Biochemical and Biophysical Research Communications 685 (2023) 149154

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The potential antioxidant effect of N-acetylcysteine on X-ray ionizing radiation-induced pancreas islet cell toxicity

Hamit Yilmaz [a,*], Filiz Mercantepe [b], Levent Tumkaya [c], Tolga Mercantepe [c], Adnan Yilmaz [d], Sema Yilmaz Rakici [e]

[a] Department of Biophysics, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[b] Department of Endocrinology and Metabolic Diseases, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[c] Department of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[d] Department of Biochemistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[e] Department of Radiation Oncology, Faculty of Medicine, Recep Tayyip Erdogan University, 53100, Rize, Turkey

ARTICLE INFO

Keywords:
N-Acetylcysteine
Glucagon
Insulin
Langerhans islet Cells
Oxidative stress
X-ray irradiation

ABSTRACT

Purpose: Previous research has highlighted the impact of X-ray irradiation-induced organ damage, on cancer patients after radiation therapy. The ionizing radiation-induced oxidative stress causes injury to the pancreatic islet cells of Langerhans. We used histopathological, immunohistochemical, and biochemical analyses to examine α- and β-cells in the islets of Langerhans in rats undergoing whole-body x-ray ionizing radiation, a group of which was treated with NAC.

Material and methods: Twenty-four male rats were randomly divided into 3 groups, one control, and two experimental groups. Group I (Control) was administered only saline solution (0.09% NaCl) by oral gavage for 7 days. Group II (IR) was administrated whole body single dose 6 Gray ionizing radiation (IR) and saline solution (0.09% NaCl) by oral gavage for 7 days. Group III (IR + NAC) was administered 300 mg/kg NAC (N-acetylcysteine) by oral gavage for 7 days, 5 days before, and 2 days after 6 Gray IR application.

Results: In the X-ray irradiation group, we observed diffuse necrotic endocrine cells in the islets of Langerhans. In addition, we found that Caspase-3, malondialdehyde (MDA) levels increased, and insulin, glucagon, and glutathione (GSH) levels decreased in the IR group compared to the control group. In contrast, we observed a decrease in Caspase-3, and MDA levels in necrotic endocrine cells, and an increase in insulin, glucagon, and GSH levels in the IR + NAC group compared to the IR group.

Conclusion: This study provides evidence for the beneficial effects of N-acetyl cysteine on islets of Langerhans cells with X-ray ionizing-radiation-induced damage in a rat model.

1. Introduction

Ionizing radiation (IR) exposure may occur via a variety of environmental agents, the most prominent of which are cancer therapy and imaging methods [1]. Exposure to ionizing radiation results in cell damage, membrane lipid peroxidation, and ultimately, cell death and apoptosis due to an increase in reactive oxygen species that is caused by DNA double-strand breaks [2]. Despite the important advances in x-ray irradiation, which is commonly used in cancer treatment, and the organ-protective, tumor-focused protocols; the acute and chronic damage caused by ionizing radiation in healthy tissues remains to be an important concern for clinicians and cancer survivors [3]. This concern has driven researchers toward the development of safe and effective radioprotective agents, especially due to the increased survival resulting from the advances in cancer treatment.

In recent years, an increasing number of animal studies and human studies involving cohorts of child cancer survivors have created a causal relationship between ionizing radiation and the development of diabetes mellitus (DM) [4–7]. Although the molecular mechanism of this relationship has not been elucidated, there is consensus that the main underlying pathology is damage to insulin-secreting beta cells in pancreatic islets of Langerhans due to ionizing radiation-induced oxidative stress [8,9]. This hypothesis is confirmed as the protective effects of antioxidants in ionizing radiation-induced damage have been

* Corresponding author. Recep Tayyip Erdogan University Faculty of Medicine, Department of Biophysics, 2 Nolu Sehitler Street, 53020, Rize, Turkey.
E-mail address: hamit.yilmaz@erdogan.edu.tr (H. Yilmaz).

https://doi.org/10.1016/j.bbrc.2023.149154
Received 1 August 2023; Received in revised form 12 October 2023; Accepted 25 October 2023
Available online 27 October 2023
0006-291X/© 2023 Elsevier Inc. All rights reserved.

demonstrated. Therefore, it is of major importance to develop safe and effective adjuvant antioxidant agents without a tumor-protective effect that can be used clinically to eliminate the damage induced by ionizing radiation exposure in healthy tissues.

Ionizing radiation increases the production of reactive oxygen species [10]. The most important intracellular antioxidant that can be endogenously produced in all cells is glutathione (GSH), which is capable of scavenging endogenously and exogenously produced free radicals and detoxifying electrophiles. In oxidative stress conditions, its levels rapidly deplete as it detoxifies free radicals [11]. GSH mainly comprises three amino acids (cysteine, glutamic acid, and glycine). N-acetylcysteine (NAC) is an amino acid derivative that has been well acknowledged in the medical field for its mucolytic capabilities, particularly in the realm of respiratory ailments such as chronic bronchitis. Additionally, its therapeutic efficacy in cases of paracetamol intoxication is of significant importance [12]. Over the course of time, recent studies have provided insights into the potential advantages of this substance that extends beyond its ability to dissolve mucus [13]. The potential of N-acetylcysteine (NAC) as a preventative agent in many health disorders is a subject of great interest and promise in current research. The antioxidative, anti-inflammatory, cardioprotective, and neuroprotective properties of this substance have prompted scholars to explore its potential preventive effects on a wide range of ailments, including neurodegenerative diseases, psychiatric disorders, cardiovascular events, and acute organ injury [13]. NAC fundamentally resembles GSH and consists of a precursor molecule (L-cysteine) for GSH production [14]. In oxidative stress conditions where GSH is depleted, NAC serves as a substrate source for GSH production [15]. A multitude of studies have demonstrated NAC to play a cytoprotective role by preventing DNA damage [16,17].

Our review of the literature showed us that there were not any studies reporting the effects of NAC therapy on ionizing radiation-induced damage in pancreatic islets of Langerhans cells. Based on the idea that NAC therapy could offer a solution in conditions of injury where the depletion of GSH levels has a key role, we aimed to observe the potential damage caused by ionizing radiation in pancreatic islet cells and explore the effect of NAC pretreatment against this potential damage in islet cells. For this purpose, we used histopathological, immunohistochemical, and biochemical analyses to examine α- and β-cells in the islets of Langerhans in rats undergoing whole-body x-ray ionizing radiation, a group of which was treated with NAC.

2. Materials and methods

2.1. Animals and experimental design

This study was performed with the approval of Recep Tayyip Erdoğan University Animal Research Ethics Committee (Rize, Türkiye; Approval number: 2023/22). The animals were cared for according to the guidelines of the Institutional Animal Care and Use Committee (IACUC). This study used 24 male, 3-to-4-month-old Sprague-Dawley rats (280 ± 26 gr) procured from Recep Tayyip Erdoğan University Animal Research Unit. All rats were housed in cages of eight, under optimal laboratory conditions (50–55% humidity rate, temperature 22 ±2 °C, 12-h dark:12-h light period). Subjects that freely roamed in the cage were fed with pellet chow consisting of the same protein rate (20%) and daily water. Eight rats were randomly selected from subjects with similar biological and physiological characteristics to compose each of the three groups, which included one control and two experimental groups. Group I (Control) was given only saline solution (0.09% NaCl) by oral gavage for 7 days. Group II (IR) received 6 Gray whole-body ionizing radiation (IR) in a single dose and (0.09% NaCl) by oral gavage for 7 days. Group III (IR + NAC) was given 300 mg/kg NAC (N-acetyl cysteine) regularly for 5 days before receiving 6 Gray IR and for 2 days after irradiation at the same dose over 7 days in total [17]. NAC administration was performed via oral gavage. The dosage of NAC was determined based on prior studies documented in the literature [17–19].

