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Original Article

Pre-irradiation effects of ectoine on radiation-induced cardiotoxicity in female Swiss albino mice model

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ABSTRACT

Ectoine is a compatible solute that acts as a natural protectant. *In the mice model*, a single post-irradiation ectoine dose showed protective effects by modulating both inflammatory and oxidative stress pathways. The effect of ectoine has never been tested on mice cardiac tissue, thus the current study aimed to explore the pre-irradiation effect(s) of ectoine on radiation-induced cardiotoxicity. Forty female Swiss albino mice (17.6-23.1 g); controls (injected intraperitoneally for ten days with 0.2 mL saline), ectoine groups injected with 20 mg/kg of ectoine for ten days), irradiated groups (injected intraperitoneally for ten days with 0.2 mL saline then received six Gy whole body x-irradiation single dose), ectoine irradiated groups (injected with ectoine for ten days then irradiated). Animals were sacrificed on days seven, and 14 (five animals each). Hearts were examined for histological changes and immune-stained for Bax. Ectoine concentration in hearts was measured by HPLC. Serum cardiac troponin T, Total antioxidant capacity, and apoptosis-inducing factor were evaluated by mice with ready-to-use ELISA kits. Heart histological changes were documented in 40% of the 7- & 14-days post-irradiation. Ectoine concentrations (0.63 x 10^{-4} mg/mg of heart weight) were higher in ectoine groups than ectoine irradiated groups (0.011 x 10^{-4} mg/mg) 14-days post-treatment. Serum troponin T significantly differed between the 14 days groups (p = 0.032). Apoptosis inducible factor significantly increased in ectoine irradiated group (at 14 days) than those of control (p = 0.014), irradiated (p = 0.020), and ectoine (p = 0.033) groups. Bax showed strong to moderate immunostaining in ectoine and irradiated groups. In conclusion, Ectoine has pre-irradiation partial protective effects on heart cytotoxicity.

Keywords: *Antioxidants; Apoptosis; Cardiotoxicity; Ectoine; Radiation; Troponin*.

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1. INTRODUCTION

Radiation-induced heart disease (RIHD) is a common complication of radiotherapy (RT) where heart exposure remains unavoidable **[1-3]**. Studies showed that there is an increased risk (about 4–16% per Gy of mean heart dose) for RIHDs even at low doses of radiation (> 0.5 Gy) **[3-5]**.

Although several biomarkers have been studied for their capability to reveal early heart changes after radiotherapy, there is still a lack of robust results regarding the establishment of characteristic biomarkers capable of revealing early cardiotoxicity" **[6]**. Cardiac tropomyosin (cTnT) isoform regulates actin-myosin interaction and muscle contraction in myocytes **[7, 8]**. cTnT is released into serum in proportion to the degree of myocyte membrane damage. Thus, it acts as a specific sensitive indicative biomarker of subclinical myocardial damage and RIHD **[9-11]**. cTnT is also proven as an effective biomarker in monitoring cardiac injuries in various laboratory animal models including the mouse model **[12-14]**.

RIHDs occur via several mechanisms including oxidative stress and cell death **[3-5]**. Antioxidants play an important role in preventing the formation and scavenging of free radicals and other oxidizing species **[5]**. Compatible solutes and their derivatives showed variable antioxidant capacities. Ectoine is expressed by halophile bacteria to overcome the challenges of their environments. As a compatible solute, ectoine does not interfere with the cell's metabolism even at high molar concentrations and was proven to play a role as a natural cellular protectant against the damaging effects of radiation **[15-18]**.

Although one *in vitro* study showed that ectoine protects the enzyme lactate dehydrogenase against oxidation with hydrogen peroxide **[19]**, the ectoine antioxidant effect was debated and considered very poor **[20]** until recently proven otherwise **[16, 17, 21]**. *In vitro*, ectoine prevented DNA single and double-strand breaks by electron irradiations via its scavenging and electron characteristics **[22, 23]**. Moreover, ectoine exhibited a concentration-dependent radioprotective effect against radiation-mediated oxidative damage **[24]**. *In vivo*, ectoine showed post-irradiation protective effects which are modulated via antioxidant and anti-inflammatory effects **[16, 17]**.

Oxidative/nitrative stress in RIHDs can also induce apoptosis via various mechanisms **[25]**. Apoptosis-inducing factor (AIF) is a dual-function protein working both to support normal oxidative phosphorylation and as a key apoptotic factor. Calpains and cathepsins cleave AIF in the mitochondria and affect its release during death signaling. Once released, AIF forms complexes with pro-apoptotic proteins and is delivered to the nucleus where it takes apart in DNA cleavage and chromatin condensation **[26]**. Ectoine increased the nuclear inclusions and decreased both cytoplasmic inclusion and the total amount of aggregates as a protective mechanism against polyglutamine-induced toxicity in neuro2a cells **[26]**. It also prevented the antiapoptotic reactions and reduced the life span of lung infiltrating neutrophils **[27]**. Recently, ectoine significantly reduced the rate of apoptosis in hypoxia-treated retinal ganglion cells (RGCs) **[28]**.

