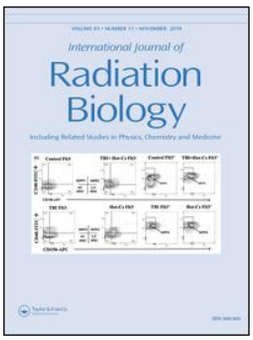

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ORIGINAL ARTICLE

Protective effect of N-acetyl cysteine against radiotherapy-induced cardiac damage

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ABSTRACT

Purpose: Although radiotherapy (RT) is an important component of cancer treatment, it induces adverse tissue reactions in the around of cancer tissue. Therefore, radioprotectives are needed to protect normal tissues. The aim of this study was to investigate the radioprotective effect of N-acetylcysteine (NAC) on RT-induced cardiac damage in rats for the acute term.

Materials and methods: The animals were divided into four groups. The rats in control group were injected with saline for 7 d; the rats in NAC group were injected NAC at dose of 240 mg/kg d for 7 d; the rats in RT group were injected with saline for 7 d plus was irradiated 1 h after the last injection and the rats in NAC + RT group were injected with NAC for 7 d and irradiated 1 h after the last NAC dose. The electrocardiogram was recorded and evaluated PR interval, QRS duration, QT interval, T wave alterations and heart rate. Serum interleukin-4, interleukin-6, tumor necrosis factor-alpha, interleukin 1 beta, galectin-3 levels and creatine kinase and creatine kinase isoenzyme-MB activities were determined in all groups. Also, tissue malondialdehyde (MDA) and nitric oxide levels, superoxide dismutase, catalase and glutathione peroxidase activities were determined. In addition, histological changes of heart were evaluated. All measurements were performed 24 h after RT.

Results: In the RT group, findings supporting cardiac injury were observed in the electrocardiogram. Also, cytokine levels and oxidative stress were significantly increased. Pretreatment of rats with NAC ameliorated cardiac injury induced by RT.

Conclusions: Our findings suggested that NAC may be a potential radioprotector which is capable of preventing cardiac damage.

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Radiotherapy; heart; N-acetyl cysteine; cytokines; antioxidants; radioprotector

Introduction

Radiotherapy (RT) is one of the commonly used methods for cancer treatment. RT kills cancer cells, but also damages healthy cells and causes various side effects on cancer patients. During the RT treatment of thoracic cavity malignant tumors; mainly lung, breast and Hodgkin lymphoma, heart is heavily exposed to ionizing radiation (Andratschke et al. 2011; Barjaktarovic et al. 2013). Cancer-related heart disease has become the leading cause of death in the world (Menezes et al. 2018). After RT, pericardial diseases, myocarditis, cardiomyopathy, valvular diseases, coronary artery disease and conduction abnormalities may occur in long-term survival (Kruse et al. 2003; Madan et al. 2015). Long before the onset of cardiac changes occurring many years after RT, some subclinical cardiac changes may occur during hours, days, weeks, months or early years after RT (Jacob et al. 2016). These can be detected based on either functional dysfunction or structural impairment measurements (Jacob et al. 2016). Although RT techniques developed in recent years have reduced the average radiation exposure of the heart, epidemiological data show that exposure to low dose RT also leads to heart damage (Zhu et al. 2018). In a population-based study, investigating a threshold dose below which optimal therapeutic effects are achieved with the least cardiotoxicity, 2168 women with breast cancer were exposed to the radiation in the range of 0.03–27.72 Gy (Darby et al, 2013). Analysis results showed that rates of major coronary events such as myocardial infarction, coronary revascularization or death from ischemic heart disease increased linearly with the mean heart dose by 7.4% per gray (Darby et al. 2013).

In recent years, new treatment techniques were developed to reduce the early and late toxic effects of RT on the heart (Rygiel 2017). Use of pharmaceutical agents to prevent RT-related cardiac damage is one of the potential methods (Yarom et al. 1993; Inano et al. 1997; Baker et al. 2011). Most of these agents capture free radicals and protect the tissue against radiation damage (Raviraj et al. 2014). One of

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these agents, black grape juice, when administered 1 week before starting irradiation in a rat model, reduced adverse cardiac effects (de Freitas et al. 2013). Another agent used as a cardioprotective in irradiated mouse is hydrogen-saturated water. It was reported that administration of water saturated with molecular hydrogen starting 24 h before single-dose local heart irradiation reduced chronic myocardial injury *via* free radical scavenging (Qian et al. 2010). In another study, it was reported that vitamin C and vitamin E exhibited efficient cardioprotection in patients with malignancies at 3 weeks after RT (Ma et al. 2019).

N-acetylcysteine (NAC), an acetylated cysteine residue, is a cheap and easily accessible drug with few side effects. It has been used for several decades in clinics as a mucolytic agent. NAC also acts as a scavenger of free radicals due to its direct interaction with reactive oxygen species (Tahan et al. 2007; Uraz et al. 2013; Lasram et al. 2014; Mohammed et al. 2019). In addition, NAC is a potent anti-inflammatory compound (Csontos et al. 2012; Cazzola et al. 2017) and inhibits expression of proinflammatory cytokines (Pei et al. 2018).

There are some studies on the protective effect of NAC against cardiac injury (Mansour et al. 2015; Nagoor Meeran and Mainzen Prince 2011). To the best of our knowledge, there are no studies in the literature investigating the effect of NAC on acute RT-induced cardiac injury. Based on this knowledge, we designed this study to evaluate the protective effects and possible mechanisms of NAC in RT-induced cardiac damage by using electrophysiological, biochemical and histological methods for an acute term.

Material and methods

Animals

A total of 30 adult female Wistar albino rats weighing 200–250 g were housed in polycarbonate cages in the Experimental Animal Center of Mersin University (Mersin, Turkey). They were kept under standard conditions (at 25 ± 1.5 °C and 55% humidity with 12 h/12 h light/dark cycles) and they had free access to food and water. The study was approved by Mersin University Experimental Animals Local Ethics Committee and carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (Reference No: 26.12.2016/16/50).

Chemicals and kits

NAC was provided as a pure dry powder (Bilim İlaç Sanayi, İstanbul, Turkey). Other chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co, St. Louis, MO). NAC reconstituted in physiological saline and administered at pH 7.2.

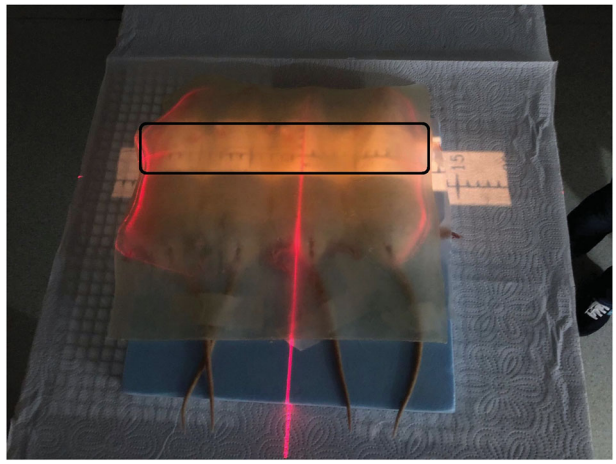

Figure 1. Irradiation of rats. The area within the black rectangle was irradiated.

