Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2024, 17(3), 811-825
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2024, 17(3), 811-825
DOI: 10.18185/erzifbed.1555393
Research Article

Radioprotective Effect of Umbelliferon Against Radiation-Induced Myocardial Damages

Nurdan DUMLU[1], Fatime GEYİKOĞLU[1], Suat ÇOLAK[2*]

[1]Department of Biology, Faculty of Sciences, Atatürk University, 25240, Erzurum, Turkey
[2]Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, 24002, Erzincan, Turkey

Received: 24/09/2024, **Revised:** 11/10/2024, **Accepted:** 01/11/2024, **Published:** 31/12/2024

Abstract

Radiation at the high dose may attenuate myocardial functions while presenting the therapeutic effect on cancer cells and thus can life-threatening. There are numerous phytotherapeutic studies in the literature to reduce or eliminate tissue damage. Therefore, the current study was planned to evaluate the role of umbelliferone radiation-induced cardiac injury using biochemical and histological data. Rats will be divided into eight different groups as control, radiation and treatment groups. Radiation exposure to the rats induced oxidative stress, inflammation, pathological changes and vascular dysfunction in cardiac tissue. Whereas umbelliferone in high dose (100 mg/kg) pretreatment supported the anti-oxidant activity and also reduced inflammatory response and histopathologic damage in heart tissue against radiation-associated toxicity. In conclusion, it was shown that umbelliferon (100 mg/kg) pretreatment can be used against the side effects of radiotherapy.

Keywords: Radiation, umbelliferone, oxidative stress, cardiotoxicity, rat

Umbelliferon'un Radyasyona Bağlı Miyokard Hasarlarına Karşı Radyoprotektif Etkisi

Öz

Yüksek dozda uygulanan radyasyon, kanser hücreleri üzerinde tedavi edici etki gösterirken miyokardiyal fonksiyonları zayıflatmakta ve yaşamı tehdit etmektedir. Literatürde doku hasarını azaltmak veya gidermek için çok sayıda fitoterapötik çalışma vardır. Bu çalışma ile biyokimyasal ve histolojik yöntemler kullanarak umbelliferonun radyasyona bağlı kalp hasarındaki rolünün değerlendirilmesi hedeflenmiştir. Bu amaçla, Sıçanlar kontrol, radyasyon ve tedavi grupları olmak üzere sekiz farklı gruba ayrılmıştır. Sıçanların kalp doku örneklerinde radyasyon maruzuyetinin oksidatif streste artma, inflamasyon, patolojik değişiklikler ve vasküler disfonksiyona neden olduğu görülmüştür. Yüksek dozda (100 mg/kg) umbelliferon uygulanan grupta ise anti-oksidan aktivite desteklenmiş ve radyasyonla ilişkili toksisiteye karşı kalp dokusunda inflamatuar yanıtı ve histopatalojik hasarı azalttığı görülmüştür. Sonuç olarak, umbelliferon (100 mg/kg) ön tedavisinin radyoterapinin yan etkilerine karşı kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Radyasyon, umbelliferon, oksidatif stres, kardiyotoksisite, sıçan

*Corresponding Author: scolak@erzincan.edu.tr
Nurdan DUMLU, https://orcid.org/0009-0009-1658-6484
Fatime GEYİKOĞLU, https://orcid.org/0000-0003-2488-1757
Suat ÇOLAK, https://orcid.org/0000-0001-8320-3038

1. Introduction

According to the report published by the World Health Organisation in 2021, cardiovascular diseases are in the first place among the causes of death. In second place are respiratory system diseases due to the Covid-19 pandemic that we experienced between 2019-2021. Cancer cases, which were in the top 3 among the causes of death in previous years, decreased to 6th place among the causes of death with the global epidemics [1]. By 2035, It is estimated that there will be 24 million new cancer cases worldwide [2]. Surgery, chemotherapy, radiotherapy methods and their combinations are still frequently used in cancer treatments. Radiotherapy (RT), one of them, remains the most widely used method of cancer treatment and is estimated to be given to a round 60% of cancer patients [3]. Although ionizing radiation therapy has beneficial effects on living cells, it has also been reported that it has many side effect that develop depending on dose and duration and finally this effects reduced quality of life [4]. Furthermore, these adverse effects have also been linked to developing metabolic disorders, which may cause to serious complications such as renal failure, impairment in lung function, liver injury, neuropathy, atherosclerosis or cardiac damage [5-9].

Many of these toxic effects result from excessive Reactive Oxygen Species (ROS) that induce oxidative stress caused by ionising rays used in radiotherapy [10]. Our body has an antioxidant enzymatic defense system such as catalyze (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). These prevent oxidative stress by directly scavenging reactive oxygen species or reducing their effect [11]. In cases where this system is insufficient, the intake of primarily plant-derived dietary antioxidants is thought to play an important role in protecting human health [12-14].

The coumarins are biologically active compounds that have heterocyclic structure and belonging to the class of benzopyrone. Coumarins and their derivatives have recently been studied extensively and found to have many biological properties, including antibacterial, anti-inflammatory, antioxidant, antitumor, antiviral, and especially anticoagulant [15-17]. Umbelliferone (UMB), known as 7-hydroxycoumarin, is a naturel coumaric compound that found in many plant [18]. Although there are many theories to explain how umbelliferone works, the most widely accepted is that it has antioxidant properties [19]. Studies have reported that it is effective against myocardial ischaemia damage [20], liver damage [21], testicular dysfunction [22] and diabetic nephropathy [23].

