 15 mg/kg and 20 mg/kg of doxorubicin induced a statistically significant decrease of SOD, GSH-Px, and GST activity compared to the control group. The above stated confirms that, in fact, both applied doses disrupt the enzymatic antioxidative deffence of cardiomyocytes and establish an animal model of doxorubicin-induced oxydative injury. It should be pointed out that a greater reduction in SOD activity was achieved with 20 mg/kg compared to 15 mg/kg although the differences were not statistically significant, while the same dose induced a statistically significant reduction of GST compared to the G15 model. SOD plays an important role in the antioxidant system by converting superoxide radicals into hydrogen peroxide and molecular oxygen. Decreased levels of SOD after doxorubicin treatment intensify oxidative damage to cells by the accumulation of superoxide radicals (10). On the other hand, lower doses of doxorubicin in the G15 group induced the reduction of GR (statistically significant) and GSH-Px more efectivelly. Like the literature data (19,20), our study also indicates that doxorubicin application in both doses induced the dysregulation of the antioxidative enzymes activity and weakening of the antioxidative defence of the myocardium, regardless of the different impact of doses on specific enzymes. Knowing that in both models a reduction in the activity of antioxidant enzymes was induced, but that the intensity of lipid peroxidation was more pronounced after the administration of 20 mg/kg, it can be considered that this model is perhaps more suitable for studies focused on the pathogenesis of doxorubicin-induced oxidative stress and potential antioxidative medications.

By reviewing the literature, the most common histological manifestations of toxicity on the myocardium are: the disorganization of cardiomyocytes and myofilaments, vacuolization and necrosis, changes in the appearance of the nucleus and infiltration by neutrophils (21-26). In our research we detected interstitial edema and cardiomyocyte edema in some places, which were more pronounced in G20. Also, there are pronounced changes in the appearance of cardiomyocyte nuclei that were equally represented in both models, starting from mild hyperchromasia to the reduction in size, karyopyknosis, irregularity of the nuclear membrane and presence of the perinuclear halo. Of all the observed histological parameters of myocardial damage, the presence of intracytoplasmic vacuoles was the most prevalent–it was observed (in varying degrees) in all animals in both models.

The occurrence of these histomorphological changes is a consequence of the action of doxorubicin through different mechanisms, so the appearance of vacuoles is attributed to the swelling of membranous organelles due to the redistribution of electrolytes and water (27). Among cellular organelles, DOX has the greatest affinity for mitochondria that accumulate it, and this is precisely the reason for the mentioned disorganization of cardiomyocytes and myofilaments. The most serious and irreversible consequences of mitochondrial and cardiomyo-

cyte damage, in general, are necrosis (more pronounced in G20) (indicated by changes in the appearance of the nuclei) which was equally represented in both models.

CONCLUSIONS

Based on the obtained results, it is safe to say that both applied doses induce an animal model of acute doxorubicin induced oxydative stress and acute doxorubicin-induced cardiotoxicity. However, the decision on the model applied should be made according to the focus of the study, and the nummber of animals included. The model induced by the aplication of 20 mg/kg is more suitable for studies focused on the pathogenesis of doxorubicin-induced oxidative stress and potential antioxidative medications, but due to the high mortality of the animals and a smaller amount of serum collected, it requires more animals to be subjected to the treatment. The model induced by the application of 15 mg/kg induced a disruption in anzymatic antioxidative deffense but at the same time more pronounced muocardium tissue injury, so it is adequate if the studies are based on cardiomyocyte and myocardial tissue morphology.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