2.2. Drugs & chemicals

Before being sacrificed, the animals were anesthetized with Ketamine HCL ((Ketalar®, 50 mg/kg, Pfizer İlaçları Ltd. Şti, Istanbul, Turkey) and Xylazine HCL ((Rompun®, 5 mg/kg, Bayer, USA). N-acetyl cysteine (ASIST 4% 150-mL 60g granule, Hüsnü Arsan İlaçları A.S., İstanbul, Türkiye).

2.3. X-ray irradiation procedure

Prior to irradiation, all rats were fixed in a prone position under anesthesia (IM, 50 mg/kg ketamine and 5 mg/kg xylazine). In this study, the ELEKTA Synergy model linear accelerator device was used, generating photon energy of 6 MV. A dose of 6 Gy was planned to be delivered to the rat, and bolus material was placed 1 cm above and below the anesthetized rat for simulation computed tomography. Simulation images with applied bolus were obtained, and the rat contours were delineated. The CMS XIO planning system and Convolution algorithm defined a field size of 40 × 40 cm^2. Care was taken to position the rat at least 3 cm away from the edges of the irradiation field, and the irradiation dose was calculated. Doses were calculated at gantry angles of 0 and 180°, with a Source-to-Axis Distance (SAD) of 100 cm and a Source-to-Skin Distance (SSD) of 97.5 cm to ensure dose homogeneity (4 Gy/min). Irradiation was performed in pairs, adhering to simulation conditions. Group 2 and Group 3 received whole-body 6 Gy x-rays in a single fraction [20,21]. All subjects were sacrificed under anesthesia 48 h after irradiation.

2.4. Biochemical analysis

2.4.1. Tissue sampling and homogenization

One gram of pancreatic sample was added with 9 ml of a working solution consisting of 0.15 M KCl to be homogenized on ice. The homogenates were then transferred to Eppendorf tubes and homogenized on ice at 9500 rpm for several minutes. In the next stage, the homogenates were centrifuged at 4000×*g* for 10 min at +4,°C and supernatants were separated [22]. MDA and GSH levels were spectrophotometrically measured from the separated supernatants.

2.4.2. Malondialdehyde (MDA) analysis

The determination of MDA, which is produced by lipid peroxidation, was performed by spectrophotometrically measuring the concentration of the pink-colored mixture on heating with TBA solution. After incubation, 900 μL from the assay tubes was added to 600 μL of TCA solution and centrifuged at 3000 rpm for 10 min. Following centrifugation, the obtained 900 μL of supernatant was added to 300 μL of freshly prepared TBA solution, and the resulting new mixture was left to incubate in boiling water for 15 min. Absorbance was measured at 532 nm and lipid peroxide contents were given as nmol/gr tissue [23].

2.4.3. Glutathione (GSH) analysis

Ellman's reagent method was used to measure glutathione (GSH) levels in pancreatic tissue [24]. 200 μL 3 M Na2HPO4 and 50 μL DTNB (4 mg DTNB, prepared in 1% 10 mL sodium citrate solution) were added to the 50 μL supernatant. The obtained *mixture* was vortexed and measured spectrophotometrically at 412 nm.

2.5. Histopathological analysis

In histopathological analysis, the following procedures were sequentially performed: Pancreatic tissues excised from the rats were divided into pieces of an approximate volume of 1.5 cm^3 and fixed by being kept in 10% neutral formalin solution (Sigma-Aldrich, Saint Louis, MO, USA) for 24 h. In the next stage, in line with the stages of routine

histological tissue processing, pancreatic tissue specimens were dehydrated by being kept in an ascending series of ethanol (50%, 70%, 80%, 90%, 96%, 100%, 100%; Merck GmbH, Darmstadt, Germany) using a tissue processor (Shandon Citadel 2000, Thermo Scientific, Germany). After dehydration, the specimens were cleared by being passed through two series of xylol solutions (Merck GmbH, Darmstadt, Germany). In the next stage, the specimens were embedded into tissue embedding cassettes (Isolab GmbH, Germany) using a tissue embedder (Leica, EG1150, Germany), and sections of 4–5 μm were obtained by trimming the tissue to sections of 30 μm using a rotary microtome (Leica RM2525, Germany). The obtained sections were stained with Harris hematoxylin and Eosin (Eosin G Merck GmbH, Darmstadt, Germany) using a tissue stainer (Leica ST5020, Germany) according to the standard procedure. In the final stage; the sections were analyzed and photographed using a light microscope (Olypmus BX51, Olympus Corp, Japan) with an attached digital camera (Olypmus DP71, Olympus Corp, Japan).

2.6. Immunohistochemical (IHC) analysis

Pancreatic tissue sections were cut into sections of 1–2 μm using a rotary microtome (Leica Biosystems, RM2525, Germany). Pancreatic tissue sections that were transferred to positively charged slides and incubated with insulin primary antibody (ab181547, Abcam, UK), glucagon antibody (ab92517, Abcam, UK), caspase-3 primary antibody (ab2302, Abcam, UK), and an appropriate secondary antibody (Goat Anti-Rabbit IgG H&L (HRP), ab97051, Abcam, UK)), were incubated using a fully automated closed-system with the Leica Bond Max IHC/ISH stainer (Leica microsystem, Melbourne, Australia) according to the manufacturer's instructions. In the next stage, the sections were counterstained with Harris Hematoxylin (Merck GmbH, Darmstadt, Germany).

2.7. Semi-quantitative analysis

Findings of necrosis in endocrine cells, loss of volume in the islets of Langerhans, and edematous areas were evaluated and scored as shown in Table 1 according to studies that histopathologically analyzed X-ray irradiation-induced pancreatic damage in H&E-stained pancreatic tissue sections [25,26]. Immunopositive cells in pancreatic tissue sections that underwent IHC were scored as shown in Table 2 [27]. In semi-quantitative analyses, 20 randomly selected areas from sections of each rat were scored by two histopathologists blinded to the study groups using x20 objective magnification.

Table 1
Pancreas pathological damage score (PPDS).

Score	Findings
Necrotic Endocrine Cells	
0	≤5%
1	6%25%
2	26%–50%
3	51%–75%
4	>75%
Langerhans Islet Narrow	
0	1
1	1–1.5
2	2–2.5
3	2.5–3
4	>3
Edema	
0	≤5%
1	6%–25%
2	26%–50%
3	51%–75%
4	>75%

Table 2
Immunpositivity Scoring Methods Results median (25%–75% interquartile range)).

Score	Findings
0	≤5%
1	6%–25%
2	26%–50%
3	51%–75%
4	>75%

3. Results

3.1. Biochemical results

Pancreatic tissue MDA level measurements were performed to determine lipid peroxidation. In this context, we observed that the MDA level of pancreatic tissue in the IR group was significantly increased compared to the control group (Table 3, $p = 0.001$). On the other hand, we found that pancreatic tissue MDA level was significantly reduced in the IR + NAC group compared to the IR group. (Table 3, $p = 0.001$).

