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Effects of 2100 MHz radio frequency radiation on the viscosity of blood and oxidative stress parameters in hypertensive and normal rats

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► ***Original article***

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ABSTRACT

Background*:** With the development of technology, human beings are increasingly under the exposure of Radio Frequency Radiation (RFR) (due to wireless technologies, mobile phones, base stations etc). The aim was to investigate the possible effects of Radio Frequency Radiation (RFR) on blood, aorta and heart tissue in hypertensive and non-hypertensive rats with various parameters. ***Materials and Methods*:** Male Wistar Albino rats were divided into 4 groups: 1.Control (C), 2.Hypertension (H), 3.RFR, 4.RFR+Hypertension (RFR+H). 60 mg/kg L-Nitroarginine Methyl Ester (L-NAME) was administered for 1 month by oral gavage to induce hypertension. The rats were exposed to 2100 MHz RFR for 60 minutes/day, 5 days/ week for 8 weeks. Blood hematocrit, blood and plasma viscosity, malondialdehyde (MDA), total nitric oxide (NOX), and Total sulphydril groups (RSH) / glutathione (GSH) levels in plasma and heart tissue were determined. Histological examination of the aortic wall and left ventricular muscle was performed. ***Results: RFR exposure resulted in a marked increase in cardiac and plasma MDA and NOX levels and a decrease in RSH/GSH levels ($p<0.05$). Blood viscosity increased in the hypertensive group with RFR exposure ($p<0.05$). There was an increase in left ventricular weight and number of muscle cells in hypertensive groups ($p<0.05$). ***Conclusion:*** According Our study suggests that exposure to RFR causes more negative effects on hypertension in terms of oxidative stress and antioxidants in the heart and plasma. Also, RFR exposure showed more prominent left ventricular fibrosis and aortic degenerative effects in hypertensive groups.

Keywords: *Natural Hypertension, radiation, oxidative stress, viscosity, rats.*

INTRODUCTION

Hypertension is a major risk factor for cardiovascular diseases [1] and defined as a persistent elevation of systolic blood pressure of 140 mmHg or greater and/or diastolic blood pressure of 90 mmHg or greater [2]. Oxidative stress is important pathogenesis of essential hypertension and leads to arterial damage [3, 4]. This was proved by an elevation of lipid peroxidation in hypertensive patients [3]. The increased oxidative stress will reduce the bioavailability of NOx, a potent vasodilator [4] and this reduction contributes to the development of hypertension. Radio-frequency radiation (RFR) sources, which we are more intensely exposed to with the increase in the use of mobile phones and base stations, are also used in medical systems such as diathermy and MRI units for treatment purposes.

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Oxidants can be generated in numerous exogenous ways such as chemicals and radiation exposure through redox-catalysis and encompass reactive oxygen species (ROS), reactive nitrogen species (RNS), sulfur-centered radicals and various others [5]. MDA, the end product of the lipid peroxidation is a highly reactive three carbon dialdehyde and one of the most intensively aldehydes formed during lipid per-oxidation. RNS, such as NO, a free radical and peroxynitrite (ONOO−) that is an extremely potent cellular oxidant produced with the reaction of NO with superoxide (O2•−) act together with other ROS to damage cells, causing nitrosative stress [6].

GSH is known to function as an important antioxidant in the detoxification of xenobiotics, carcinogens, free radicals and maintenance of immune functions as a nonprotein thiol [7]. By this way, GSH also acts as a cosubstrate in the enzymatic repair of radiation damage [8]. Considerable evidence points to the fact that intracellular non-protein sulphydryl compound play an important role in the cellular response to ionizing radiation, and the alterations in blood glutathione levels were also suggested to be used as an index of tumor response to therapy [7, 9].

The viscosity of blood is one of the important factors in blood pressure. Increased blood viscosity rises blood pressure [10-12].

Blood is a complex fluid whose flow properties are significantly affected by the arrangement, orientation, and deformability of red blood cells. Blood is a liquid that consists of plasma and particles, such as the red blood cells. The viscosity of blood thus depends on the viscosity of the plasma, in combination with the hematocrit [13].

Mobile phone electromagnetic radiation may cause a conformational change on hemoglobin structure [13] and this results in deformation of the erythrocytes.

There are four parameters affecting blood viscosity. These are hematocrit level, erythrocyte deformability, erythrocyte aggregation and plasma viscosity. The increase in hematocrit increases blood oxygen transport capacity and oxygen transport to tissues. On the other hand, increasing the blood viscosity of the hematocrit may impair tissue perfusion by increasing resistance to flow. For this reason, hematocrit value is important for optimal oxygen transport to tissues [10]. With increasing blood viscosity, the heart needs to generate greater pressure for pumping blood. Studies have also shown that high blood viscosity causes atherosclerosis. Increase in blood viscosity has negative effects on both heart and vein [11-12].