In recent years, the discovery of new agents of natural and herbal origin has become increasingly popular among researchers due to the lack of complete treatment of human diseases and undesirable side effects. From this point of view, this study aimed to determine whether umbelliferone pretreatment reduces radiation-induced myocardial damage. Thus, our study for the first time reveals the novel therapeutic role of umbelliferone in cardiac tissue against radiation-induced injury.

2. Materials and Methods

Umbelliferone and chemicals

Umbelliferone (purity ≥98%) and other chemicals have been purchased from Merck Healthcare KGaA (Darmstadt, Germany). Thromboxane B2 (TXB2) was obtained from Cell Signaling Technology (Danvers, MA, USA). TAC (Randox, UK), TOS commerical kits (Rel Assay Diagnostics; Mega Tip, Gaziantep, Turkey) were purchased from commercial sources.

Animals care and maintenance

In this experimental study, fifty six male Spraque Dawley rats (weighing 280-300 g) were obtained from Animal Center of Atatürk University. The animals were housed in an air-conditioned room (20- 24°C) with 12-hour light-dark cycle. The rats were provided free access to standard feed and water ad libitum. All experimental procedures were approved by Institutional Ethical Committee for Animal Care and Use at Atatürk University, Erzurum, Turkey (Code number: 263, 26.12.2019B.30.2.ATA.0.23.85-11) and were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals [24].

Experimental design

The experimental groups were formed as 7 groups with 8 animals in each group. The groups were administrated different doses as follows:
Group I: (Control group) given physiological saline,

Group II: (Radiation group) exposed a single high dose of 12 Gy of gamma rays,

Group III, IV and V: (Umbelliferone groups) Administered umbelliferone (30, 60 and 90 mg/kg body weight/day) alone,

Group VI, VII and VIII: (treatment groups) received umbelliferone orally (30, 60 and 90 mg/kg body weight/day) for 5 days prior to radiation exposure.

The umbelliferone doses were determined based on the literature and our pilot studies [25].After treatment for 24 h, the animals were anesthetized by injections of 12 mg/kg xylazine and 80 mg/kg ketamine and were sacrificed to collect heart tissues. The tissue samples were carefully collected and divided into two parts: one part was fixed in 10% formalin solution for histopathological studies and the second part was stored as a lysate for biochemical and other analysis.

Ionizing radiation exposure

The cardiac irridation model was established as previously described by researchers [26,27]. According to the this procudure, a Theratron 780-C source (AECL, Ontario, Canada) with a dose rate of 0.78 Gy/min was used to deliver a single 12 Gy dose to whole body. The applications were carried out in the Radiotherapy Unit of Atatürk University Medical College

Hospital, Erzurum, Turkey. A tissue-equivalent ionisation chamber was used to calibrate the exposure setup by physical measurement. The homogenous irradiation field measured 30 × 30 cm, exhibiting uniformity within 1%. Each rat was placed in a ventilated circular holder during irradiation to minimize movement, ensuring that the whole body received a uniform dose of radiation.

Total Antioxidant Capacity and Total Oxidant Status

Total antioxidant capacity (TAC) and total oxidative status (TOS) assay kits were used to measure oxidative stress and resistance to oxidants. Measurements were conducted according to the protocols of TAC and TOS kits [28,29]. The TAC and TOS results were given as mmol trolox equivalent/L and μmol H_2O_2 equivalent/L, respectively.

TNF-a and Thromboxane B2 (TXB2)

Rat heart tissue samples (approximately 2 g) were weighed and then stored at -80 °C. After, tissue samples were homogenized into phosphate-buffered saline (PBS) and centrifuged at 10,000g, 4 °C for 10 minutes. The supernatants (40 μl) were collected and promptly transported to −80 °C. Thromboxane B2 (TXB2) and tumor necrosis factor alpha (TNF-a) levels were tested using commercially available ELISA kits, according to the manufacturer's instructions.

Histopathological studies

Rat heart tissue samples were fixed in buffered 10% formalin for 48 h and then embedded in paraffin blocks. The heart tissues were sectioned at 5 μm and stained using Haematoxylin-Eosin (H&E), Congo Red and Periodic Acid-Schiff (PAS) methods. The slides were examined under a light microscope. Changes in heart tissue were scored in twenty different areas for each section. The cardiac lesions (congestion, infiltration, haemorrhage and apaptotic) in the groups were scored out of a maximum of 18. These lesions were scored as 1 if less than 30%, 2 if between 30-60% and 4-5 if more than 60% [30].

Statistical Method

Data analysis of TAC, TOS, TNF-a, TXB2 parameters was performed using SPSS 24.0 for Windows software (SPSS Inc., Chicago, IL). Data are expressed as means ± SE. Since, all results followed a normal distribution comparisons between the groups were carried out using one-way parametric ANOVA. The data were commented according to the significance level of 0.05.

Results and Discussion

Evaluation of biochemical parameters

TAC, TOS, TNF-a, TXB2 tissue levels in all experimental groups are presented in Table I according to the groups. In the RAD group (group II), TOS levels in rat heart tissue increased while TAC levels decreased compared to the control group. This is a clear indication that the

oxidative balance in the heart tissue was impaired. However, no significant difference was found between the control and RAD+UM25 and RAD+UM50 treated groups. Furthermore, it was observed that these levels returned to control levels in UM100-treated rats.