Experimental design

The animals were divided into four groups as control, NAC, RT and NAC + RT. The rats in the control group ($n = 6$) were injected with 0.9% saline solution as intraperitoneally (i.p.) for seven consecutive days. The rats in the NAC group ($n = 8$) were pretreated with NAC by ip injection at a dose of 240 mg/kg bodyweight for seven consecutive days (Atis et al. 2006; Çağlar et al. 2013). The rats in the RT group ($n = 8$) were exposed to radiation at dose of 20 Gy. The rats in the NAC + RT group ($n = 8$) were pretreated by i.p. NAC injection at a dose of 240 mg/kg bodyweight for seven consecutive days before they were exposed to radiation at dose of 20 Gy. Twenty four hours after the RT, ECG was recorded from all animals and blood samples were collected. Then all rats were sacrificed following over dose anesthesia and heart tissue was isolated for biochemical and histological evaluations.

Radiotherapy

Before irradiation, the rats in the RT and NAC + RT groups were anesthetized with an intramuscular injection of ketamine hydrochloride (Ketalar®, 50 mg/kg, Eczacıbaşı, Istanbul, Turkey) and xylazine hydroxyl chloride (Rompun®, 5 mg/kg, Bayer, Pittsburgh, PA). Rats were immobilized in a supine position and thoracic regions were irradiated with 6 MV photon energy using linear accelerator (Siemens, Primus, Germany). A 1 cm bolus was placed on the rat's skin for up to build- up the region and homogeneous dose distribution (Figure 1). Total 20 Gy radiation was given, 10 Gy anterior direction and 10 Gy posterior direction, as a single fraction (Cilliers et al. 1989; Dalloz et al. 1999; Gürses et al. 2014).

Electrocardiography (ECG) recordings

ECG was recorded 24 h after the RT from the control and experimental groups. The ECG records were obtained by anesthetizing the rat using ketamine hydrochloride (Ketalar®, 50 mg/kg, Eczacıbaşı, Istanbul, Turkey) and xylazine hydrochloride (Rompun®, 5 mg/kg, Bayer,

Pittsburgh, PA). Disposable disc-shaped small Ag/AgCl electrodes were used for recordings. Electrode impedance was considered to be less than 5 kΩ. The ECG signals were continuously recorded for at least 15 min in lead I using BIOPAC MP 100 Acquisition System version 3.5.7 (Santa Barbara, CA) from all groups. Data were transferred to the computers translating to the numerical signals by 16-bit A/D converter for the off-line analysis. The sampling rate was chosen as 500 sample/s. BIOPAC Acknowledge Analysis Software (ACK 100 W) was used to measure ECG signals. The ECGs were analyzed for heart rate, P, QRS and T wave changes, PR and QT intervals, ST-segment changes, T wave changes and pathologic Q waves.

Biochemical analysis

Tissue inflammatory factors and cardiac biomarkers

Interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1 β), galectin-3 (GAL-3) levels and creatine kinase (CK) and creatine kinase isoenzyme-MB (CK-MB) activities were measured for each 100 µL serum sample by using ELISA kit (MyBioSource, San Diego, CA) according to manufacturer's instructions.

Tissue protein content

Total protein content in tissue homogenates was measured by the Lowry method (Lowry et al. 1951). In the alkaline solution, copper ion (Cu^{2+}) forms a complex with peptide bonds in proteins. Folin–Ciocalteus–Phenol Reagent is reduced by a copper-protein complex, resulting in a dark blue color. The intensity of the color formed was directly proportional to the protein concentration and was measured spectrophotometrically at 750 nm. Bovine serum albumin was used as standard.

Malondialdehyde (MDA) level

Malondialdehyde (MDA) levels were measured according to the method developed by Yagi (1998). This method is based on the principle that the pink color formed during the reaction between lipid peroxidation products and thiobarbituric acid is measured at 532 nm by UV–VIS spectrophotometer. MDA levels are expressed as nmol/mg protein.

Superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) activity was determined by measuring superoxide radicals which are released by xanthine oxidase in the presence of xanthine and nitroblue tetrazolium (NBT) at 560 nm *via* spectrophotometrically according to Sun et al. SOD activity was expressed as U/mg protein (Sun et al. 1988).

Catalase (CAT) activity

This method is based on the monitoring of degradation of H_2O_2 substrate by catalase (CAT) activity at 240 nm for 1 min. The CAT activity was expressed as U/mg protein (Aebi 1984).

Nitric oxide (NO) level

The amount of nitric oxide (NO) was determined by Vanadium-3-chloride-Gries Reaction Method according to Miranda et al. An aliquot of 100 mL vanadium chloride solution was added to 100 mL of each sample followed by 50 mL sulfanilamide and 50 mL NEDD solutions. The mixture was incubated at 37 °C for 30 min and then measured at 550 nm *via* spectrophotometer (Miranda et al. 2001).

Glutathione peroxidase (GSH-Px) activity

This method is based on the principle of spectrophotometric measurement of NADPH formed during the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) by GSH-Px enzyme at 340 nm wavelength via a spectrophotometer. Enzyme activity was expressed as U/mg protein (Paglia and Valentine 1967).

Histopathological evaluation

After ECG recordings, the animals were anesthetized using ketamine (100 mg/kg; ip) and xylazine (10 mg/kg; ip) and sacrificed. Following sacrifice, the hearts were immediately excised. For microscopic evaluation of the heart tissue samples were fixed with 10% formalin and embedded in paraffin. Tissue blocks were sectioned into 5 μm-thick slices by rotary microtome (Leica 2255), and stained by hematoxylin and eosin (H&E) and Masson's Trichrome (MS). For transmission electron microscopic (TEM), evaluation of the heart tissue samples were fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated in gRTed alcohol series, cleared with propylene oxide and embedded in epon. Thin sections (50–70 nm) were cut by a ultramicrotome (Leica UCT-125) and contrasted with uranyl acetate and lead citrate. Sections were examined and photographed under a transmission electron microscope (JEOL JEM-1011).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 20.0 (IBM, Istanbul, Turkey). The Kolmogorov–Smirnov test was used to check normality of variables. Statistical analysis for comparisons of groups was evaluated by using ANOVA followed by the Tukey *post hoc* test. Data were expressed as mean ± standard deviation (SD). $p < .05$ was considered statistically significant.

Results

ECG analysis

Visual evaluation

ECG analysis recordings collected from NAC, NAC + RT and control groups were shown in Figure 2. In these groups, all rats had normal ECG wave intervals (Figure 2(A–C)). For two rats (25%) in the RT group, no P wave (reflects depolarization of atria) was observed (Figure 3(A)). Also, in some rats increased, T wave amplitude (50%) was observed (Figure 3(B,C)).