In some studies, exposure to radiation has been demonstrated to increase oxidative stress in tissues and decrease antioxidant capacity. However, studies that research into the effects of exposure to radiation in hypertensives are inadequate.

Our objective in this study is to explore whether hypertensive rats are more negatively affected by exposure to radiation compared to non-hypertensive rats. Therefore this study was designed to investigate the effects of 2100 MHz-modulated RFR in hypertensive and non-hypertensive rats and determine the relationship between the oxidative mechanisms based on the MDA, GSH/RSH and NOx levels. We also evaluated histological examinations of the heart and measured plasma viscosity, blood viscosity and hematocrit levels to determine effects of RFR in hypertensive and non-hypertensive rats.

MATERIALS AND METHODS

Experimental study design

Twenty-four male Wistar Albino rats (250±20 g) were divided into four experimental groups with 6 animals in each group. 1. Control (C): Tap water was given 1 ml / day by gavage for 1 month. 2. Hypertension (H): 60 mg/kg L-NAME dissolved in 1 ml of tap water for was administered for 1 month by oral gavage. Rats with blood pressure greater than 140/90 mmHg were considered hypertensive [14]. 3. RFR: This group was exposed to 2100 MHz RFR for 60 minutes/day, 5 days/week for 8 weeks. 4. RFR+Hypertension (RFR+H): Animals received 60 mg/kg L-NAME dissolved in 1 ml of tap water for 1 month via oral gavage and exposed to 2100

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MHz RFR for 60 minutes/day, 5 days/week for 8 weeks. Systolic and diastolic blood pressure was measured weekly by non-invasive indirect blood pressure system. The rats were kept in polyethylene cages in a well-ventilated room at 27 °C with a 12:12-hour light-dark cycle and had free access to water and food throughout the study. After 3 months all rats were sacrificed by taking blood from their hearts under anesthesia with intramuscular Rompun (5mg/kg) + Ketamine (45mg/kg). All animal management and handling procedures, in addition to the study design, were approved by the university ethical and research committee (Approval No: G.Ü.ET-16.006).

Exposure system

2100 MHz RF radiation was measured with an EMR 300 *(Narda, Germany)* with an electric field probe type 8.3. For RF fields; the root mean square value of electric field (ERMS) was found to be 17.25 V/m. RF environmental background level was around 0.21 V/m. SAR was calculated with the following equation: SAR = σ/ρ[ERMS 2] [W/kg] where [15] ERMS is the root mean square value of the electric field (V/m), σ is the mean electrical conductivity of the tissues in Siemens/meter (S/m) and ρ is the mass density (kg/m^3) [16]. Conductivity (0.87 S/m) and mass density (1105 kg/m^3) were derived for the equivalent tissue by using dielectric properties and mass densities of these tissues. RF exposure in the experiment resulted in a whole-body average SAR of 0.23 W/kg with an ERMS field of 17.25 V/m.

Measurement of blood pressure

A tail cuff non-invasive method was used to measure systolic and diastolic blood pressure (Biopac NIBP200-A). Animal tail non-invasive blood pressure amplifier incorporates a built-in pump that automatically inflates the blood pressure cuff to occlude the vessel in the tail of a rat. The rats were placed in an approximately body-sized plastic container prior to the blood pressure measurement. The animals were pre-warmed for 15 minutes to increase blood flow to the tails. A minimum of 5 measurements were recorded and the mean was used for analysis.

Measurement of blood hematocrit and viscosity

Hematocrit value was determined by microhematocrit method. Heparinized capillary tube (1x70 mm) and microhematocrit centrifuge was used.

The blood and plasma viscosity was measured using an oscillator flow with Vilastic Bioprofiler (Vilastic scientific, Inc., Austin, U.S.).

Heart and body weight

Animals were weighed before the sacrification. The heart was dissected out and then weighed. Heart weight-to-body weight ratio was calculated.

Histochemical study of ventricular heart and aorta

Hematoxylin-Eosin staining was performed to 4 μm thick sections. Slides were examined with Photo-light microscope (DM4000B Image Analyze System, Leica, Microsystems, Heidelberg GmbH, Heidelberg, Germany) and Leica DFC280 plus camera.