1. Mackova K, Da Cunha MGMCM, Krofta L, Albersen M, Deprest J. The importance of developing relevant animal models to assess existing and new materials. Curr Opin Urol. 2019;29(4):400-6.
2. Wolfensohn S, Lloyd M. Handbook of laboratory animal management and welfare. Oxford University Press, New York, USA, 1994.
3. Dabour MS, Abdelgawad IY, Sadaf B, Daniel MR, Grant MKO, Seelig D, et al. Losmapimod ameliorates doxorubicin-induced cardiotoxicity through attenuating senescence and inflammatory pathways. Biomed Pharmacother. 2024;179:117288. doi:10.1016/j.biopha.2024.117288.
4. Songboa M, Langa H, Xinyong C, et al. Oxidative stress injury in doxorubicin-induced cardiotoxicity. Toxicology Letters. 2019;307:41-8.
5. Al-Malky HS, Al Harthi SE, Osman AM. Major obstacles to doxorubicin therapy: Cardiotoxicity and drug resistance. J Oncol Pharm Pract. 2020;26(2):434-44.
6. Al-Jaouni S, Abdul-Hady S, El-Bassossy H, Salah N, Hagras M. Ajwa Nanopreparation Prevents Doxorubicin-Associated Cardiac Dysfunction: Effect on Cardiac Ischemia and Antioxidant Capacity. Integr Cancer Ther. 2019;18. doi:10.1177/1534735419862351.
7. Wenningmann N, Knapp M, Ande A, Vaidya TR, Ait-Oudhia S. Insights into Doxorubicin-induced Cardiotoxicity: Molecular Mechanisms, Preventive Strategies, and Early Monitoring. Mol Pharmacol. 2019;96(2):219-32.
8. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-58.
10. Stilinović N, Čapo I, Vukmirović S, Rašković A, Tomas A, Popović M, et al. Chemical composition, nutritional profile and in vivo antioxidant properties of the cultivated mushroom Coprinus comatus. R Soc Open Sci. 2020;7(9). doi:10.1098/rsos.200900.
11. McCord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244(22):6049-55.
12. Chiu PTY, Stults FH, Tappel AL. Purification of rat lung soluble glutathione peroxidase. Biochim. Biophys. Acta. 1976;445:558-660.
13. Glatzle D, Vulliemuier JP, Weber F, Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. Experientia. 1974;30:665-7. doi:10.1007/BF01921531.
14. Bosanac M, Amidzic J, Stefanovic M, Radic J, Kolarov-Bjelobrk I, Janicic S, et al. Can pumpkin save us of doxorubicin induced cardiotoxicity? Int. J. Morphol. 2023;41(1):231-6.
15. Robinson NB, Krieger K, Khan FM, Huffman W, Chang M, Naik A, et al. The current state of animal models in research: A review. Int J Surg. 2019;72:9-13.
16. Kullenberg F, Peters K, Luna-Marco C, Salomonsson A, Kopsida M, Degerstedt O, et al. The progression of doxorubicin-induced intestinal mucositis in rats. Naunyn Schmiedebergs Arch Pharmacol. 2023;396(2):247-60.
17. Shen RL, Pontoppidan PE, Rathe M, Jiang P, Hansen CF, Buddington RK, et al. Milk diets influence doxorubicin-induced intestinal toxicity in piglets. Am J Physiol Gastrointest Liver Physiol. 2016;311(2). doi:10.1152/ajpgi.00373.2015.
18. Zare MFR, Rakhshan K, Aboutaleb N, Nikbakht F, Naderi N, Bakhshesh M, et al. Apigenin attenuates doxorubicin induced cardiotoxicity via reducing oxidative stress and apoptosis in male rats. Life Sci. 2019;232. doi:10.1016/j.lfs.2019.116623.
19. Al-Amir H, Janabi A, Hadi NR. Ameliorative effect of nebivolol in doxorubicin-induced cardiotoxicity. J Med Life. 2023;16(9):1357-63.
20. Sheibani M, Nezamoleslami S, Faghir-Ghanesefat H, Emami AH, Dehpour AR. Cardioprotective effects of dapsone against doxorubicin-induced cardiotoxicity in rats. Cancer Chemother Pharmacol. 2020;85(3):563-71.
21. Shosha MI, El-Ablack FZ, Saad EA. Glycine protects against doxorubicin-induced heart toxicity in mice. Amino Acids. 2023;55(5):679-93.
22. Nagy A, Börzsei D, Hoffmann A, Török S, Veszelka M, Almási N, et al. A Comprehensive Overview on Chemotherapy-Induced Cardiotoxicity: Insights into the Underlying Inflammatory and Oxidative Mechanisms. Cardiovasc Drugs Ther. 2024. doi:10.1007/s10557-024-07574-0.
23. Reis-Mendes A, Ferreira M, Padrão AI, Duarte JA, Duarte-Araújo M, Remião F, et al. The Role of Nrf2 and Inflammation on the Dissimilar Cardiotoxicity of Doxorubicin in Two-Time Points: a Cardio-Oncology In Vivo Study Through Time. Inflammation. 2024;47(1):264-84.
24. Ekinci Akdemir FN, Yildirim S, Kandemir FM, Tanyeli A, Küçükler S, Bahaeddin Dortbudak M. Protective effects of gallic acid on doxorubicin-induced cardiotoxicity; an experimantal study. Arch Physiol Biochem. 2021;127(3):258-65.
25. Alharbi FK, Alshehri ZS, Alshehri FF, Alhajlah S, Khalifa HA, Dahran N, et al. The role of hesperidin as a cardioprotective strategy against doxorubicin-induced cardiotoxicity: The antioxidant, anti-inflammatory, antiapoptotic, and cytoprotective potentials. Open Vet J. 2023;13(12):1718-28.
26. Ahmed AZ, Mumbrekar KD, Satyam SM, Shetty P, D'Souza MR, Singh VK. Chia Seed Oil Ameliorates Doxorubicin-Induced Cardiotoxicity in Female Wistar Rats: An Electrocardiographic, Biochemical and Histopathological Approach. Cardiovasc Toxicol. 2021;21(7):533-42.
27. Balli E, Mete UO, Tuli A, Tap O, Kaya M. Effect of melatonin on the cardiotoxicity of doxorubicin. Histol Histopathol. 2004;19:1101-8.