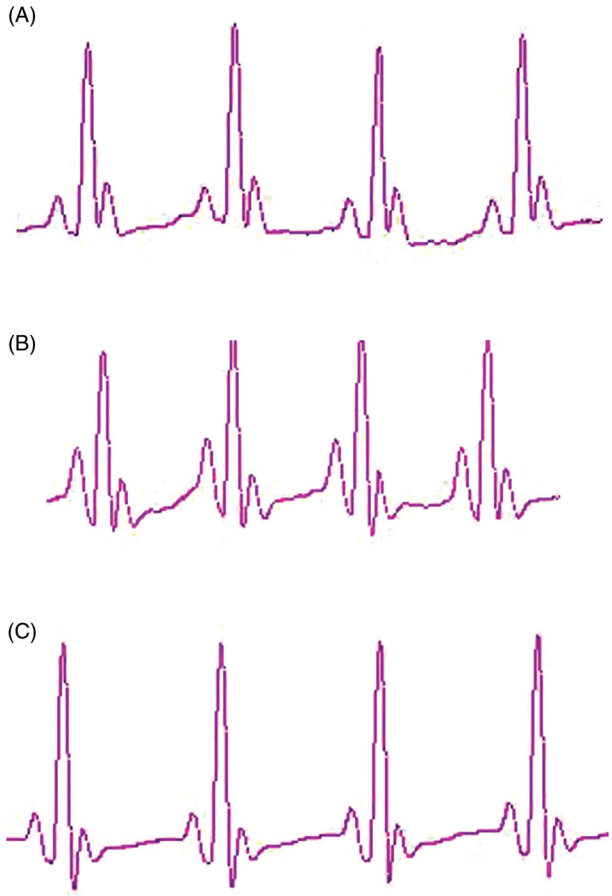

Figure 2. Samples of ECG recordings from control (A), NAC (B) and NAC + RAD groups. In all groups ECG records were normal. Horizontally (time): 5 mm = 50 ms; vertically (amplitude): 5 mm = 1.5 mV.

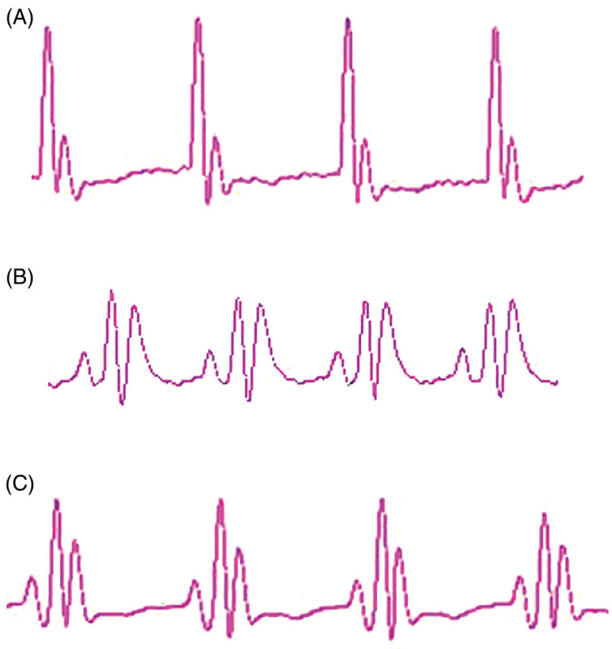

Figure 3. Samples of ECG recordings from RT group. Absent P wave (A), tall T wave (B) and decreased heart rate (C). Horizontally (time): 5 mm = 50 ms; vertically (amplitude) 5 mm = 1.5 mV.

Quantitative analysis

ECG data were shown in Table 1. ECG analysis results showed that atrioventricular conduction interval (PQ interval) was not significantly changed in NAC, RT and NAC + RT groups compared to the control group. Similarly, there were no changes in ventricular conduction time (QRS complex width) among groups. T wave amplitude in the RT group significantly increased compared to the control, NAC and NAC + RT groups. In the RT group, the T amplitude increased by 2.98 fold compared to the control group, but NAC treatment caused T amplitude to return to the control's value in the NAC + RT group (Table 1). There were no significant differences among the control, NAC and NAC + RT groups. The heart rates in the RT group were observed to be lower than the control, NAC and NAC + RT groups. Heart rate decreased by 14% in RT group compared to control group but NAC treatment reduced this ratio to 3% in the NAC + RT group. There were no significant differences between the control group and NAC, NAC + RT groups.

Light and electron microscopic findings

As a result of microscopic examination of H&E and MS staining, cardiac muscle cells had normal morphological features in all groups (Figures 4 and 5). In the electron microscopic examinations, the heart muscle cells had normal morphological structures in all groups. Myofibrils exhibited a regular sarcomere organization. Cytoplasmic organelles showed a normal structure in control group. Similar findings were observed in the NAC, RT and RT + NAC groups but there were non-significant expansions in sarcoplasmic reticulum cisterns in some muscle cells (Figure 6).

Biochemical findings

Biochemical findings were shown in Table 2. IL-4, IL-6, IL1-β and TNF-α levels were significantly elevated in the RT group compared to the control, NAC and NAC + RT groups ($p<.05$), but significantly decreased by NAC treatment in the NAC + RT group. CK and GAL-3 levels were not changed in all groups. CK-MB levels were significantly increased by 71.5% in RT group compared to control group, but in the NAC + RT group, NAC treatment reduced this ratio to 33%. There were no significant differences among other groups. The SOD and GPx levels were not observed to be significantly variable among groups. Only in the RT group the MDA levels were higher than the control, NAC and NAC + RT groups ($p<.05$), and no significant change was observed among control, NAC and NAC + RT groups ($p>.05$). MDA levels increased by 84% in RT group compared to control group, however, in the NAC + RT group, NAC treatment reduced this ratio to 46%. CAT activities were observed to be decreased in both RT and NAC + RT groups compared to control group ($p<.05$). In the RT group, NO levels were lower than the control, NAC and NAC + RT groups ($p<.05$). There was no significant difference among control, NAC and NAC + RT groups ($p>.05$).

Table 1. ECG parameters in control and experimental groups.

Variables	Control ($n = 6$)	NAC ($n = 8$)	RT ($n = 8$)	RT + NAC ($n = 8$)
T wave amplitude (mV)	1.5 ± 0.02	1.4 ± 0.04	4.47 ± 0.04[a,b,c]	1.43 ± 0.02
PQ interval (ms)	55.2 ± 14.3	51.5 ± 7.1	45.1 ± 5.9	49.3 ± 15.7
Duration of QRS (ms)	39.1 ± 8.2	35.4 ± 6.1	40.0 ± 6.3	38.4 ± 4.3
QT interval (ms)	70.3 ± 16.2	71.7 ± 8.0	65.4 ± 13.1	74.7 ± 7.2
Heart rate (beats/min)	276.6 ± 32.1	266.9 ± 28.9	237.7 ± 26.3[a,b,c]	268.1 ± 23.7

[a]Different from control group ($p < .05$), [b]Different from NAC group ($p < .05$), [c]Different from NAC + RT group ($p < .05$).