We also evaluated the anti-inflammatory activity of UMB by determining the TNF-a value. While the level of TNF-a was significantly up-regulated in the radiation-exposured group, the group pretreated with UMB100 showed a marked down-regulation of TNF-a in the heart tissue sample ($p < 0.05$).

TXB2 levels were significantly higher in the irradiated group compared to the other groups ($p < 0.05$). This increase is an important evidence of vascular injury. In the rats treated with different doses of UMB, the level of TBX2 did not change level of the control group ($p > 0.05$).

Table 1. Effects of Umbelliferone on TAC, TOS, TNF-α and TXB2 levels

Groups	TAC (mmol Trolox Equiv./L)	TOS (μmol H_2O_2 Equiv./L)	TNF-a (pg/mg tissue)	TXB2 (pg/mg)
Control	7.94±0.80	5.01±0.24	3.90±0.29	429±19
Rad	3.90 ±0.10^{a}	8.10±0.01^{a}	6.30±0.27^{a}	645±15^{a}
Umb25	8.17±0.12 ^{b}	5.13±0.06^{b}	3.81±0.12^{b}	411±19
Umb50	8.29±0.27 ^{b}	5.13±0.11 ^{b}	3.78±0.05^{b}	407±13
Umb100	8.42±0.10^{b}	5.05±0.19^{b}	3.83±0.12^{b}	404±8
Umb25+Rad	4.80±0.16 ^{a,b,c}	4.79±0.05^{a,b,c}	5.10±0.15^{a,b,c}	596±13^{a,b}
Umb50+Rad	4.64±0.14 ^{a,b,c}	4.50±0.05^{a,b,c}	4.40 ±0.17^{a,b,c}	495±16^{a,b,c}
Umb100+Rad	7.57±0.23 ^{b,c,d}	4.97±0.01^{b,c,d}	3.83±0.08^{b,c,d}	469±12^{a,b,c,d}

Data are presented as means ± SD (n=7). ^{a,b,c,d} The groups in the same column with different letters are statistically significant ($p < 0,05$). Abbreviation used: Rad: Radiation, Umb25: 25 mg/kg Umbelliferone, Umb50: 50 mg/kg Umbelliferone; Umb100: 100 mg/kg Umbelliferone

Many researchers are currently working on the development of new methods in cancer treatment. But, as of today, radiotherapy is one of the most effective and preferred methods in cancer treatment. Unfortunately, although the number of patients treated with this method is increasing day by day, the side effects of the method have not been reduced [31,32]. In a clinical application, ionizing radiation such as gamma rays or X-rays are frequently preferred. However,

The type and dose of radiation have an important role in the development of cardiotoxicity seen with radiation [33].

It has been demonstrated that exposure to ionizing radiation causes oxidative stress by increases ROS production, reduces antioxidant capacity, and inducing apoptosis [34, 35]. The main impacts of oxidative stress on cardiotoxicity induced by radiation involve several kinds of molecular pathways and oxidative damage to organic macromolecules such as proteins, lipids, and DNA. Excessive production of ROS also contributes to this impact. [31, 33]. Many of these possible effects have been ameliorated by the presence of different antioxidants, both enzymatic and non-enzymatic, including GSH-Px, SOD, vitamin E, melatonin and zinc [35]. The TOS is frequently included in the set of parameters used to characterize cellular stress responses [36]. From our experimental data, we found that the level of TOS in rat heart tissues increased significantly with the application of irridation. On the contrary, it was noticed that TAC levels decreased. This indicates that irradiation increases oxidative stress in rat heart tissue and that this stress depletes antioxidant stores. In the current study, high dose of umbelliferone pretreatment increased TAC levels through umbelliferone supplementation, which is important for the redox modulation of heart tissue after high-dose radiation exposure. Considering the data, the results of our study clearly demonstrate that umbelliferone has a radioprotective effect on the rat heart by decreasing the formation of reactive oxygen species

An essential intermediate and second messenger such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) are formed by irradiation-induced ROS [37]. Multifunctional proinflammatory cytokine TNF-α is primarily created by activated mononuclear phagocytes, however it can also be produced occasionally by T lymphocytes, smooth muscle cells, and activated polymorphonuclear endothelial cells [38]. The anti-inflammatory effect of umbelliferone detected by biochemically measuring expression of TNF-a levels on irridation-induced cardiac tissue damage. There are some articles indicating that inflammation occurs after irridation applied to rat heart tissue [39,40]. The results of our study are similar to those of these studies, and there was a significant increase in TNF-A levels in the patient group of animals. However, pretreatment with high dose of umbelliferone prevented significant increases in TNF-a in cardiac muscle cells and markedly reduced inflammation induced by acute irradiation. TNF-a and other cytokines promote inflammation not only through the recruitment of white blood cells but also through the activation of NF-κB. [41]. The inhibition of TLR/NF-κB signaling during myocardial ischemia is believed to be connected to umbelliferone's radioprotective function [42]. We attempted to demonstrate in this experimental investigation that umbelleriferone, in addition to its antioxidant properties, plays an impotant role in the inflammatory response in irradiation-induced heart damage.