Altogether, the current study was designed to investigate the possible protective effect(s) of pretreatment with ectoine on the heart of x-irradiated female mice.

2. Material and methods

2.1. Animals and treatment

The study included 40 female virgin Swiss albino mice (17.6–23.1 g) as the experimental animal model. The mouse model represents the model of choice to investigate the RIHDs due to its multiple characteristics and experience in its use **[4]**. Breast cancer (BC) affects mainly females, and radiotherapy (RT) improves its prognosis. However, BC-RT irradiation increases the risk for RIHDs. Therefore, a female animal model is selected to imitate the clinical situation (from a gender perspective) for further applications in clinical settings. Mice were obtained from the animal house of the Institute of

Graduate Studies and Research (IGSR), Alexandria University, Alexandria, Egypt, and kept under observation for one week before study with food and water *ad libitum*. All procedures were performed in accordance with ethical rules approved (ALEXU-IACUC Ethical approval number AU-01219073024) by the ethics committee of the IGSR for medical research on small animals and the study is reported following ARRIVE guidelines (https://arriveguidelines.org). Animals were randomly divided into four groups as follows:

Control groups (10 animals): animals received intraperitoneal injection(s) of 0.2 mL saline (one dose/day). Five animals were sacrificed on day 7, and the other 5 on day14.

Ectoine groups (10 animals): animals received intraperitoneal fractionated doses of ectoine (Biotop, Germany; the injected ectoine is dissolved in saline; 20 mg/kg/day for 10 days) and animals were then sacrificed on days 7, and 14 after the end of the treatment period (five animals at each time point).

Irradiated groups (10 animals): animals received an intraperitoneal injection(s) of 0.2 mL saline (one dose/day) then a single whole-body x-ray dose of 6 Gy with a dose rate of 200 cGy/ min and using the 3D conformal RT technique and sacrificed on day 7, and 14 after irradiation (five animals at each time point) to assess the preclinical short-term effects. Animal x-ray irradiations were performed using a linear accelerator (PRIMUS, SIEMENS; 6 MV). The source-to-target distance was set to 100 cm and the radiation field was 40 cm x 40 cm and a wax block (one cm thick) was used for tissue compensation. Animals were sedated during irradiation using an inhalation anesthetic (diethyl ether).

Ectoine irradiated groups (10 animals): animals received an intraperitoneal fractionated dose of ectoine (20 mg/kg for 10 days) then a single whole-body x-ray dose of 6 Gy and animals were sacrificed on days 7, and 14 after irradiation (five animals at each time point).

At the designated time points the animals were sacrificed by cervical dislocation (without prior anesthesia). Immediately after scarification, blood samples were collected from the descending Aorta through a laparotomy incision and serum was separated. Hearts were removed quickly, washed with ice-cold saline, properly labeled, and divided into parts for further investigations.

2.2. Histological investigations

Histopathological examination and Bax immunohistochemical analysis were performed and scored according to Abdel-Daim and his co-authors **[29]**.

2.3. Determination of ectoine concentration in heart homogenate by HPLC

Heart tissues were pooled and homogenized in saline using tissue laser LT (QIAGEN Hilden, Germany) and stainless-steel carbide beads (QIAGEN Hilden, Germany). The disruption was carried out in high-speed (50 Hz) shaking steps for 10 minutes followed by centrifugation at 10,000 rpm for 15 min. Pellets of cellular debris were discarded, and the supernatant was used to detect ectoine using HPLC according to Brands *et al*., **[21]**. Briefly, HPLC was applied using an Agilent Eclipse XBD-C18 (150 × 4.6 mm, 5 μm) column which is manufactured by Agilent Technologies (Polo Alto, CA, USA) to detect ectoine concentration in filtered tissue lysates. The HPLC system (Agilent 1200 series chromatographic system; Polo Alto, CA, USA), was equipped with a diode array detector (DAD), and a UV detector set at 210 nm. Measurements were performed in 80% (v/v) acetonitrile as a mobile phase with a flow rate of one mL/min at room temperature and the injection volume was

20 µL. Standards of different concentrations of ectoine dissolved in saline were used to establish a standard curve. This step was done to assure the ectoine internalization and to examine its possible accumulation.

2.4. Determination of cTnT, T-AOC, and AIF in serum by ELISA

Mouse ELISA ready-to-use kits (Shanghai Coon Koo Biotech Co., Ltd, China) were used to detect; mouse cardiac troponin T (cTnT; CK-bio-15841; as a marker of cardiotoxicity), mouse total antioxidant capacity (T-AOC; CK-bio-20382; as a marker of oxidative stress), and mouse apoptosis-inducing factor (AIF; CK-bio-15722; as a marker of apoptosis and mitochondrial bioenergetics).