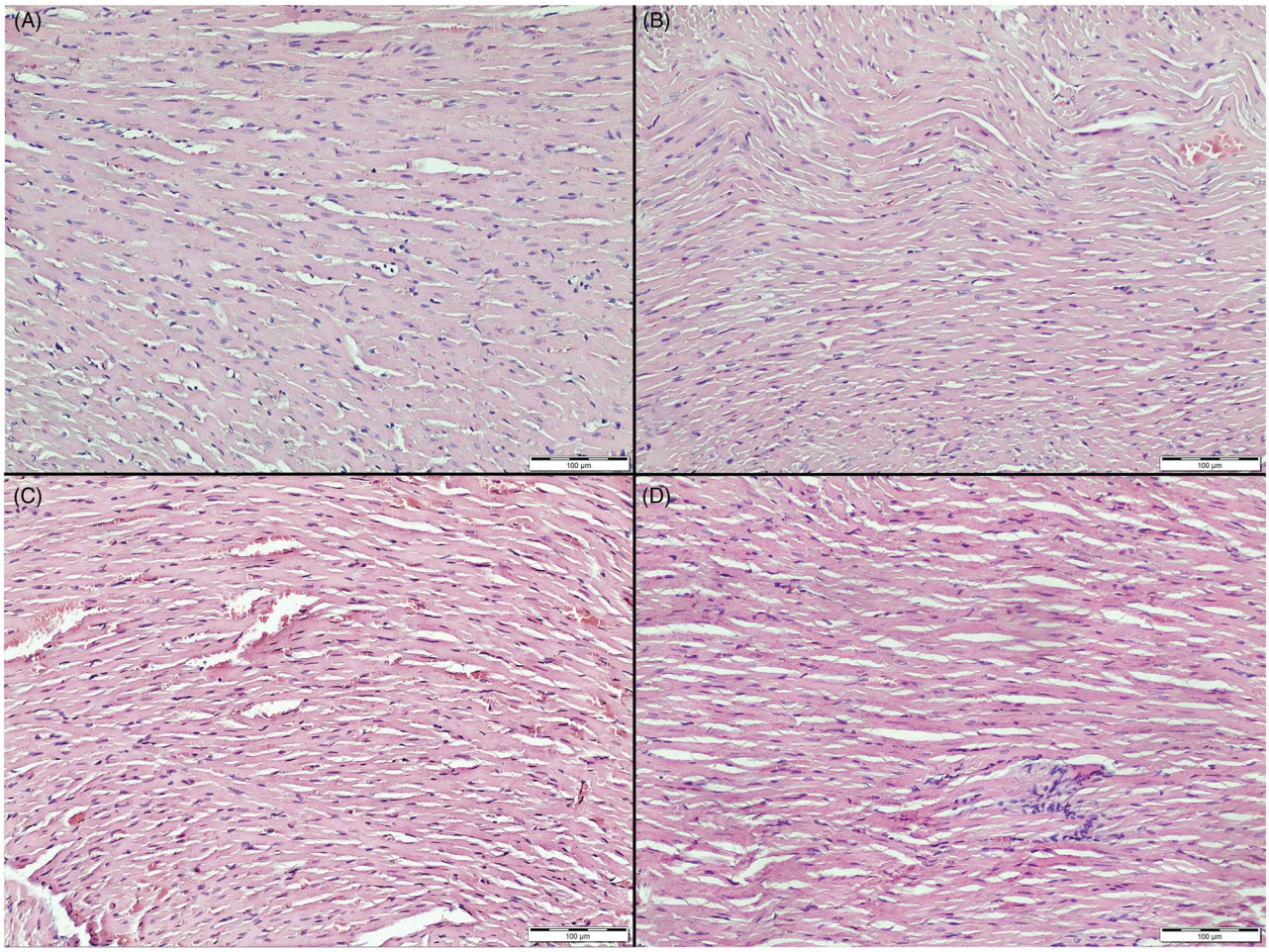

Figure 4. Light micrographs of myocardial fibers (H&E staining ×100). Control group (A), NAC group (B), RT group (C), RT + NAC group (D). Normal myocardial cells were observed in all groups.

Discussion

The cardiovascular complications of radiation exposure such as pericardial diseases, cardiomyopathy, vascular injury, valvular diseases and conduction abnormalities are an important cause of morbidity and mortality following radiation therapy for cancer (Finch et al. 2014). The current approach to prevent cardiac complications is to reduce cardiac exposure during RT (Boerma et al. 2016). In this study, we investigated the protective effect of NAC against RT-induced cardiac damage by using electrophysiological, biochemical and histological methods. We demonstrated for the first time that NAC treatment before RT treatment prevented the RT-induced cardiac damage in rats.

Electrocardiogram is the preferred method for the assessment of the cardiovascular complications of radiation exposure, for instance, myocardial ischemia, myocardial dysfunction and conduction abnormalities (Ma et al. 2019). In this study, we recorded ECG of rats in all groups. In ECG analysis, we observed absence of P wave (25%), high T waves (50%) and low heart rate in the RT group 24 h after single exposure to RT. These findings imply cardiac abnormality (Murray and Colombo 2014). In ECG recording, the P wave reflects depolarization of the atria (Konopelski and Ufnal 2016). Absence of P wave is conducted related to atrial fibrillation and sinoatrial block (Longmore et al. 2014; Korosoglou et al. 2018). The T wave on electrocardiogram represents ventricular repolarization. Tall T-waves can be an early sign of ST-elevation myocardial infarction. Similarly, decreased heart rate signs acute myocardial infarction (Kenny and Brown 2019). These findings were supported by a 71% increase in CK-MB activity in the RT group