In the measurements of pancreatic GSH level, we observed that the pancreatic tissue GSH level of the IR group decreased significantly compared to the control group. (Table 3, $p = 0.02$). However, we observed that the pancreatic tissue GSH level in the IR + NAC group was elevated compared to the IR group. (Table 3, $p = 0.012$).

3.2. Histopathological results

Light microscopic examination of pancreatic tissue sections determined normal cells in the islets of Langerhans in the control group (Table 4, Fig. 1a and b, p = 0.001, PPDS 0(0-0)). In contrast, in pancreatic tissue sections of the IR group, there were necrotic cells accompanied by abundant cytoplasmic vacuoles in the islets of Langerhans. In addition, we determined a decrease in the surface area of the islets of Langerhans and the presence of extensive edematous areas (Table 4, Fig. 1c–d, p = 0.001, PPDS: 6(6-6)). In the IR + NAC group, we observed a decrease in the necrotic endocrine cells and edematous areas in the islets of Langerhans. In addition, we observed a reduction in the shrinkage found in the islets of Langerhans of the IR + NAC group (Table 4, Fig. 1e–f, p = 0.001, PPDS: 0.5(0–2)).

3.3. Immunohistochemical results

In the control group, we determined extensive immunohistopositivity in beta cells in the islets of Langerhans (Table 5, Fig. 2a, p = 0.001, Insulin positivity score 3(3-3). In contrast, in beta cells of the IR group, there was a decrease in insulin positivity (Table 5, Fig. 2b, Insulin positivity score 0(0–1). In the IIR + NAC group, we found extensive insulin positivity in beta cells in the islets of Langerhans (Table 5, Fig. 2c, p = 0.001, Insulin positivity score 3(2–3).

In the control group, we determined alpha cells with extensive immunopositivity in the islets of Langerhans (Table 5, Fig. 3a, p = 0.001, Glucagon positivity score 2.5(2–3). In contrast, in alpha cells of the IR group, there was a decrease in glucagon positivity (Table 5, Fig. 3b,

Table 3
Biochemical Analysis Results (mean ± standart deviation).

Group	MDA (nmol/g tissue)	GSH (nmol/g tissue)
Control	12.23 ± 0.79	3.38 ± 0.27
IR	16.68 ± 0.78[a]	2.42 ± 0.47[c]
IR + NAC	14.46 ± 0.67[a,b]	3.45 ± 0.76[d]

[a]p = 0.001: Compared to the Control group.
[b]p = 0.001: Compared to the IR group.
[c]p = 0.02: Compared to the Control group.
[d]p = 0.012: Compared to the IR group,One-Way ANOVA-Tukey HSD.

Table 4
Pancreas Pathological Damage Score (PPDS) Score Results (median-25%–75% interquartile range).

Group	Necrocit Endocrine Cells	Langerhans Islets Narrow	Edema	PPDS
Control	0(0–0)	0(0–0)	0(0–0)	0(0–0)
IR	2(2-2)[a]	2(2–3)[a]	2 (1–2)[a]	6(6-6)[a]
IR + NAC	0(0–1)[b]	0(0–1)[b]	0 (0–1)[b]	0.5 (0–2)[b]

[a] $p = 0.001$ versus to Control Group.
[b] $p = 0.001$ versus IR,Kruskal Wallis test with Bonferroni corretions.

Glucagon positivity score 0(0-0). In the IR + NAC group, we found an increase in alpha cells showing extensive glucagon positivity in the islets of Langerhans (Table 5, Fig. 3c, p = 0.001, Glucagon positivity score 2 (2–3).

In the control group, we determined normal endocrine cells in the islets of Langerhans (Table 5, Fig. 4a, p = 0.001, Caspase-3 positivity score: 0(0-0). In the IR group, we observed an increase in endocrine cells showing extensive caspase-3 positivity in the islets of Langerhans (Table 5, Fig. 4b, p = 0.001, Caspase-3 positivity score 2(2-2). In the IR + NAC group, we found a decrease in endocrine cells showing extensive caspase-3 positivity in the islets of Langerhans (Table 5, Fig. 4c, p = 0.001, Caspase-3 positivity score 0.5(0–1)).

4. Discussion

The results of the present study show that whole-body X-ray

Table 5
Immunpositivity Score Results (median-25%–75% interquartile range).

Group	Insulin Positivity Score	Glucagon Positivity Score	Caspase-3 positivity Score
Control	3(3–3)	2.5(2–3)	0(0–0)
IR	0(0–1)[a]	0(0-0)[a]	2(2-2)[a]
IR + NAC	3(2–3)[b]	2(2–3)[b]	0.5(0–1)[b]

[a] $p = 0.001$ versus to Control Group.
[b] $p = 0.001$ versus IR,Kruskal Wallis test with Bonferroni corretions.