2.5. Data and statistical analysis

Statistical analyses were performed using IBM SPSS statistics for Windows, version 20 (Armonk, NY: IBM Corp. 2011). Data are presented as mean ± standard deviation (Mean±SD). Student t-test was used for comparing between 7-Days and 14-Days groups. ANOVA test was used for comparing groups within the same time interval. Pairwise comparison between every two groups was done using Post Hoc Test (Tukey). The significance was judged at the 5% level.

3. Results

3.1. Death rate and body weight

Death rate ranged from 20% (in ectoine and ectoine irradiated groups 7 days and 14 days post-treatment groups: respectively) to 40% (in irradiated 14 days post-treatment group). Both controls (p = 0.010) and ectoine (p = 0.003) groups had significant increase in 14-days post-treatment body weight **(Table 1)**.

Table 1. Body weights of the studied groups before and after treatments

Groups	Body weight (g) M ± SD				p_1 (7 vs. 14)	
	7 Days		14 Days			
	Before	After	Before	After	Before	After
Controls	20.46±1.76	24.68±5.86	21.30±1.03	25.74^{a}±2.54	0.383	0.72
Irradiated	21.04±0.48	20.56±0.60	21.66±1.20	20.63^{b}±1.69	0.331	0.93
Ectoine	18.84±1.05	20.68±0.75	20.28±1.69	24.64^{ab}±0.75	0.144	<0.001*
Ectoine irradiated	20.22±2.30	19.56±3.11	20.40±0.51	21.43^{ab} ±3.44	0.872	0.421
^{F}p	0.189	0.139	0.222	0.021*		

p_1: p-value for Student t-test for comparing between 7 Days and 14 Days; ^{F}p: p-value for ANOVA test for comparing between Groups; Pairwise comparison between every 2 groups, was done using Post Hoc Test (Tukey); Means with Common letters (a,b,….) are not significant (i.e. Means with Different letters are significant); *: Statistically significant at $p \leq 0.05$.

3.2. Histological investigations

H&E staining of heart tissues revealed normal histological architecture, with no identifiable degenerative, necrotic, or apoptotic cells in controls **(Fig. 1)**. Histological alterations were detected in ectoine, irradiated, and ectoine irradiated groups (for both 7- and 14-days groups) **(Fig. 1)**. Ectoine treated groups showed dilated congested blood vessels, separation of muscle fibers with interstitial edema, cytoplasmic vacuolization, focal hyaline degeneration, and focal degenerative changes of muscle fibers. Ectoine irradiated groups showed mainly stromal focal edema and lymphocytic infiltration. The cardiotoxic effects in irradiated groups were less severe in the 14-days than 7-days postx-ray irradiated group **(Fig. 1)**. Heart muscle fibers showed separation, degeneration, fragmentation, cytoplasmic vacuolization, scattered pyknotic nuclei, interstitial edema and mild lymphocytic infiltration in 40% of the 7-days post-x-ray (6 Gy) whole body irradiated group **(Fig. 1)**. Dilated congested blood vessels within the stroma, hemorrhage, extravasated RBCs of myocardial tissue were detected as well. Similarly, heart muscle fibers' separation, cytoplasmic vacuolization, focal lymphocytic infiltration, and vacuolated endothelial cells were detected in 40% of the 14-day post-x-ray (6 Gy) whole body irradiated group **(Fig. 1)**.

Bax-immunostaining showed weak staining in controls **(Fig. 2)**. Both Irradiation and ectoine enhanced apoptosis as indicated by moderate/strong heterogenic staining. Apoptosis normalized to weak staining only in ectoine groups 14-days post-treatment **(Fig. 2)**. Ectoine irradiated group had normal apoptosis as indicated by negative/weak heterogenic staining (7-days) and weak staining (14-days) post-treatment **(Fig. 2)**.

3.3. HPLC

Ectoine accumulated and was still detectable in heart tissues after 14 days post-treatment. Ectoine concentrations (0.63×10^{-4} mg/mg of heart weight) were higher in ectoine groups than ectoine irradiated groups (0.011×10^{-4} mg/mg of heart weight) 14-days post treatment which might indicate destructive or enhanced radiation-induced effect on ectoine clearance.

3.4. Serology

3.4.1. Troponin

A statistically significant difference was observed in troponin (cTnT) between all groups 14 days post-treatment **(Table 2)**.