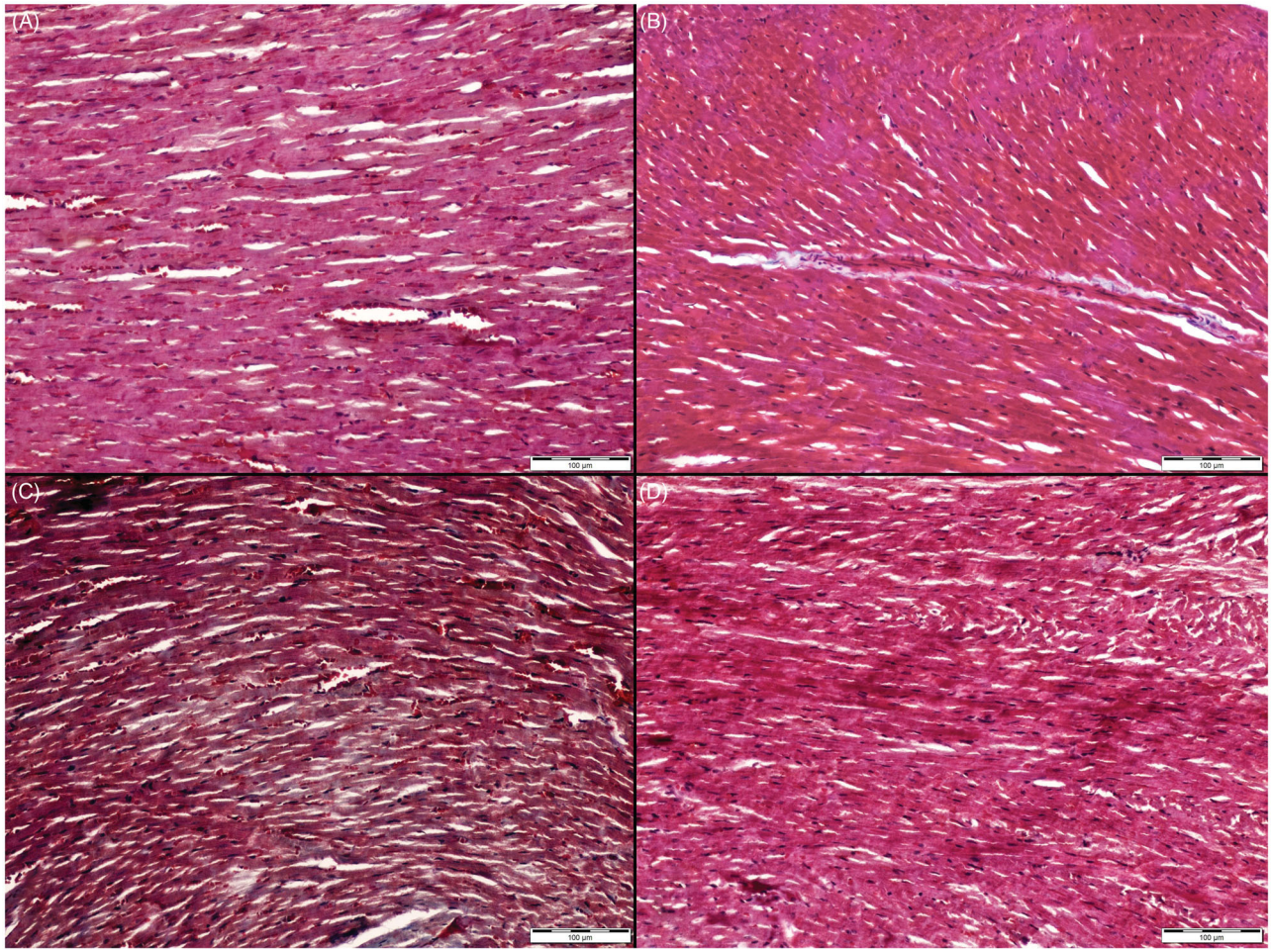

Figure 5. Light micrographs of myocardial fibers (MS staining ×100). Control group (A), NAC group (B), RT group (C), RT + NAC group (D). The muscle cells have normal morphologic characteristics in all groups. X100.

compared to the control group. The MB isoform of the CK enzyme is found primarily in cardiac tissue and its activity is not only useful as an index for early detection of acute myocardial infarction, but also for all types of myocardial damage (Przybyszewski and Widel 1996). CK and GAL-3, which are other cardiac damage markers, were not significantly different among the groups. In our study, ECG findings and CK-MB activity suggest that 20 Gy single-dose RT has the potential to trigger myocardial infarction in rats but NAC treatment alleviated all these findings that are sign of heart damage. Animal studies have shown that dose of RT plays an important role in cardiac injury (Boerma et al. 2016). In rats which are exposed to a dose of 35–40 Gy resulted in the pericardium and epicardium responded to irradiation with exudative pericarditis after 4 months and animals that exposed 10–15 Gy developed only minor damage after 1 year (Lauk et al. 1985). Higher doses of radiation previously led to severe heart failure earlier (Gillette et al. 1992).

Cytokines are important signaling factors produced by endothelial cells in response to irradiation (Venkatesulu et al. 2018). Increased levels of IL-1, IL-4, IL-6, IL-8, IL-13, IL-33, TGF-β and TNF-α have been observed in several irradiation studies (Yahyapour et al. 2018). One group of investigators showed that rats exposed to 10 Gy of whole-body irradiation had increased levels of IL-1β, IL-6 and TNF-α mRNA at 3 and 6 h after irradiation (Linard et al. 2004). Irradiation of endothelial cells in another study led to elevated levels of IL-6 and IL-8 (Meeren et al. 1997). In this study, we observed that cytokine levels significantly increased in the RT group when compared to the control group the increase in Il-4, IL-6 and IL-1β levels were 6%, 26.6% and 63%, respectively. It was found in previous studies that ionizing radiation applications increased secretion of cytokines (Sun and Liu 2000; Liu et al. 2002; Schaue et al. 2012). Cytokine production is time-dependent and usually peaks at 4–24 h after irradiation and then decreases to basal levels from 24 h to several days (Di Maggio et al. 2015). In our study, cytokine levels, measured 24 h after RT, observed to be elevated compared to the control group. However, NAC treatment reduced these levels below the levels of control group. A few studies have evaluated the levels of cytokine production in the rats exposed to a high or fractionated dose of RT. In a previous study, the authors evaluated the cytokines secretion profile of fibrosarcoma, glioblastoma, lung adenocarcinoma and breast adenocarcinoma in order to compare their cytokine profiles after an acute and fractionated doses of RT (Desai et al. 2013). They

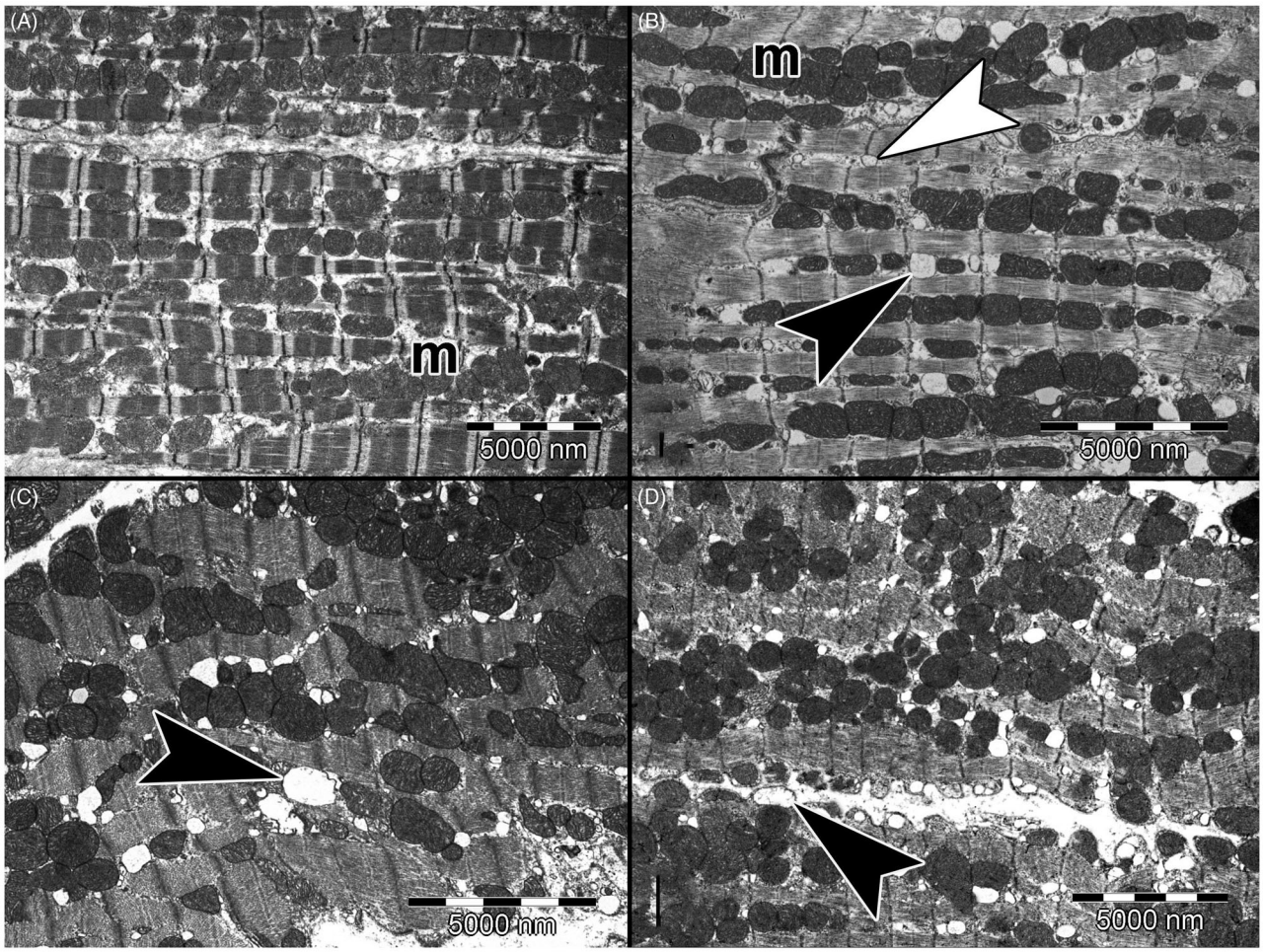

Figure 6. Transmission electron micrographs of myocardial fibers. Ultrastructurally normal miyofibriller organization and cytoplasmic organelles were observed in the control group (A), NAC group (B) RT group (C) and RT + NAC group (D). White arrowhead: Normal sarcoplasmic reticulum cisterns, black arrowhead: expansions in the sarcoplasmic reticulum m: normal mitochondrion. (A) X6000, (B) X5000, (C) X7500 and (D) X6000.