Strong blood vessel constrictor Thromboxane A2 (TxA2) has been linked to the etiology of a number of cardiovascular disorders. The half-life of TxA2 is very short and therefore is rapidly metabolized to form the relatively stable but inactive TXB2 [38, 43]. In this work, the concentration of TXB2 level was measured to reflect the quantity of TXA2. In the current investigation, it was indicated that, in contrast to the control values, TXB2 levels were considerably enhanced following acute radiation exposure. On the other hand, umbelliferone injection dramatically reduces the rising radiation-mediated TXB2 levels in rats. This finding

suggested that radiation exposure causes the secretion of pro-inflammatory cytokines and may reduce vascular damage in the heart tissue. This finding could point to the potential of umbelliferone as a novel endovascular treatment for heart damage.

Evaluation of histopathological changes

Histological examinations were performed on cardiac tissue from irridated rats using different staining techniques, and examples of result are given in Figs. 1, 2 and 3. Firstly, H&E staining results showed that the heart tissues of the control group rats had a normal morphology and appearance. In the radiated patient groups, there were more light stained and necrotic areas throughout the cardiac muscle tissue. This is an indication of weakened cardiac muscle tissue. The presence of cylindrical shaped cell nuclei, oedema, congestion and infiltration were among other important findings. Severe degeneration was also observed around the vessels, On the opposite, in the Rad+UMB100 group, the heart tissue had a well-preserved appearance and shape. The pathological changes, such as the presence of necrotic areas, congestion and edema, were observed less frequently. The results in UMB25 and UMB50 groups were not given because they were similar to the patient group (Fig 1).

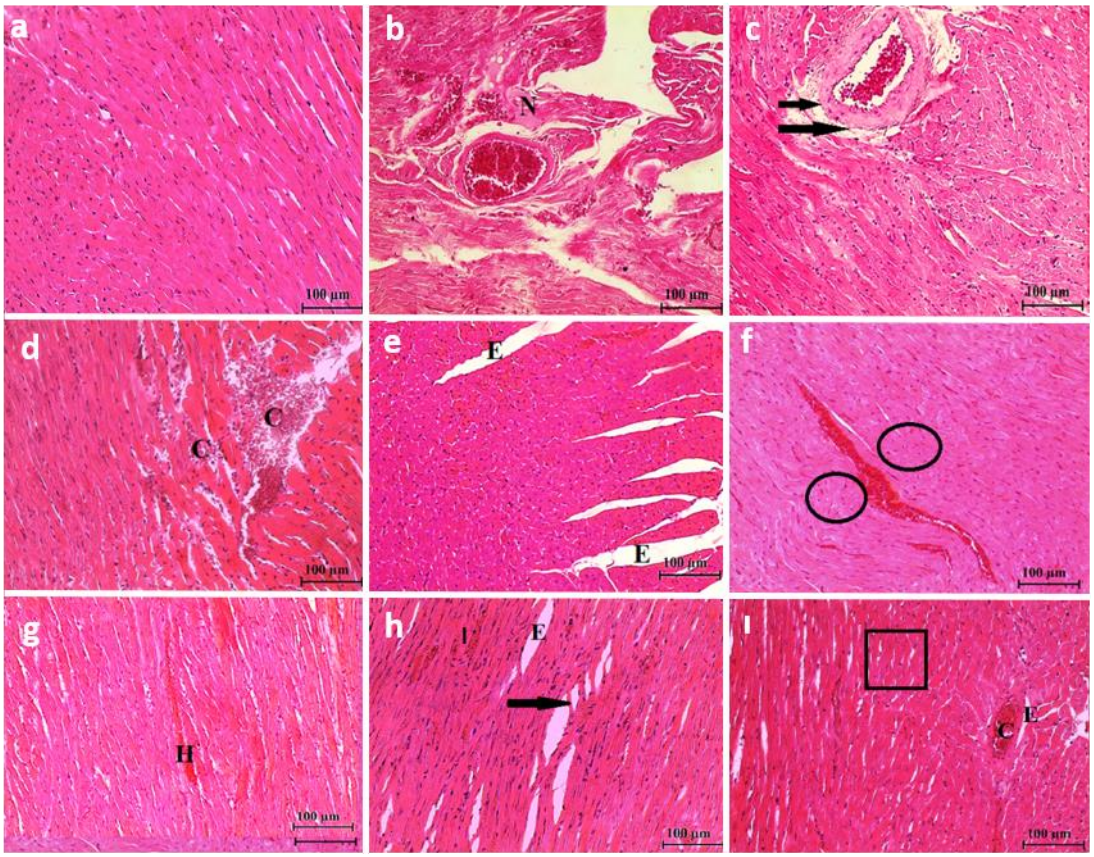

Fig. 1. Heart tissue section. **a)** The control group had normal architecture; The heart tissue of Rad group was shown a severe damage in figure b-h. **C;** Congestion, **N;** Nekrosis, **E;** Edema, **H;** Haemorrhage, **I;** Infiltration, **double black arrows;** Artery wall damage, **black arrows;** Reduced edema area, **Circle**; Apoptotic cells, **Frame;** Vacuolization **ı)** Slight renal injury in group given umbelliferone (100 mg/kg) before irradiated,. (H&E), Bar: 100 µm

According to the PAS staining results, the presence of light stained areas and a decrease in glycogen content were observed in rat heart tissues exposed with radiation compared to the control group. PAS staining also revealed edema and vessel wall degeneration, which were present in H&E staining (Fig 2).