Table 2. The effect of ectoine on serum Cardiac Troponin- T (cTnT) in the model of irradiation-induced cardiotoxicity in female albino mice

Groups	7 Days	14 Days	t	p_1
	Mean ± SD (ng/mL)			
Controls	33.4 [a] ± 11.1	25.4 [b] ± 4	1.526	0.171
Irradiated	58.1 [a] ± 62.1	80.2 [ab] ± 52.4	0.511	0.628
Ectoine	27.7 [a] ± 5.17	30.3 [b] ± 10.1	0.465	0.656
Ectoine irradiated	37.2 [a] ± 21	91.8 [a] ± 57.3	1.999	0.086
F (p)	0.650 **(**0.596**)**	4.005 **(**0.032***)**		

p_1: p-value for **Student t-test** for comparing between 7 Days and 14 Days; F: F for ANOVA test, pairwise comparison bet. every 2 groups were done using Post Hoc Test (LSD); p: p-value for comparing between the different studied groups (Means with Common letters are not significant (i.e. Means with Different letters are significant))

*: Statistically significant at $p \leq 0.05$

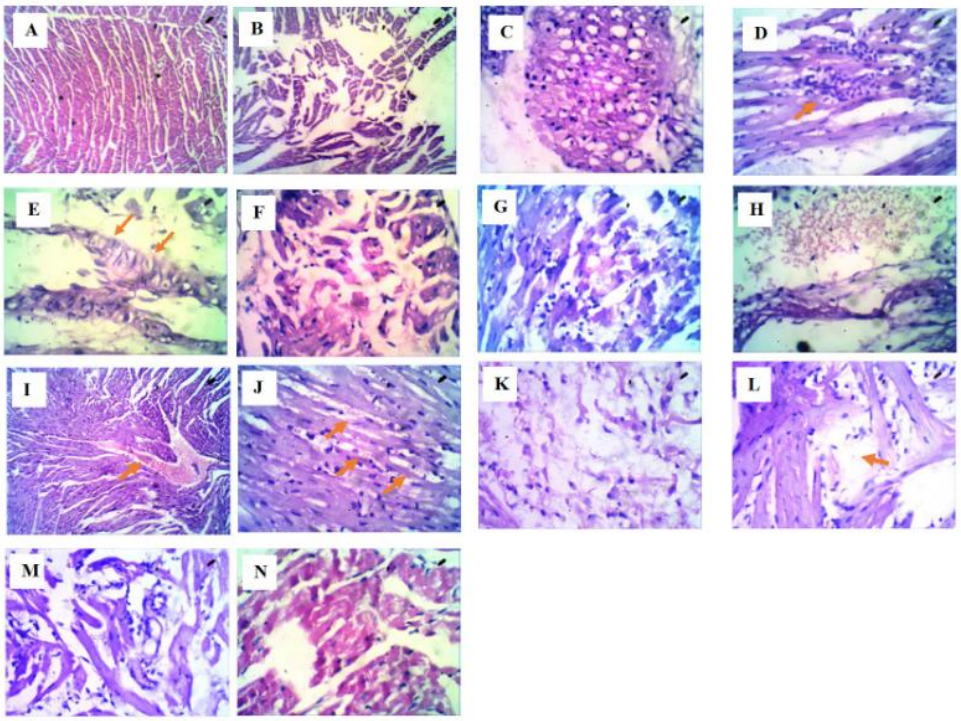

Fig. 1. Histological examinations of heart tissues. A: Control 7 days (normal myocardial smooth muscle fibers); B: Irradiated 7 days (separation of muscle fibers); C: Muscle fibers showing cytoplasmic vacuolization; D: The stroma shows focal lymphocytic infiltrate (arrow); E: Thick walled blood vessels showing vacuolated endothelial cells (arrows); F: Atrophic muscle fibers; G: Muscle fibers showing nuclear internalization; H: Dilated congested blood vessels within the stroma; I: Myocardial tissue showing the area of hemorrhage (arrow); J: Myocardial tissue showing extravasated RBCs (arrows); K: Ectoine plus irradiation 7 days (the stroma shows focal edema and lymphocytic infiltrate); L: Ectoine 14 days (focal interstitial edema (arrow); M: Ectoine 14 days (mild lymphocytic infiltrate indicated by the arrow); N: Ectoine 7 days (muscle fibers showing focal hyaline degeneration indicated by the arrow). (H&EX100-400).