Table 2. Anti-inflammatory and antioxidant parameters in control and experimental groups.

Variable	Control ($n = 6$)	NAC ($n = 8$)	RT ($n = 8$)	RT + NAC ($n = 8$)
IL-4 (pg/mL)	1832.1 ± 301.5	1116.1 ± 123.9[a]	1942.1 ± 238.7[a,b,c]	1329.9 ± 163.5[a]
IL-6 (pg/mL)	728.1 ± 21.4	266.82 ± 11.5[a,c]	922.9 ± 93.7[a,b,c]	405.59 ± 85.2[a,b]
IL-1β (ng/mL)	1.1 ± 0.2	0.91 ± 0.12	1.80 ± 0.26[a,b,c]	0.97 ± 0.28
TNF-α (pg/mL)	811.6 ± 107.7	687.76 ± 91.46	956.01 ± 201.6[a,b,c]	786.43 ± 135.4
CK (ng/mL)	1.17 ± 0.16	1.56 ± 0.65	1.38 ± 0.31	1.61 ± 0.43
CK-MB (ng/mL)	10.2 ± 0.83	11.1 ± 2.2	17.5 ± 6.4[a,b,c]	13.6 ± 3.5
GAL-3 (ng/mL)	8.7 ± 1.2	8.5 ± 0.8	8.8 ± 0.1	9.1 ± 0.4
SOD (U/mg protein)	8.2 ± 0.7	8.1 ± 1.9	7.5 ± 2.0	7.4 ± 1.4
MDA (nmol/mg protein)	60.5 ± 2.6	52.5 ± 6.1	111.5 ± 43.7[a,b,c]	83.2 ± 23.2
CAT (U/mg protein)	105.4 ± 10.7	113.1 ± 13.9	85.9 ± 2.4[a]	88.8 ± 17.7[a,b]
GPx (U/mg protein)	2.20 ± 0.2	2.5 ± 0.3	2.3 ± 0.8	1.90 ± 0.4
NO (nmol/mg protein)	16.90 ± 1.4	17.1 ± 0.6	14.0 ± 1.2[a,b,c]	17.1 ± 1.7

[a]Different from control group ($p < .05$), [b]Different from NAC group ($p < .05$), [c]Different from NAC + RT group ($p < .05$).

observed that most of the cytokines have increased and the magnitude of this increase was greater in acute doses than fractionated doses. We used an acute dose (20 Gy) in our study.

Connection between cytokines and free radicals is important because many cytokines can be induced by increased free radical formation (Elmarakby and Sullivan 2012). In this study, to determine oxidative stress caused by RT, heart MDA, NO levels, and SOD, CAT, GPx activities were measured in all groups. In the RT group, mean MDA level increased by 80.9%, NAC treatment reduced this increase to 37.5% compared to control. MDA, the lipid peroxidation product, is reliable markers that determine oxidative stress in clinical situations (Niki 2014). CAT is an antioxidant enzyme and catalyzes the conversion of toxic hydrogen peroxide into oxygen and water (Purwar et al. 2011). In this study, CAT activity in the RT group decreased by 18.5%. NAC treatment reduced this decrease to 15.7%. Other antioxidant enzyme activities (SOD and GPx) did not differ significantly between the groups. NO levels reduced by 17.1% in the RT group compared to the control group. Administration of NAC recovered this level to the level of

control group. Generally, membranes are abundant in phospholipids which are particularly sensitive to oxidative stress. Membrane lipid peroxidation due to oxidative stress leads to structural and functional damage. Because of particular sensitivity to lipid peroxidation and insufficient antioxidant defense, cardiomyocytes are especially vulnerable to the oxidative activity of free radicals generated by ionizing radiation (Tapio 2016). Increased oxidative stress induced by radiation in the heart has also been reported in previous studies. Recently, it has been shown that 3 Gy total body irradiation for 24 h caused increased MDA and protein carbonyl levels in the cardiac tissue of C57BL/6 mice (Azimzadeh et al. 2011). In another study, the rats were exposed to a single dose of 6 Gy of whole body gamma-irradiation and a significant increase in MDA level has been observed for the irradiated rats (de Freitas et al. 2013). Similarly, it has been reported that gamma-irradiation (7 Gy) induced significant increase in MDA level and xanthine oxidase and adenosine deaminase activities, and significant decrease in total nitrate/NO level and GPx, SOD and CAT activities at 8 and 15 d after irradiation in heart tissue (Mansour and Tawfik 2012). It has been suggested that this induced increase in oxidative stress was dependent on dysfunctional mitochondrial electron transport (Barjaktarovic et al. 2011). On the other hand, it has been reported that increased oxidative stress reduced NO production and bioavailability in endothelial cells (Tapio 2016). The interaction of nitric oxide with free radicals leads to the production of peroxynitrite, which can cause nitrosylation of tyrosine residues of proteins and impair protein function. Thus, radiation-induced increases in oxidative stress affect the structure of the endothelium (Venkatesulu et al. 2018). Radiation-induced endothelial cell damage is considered to be the primary and major cause of myocardial damage (Wang et al. 2019). The results of this study showed that RT administration increases both cytokine release and oxidative stress. Oxidative stress leads to endothelial cell dysfunction and reduces nitric oxide production. NAC, which is well known for its antioxidant and anti-inflammatory properties, has greatly mitigated these effects of RT.

In our study, light microscopic and ultrastructural examination of the heart tissue was also performed to determine the relationship between ECG changes and biochemical changes with morphological changes in the heart. In this study, both light and electron microscopic findings showed that RT did not lead to a change in the structure of the heart cells. In ultrastructural evaluation, cardiac muscle fibers and all cytoplasmic organelles including mitochondria had normal appearance. In previous studies, it has been suggested that radiation-induced pathological changes begin immediately after exposure to radiation, but histological changes may occur in weeks, months or even years after treatment (Stone et al. 2003; Barjaktarovic et al. 2011). In a study, the time-dependent effects of radiation on cardiac ultra-structure were investigated (Cilliers et al. 1989). Wistar rats were exposed to 20 Gy radiation to a field including the heart and the lung. The hearts were excised at varying time intervals (1 h–180 d) and the ultrastructure of heart studied. The most pronounced change observed in the myocyte was that of intercalated disc damage which reached a peak at 30 d post-irradiation. Mitochondrial damage, characterized by swelling and fenestration in areas of myofibrillar contracture, was focal and relatively scarce (Cilliers et al. 1989). In another study, the ultrastructural changes in mouse cardiac muscle were investigated from 4 d to 1 year after irradiation (Yang et al. 1976). Myofibrillary and capillary degeneration were the most severe in 1–3 months of irradiation. Heart muscle showed focal myofibrillolysis in some myocytes, myofibrillary degeneration with loss of all myofibrils, presence of lipid bodies and lysosomal-like bodies, and partially vacuolated mitochondrial areas in myocytes (Yang et al.1976). In this study, the absence of any structural changes in the heart cells may be related to the investigation of the very early (at 24 h after irradiation) effects of radiation.