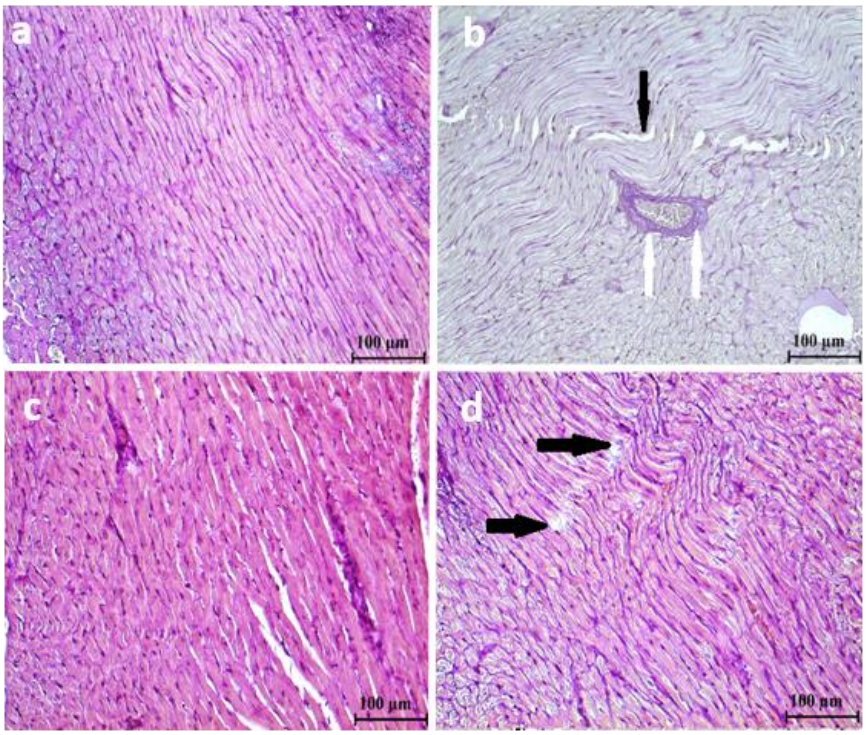

Fig. 2. Heart tissue section staining with PAS. **a)** The control group had normal architecture. **b)** Reduced glycogen content in the irradiated group. **Black arrow;** Muscle cell degeneration, **White arrow;** Intense PAS-positive staining around the vessel. (PAS), Bar: 100 µm.

With Congo red staining method, several histopathologic changes such as marked protein accumulation was observed in cardiac muscle cells and vascular endothelium. However, this was not observed in the UMB100-treated patient groups and histological results were similar to the control group (Fig 3).

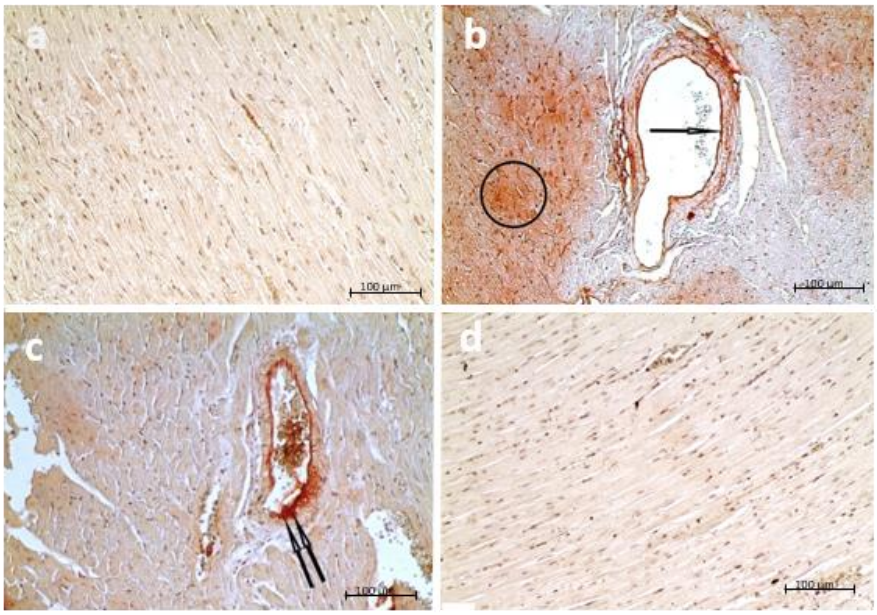

Fig 3. a) The control group had normal architecture. **b and c)** The amyloid aggregations in heart tissue (Circle) and vasculer area (Arrow) **d)** Normal histological appearance of kidney in group given umbelliferone before irradiated. (Congo red), Bar: 100 µm.

In order to provide more accurate and clear evidence of histopathological findings in rat heart tissue, a semi-quantitative scoring system was performed and the results are presented in Table 2. As a result of radiation exposure, congestion, inflammation, haemorrhage and apoptotic cells increased in myocardial tissue and this result was statistically significant ($p < 0.05$). Whereas in the Umb100 pretreated group, pathological conditions could be restored.