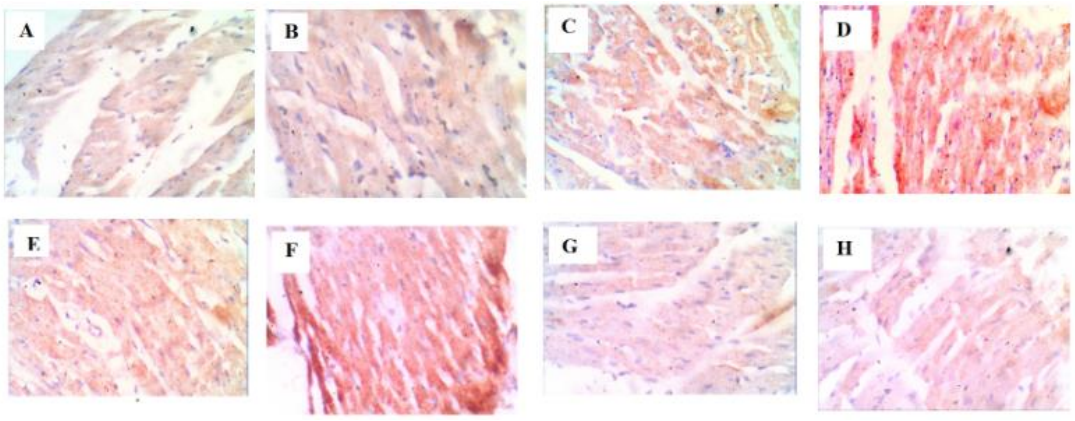

Fig. 2. Immunohistochemical cytoplasmic staining of Bax. A: Control (7 days)/weak staining; B: Control (14 days)/weak staining; C: Irradiated (14 days)/moderate staining; D: Irradiated (14 days)/strong staining; E: Ectoine treated (7 days)/moderate staining; F: Ectoine treated (14 days)/strong staining; G: Ectoine plus irradiation (7 days) showing weak cytoplasmic staining. H: Ectoine plus irradiation (14 days) showing weak cytoplasmic staining (IHCX400).

Fig. 3. Body weights of the studied groups before and after treatments.

p: p-value for Paired t-test for comparing between Before and After

3.4.2. T. AOC

T. AOC significantly increased (p = 0.007) in all groups as compared to controls after 7 days x-ray irradiation **(Table 3)**; controls (7-days) showed weight independent significant decrease in T.AOC than irradiated (p = 0.009), ectoine (p = 0.005), and ectoine plus irradiated (p = 0.002) groups. This transient increase was absent at 14 days post-treatment. However, there was a significant increase in the T. AOC of the control group of 14 days than that of 7 days (p = 0.001) post-treatment.

3.4.3. AIF

A significant increase in AIF was observed at 14 days post-treatment in the ectoine irradiated group only **(Table 4)**.

Table 3. The effect of ectoine on serum Total Antioxidant Capacity (T. AOC) in the model of irradiation-induced cardiotoxicity in female albino mice

Groups	7 Days	14 Days	t	p_1
	Mean ± SD (U/mL)			
Controls	84.17^{b} ± 19.52	147.62^{a} ± 12.53	5.603	0.001^{*}
Irradiated	125.7^{a} ± 32.71	129.8^{a} ± 7.03	0.273	0.797
Ectoine	132.8^{a} ± 11.24	147.56^{a} ± 15.17	1.612	0.151
Ectoine irradiated	136.0^{a} ± 16.36	140.5^{a} ± 29.53	0.290	0.780
F (p)	5.862 (0.007*)	0.711 (0.564)		

p_1: p-value for Student t-test for comparing between 7 Days and 14 Days; F: F for ANOVA test, pairwise comparison between every 2 groups were done using Post Hoc Test (LSD); p: p-value for comparing between the different studied groups (Means with Common letters are not significant (i.e. Means with Different letters are significant))
*: Statistically significant at $p \leq 0.05$

Table 4. The effect of ectoine on serum Apoptosis Inducing factor (AIF) in the model of irradiation-induced cardiotoxicity in female albino mice

Groups	M ± SD (pg/mL)		t	p_1 (7 vs. 14)
	7 Days	14 Days		
Controls	760.5^a ± 57.96	723.5^b ± 48.89	1.017	0.343
Irradiated	771.4^a ± 56.37	716.2^b ± 43.60	1.442	0.199
Ectoine	776.0^a ± 54.47	846.4^b ± 194.3	0.773	0.476
Ectoine irradiated	711.2^a ± 27.99	1345.3^a ± 570.2	2.222	0.112
F (p)	1.681 (0.214)	3.621 (0.045*)		

p_1: p-value for Student t-test for comparing between 7 Days and 14 Days; F: F for ANOVA test, pairwise comparison between every 2 groups were done using Post Hoc Test (LSD); p: p-value for comparing between the different studied groups (Means with Common letters are not significant (i.e. Means with Different letters are significant))
*: Statistically significant at $p \leq 0.05$

4. Discussion

Results show for the first time the cardiotoxic effects of ectoine and ectoine plus x-rays irradiation (as documented by histopathological changes) in female mice animal models. Tachycardia in *Daphnia magna* was documented previously at low concentrations of ectoine (2.5 & 4 mg/L) while higher concentrations of 20 & 25 mg/L caused bradycardia and decreased survival rate **[30]**. Ectoine toxicity at 50 mg/L (lethal dose) was not mediated by catalase or nitric oxide **[30]**. Recent studies showed that ectoine alleviated the slow heart rate induced by ethanol or DMSO treatment in the *Daphnia magna* model and each stressor showed a different antioxidative-dependency effect **[31, 32]**. A link between tachycardia and induced cardiomyopathy was reported in animal models and humans **[33, 34]**. Since ectoine is present in nasal and eye drops used by athletics for various cardiac demands **[35, 36]** and many sudden deaths occur among them for unknown reasons, it is highly recommended to further investigate the dose range and time course of the ectoine-induced cardiotoxic effect(s) on various parts of their hearts.