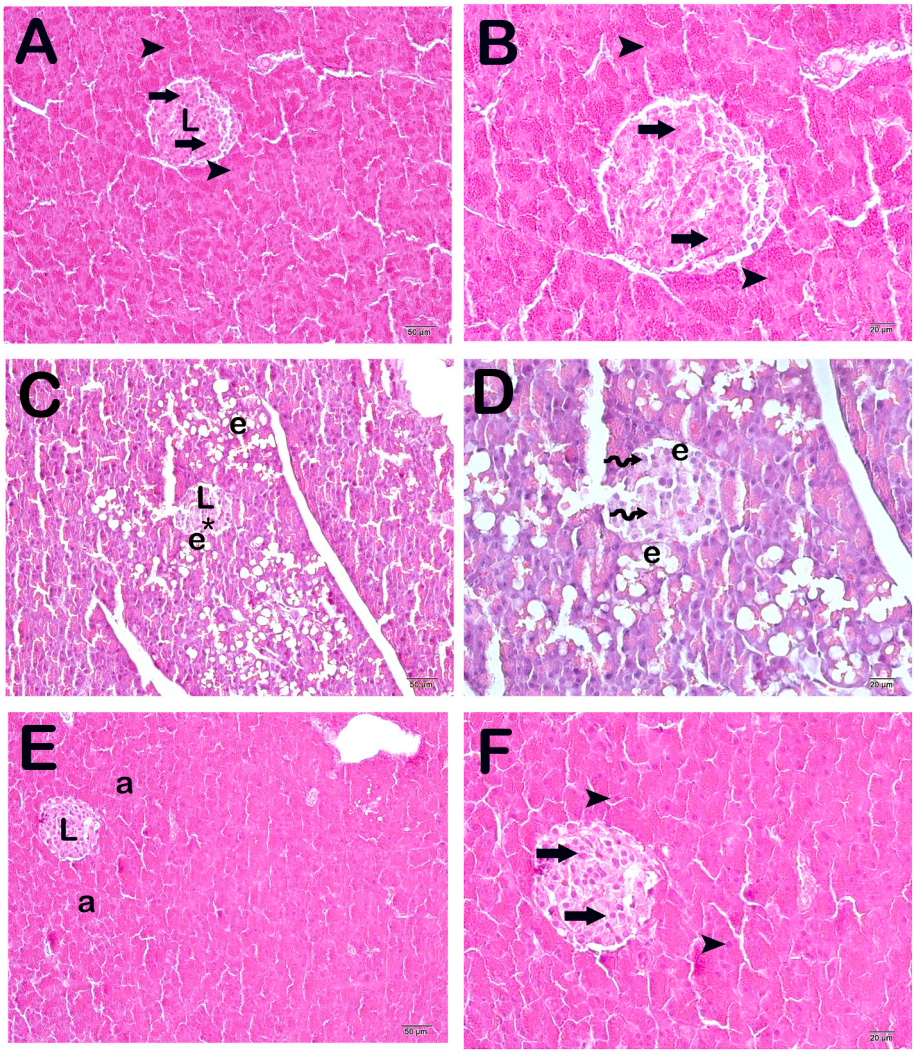


Fig. 1. The representative light microscopic screenshot of sections of pancreatic tissue. H&E staining.
Langerhans Islets (L), Eczocrin asinuse (a)
A(x20)-B(x40) Control Group: Islets of Langerhans (L) consisting of normal endocrine cells (arrow) are observed (PPDS median: 0(0-0)). **C(x20)-D(x40) IR Group:** Islets of Langerhans (asterisk) with reduced volume (narrow) consisting of necrotic endocrine cells (spiral arrow) are observed. In addition, widespread edematous areas are observed in the islets of Langerhans and exocrine acini (PPDS median: 6(6-6)). **E(x20)-F(x40) IR + NAC Group:** It is observed that the necrotic endocrine numbers and edematous areas in the islets of Langerhans are decreased (PPDS median: 0.5(0–2)).

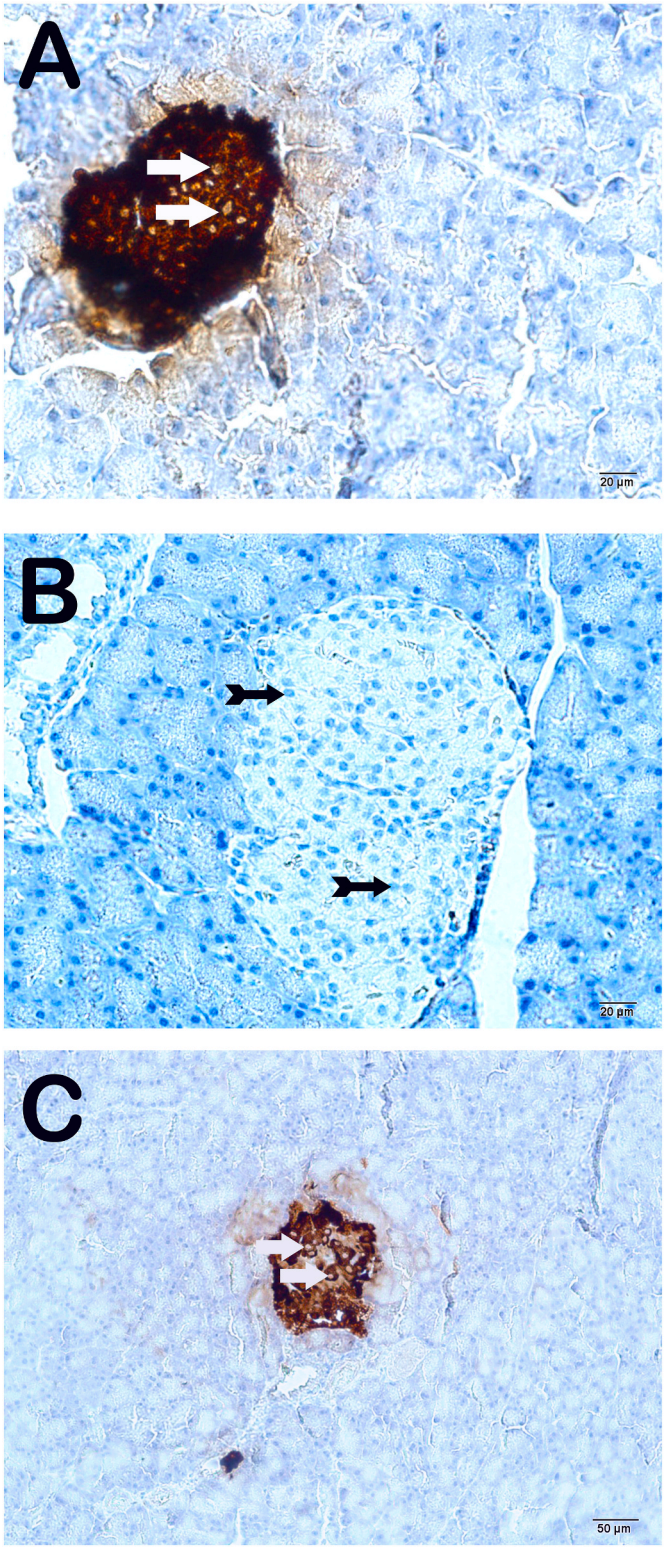


Fig. 2. Representative light microscopic screenshot of Pancreatic tissue incubated with insulin primary antibody.
A(x40) Control Group: Beta cells (white arrow) with intense Insulin positivity are observed in the islets of Langerhans (Insulin Positivity Score: 3(3-3)). **B (x40) IR Group:** It is observed that insulin positivity is decreased from beta cells in the islets of Langerhans (Insulin Positivity Score: 0(0–1)). **C(x40) IR + NAC Group:** Increased insulin positivity is observed in beta cells in the center of the islets of Langerhans (Insulin Positivity Score: 3(2–3)).