Conclusion

In this study, our data have shown that pretreatment by NAC before irradiation therapy prevented RT-induced cardiac damage in rats by decreasing the cytokine releasing and oxidative stress. The NAC effectively restored heart function. As a result, it has been thought that NAC may be used as radio-protector for heart before RT applications, especially in patients with breast cancer. However, clinical studies should be conducted to further clarify this issue.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Aebi H. 1984. Catalase in vitro. Meth Enzymol. 105:121–126.

Andratschke N, Maurer J, Molls M, Trott KR. 2011. Late radiation-induced heart disease after radiotherapy. Clinical importance, radiobiological mechanisms and strategies of prevention. Radiother Oncol. 100(2):160–166.

Atis S, Nayci A, Ozge A, Comelekoglu U, Gunes S, Bagdatoglu O. 2006. N-acetylcysteine protects the rats against phrenic nerve dysfunction in sepsis. Shock. 25(1):30–35.

Azimzadeh O, Scherthan H, Sarioglu H, Barjaktarovic Z, Conrad M, Vogt A, Calzada-Wack J, Neff F, Aubele M, Buske C, et al. 2011. Rapid proteomic remodeling of cardiac tissue caused by total body ionizing radiation. Proteomics. 11(16):3299–3311.

Baker JE, Moulder JE, Hopewell JW. 2011. Radiation as a risk factor for cardiovascular disease. Antioxid Redox Signal. 15(7):1945–1956.

Barjaktarovic Z, Schmaltz D, Shyla A, Azimzadeh O, Schulz S, Haagen J, Dörr W, Sarioglu H, Schäfer A, Atkinson MJ, et al. 2011. Radiation–induced signaling results in mitochondrial impairment in mouse heart at 4 weeks after exposure to X-rays. PLoS One. 6(12): e27811.

Barjaktarovic Z, Shyla A, Azimzadeh O, Schulz S, Haagen J, Dörr W, Sarioglu H, Atkinson MJ, Zischka H, Tapio S. 2013. Ionising radiation induces persistent alterations in the cardiac mitochondrial function of C57BL/6 mice 40 weeks after local heart exposure. Radiother Oncol. 106(3):404–410.

Boerma M, Sridharan V, Mao XW, Nelson GA, Cheema AK, Koturbash I, Singh SP, Tackett AJ, Hauer-Jensen M. 2016. Effects of ionizing radiation on the heart. Mutat Res. 770 (Pt B):319–327.

Çağlar Y, Özgür H, Matur I, Yenilmez E D, Tuli A, Gönlüşen G, Polat S. 2013. Ultrastructural evaluation of the effect of N-acetylcysteine on methotrexate nephrotoxicity in rats. Histol Histopathol. 28(7): 865–874.

Cazzola M, Calzetta L, Facciolo F, Rogliani P, Matera MG. 2017. Pharmacological investigation on the anti-oxidant and anti-inflammatory activity of N-acetylcysteine in an ex vivo model of COPD exacerbation. Respir Res. 18(1):26.

Cilliers GD, Harper IS, Lochner A. 1989. Radiation-induced changes in the ultrastructure and mechanical function of the rat heart. Radiother Oncol. 16(4):311–326.

Csontos C, Rezman B, Foldi V, Bogar L, Drenkovics L, Röth E, Weber G, Lantos J. 2012. Effect of N-acetylcysteine treatment on oxidative stress and inflammation after severe burn. Burns. 38(3):428–437.

Dalloz F, Maingon P, Cottin Y, Briot F, Horiot JC, Rochette L. 1999. Effects of combined irradiation and doxorubicin treatment on cardiac function and antioxidant defenses in the rat. Free Radic Biol Med. 26(7-8):785–800.

Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Brønnum D, Correa C, Cutter D, Gagliardi G, Gigante B, et al. 2013. Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med. 368(11):987–998.

de Freitas RB, Boligon AA, Rovani BT, Piana M, de Brum TF, da Silva Jesus R, Rother FC, Alves NM, Teixeira da Rocha JB, Athayde ML, et al. 2013. Effect of black grape juice against heart damage from acute gamma TBI in rats. Molecules. 18(10):12154–12167.

Desai S, Kumar A, Laskar S, Pandey BN. 2013. Cytokine profile of conditioned medium from human tumor cell lines after acute and fractionated doses of gamma radiation and its effect on survival of bystander tumor cells. Cytokine. 61(1):54–62.

Di Maggio FM, Minafra L, Forte GI, Cammarata FP, Lio D, Messa C, Gilardi MC, Bravatà V. 2015. Portrait of inflammatory response to ionizing radiation treatment. J Inflamm. 12(1):14.

Elmarakby AA, Sullivan JC. 2012. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovasc Ther. 30(1):49–59.

Finch W, Shamsa K, Lee MS. 2014. Cardiovascular complications of radiation exposure. Rev Cardiovasc Med. 15(3):232–244.

Gillette SM, Gillette EL, Shida T, Boon J, Miller CW, Powers BE. 1992. Late radiation response of canine mediastinal tissues. Radiother Oncol. 23(1):41–52.

Gürses I, Özeren M, Serin M, Yücel N, Erkal HŞ. 2014. Histopathological evaluation of melatonin as a protective agent in heart injury induced by radiation in a rat model. Pathol Res Pract. 210(12):863–871.

Inano H, Suzuki K, Onoda M, Wakabayashi K. 1997. Anti-carcinogenic activity of simvastatin during the promotion phase of radiation-induced mammary tumorigenesis of rats. Carcinogenesis. 18(9): 1723–1727.

Jacob S, Pathak A, Franck D, Latorzeff I, Jimenez G, Fondard O, Lapeyre M, Colombier D, Bruguiere E, Lairez O, et al. 2016. Early detection and prediction of cardiotoxicity after radiation therapy for breast cancer: the BACCARAT prospective cohort study. Radiat Oncol. 11(1):1–10.

Kenny BJ, Brown KN. 2019. ECG T Wave. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK538264/.

Konopelski P, Ufnal M. 2016. Electrocardiography in rats: a comparison to human. Physiol Res. 65(5):717–725.

Korosoglou G, Marwan M, Giusca S, Schmermund A, Schneider S, Bruder O, Hausleiter J, Schroeder S, Leber A, Limbourg T, et al. 2018. Influence of irregular heart rhythm on radiation exposure, image quality and diagnostic impact of cardiac computed tomography angiography in 4339 patients. J Cardiovasc Comput Tomogr. 12(1):34–41.

Kruse JJ, Strootman EG, Wondergem J. 2003. Effects of amifostine on radiation-induced cardiac damage. Acta Oncol. 42(1):4–9.

Lasram MM, Lamine AJ, Dhouib IB, Bouzid K, Annabi A, Belhadjhmida N, Ahmed MB, El Fazaa S, Abdelmoula J, Gharbi N. 2014. Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats. Life Sci. 107(1–2):50–58.