The question that arises now is "why there was no severe combined effect in the irradiated group who received ectoine before irradiation if ectoine by itself causes ischemic action(s) on the heart? Our HPLC detection of ectoine showed less accumulation of ectoine after irradiation. Radiation generates hydroxyl free radicals via indirect interactions (ref), which cleaves ectoine into two products and leads to further oxidation at C-terminus *in vitro* **[21]**. This interaction would explain why accumulated ectoine exhibited a protective effect via scavenging the hydroxyl free radicals after irradiation instead of allowing synergetic damaging cardiotoxic effects. This assumption was further supported by our Bax-immunostaining results which inferred ischemic action on heart tissues which was elicited by irradiation or ectoine treatments and aborted by ectoine pretreatment of irradiated groups (showed by negative to weak Bax-staining).

Although significant variation between groups was detected 14 days after treatments, serum troponin did not reflect any damaging cardiac effect as it was expected. Troponin seems to be a problematic biomarker **[37]**. It is known now that troponin has both cytosolic and structural distributions and after injury, its release into the blood exhibits a biphasic pattern with a short clearance of two hours and prolonged

clearance of five to seven days **[38]**. Also, ischemia and reperfusion degrade troponin into many fragments that appear in serum with no structural damage. Troponin > 30 ng/L is suggested to indicate demand ischemia in the context of other serious pathologies" **[39]**. cTnT ≥ 0.01 mg/L showed to be associated with many factors including body mass index (BMI) and arrhythmia in the general population **[40]**. Also, an increase in the body weight in both controls and ectoine 14-days post-treatment was observed. Moreover, apoptosis is another factor that affects the appearance of troponin in serum. Thus, the appearance of troponin in our present study would be related to apoptosis as well. This is supported by the fact that a significant increase in AIF was observed at 14 days post-treatment in the ectoine irradiated group than in other groups.

The low total antioxidant capacity detected in the controls (after 7 days of x-ray irradiation) could indicate oxidative stress or increased susceptibility to oxidative damage in that group.

Conclusion

In conclusion, ectoine has pre-irradiation partial protective effects on the heart cytotoxicity and this effect may be due to modulation of apoptosis while x-ray irradiation alone had cardiotoxic effects.

Further studies should be performed to investigate:

a- The dose range and time course of ectoine cardiotoxicity on other (small and larger) animal models as well as athletes who are using ectoine-containing drugs and supplements.

b- Pharmacokinetics and pharmacodynamics of ectoine on various systems.

c- Whether the radio-modification effect of ectoine is tissue-specific or concentration-dependent.

d- Possible ectoine toxicities on other major organs.

e- Pre-irradiation versus post-irradiation effects of ectoine on the heart and other systems.

List of abbreviations

RIHD, Radiation-induced heart disease; RT, Radiotherapy; cTnT, Serum cardiac troponin T; AIF, Apoptosis-inducing factor; RGCs, Retinal ganglion cells; IGSR, Institute of Graduate Studies and Research; T-AOC, Total antioxidant capacity; SD, Standard deviation; BMI, Body mass index;

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with ethical rules approved by the ethics committee of the Institute of Graduate Studies and Research (IGSR; Alexandria University, Egypt) for medical research on small animals and the study is reported following ARRIVE guidelines (https://arriveguidelines.org).

Consent to participate: not applicable

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Availability of data and materials: All data generated or analyzed during this study are included in this published article.

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Author contributions

SN, BH, SS, SL, and MK: Laboratory investigations and interpretation of data.

EE & EE: Conception, laboratory investigations, interpretation of data, preparation of the

manuscript, and revision for important intellectual content

"All authors have read and approved the manuscript"

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5. REFERENCES

1. Marlière, S., Vautrin, E., Saunier, C., Chaikh, A. and Gabelle-Flandin, I. Radiation-related heart toxicity: Update in women. In Annales de Cardiologie et D'angeiologie; 2016; 65(6), pp. 411-419.

2. Kammerer, E., Le Guévelou, J., Jacob, S., Geffrelot, J., Danhier, S., Saloux, E., Sichel, F., Laurent, C., Levy, C. and Thariat, J. Cardiac complications of breast radiation therapy. Bulletin du cancer 2019; 106(4), pp.379-388.

3. Livingston, K., Schlaak, R.A., Puckett, L.L. and Bergom, C. The role of mitochondrial dysfunction in radiation-induced heart disease: from bench to bedside. Frontiers in cardiovascular medicine 2020; 7, p.20.

4. Baselet, B., Rombouts, C., Benotmane, A.M., Baatout, S. and Aerts, A. Cardiovascular diseases related to ionizing radiation: the risk of low-dose exposure. International Journal of Molecular Medicine 2016; 38(6), pp.1623-1641.

5. Wang, H., Wei, J., Zheng, Q., Meng, L., Xin, Y., Yin, X. and Jiang, X. Radiation-induced heart disease: a review of classification, mechanism, and prevention. International journal of biological sciences 2019; 15(10), p.2128.