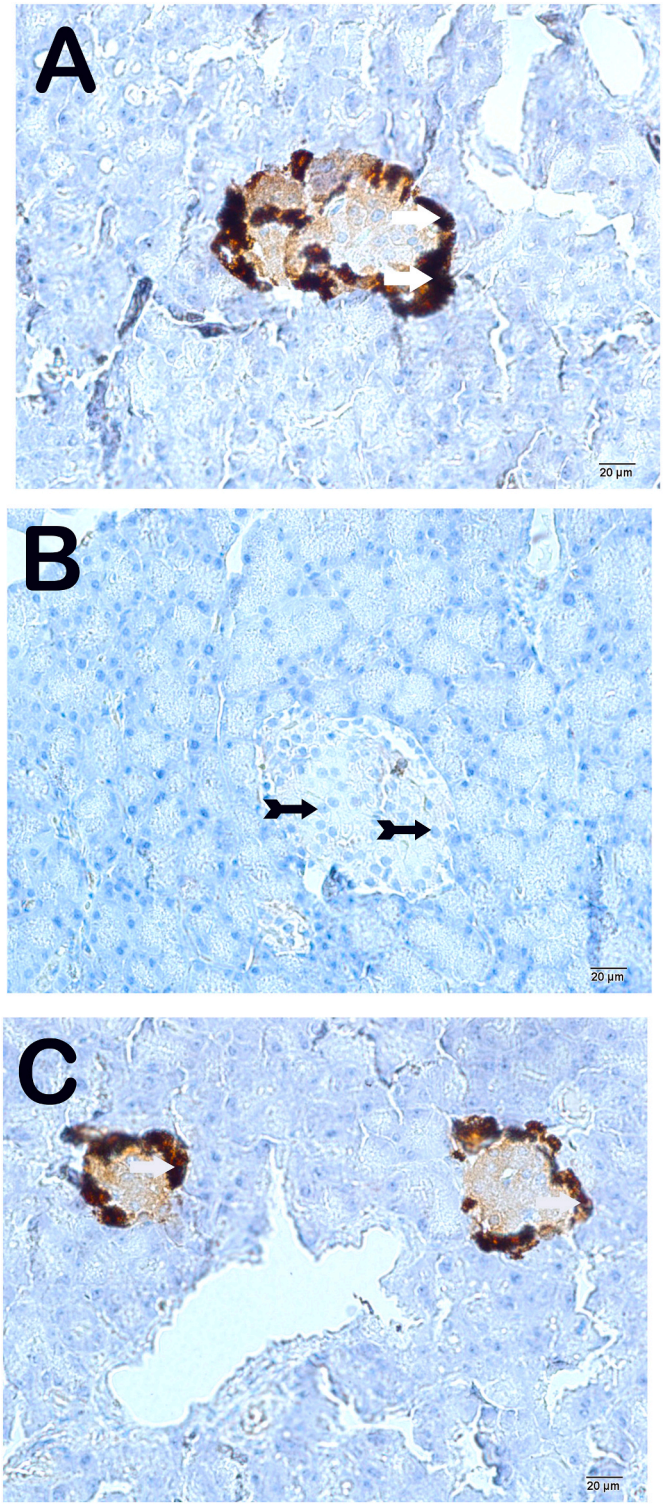


Fig. 3. Representative light microscopic screenshot of Pancreatic tissue incubated with Glucagon primary antibody.
A (x40) Control Group: Alpha cells (white arrow) with intense Insulin positivity are observed in the islets of Langerhans (Glucagon Positivity Score: 2.5 (2–3)). **B(x40) IR Group:** It is observed that Glucagon positivity is decreased from alpha cells in the islets of Langerhans (Glucagon Positivity Score: 0(0-0)). **C(x40) IR + NAC Group:** Increased glucagon positivity is observed in alpha cells in the peripheral area of the islets of Langerhans (Glucagon Positivity Score: 2(2–3)).

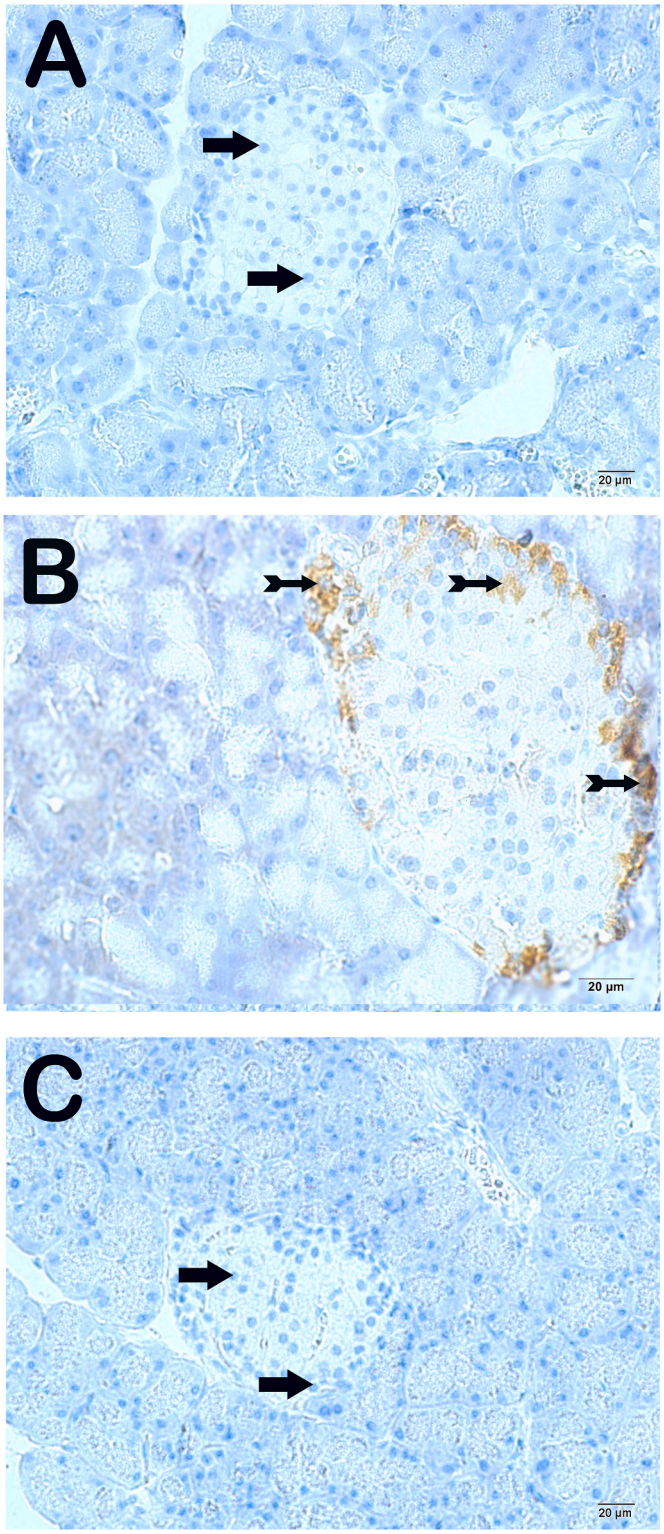


Fig. 4. Representative light microscopic screenshot of Pancreatic tissue incubated with Caspase-3 primary antibody.
A (x40) Control Group: Normal endocrine cells are observed to be immunonegative in the islets of Langerhans (Caspase-3 Positivity Score: 0(0-0)). **B (x40) IR Group:** Apoptotic endocrine cells (tailed arrow) with intense Caspase-3 positivity are observed in the islets of Langerhans (Caspase-3 Positivity Score: 2(2-2)). **C(x40) IR + NAC Group:** It is observed that Caspase-3 positivity is decreased in endocrine cells in the islets of Langerhans. (Caspase-3 Positivity Score: 0.5(0–1)).

irradiation in rats caused damage and cell apoptosis in the pancreatic islet of Langerhans cells; however, this damage is alleviated by NAC pretreatment. Moreover, the index of the oxidative status induced by ionizing radiation could be shifted back to an antioxidant status with NAC treatment as shown by the elevated levels of GSH. Moreover, the reduced MDA levels in the IR + NAC group indicate that NAC prevents lipid peroxidation in cell membranes, probably through an antioxidative effect. Ionizing radiation results in oxidative damage and inflammation by increasing the production of reactive oxygen species [10]. The occurrence of oxidative stress can result in the oxidation of many cellular components such as cell membranes, DNA, proteins, and lipids [28]. Consequently, this oxidative damage can give rise to compromised cellular activities, DNA damage, and ultimately, cell death through the process of apoptosis [29]. The persistence of oxidative stress, particularly inside organs and tissues, has the potential to contribute to the advancement of several diseases [28,29]. The implementation of techniques aimed at effectively managing oxidative stress and enhancing antioxidant defenses is crucial in the prevention and mitigation of tissue damage and its subsequent health implications. Antioxidants have the potential to mitigate the effects of oxidative stress.

Pancreatic islet cells, particularly beta cells, are highly susceptible to oxidative stress due to their low antioxidant enzyme capacity [14]. Inflammation and oxidative stress constitute an important mechanism in the development of diabetes [14,30]. Prospective follow-up of child survivors of cancer who received radiotherapy for cancer during childhood revealed that they were more likely to develop diabetes compared to both their twin siblings and the general population and that abdominal radiation exposure and the radiation dose had a causal relationship with the development of diabetes [4,7,31]. A prospective examination of radiation workers showed that these individuals developed diabetes at higher rates [1]. Furthermore, experimental animal model studies proved that IR-induced damage occurred in both the endocrine and exocrine pancreas and that insulin resistance developed [5,22,32].