Lauk S, Kiszel Z, Buschmann J, Trott K. 1985. Radiation-induced heart disease in rats. Int J Radiation Oncol Biol Phys. 11(4):801–808.

Linard C, Marquette C, Mathieu J, Pennequin A, Clarençon D, Mathé D. 2004. Acute induction of inflammatory cytokine expression after γ-irradiation in the rat: effect of an NF-κB inhibitor. Int J Radiat Oncol Biol Phys. 58(2):427–434.

Liu XD, Ma SM, Liu SZ. 2002. Effect of ionizing radiation on IL-12 production and NF-B activation in mouse peritoneal macrophages. Chin J Radiol Med Prot. 22:7–10.

Longmore M, Wilkinson IB, Baldwin A, Wallin E. 2014. Oxford handbook of clinical medicine 9th edition page 90. Oxford: Oxford University Press. ISBN 978-0-19-852558-5.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1): 265–275.
Ma CX, Zhao XK, Li YD. 2019. New therapeutic insights into radiation-induced myocardial fibrosis. Ther Adv Chronic Dis. 10:1–10.
Madan R, Benson R, Sharma DN, Julka PK, Rath GK. 2015. Radiation induced heart disease: pathogenesis, management and review literature. J Egypt Natl Canc Inst. 27(4):187–193.
Mansour HH, El Kiki SM, Hasan HF. 2015. Protective effect of N-acetylcysteine on cyclophosphamide-induced cardiotoxicity in rats. 2015. Environ Toxicol Pharmacol. 40(2):417–422.
Mansour HH, Tawfik SS. 2012. Early treatment of radiation-induced heart damage in rats by caffeic acid phenethyl ester. Eur J Pharmacol. 692(1–3):46–51.
Meeren A, Bertho JM, Vandamme M, Gaugler MH. 1997. Ionizing radiation enhances IL-6 and IL-8 production by human endothelial cells. Mediators Inflamm. 6(3):185–193.
Menezes KM, Wang H, Hada M, Saganti PB. 2018. Radiation matters of the heart: a mini review. Front Cardiovasc Med. 5:8310.
Miranda KM, Espey MG, Wink DA. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 5(1):62–71.
Mohammed WI, Radwan RA, Elsayed HM. 2019. Prophylactic and ameliorative effect of N-acetylcysteine on doxorubicin-induced neurotoxicity in Wister rats. Egypt J Basic Clin Pharmacol. 9:1–13.
Murray GL, Colombo J. 2014. Ranolazine improves autonomic balance in heart failure when added to guideline-driven therapy. Heart Int. 9(2):59–65.
Nagoor Meeran MF, Mainzen Prince PS. 2011. Protective effects of N-acetyl cysteine on lipid peroxide metabolism on isoproterenol-induced myocardial infarcted rats. J Biochem Mol Toxicol. 25(3): 151–157.
Niki E. 2014. Biomarkers of lipid peroxidation in clinical material. Biochim Biophys Acta. 1840(2):809–817.
Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 70(1):158–169.
Pei Y, Liu H, Yang Y, Yang Y, Jiao Y, Tay FR, Chen J. 2018. Biological activities and potential oral applications of N-acetylcysteine: progress and prospects. Oxid Med Cell Longev. 22:1–14.
Przybyszewski WM, Widel M. 1996. Activity of creatine kinase MB-isoenzyme in rat serum after heart irradiation and/or farmorubicin (4′-epidoxorubicin) treatment. Cancer Lett. 100(1–2):145–150.
Purwar N, McGarry JM, Kostera J, Pacheco AA, Schmidt M. 2011. Interaction of nitric oxide with catalase: structural and kinetic analysis. Biochemistry. 50(21):4491–4503.
Qian L, Cao F, Cui J, Wang Y, Huang Y, Chuai Y, Zaho L, Jiang H, Cai J. 2010. The potential cardioprotective effects of hydrogen in irradiated mice. J Radiat Res. 51(6):741–747.
Raviraj J, Bokkasam VK, Kumar VS, Reddy US, Suman V. 2014. Radiosensitizers, radioprotectors, and radiation mitigators. Indian J Dent Res. 25(1):83–90.
Rygiel K. 2017. Cardiotoxic effects of radiotherapy and strategies to reduce them in patients with breast cancer: an overview. J Can Res Ther. 13(2):186–192.
Schaue D, Kachikwu EL, McBride WH. 2012. Cytokines in radiobiological responses: a review. Radiat Res. 178(6):505–523.
Stone HB, Coleman CN, Anscher MS, McBride WH. 2003. Effects of radiation on normal tissue: consequences and mechanisms. Lancet Oncol. 4(9):529–536.
Sun YM, Liu SZ. 2000. Changes in TNF alpha expression in mouse peritoneal macrophages of mice after whole-body irradiation. Radiat Prot. 18:119–125.
Sun Y, Oberley LW, Li Y. 1988. A simple method for clinical assay of superoxide dismutase. Clin Chem. 34(3):497–500.
Tahan G, Tarcin O, Tahan V, Eren F, Gedik N, Sahan E, Biberoglu N, Guzel S, Bozbas A, Tozun N, et al. 2007. The effects of N-acetylcysteine on bile duct ligation–induced liver fibrosis in rats. Dig Dis Sci. 52(12):3348–3354.
Tapio S. 2016. Pathology and biology of radiation-induced cardiac disease. J Radiat Res. 57(5):439–448.
Uraz S, Tahan G, Aytekin H, Tahan V. 2013. N-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. Scand J Clin Lab Invest. 73(1):61–66.
Venkatesulu BP, Mahadevan LS, Aliru ML, Yang X, Bodd MH, Singh PK, Yusuf SW, Abe JI, Krishnan S. 2018. Radiation-induced endothelial vascular injury: a review of possible mechanisms. JACC Basic Transl Sci. 3(4):563–572.
Wang H, Wei J, Zheng Q, Meng L, Xin Y, Yin X, Jiang X. 2019. Radiation-induced heart disease: a review of classification, mechanism and prevention. Int J Biol Sci. 15(10):2128–2138.
Yagi K. 1998. Simple procedure for specific assay of lipid hydroperoxides in serum or plasma. Methods Mol Biol. 108:107–110.
Yahyapour R, Amini P, Saffar H, Rezapoor S, Motevaseli E, Cheki M, Farhood B, Nouruzi F, Shabeeb D, Eleojo Musa A. 2018. Metformin protects against radiation-induced heart injury and attenuates the up-regulation of dual oxidase genes following rat's chest irradiation. Int J Mol Cell Med. 7(3):193–202.
Yang VV, Stearner SP, Tyler SA. 1976. Radiation-induced changes in the fine structure of the heart: comparison of fission neutrons and60 Co γ rays in the mouse. Radiat Res. 67(2):344–360.
Yarom R, Harper IS, Wynchank S, van Schalkwyk D, Madhoo J, Williams K, Salie R, Genade S, Lochner A. 1993. Effect of captopril on changes in rats' hearts induced by long-term irradiation. Radiat Res. 133(2):187–197.
Zhu Q, Kirova YM, Cao L, Arsene-Henry A, Chen J. 2018. Cardiotoxicity associated with radiotherapy in breast cancer: a question-based review with current literatures. Cancer Treat Rev. 68: 9–15.