Table 2. Comparison of histomorphologic scores between groups in heart tissue

Groups	Congestion	Infiltration	Haemorrhage	Apoptosis
Control	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00
Rad	5,49±0,50^{a}	4,81±0,62^{a}	4,50±0,91^{a}	4,86±0,64^{a}
Umb25	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}
Umb50	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}
Umb100	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}	0,00±0,00^{b}
Umb25 +Rad	3,96±0,34^{a,b}	4,69±0,47^{a,b}	4,43±0,73^{a,b}	4,72±0,52^{a,b}
Umb50+Rad	3,84±0,41^{a,b}	3,00±0,26^{a,b,c}	1.80±0,44^{a,b,c}	1,70±0,40^{a,b,c}
Umb100+Rad	1,41±0,19^{a,b,c}	1,60±0,11^{a,b,c}	1,57±0,21^{a,b,c}	1,92±0,24^{a,b,c}

Data are presented as means ± SD (n=7). ^{a,b,c,d} The groups in the same column with different letters are statistically significant (p< 0,05). Abbreviation used: Rad: Radiation, Umb25: 25 mg/kg Umbelliferone, Umb50: 50 mg/kg Umbelliferone; Umb100: 100 mg/kg Umbelliferone

Cardiac dysfunction may result from radiation-induced cardiac fibrosis and remodeling [9]. Our pathological findings demostrated numerous tissue damage such as widespread interstitial edema, vacuolization, degenerations in arteries, widespread hemorrhagic formations, degenerations in contraction bands, congestion in large and small capillaries, muscle fiber degenerations, intense infiltration areas, decrease in glycogen content in muscle cells, amyloid accumulations in the lumens of vessels and between muscle fibers. In line with our result, El-Benhawy et al., (2021) found that, radiation exposure resulted in rregular longitudinal cardiac muscle fibers with areas of destructive and necrotic myocytes, interstitial mononuclear cellular infiltration, dilated congested blood vessels and dilated spaces between the cardiac muscle fibers in heart tissue [44]. Also, with the work done by Sarhan and Naoum who showed distorted cardiac muscle fibres of the irradiated rats with deeply stained nuclei (pyknotic) and highly thickened and elongated arterial Wall [45].

In addition to all the findings, Hemnani and Parihar (1998) reported that the production of superoxide anions and their derivatives, which cause peroxidation of lipids in cell membranes, is responsible for the radiation-induced cardiotoxicity mechanism [46]. Damaged cardiac tissue observed in the present study may be due to increased oxidative stress. Radiation-induced

histopathological changes in rat heart tissue are significantly attenuated by pretreatment with umbelliferone.

Conclusion

As a result, Current study reflects new and beneficial evidence about the role of umbelliferone agianist heart injury before radiation exposure. Umbelliferone has a beneficial role in reducing oxidative stress and cardio-toxicity induced by radiation exposure. Moreover, It has also been shown to have a protective effect on biochemical, inflammation and pathological parameters. Researchers should focus more on the development of new therapeutic agents for patients harmed by IR exposure during radiotherapy.

Ethics in Publishing

All experimental procedures were performed according to approval of the Institutional Ethical Committee for Animal Care and Use at Atatürk University, Erzurum, Turkey (Code number: 263, 26.12.2019B.30.2.ATA.0.23.85-11)

Author Contributions

ND and FG were responsible for the design and execution of the experiments. FG and SC performed data analysis, drafting and editing.

Acknowledgements

No financial support has been provided for the carrying out of this study. There is no conflict of interest regarding the publication of this paper.

References

[1] https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death, 16.09.2024

[2] Pilleron, S., Sarfati, D., Janssen-Heijnen, M., Vignat, J., Ferlay, J., Bray, F., & Soerjomataram, I. (2019) Global cancer incidence in older adults, 2012 and 2035: a population-based study. International Journal of Cancer, 144(1), 49-58.

[3] Simcock, R., Fallowfield, L., Monson, K., Solis-Trapala, I., Parlour, L., Langridge, C., Jenkins, V. Arix. (2013) A Randomised Trial of Acupuncture V Oral Care Sessions in Patients with Chronic Xerostomia Following Treatment of Head and Neck Cancer. Annual Oncology, 24 (3), 776–783.

[4] Konak, M., Cincik, H., Erkul, E., Kucukodaci, Z., Gungor, A., Ozdemir, S., Cekin, E., Arisan, V., Mutluoglu, M., Salihoglu, M. (2016) The Protective Effects of Different Treatments on Rat SalivaryGlands after Radiotherapy. European Archives of Oto-Rhino-Laryngology, 273 (12), 4501–4506.

[5] Huang, Y., Zhang, W., Yu, F., Gao F. (2017) The Cellular and Molecular Mechanism of Radiation-Induced Lung Injury. Medical Science Monitor,15(23), 3446-3450.

[6] Azmoonfar, R., Khosravi, H., Rafieemehr, H., Mirzaei, F., Dastan, D., Ghiasvand, M. R., Khorshidi, L., Pashaki, A. S. (2023) Radioprotective effect of Malva sylvestris L. against radiation-induced liver, kidney and intestine damages in rat: A histopathological study. Biochemistry and Biophysics Reports, 34, 101455.

[7] Aras, S., Efendioğlu, M., Wulamujiang, A., Ozkanli, S. S., Keleş, M. S., Tanzer, İ.O. (2021) Radioprotective effect of melatonin against radiotherapy-induced cerebral cortex and cerebellum damage in rat. International Journal of Radiation Biology, 97(3), 348-355.