Cells are protected from oxidative damage by antioxidant defense systems [33]. In conditions of excessive oxidative stress or depleted antioxidant capacity, the high levels of reactive oxygen species lead to various pathological processes [2,34]. GSH, a cellular detox found in all cells, is considered the most important member of the antioxidant defense system [12]. In conditions of excessive oxidative stress, GSH is depleted due to its increased use, and cells are rendered defenseless against reactive oxygen species. GSH mainly comprises three peptides (cysteine, glutamic acid, and glycine) [35]. The GSH concentration in cells depends on the levels of the used substrates and the synthesizing enzymes [11]. The cell is generally rich in glycine and glutamate content; however, the amount of cysteine is limited [36]. NAC serves as a cysteine substrate for GSH and supplies cysteine in situations where GSH levels are low [37]. Although this is not the only mechanism underlying the organ protective quality of NAC, it is certainly responsible for a significant portion of this quality. Numerous studies prove this NAC's antioxidant quality [15–17]. Accordingly, our study also showed that GSH increased in the NAC group, while MDA levels that indicate lipid peroxidation decreased. These results support the use of NAC as an agent that can improve oxidative stress in the endocrine pancreatic tissue with IR-induced damage. The results of our study are consistent with both data from the literature and the results of other studies on this matter.

There are studies in the literature showing that NAC has favorable effects on both insulin resistance and diabetes [14,30,35,36,38]. This effect is thought to derive from its protective role against the oxidative stress that develops due to glucotoxicity and lipotoxicity. Two different animal model studies have shown that the high-fat-fed offspring of rats receiving NAC during pregnancy and breastfeeding had improved insulin sensitivity and glucose tolerance in isolated islet cells, with higher beta cell function and survival [30,35]. NAC was also demonstrated to offer protection via an antioxidant effect in pancreatic damage related to ischemia/reperfusion [36]. Two different animal studies that were quite similar showed that NAC treatment along with bone marrow

mesenchymal stem cell treatment for severe acute pancreatitis ameliorated acute pancreatitis and improved the degeneration, apoptosis, and necrosis of the islets of Langerhans [11,39]. NAC prevents lipid peroxidation and maintains the integrity of the cell membrane, which is the major site of oxidation reactions, by protecting the cell membrane against oxidative stress, and hence, inhibits the activation of the caspase cascade that initiates apoptosis [15,40]. Our result that the elevated MDA levels and caspase-3 positivity in the IR group decreases in the IR + NAC group is supported by these data.

In this study, we administered NAC to the same subject group in two conditions: before and after IR. There is evidence in the literature suggesting that NAC treatment is effective in conditions that may lead to GSH depletion, but not in environments where GSH is found at normal levels [15]. The GSH pool is not depleted before the application of IR. However, it is quite likely that the GSH pool would be depleted after the application of IR. The administration of NAC before IR and after IR to two separate groups will be the subject of another study we plan to perform to understand whether the protective effect of NAC derives from the time of application or the poor antioxidant capacity of the pancreas. Clarifying this matter will also help us gain more information regarding the effect mechanism of NAC. Because finding that it is effective in both conditions would suggest the presence of mechanisms independent of GSH levels.

Our study is the first study that has shown that NAC improves IR-induced damage in pancreatic Langerhans cells. The current work is novel since it examines the endocrine pancreas, insulin- and glucagon-secreting islet cells, and the impact of n-acetylcysteine on these cells in pancreatic damage caused by x-ray ionizing radiation. Accordingly, certain limitations of our study should be taken into consideration. Studies show that IR-induced islet cell damage is a relatively late complication and that DM may develop years after IR exposure [7,31]. However, our study investigated the acute effects of IR on pancreatic islet cells. It is not known how much of this acute damage progresses to the chronic phase. Therefore, studies that will investigate the chronic effects of IR on the endocrine pancreas are highly needed. This will be the subject of our future studies. Also, it is not known whether the protective effects of the NAC treatment will continue in the long term. In addition, although this study demonstrated damage in pancreatic islet cells after IR, it did not biochemically determine whether or not diabetes developed. Future studies should include fasting plasma glucose, insulin, and c-peptide levels to determine endocrine pancreatic function after IR. Lastly, IR was applied to healthy animals rather than those with an induced model of cancer. Certain growth factors and molecules produced in cancerous tissues may endogenously cause different cellular reactions by affecting various pathways. This may result in a difference between the responses of cancerous tissues and healthy tissues to the same molecule. Clinical studies on individuals with cancer and animal studies with induced cancer models are needed to elucidate this matter. In order to gain a deeper understanding of the protective effects of N-acetylcysteine (NAC) in endocrine pancreas damage induced by x-ray ionizing radiation, it is imperative to do additional molecular investigations that explore potential molecular processes involving oxidative stress. Further studies are needed to better determine the pathways through which the therapeutic effect is achieved.

5. Conclusion

The adverse effects of ionizing radiation from X-rays on pancreatic islet cells highlight the necessity for the development of preventive and therapeutic interventions to mitigate this harmful radiation-induced toxicity. N-acetylcysteine (NAC) has been extensively studied for its antioxidant capabilities and is considered a promising candidate for mitigating cell damage caused by radiation exposure. The findings of our investigation have indicated that N-acetylcysteine (NAC) has the potential to mitigate the detrimental impact of ionizing radiation on islet cells through its ability to fight oxidative stress. Moreover, the administration of N-acetylcysteine (NAC) may have potential consequences in the preservation of both endocrine and exocrine functions of the pancreas, specifically in clinical situations when there is repetitive exposure to radiation. This is due to its ability to mitigate radiation-induced pancreatic damage.

Consent form

All the authors have read and approved the manuscript in all respects for publication.

Author contributions

All authors take public responsibility for the content of the submitted work. The authors confirm their contribution to the paper as follows: Hamit Yılmaz, Filiz Mercantepe, designed this study, Conceptualization, Investigation, Formal analysis, Writing – of the original draft, and Supervision. Hamit Yılmaz, Filiz Mercantepe, Levent Tumkaya, Tolga Mercantepe analysis, Writing – original draft. Adnan Yilmaz: Formal analysis, Investigation. Sema Yilmaz Rakıcı, revision and language analysis. The authors declare that all data were generated in-house and that no paper mill was used.

Funding sources

No financial resources were received at any stage of this study.

Notes on contributors

Hamit Yilmaz is a scientist at the Biophysics Department at Recep Tayyip Erdogan University. His research focuses on radiation-induced normal tissue injury.

Filiz Mercantepe is a scientist and medical doctor at the Endocrine and Metabolism Department, at Recep Tayyip Erdogan Education and Training Hospital. She focuses on endocrine and metabolism diseases.

Levent Tumkaya is a scientist at the Histology and Embryology Department at Recep Tayyip Erdogan University. His research focuses on electromagnetic field-induced tissue injury.

Tolga Mercantepe is a scientist at the Histology and Embryology Department at Recep Tayyip Erdogan University. His research focuses on gamma radiation-induced tissue injury.

Adnan Yilmaz is a senior Biochemistry Department scientist at Recep Tayyip Erdogan University. His research focuses on oxidative stress mechanisms.

Sema Yilmaz Rakıcı is a scientist Department of Radiation Oncology at Faculty of Medicine, Recep Tayyip Erdogan University.