6. Gkantaifi, A., Papadopoulos, C., Spyropoulou, D., Toumpourleka, M., Iliadis, G., Kardamakis, D., Nikolaou, M., Tsoukalas, N., Kyrgias, G. and Tolia, M. Breast radiotherapy and early adverse cardiac effects. The role of serum biomarkers and strain echocardiography. Anticancer Research 2019; 39(4), pp.1667-1673.

7. Zot, A.S. and Potter, J.D. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. Annual review of biophysics and biophysical chemistry 1987; 16(1), pp.535-559.

8. Marques, M.D.A. and de Oliveira, G.A. Cardiac troponin and tropomyosin: structural and cellular perspectives to unveil the hypertrophic cardiomyopathy phenotype. Frontiers in physiology 2016; 7, p.429.

9. Sarko, J. and Pollack Jr, C.V. Cardiac troponins. The Journal of emergency medicine 2002; 23(1), pp.57-65.

10. Gomes, A.V., Barnes, J.A., Harada, K. and Potter, J.D. Role of troponin T in disease. Molecular and cellular biochemistry 2004; 263(1), pp.115-129.

11. Klee, N.S., McCarthy, C.G., Martinez-Quinones, P., and Webb, R.C. Out of the frying pan and into the fire: damage-associated molecular patterns and cardiovascular toxicity following cancer therapy. Therapeutic advances in cardiovascular disease 2017; 11(11), pp.297-317.

12. O'Brien, P.J., Dameron, G.W., Beck, M.L., Kang, Y.J., Erickson, B.K., Di Battista, T.H., Miller, K.E., Jackson, K.N. and Mittelstadt, S. Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. Comparative Medicine 1997; 47(5), pp.486-495.

13. Aartsen, W.M., Pelsers, M.M.A.L., Hermens, W.T., Glatz, J.F.C., Daemen, M.J.A.P. and Smits, J.F.M. Heart fatty acid binding protein and cardiac troponin T plasma concentrations as markers for myocardial infarction after coronary artery ligation in mice. Pflügers Archiv 2000; 439(4), pp.416-422.

14. Metzler, B., Hammerer-Lercher, A., Jehle, J., Dietrich, H., Pachinger, O., Xu, Q. and Mair, J. Plasma cardiac troponin T closely correlates with infarct size in a mouse model of acute

myocardial infarction. Clinica chimica acta 2002; 325(1-2), pp.87-90.

15. Buenger, J. and Driller, H. Ectoin: an effective natural substance to prevent UVA-induced premature photoaging. Skin pharmacology and physiology 2004; 17(5), pp.232-237.

16. Sarhan, D.A., Shehata, R.R., Kandil, Y.I., Kotb, M.A. and El-Bassiouni, E.A. Influence of whole body x-irradiation on the liver and possible protective effect by the compatible solute ectoine in a mouse model. Eur J Biophysics 2016; 4(3), pp.16-21.

17. Refaat, R., Sarhan, D., Kotb, M., El-Abd, E. and El-Bassiouni, E. Post-irradiation protective effects of ectoine on brain and testicles in male mice. Pharmacological Reports 2018; 70(2), pp.304-308.

18. Rieckmann, T., Gatzemeier, F., Christiansen, S., Rothkamm, K., & Münscher, A. The inflammation-reducing compatible solute ectoine does not impair the cytotoxic effect of ionizing radiation on head and neck cancer cells. Scientific Reports 2019; 9(1), 1–8. https://doi.org/10.1038/s41598-019-43040-w

19. Andersson, M.M., Breccia, J.D. and Hatti-Kaul, R. Stabilizing effect of chemical additives against oxidation of lactate dehydrogenase. Biotechnology and applied biochemistry 2000; 32(3), pp.145-153.

20. Cornacchione, S., Neveu, M., Talbourdet, S., Lazou, K., Viron, C., Renimel, I., de Queral, D., Kurfurst, R., Schnebert, S. and Heusele, C. In vivo skin antioxidant effect of a new combination based on a specific Vitis vinifera shoot extract and a biotechnological extract. Journal of drugs in dermatology 2007; 6(6), p.s8.

21. Brands, S., Schein, P., Castro-Ochoa, K.F. and Galinski, E.A. Hydroxyl radical scavenging of the compatible solute ectoine generates two N-actinides. Archives of biochemistry and biophysics 2019; 674, p.108097.

22. Hahn, M.B., Meyer, S., Schröter, M.A., Kunte, H.J., Solomun, T. and Sturm, H. DNA protection by ectoine from ionizing radiation: molecular mechanisms. Physical Chemistry Chemical Physics 2017; 19(37), pp.25717-25722.

23. Schröter, M.A., Meyer, S., Hahn, M.B., Solomun, T., Sturm, H. and Kunte, H.J. Ectoine protects DNA from damage by ionizing radiation. Scientific reports 2017; 7(1), pp.1-7.