[8] Kaplan, M., Demir, E., Yavuz, F., Kaplan, G. I., Taysi, M. R., Taysi, S., Sucu, M.M. (2022) Radioprotective effect of nigella sativa oil on heart tissues of rats exposed to irradition. International Journal of Cardiovascular Sciences, 35(2), 214-219.

[9] Zhang, J., He, X., Bai, X., Sun, Y., Jiang, P., Wang, X., Li, W., Zhang, Y. (2020) Protective effect of trimetazidine in radiation-induced cardiac fibrosis in mice. Journal of Radiation Research, 61(5), 657-665.

[10] Adaramoye, O., Okiti, O., Farombi, E. (2011) Dried fruit extract from Xylopiaaethiopica (Annonaceae) protects Wistar albino rats from adverse effects of whole body radiation. Experimental and Toxicologic Pathology, 63 (7-8), 635-39.

[11] Şener, G., Kabasakal, L., Atasoy, B.M., Erzik, C., Velioğlu-Öğünç, A, Çetinel, Ş., Contuk, G., Gedik, N., Yeğen, B.Ç. (2006) Propylthiouracil-induced hypothyroidism protects ionizing radiation-induced multiple organ damage in rats. Journal of Endocrinology, 189 (2), 257-62.

[12] Benzie, I.F. (2000) Evolution of antioxidant defence mechanisms. European Journal of Nutrition, 39, 53-61.

[13] McCord, J.M. (2000) The evolution of free radicals and oxidative stress. The American Journal of Medicine, 108(8), 652-659.

[14] Ames, B.N. (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutation Research/Fundamental And Molecular Mechanisms Of Mutagenesis, 475(1-2), 7-20.

[15] Kasaian, J., Mohammadi, A. (2018) Biological activities of farnesiferol C: a review. Journal of Asian Natural Products Research, 20 (1), 27-35.

[16] Garg, S.S., Gupta, J., Sharma, S., Sahu, D. (2020) An insight into the therapeutic applications of coumarin compounds and their mechanisms of action. European Journal of Pharmaceutical Sciences, 152, 105424.

[17] Küpeli Akkol, E., Genç, Y., Karpuz, B., Sobarzo-Sánchez, E., & Capasso, R. (2020) Coumarins and coumarin-related compounds in pharmacotherapy of cancer. Cancers, 12 (7), 1959.

[18] Mazimba, O. (2017) Umbelliferone: Sources, chemistry and bioactivities review. Bulletin of Faculty of Pharmacy, Cairo University, 55 (2), 223-232.

[19] Althunibat, O. Y., Abduh, M. S., Abukhalil, M. H., Aladaileh, S. H., Hanieh, H., & Mahmoud, A. M. (2022) Umbelliferone prevents isoproterenol-induced myocardial injury by

upregulating Nrf2/HO-1 signaling, and attenuating oxidative stress, inflammation, and cell death in rats. Biomedicine & Pharmacotherapy, 149, 112900.

[20] Dikmen, M. (2016) Antiproliferative and apoptotic effects of aprepitant on human glioblastoma U87MG cells. Marmara Pharmaceutical Journal, 21(1), 156-164.

[21] Mahmoud, A. M., Hozayen, W. G., Hasan, I. H., Shaban, E., & Bin-Jumah, M. (2019) Umbelliferone ameliorates CCl 4-induced liver fibrosis in rats by upregulating PPARγ and attenuating oxidative stress, inflammation, and TGF-β1/Smad3 signaling. Inflammation, 42, 1103-1116.

[22] Alotaibi, M. F., Al-Joufi, F., Abou Seif, H. S., Alzoghaibi, M. A., Djouhri, L., Ahmeda, A. F., Mahmoud, A. M. (2023) Umbelliferone Inhibits Spermatogenic Defects and Testicular Injury in Lead-Intoxicated Rats by Suppressing Oxidative Stress and Inflammation, and Improving Nrf2/HO-1 Signaling [Retraction]. Drug Design, Development and Therapy, 17, 1153-1154.

[23] Wang, H.Q., Wang, S.S., Chiufai, K., Wang, Q., Cheng, X.L. (2019) Umbelliferone ameliorates renal function in diabetic nephropathy rats through regulating inflammation and TLR/NF-kappaB pathway. Chinise Journal of Natural Medicine, 17(5), 346-354.

[24] Council, N.R. (2011) National Institutes of Health guide for the care and use of laboratory animals.

[25] Cruz, L. F., De-Figueiredo, G.F., Pedro, L.P., Amorin, Y.M., Andrade, J.T., Passos, T. F., Rodrigues, F. F., Souza, I.LA., Gonçalves, T.P.R., Dos Santos Lima, L.A.R., Ferreira, J.M.S., Freitas Araújo, M. G. (2020) Umbelliferone (7-hydroxycoumarin): A non-toxic antidiarrheal and antiulcerogenic coumarin. Biomedicine & Pharmacotherapy, 129, 110432.

[26] Sproull, M., Kramp, T., Tandle, A., Shankavaram, U., Camphausen, K. (2017) Multivariate analysis of radiation responsive proteins to predict radiation exposure in total-body irradiation and partial-body irradiation models. Radiation Research, 187 (2), 251-258.