Data availability statement

All data generated or analyzed during this study are included in this article. The datasets in the current study are available from the corresponding author upon reasonable request. Data is not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

Statement of ethics

This experimental study was performed with approval from the Recep Tayyip Erdogan University Animal Research Ethics Committee (Rize, Turkey) (Approval Date: 28.03.2023, Approval Number: 2023/22).

CRediT authorship contribution statement

Hamit Yilmaz: Methodology, Project administration, Writing – original draft. **Filiz Mercantepe:** Supervision, Writing – original draft,

Writing – review & editing. **Levent Tumkaya:** Formal analysis, Validation. **Tolga Mercantepe:** Methodology, Visualization. **Adnan Yilmaz:** Formal analysis, Validation. **Sema Yilmaz Rakici:** Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

None.

References

[1] S. Tapio, M.P. Little, J.C. Kaiser, N. Impens, N. Hamada, A.G. Georgakilas, D. Simar, S. Salomaa, Ionizing radiation-induced circulatory and metabolic diseases, Environ. Int. 146 (2021), 106235, https://doi.org/10.1016/j.envint.2020.106235.

[2] D. De Ruysscher, G. Niedermann, N.G. Burnet, S. Siva, A.W.M. Lee, F. Hegi-Johnson, Radiotherapy toxicity, Nat. Rev. Dis. Prim. 5 (2019), https://doi.org/10.1038/s41572-019-0064-5.

[3] D.C. Voshart, J. Wiedemann, P. van Luijk, L. Barazzuol, Regional responses in radiation-induced normal tissue damage, Cancers 13 (2021) 1–21, https://doi.org/10.3390/cancers13030367.

[4] F. de Vathaire, C. El-Fayech, F.F. Ben Ayed, N. Haddy, C. Guibout, D. Winter, C. Thomas-Teinturier, C. Veres, A. Jackson, H. Pacquement, M. Schlumberger, M. Hawkins, I. Diallo, O. Oberlin, Radiation dose to the pancreas and risk of diabetes mellitus in childhood cancer survivors: a retrospective cohort study, Lancet Oncol. 13 (2012) 1002–1010, https://doi.org/10.1016/S1470-2045(12)70323-6.

[5] C. Gemici, M. Sargin, O. Uygur-Bayramicli, A. Mayadagli, G. Yaprak, R. Dabak, M. Kocak, Risk of endocrine pancreatic insufficiency in patients receiving adjuvant chemoradiation for resected gastric cancer, Radiother. Oncol. 107 (2013) 195–199, https://doi.org/10.1016/j.radonc.2013.04.013.

[6] C. Gemici, G. Yaprak, S. Ozdemir, T. Baysal, O.O. Seseogullari, H. Ozyurt, Volumetric decrease of pancreas after abdominal irradiation, it is time to consider pancreas as an organ at risk for radiotherapy planning, Radiat. Oncol. 13 (2018) 1–7, https://doi.org/10.1186/s13014-018-1189-5.

[7] F.A. van Nimwegen, M. Schaapveld, C.P.M. Janus, A.D.G. Krol, J.M. M. Raemaekers, L.C.M. Kremer, M. Stovall, B.M.P. Aleman, F.E. van Leeuwen, Risk of diabetes mellitus in long-term survivors of Hodgkin lymphoma, J. Clin. Oncol. : Offic. J. Am. Soci. Clinic. Oncol. 32 (2014) 3257–3263, https://doi.org/10.1200/JCO.2013.54.4379.

[8] D.F. Du Toit, J.J. Heydenrych, B. Smit, T. Zuurmond, G. Louw, L. Laker, D. Els, A. Weideman, S. Wolfe-Coote, L.B. Du Toit, H. Davids, W.A. Groenewald, E. van der Merwe, S. Pistorius, The effect of ionizing radiation on the primate pancreas: an endocrine and morphologic study, J. Surg. Oncol. 34 (1987) 43–52, https://doi.org/10.1002/jso.2930340113.

[9] S. Mostoufi-Moab, K. Seidel, W.M. Leisenring, G.T. Armstrong, K.C. Oeffinger, M. Stovall, L.R. Meacham, D.M. Green, R. Weathers, J.P. Ginsberg, L.L. Robison, C. A. Sklar, Endocrine abnormalities in aging survivors of childhood cancer: a report from the Childhood Cancer Survivor Study, J. Clin. Oncol. 34 (2016) 3240–3247, https://doi.org/10.1200/JCO.2016.66.6545.

[10] G. Chen, Y. Han, H. Zhang, W. Tu, S. Zhang, Radiotherapy-induced digestive injury: diagnosis, treatment and mechanisms, Front. Oncol. 11 (2021), https://doi.org/10.3389/fonc.2021.757973.

[11] M.A. Minati, M. Libert, H. Dahou, P. Jacquemin, M. Assi, N-acetylcysteine reduces the pro-oxidant and inflammatory responses during pancreatitis and pancreas tumorigenesis, Antioxidants 10 (2021), https://doi.org/10.3390/antiox10071107.

[12] A. Alnahdi, A. John, H. Raza, N-acetyl cysteine attenuates oxidative stress and glutathione-dependent redox imbalance caused by high glucose/high palmitic acid treatment in pancreatic Rin-5F cells, PLoS One 14 (2019) 1–20, https://doi.org/10.1371/journal.pone.0226696.

[13] A. Elsayed, A. Elkomy, M. Alkafafy, R. Elkammar, S.E. Fadl, E.Y. Abdelhiee, A. Abdeen, G. Youssef, H. Shaheen, A. Soliman, M. Aboubakr, Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats, Pak. Vet. J. 42 (2022) 107–111, https://doi.org/10.29261/pakvetj/2021.035.

[14] C. Moens, C.J.F. Muller, L. Bouwens, In vitro comparison of various antioxidants and flavonoids from Rooibos as beta cell protectants against lipotoxicity and oxidative stress-induced cell death, PLoS One 17 (2022) 1–16, https://doi.org/10.1371/journal.pone.0268551.

[15] G.F. Rushworth, I.L. Megson, Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits, Pharmacol. Therapeut. 141 (2014) 150–159, https://doi.org/10.1016/j.pharmthera.2013.09.006.

[16] H.Ø. Olesen, S.E. Pors, L.B. Jensen, A.P. Grønning, C.E. Lemser, M.T.H. Nguyen Heimbürger, L.S. Mamsen, N. Getreu, S.T. Christensen, C.Y. Andersen, S. G. Kristensen, N-acetylcysteine protects ovarian follicles from ischemia-reperfusion injury in xenotransplanted human ovarian tissue, Hum. Reprod. 36 (2021) 429–443, https://doi.org/10.1093/humrep/deaa291.

[17] F. Mercantepe, A. Topcu, S. Rakici, L. Tumkaya, A. Yilmaz, The effects of N-acetylcysteine on radiotherapy-induced small intestinal damage in rats, Exp. Biol. Med. 244 (2019) 372–379, https://doi.org/10.1177/1535370219831225.

[18] W. Gao, J.-X. Liang, C. Ma, J. Dong, Q. Yan, The protective effect of N-acetylcysteine on ionizing radiation induced ovarian failure and loss of ovarian reserve in female mouse, BioMed Res. Int. (2017) 1–11, https://doi.org/10.1155/2017/4176170, 2017.

[19] E.O. Demir, G.K. Cakmak, H. Bakkal, U.O. Turkcu, N. Kandemir, A.S. Demir, O. Tascilar, N-acetyl-cysteine improves anastomotic wound healing after radiotherapy in rats, J. Invest. Surg. 24 (2011) 151–158, https://doi.org/10.3109/08941939.2011.560237.