24. Sajjad, W., Qadir, S., Ahmad, M., Rafiq, M., Hasan, F., Tehan, R., McPhail, K.L. and Shah, A.A. Ectoine: a compatible solute in radio-halophilic Stenotrophomonas sp. WMA-LM 19 strain to prevent ultraviolet-induced protein damage. Journal of applied microbiology 2018; 125(2), pp.457-467.

25. Sárközy, M., Varga, Z., Gáspár, R., Szűcs, G., Kovács, M.G., Kovács, Z.Z., Dux, L., Kahán, Z. and Csont, T. Pathomechanisms and therapeutic opportunities in radiation-induced heart disease: from bench to bedside. Clinical Research in Cardiology 2021; pp.1-25.

26. Furusho, K., Yoshizawa, T. and Shoji, S. Ectoine alters subcellular localization of inclusions and reduces apoptotic cell death induced by the truncated Machado–Joseph disease gene product with an expanded polyglutamine stretch. Neurobiology of disease 2005; 20(1), pp.170-178.

27. Sydlik, U., Peuschel, H., Paunel-Görgülü, A., Keymel, S., Krämer, U., Weissenberg, A., Kroker, M., Seghrouchni, S., Heiss, C., Windolf, J. and Bilstein, A. Recovery of neutrophil apoptosis by ectoine: a new strategy against lung inflammation. European Respiratory Journal 2013; 41(2), pp.433-442.

28. Tsai, T., Mueller-Buehl, A.M., Satgunarajah, Y., Kuehn, S., Dick, H.B. and Joachim, S.C. Protective effect of the extremolytes ectoine and hydroxyectoine in porcine organ culture. Graefe's Archive for Clinical and

Experimental Ophthalmology 2020; 258(10), pp.2185-2203.

29. Abdel-Daim, M.M., Eltaysh, R., Hassan, A. and Mousa, S.A. Lycopene attenuates tulathromycin and diclofenac sodium-induced cardiotoxicity in mice. International journal of molecular sciences 2018; 19(2), p.344.

30. Bownik, A., Stępniewska, Z. and Skowroński, T. Effects of ectoine on behavioural, physiological and biochemical parameters of Daphnia magna. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2015; 168, pp.2-10.

31. Bownik, A., Ślaska, B. and Szabelak, A. Protective effects of compatible solute ectoine against ethanol-induced toxic alterations in Daphnia magna. Journal of Comparative Physiology B 2018; 188(5), pp.779-791.

32. Bownik, A. Effects of ectoine on behavioral, physiological and biochemical parameters of Daphnia magna exposed to dimethyl sulfoxide. Science of The Total Environment 2019; 683, pp.193-201.

33. Shinbane, J.S., Wood, M.A., Jensen, D.N., Ellenbogen, K.A., Fitzpatrick, A.P. and Scheinman, M.M. Tachycardia-induced cardiomyopathy: a review of animal models and clinical studies. Journal of the American College of Cardiology 1997; 29(4), pp.709-715.

34. Kim, S.H. and Ryu, K.H. Tachycardia induced cardiomyopathy. Korean circulation journal 2019; 49(9), pp.808-817.

35. Al-Khelaifi, F., Diboun, I., Donati, F., Botrè, F., Alsayrafi, M., Georgakopoulos, C., Yousri, N.A., Suhre, K. and Elrayess, M.A. Metabolomics profiling of xenobiotics in elite athletes: relevance to supplement consumption. Journal of the International Society of Sports Nutrition 2018; 15(1), pp.1-10.

36. Al-Khelaifi, F., Donati, F., Botrè, F., Latiff, A., Abraham, D., Hingorani, A., Georgakopoulos, C., Suhre, K., Yousri, N.A. and Elrayess, M.A. Metabolic profiling of elite athletes with different cardiovascular demand. Scandinavian journal of medicine & science in sports 2019; 29(7), pp.933-943.

37. Tranbas, C. The problem(s) of troponin. Pathology 2021; 53 (S1), pp.S4-S5.

38. Wu, A.H. Release of cardiac troponin from healthy and damaged myocardium. Frontiers in laboratory medicine 2017; 1(3), pp.144-150.

39. Martín-Rodríguez, F., Sanz-García, A., Castro-Portillo, E., Delgado-Benito, J.F., del Pozo Vegas, C., Ortega Rabbione, G., Martín-Herrero, F., Martín-Conty, J.L. and López-Izquierdo, R. Prehospital troponin as a predictor of early clinical deterioration. European Journal of Clinical Investigation 2021; p.e13591.

40. Wallace, T.W., Abdullah, S.M., Drazner, M.H., Das, S.R., Khera, A., McGuire, D.K., Wians, F., Sabatine, M.S., Morrow, D.A. and de Lemos, J.A. Prevalence and determinants of troponin T elevation in the general population. Circulation 2006; 113(16), pp.1958-1965.