[27] Jacobs, E. R., Narayanan, J., Fish, B. L., Gao, F., Harmann, L. M., Bergom, C., Tracy, G., Jennifer, S., Medhora, M. (2019) Cardiac remodeling and reversible pulmonary hypertension during pneumonitis in rats after 13-Gy partial-body irradiation with minimal bone marrow sparing: effect of lisinopril. Health physics, 116 (4), 558-565.

[28] Erel, O. (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical Biochemistry, 37 (2), 112-119.

[29] Erel, O. (2005) A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry, 38 (12), 1103-1111.

[30] Akyazi, I., Eraslan, E., Gülçubuk, A., Ekiz, E. E., Çırakli, Z. L., Haktanir, D., Bala D.A., Özkurt, M., Matur, E., Özcan, M. (2013) Long-term aspirin pretreatment in the prevention of cerulein-induced acute pancreatitis in rats. World Journal of Gastroenterology, 19 (19), 2894-2903.

[31] Ping, Z., Peng, Y., Lang, H., Xinyong, C., Zhiyi, Z., Xiaocheng, W., Hong, Z., Liang, S. (2020) Oxidative stress in radiation-induced cardiotoxicity. Oxidative Medicine and Cellular Longevity, 2020(1), 3579143.

[32] Armanious, M.A., Mohammadi, H., Khodor, S., Oliver, D.E., Johnstone, P.A., Fradley, M.G. (2018) Cardiovascular effects of radiation therapy. Current Problems in Cancer, 42 (4), 433–442.

[33] Puukila, S., Lemon, J. A., Lees, S. J., Tai, T.C., Boreham, D.R., Khaper, N. (2017) Impact of ionizing radiation on the cardiovascular system: a review. Radiation Research, 188 (4.2), 539–546.

[34] Vona, R., Gambardella, L., Cittadini, C., Straface, E., Pietraforte, D. (2019) Biomarkers of oxidative stress in metabolicsyndrome and associated diseases. Oxidative Medicine and Cellular Longevity, 1, 8267234,

[35] Karslioglu, I., Ertekin, M. V., Kocer, I., Taysi, S., Sezen, O., Gepdiremen, A., & Balci, E. (2004) Protective role of intramuscularly administered vitamin E on the levels of lipid peroxidation and the activities of antioxidant enzymes in the lens of rats made cataractous with gamma-irradiation. European Journal of Ophthalmology, 14(6), 478-485.

[36] Dilber, B., Akbulut, U. E., Alver, A., Menteşe, A., Kolaylı, C. C., & Cansu, A. (2021) Plasma and erytrocyte oxidative stress markers in children with frequent breath-holding spells. Klinische Pädiatrie, 233 (04), 173-180.

[37] Elahi, M.M., Kong, Y.X., Matata, B.M. (2009) Oxidative stress as a mediator of cardiovascular disease. Oxidative Medicine and Cellular Longevity, 2(5), 259-269.

[38] Zhang, C., Chen, H., Xie, H. H., Shu, H., Yuan, W. J., & Su, D. F. (2003) Inflammation is involved in the organ damage induced by sinoaortic denervation in rats. Journal of Hypertension, 21(11), 2141-2148.

[39] Luo, N., Zhu, W., Li, X., Fu, M., Peng, X., Yang, F., Zhang, Y., Yin, H., Yang, C., Zhao, J., Yuan, X., Hu, G. (2022) Impact of Gut Microbiota on Radiation-Associated Cognitive Dysfunction and Neuroinflammation in Mice. Radiation Research, 197 (4), 350-364.

[40] Guo, D.B., Zhu, X.Q., Li, Q.Q., Li, G.M., Ruan, G.P., Pang, R.Q., Chen, Y.H., Wang, Q., Wang, J.X., Liu, J.F., Chen, Q., Pan, X.H. (2018) Efficacy and mechanisms underlying the effects of allogeneic umbilical cord mesenchymal stem cell transplantation on acute radiation injury in tree shrews. Cytotechnology, 70 (5), 1447-1468.

[41] Brasier, A. R. (2010) The nuclear factor-κB–interleukin-6 signalling pathway mediating vascular inflammation. Cardiovascular Research, 86 (2), 211-218.

[42] Gan, J., Qian, W., Lin, S. (2018) Umbelliferone alleviates myocardial ischemia: the role of inflammation and apoptosis. Inflammation, 41, 464-473.

[43] Danborno, A.M., Monnet, P. L., Orr, A.J. (2020) TxA2 Receptor Antagonist (SQ29548) attenuates Endothelium-Independent recovery from Thromboxane A2 Contraction of Isolated Rabbit Aorta. Journal of African Association of Physiological Sciences, 8(1), 8-15.

[44] El-Benhawy, S. A., Morsi, M. I., El-Tahan, R. A., Matar, N. A., & Ehmaida, H. M. (2021) Radioprotective effect of thymoquinone in X-irradiated rats. Asian Pacific Journal of Cancer Prevention, 22(9), 3005.

[45] Sarhan, H., Naoum, L. (2020) Protective role of royal jelly against gamma radiation induced oxidative stress, cardio-toxicity and organ dysfunctions in male rats. The Egyptian Journal of Hospital Medicine, 78(1), 62-67.

[46] Hemnani, T., Parihar, M.S. (1998) Reactive oxygen species and oxidative DNA damage. Indian Journal of Physiology and Pharmacology, 42, 440-452.