[20] F. Mercantepe, A. Topcu, S. Rakici, L. Tumkaya, A. Yilmaz, The effects of N-acetylcysteine on radiotherapy-induced small intestinal damage in rats, Exp. Biol. Med. 244 (2019) 372–379, https://doi.org/10.1177/1535370219831225.

[21] A. Topcu, F. Mercantepe, S. Rakici, L. Tumkaya, H.A. Uydu, T. Mercantepe, An investigation of the effects of N-acetylcysteine on radiotherapy-induced testicular injury in rats, Naunyn-Schmiedeberg's Arch. Pharmacol. 392 (2019) 147–157, https://doi.org/10.1007/s00210-018-1581-6.

[22] F. Mercantepe, L. Tumkaya, T. Mercantepe, S.Y. Rakici, S. Ciftel, S. Ciftel, Radioprotective effects of α2-adrenergic receptor agonist dexmedetomidine on X-ray irradiation-induced pancreatic islet cell damage, Naunyn-Schmiedeberg's Arch. Pharmacol. 396 (2023) 1827–1836, https://doi.org/10.1007/s00210-023-02454-0.

[23] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358, https://doi.org/10.1016/0003-2697(79)90738-3.

[24] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent, Anal. Biochem. 25 (1968) 192–205, https://doi.org/10.1016/0003-2697(68)90092-4.

[25] R. Pawlick, B. Gala-Lopez, A.R. Pepper, N. Abualhassan, A. Bruni, K. Suzuki, G. Rayat, J.F. Elliott, A.M.J. Shapiro, Low energy X-ray (grenz ray) treatment of purified islets prior to allotransplant markedly decreases passenger leukocyte populations, Islets 9 (2017) 63–72, https://doi.org/10.1080/19382014.2017.1330742.

[26] S.H. You, M.Y. Cho, J.H. Sohn, C.G. Lee, Pancreatic radiation effect in apoptosis-related rectal radiation toxicity, J. Radiat. Res. 59 (2018) 529–540, https://doi.org/10.1093/jrr/rry043.

[27] M.J. Sung, D.H. Kim, Y.J. Jung, K.P. Kang, A.S. Lee, S. Lee, W. Kim, M. Davaatseren, J.T. Hwang, H.J. Kim, M.S. Kim, D.Y. Kwon, S.K. Park, Genistein protects the kidney from cisplatin-induced injury, Kidney Int. 74 (2008) 1538–1547, https://doi.org/10.1038/ki.2008.409.

[28] M. Aboubakr, A. Farag, A. Elfadadny, M. Alkafafy, A. Soliman, M. Elbadawy, Antioxidant and anti-apoptotic potency of allicin and lycopene against methotrexate-induced cardiac injury in rats, Environ. Sci. Pollut. Control Ser. 30 (2023) 88724–88733, https://doi.org/10.1007/s11356-023-28686-4.

[29] A. Elsayed, A. Elkomy, M. Alkafafy, R. Elkammar, A. El-Shafey, A. Soliman, M. Aboubakr, Testicular toxicity of cisplatin in rats: ameliorative effect of lycopene and N-acetylcysteine, Environ. Sci. Pollut. Control Ser. 29 (2022) 24077–24084, https://doi.org/10.1007/s11356-021-17736-4.

[30] M. Michlin, L. Argaev-Frenkel, L. Weinstein-Fudim, A. Ornoy, T. Rosenzweig, Maternal n-acetyl cysteine intake improved glucose tolerance in obese mice offspring, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21061981.

[31] N.A.M.P. Nguyen, P. Vos, V. Vinh-hung, T.L. Borok, S. Dutta, U.L.F. Karlsson, H. Lee, T. Martinez, B. Jo, L.Y.M. Nguyen, N.G.A. Nguyen, S. Sallah, T.L. Borok, M. D.P.A. Borok, Nguyen, Altered Glucose Metabolism During Chemoradiation For Head And Neck Cancer, 2009 4688, 2009, pp. 4683–4687.

[32] E. Barone, A. Corrado, F. Gemignani, S. Landi, Environmental risk factors for pancreatic cancer: an update, Arch. Toxicol. 90 (2016) 2617–2642, https://doi.org/10.1007/s00204-016-1821-9.

[33] J. Frontiñán-Rubio, R.M. Santiago-Mora, C.M. Nieva-Velasco, G. Ferrín, A. Martínez-González, M.V. Gómez, M. Moreno, J. Ariza, E. Lozano, J. Arjona-Gutiérrez, A. Gil-Agudo, M. De la Mata, M. Pesic, J.R. Peinado, J.M. Villalba, L. Pérez-Romasanta, V.M. Pérez-García, F.J. Alcaín, M. Durán-Prado, Regulation of the oxidative balance with coenzyme Q10 sensitizes human glioblastoma cells to radiation and temozolomide, Radiother. Oncol. 128 (2018) 236–244, https://doi.org/10.1016/j.radonc.2018.04.033.

[34] W.W.Y. Kam, R.B. Banati, Effects of ionizing radiation on mitochondria, Free Radic. Biol. Med. 65 (2013) 607–619, https://doi.org/10.1016/j.freeradbiomed.2013.07.024.

[35] L. Argaev Frenkel, H. Rozenfeld, K. Rozenberg, S.R. Sampson, T. Rosenzweig, N-Acetyl-l-Cysteine supplement in early life or adulthood reduces progression of diabetes in nonobese diabetic mice, Curr. Dev. Nutr. 3 (2019) nzy097, https://doi.org/10.1093/cdn/nzy097.

[36] R.F. Meirelles Junior, M.S. Kubrusly, M. Bellodi-Privato, N.A.T. Molan, M.C. C. Machado, L.A.C. D'Albuquerque, Beneficial effects of N-acetyl cysteine on pancreas and kidney following experimental pancreatic ischemia-reperfusion in rats, Clinics 65 (2010) 311–316, https://doi.org/10.1590/s1807-59322010000300012.

[37] S. Yoo, J.Y. Bae, J. Moon, G. Koh, System χc- overexpression prevents 2-deoxy-D-ribose-induced β-cell damage, Free Radic. Biol. Med. 153 (2020) 17–25, https://doi.org/10.1016/j.freeradbiomed.2020.04.012.

[38] A. Alnahdi, A. John, H. Raza, Mitigation of glucolipotoxicity-induced apoptosis, mitochondrial dysfunction, and metabolic stress by n-acetyl cysteine in pancreatic β-cells, Biomolecules 10 (2020), https://doi.org/10.3390/biom10020239.

[39] D. Abdelhafez, E. Aboelkomsan, A. El Sadik, N. Lasheen, S. Ashur, A. Elshimy, G.N. B. Morcos, The role of mesenchymal stem cells with ascorbic acid and N-acetylcysteine on TNF- α, IL 1 β, and NF- κβ expressions in acute pancreatitis in albino rats, J. Diabetes Res. (2021) 2021, https://doi.org/10.1155/2021/6229460.

[40] R.H. Morales-Borges, M.J. Gonzalez, J. Duconge, D.M. Minich, N-acetyl cysteine and glutathione in health and cancer-pharmacogenomics, research, and clinical practice: hypothesis and review, Alternative Ther. Health Med. 28 (2022) 169–177. http://www.ncbi.nlm.nih.gov/pubmed/33373322.