Immunohistochemical study

Ventricular heart and aorta tissue samples obtained from the study groups were fixed in 10% neutral formalin for about 72 h. They were dehydrated in an increasing series of ethanol, and parafin embedded for conventional histological diagnosis. Cross sections (4 μm) were mounted on polylysin-coated slides (Menzer-Glaser, Braunschweig, Germany), deparaffinized with xylene and rehydrated. The slides were kept in a microwave oven in EDTA Buffer (LabVision, Fremont, CA, USA) (pH 10.0) for heat-induced antigen retrieval through microwave irradiation so as to increase the sensitivity of immunohistochemical detection. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (Fisher Scientific, Melrose Park, IL, USA) (diluted with phosphate buffered saline [PBS]) for 15 min and washed three times in PBS solution. The epitopes were stabilized by application of serum blocking solution (Lot: 1754084A, LifeTech) for 15

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minutes. The slides were incubated with primary antibodies of Collagen Type-I (ab21287, Lot: GR49709-9, Abcam) (diluted with PBS at a rate of 1/200) and Collagen Type-III (ab7778, Lot: GR261168-1, Abcam) (diluted with PBS at a rate of 2 µg/ml) for overnight at +4^{0}C. Slides were washed three times in PBS solution. After that, the biotinylated secondary antibody (Lot: 1754084A, LifeTech) was applied for 10 minutes. Slides were washed three times in PBS solution. Thereafter, streptavidin peroxidase (Lot: 1754084A, LifeTech) was applied to the slides for 10 minutes, after washing with PBS, DAB (Lot: 38703, DAB Chromogen/Substrate Kit, ScyTek) was used as a chromogen. Afterwards, all slides were counterstained with Harris's hematoxylin. Slides were examined with Photo-light microscope (DM4000B Image Analyze System, Leica, Germany) and Leica DFC280 plus camera.

Wall thickness of aortic tissues were measured from six different regions for each group and each subject. Surface area of heart tissues was also measured in cross sections for each group and each subject (10 cells were measured for each subject). Statistical variables were created as a result of these measurements.

Biochemical studies on tissue and plasma

Determination of tissue and plasma NOx

Plasma and cardiac NOx levels were obtained from Elisa reader by vanadium chloride (VCl3)/ Griess assay [17].

Before NOx determination, tissues were homogenized in five volumes of phosphate buffer saline (pH = 7) and centrifuged at 2000 × g for 5 min. After centrifugation, 0.25 ml of 0.3 M NaOH was added to 0.5 ml supernatant. The incubation of the samples for 5 min at room temperature was followed by addition of 0.25 ml of 5% (w/v) ZnSO4 for deproteiniza-tion. This mixture was then centrifuged at 3000 × g for 20 min and supernatants were used for the assays. Nitrate standard solution was serially diluted and the plates were loaded with samples (100 µl). Then Vanadium III chlo-ride (VCl3) (100 µl) and Griess reagents sulphanilamide (SULF) (50 µl) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) (50 µl) were added to each well. After incubation at 37°C for 45 min, samples were measured at 540 nm using ELISA reader.

The plasma NO· (nitrite + nitrate) levels were estimated by the method of Miranda *et al.* [17]. After the centrifugation of blood samples, the supernatants were separated. Samples were deproteinised with 0.3 M NaOH and 5% (w/v) ZnSO4, centrifuged at 14 000 rpm for 5 min, and supernatants were used for the assays. Experiments were performed at room temperature. Nitrate standard solution was serially diluted. After loading the plate with samples (100 µl), addition of vanadium III chloride (VCl3) (100 µl) to each well was rapidly followed by addition of Griess reagents, sulphanilamide (SULF) (50 µl) and N-(1-naphtyl) ethylenediamine dihydrochloride (NEDD) (50 µl). After incubation (usually 30-45 min), samples were measured at 540 nm using an ELISA reader.

Measurement of tissue and plasma lipid peroxidation

Tissue Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) [18]. Samples were homog-enized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Hei-deloph Diax 900, Germany). Following centrifugation of the homogenate at 3000 × g for 10 min (Hermle Z 323 K, Germany), 750 µl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid and heated at 100°C for 15 min. The absorbance of the samples was measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56×105 l·mol–1·cm–1.

Plasma lipid peroxide levels were estimated by the method of Kurtel *et al.* [19]. Briefly, lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). On the day of experiment, plasma was incubated at room temperature for 15 min. After incubation, 0.5 ml of supernatant was added to 1 ml of a solution containing 15%

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(w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid and 0.25 N HCl. Protein precipitant was removed by centrifugation, and the supernatants were transferred to glass test tubes containing 0.02% (w/v) butylated hydroxytoluene. The samples were then heated for 15 min at 100 °C in a boiling water bath, cooled and centrifuged to remove precipitant. The absorbance of each sample was determined at 532 nm. Lipid peroxide level was expressed in terms of MDA equivalent using an extinction coefficient of 1.56 x 105 M-1cm-1 [20].

Measurement of tissue and plasma GSH levels

The tissue GSH levels were determined by Ellman method with some modifications [21]. Briefly, after centrifugation of the ho-mogenates at 3 000 × g for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 M Na2HPO4·2 H2O solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and after mixing, the absorbance at 412nm was measured using a spectrophotometer (UV 1208, Shimadsu, Japan) at room temperature immediately. The GSH levels were calculated using an extinction coefficient of 13 600 l·mol^{-1}·cm^{-1}.

The Plasma RSH levels were determined by the method of Kurtel *et al.* [19]. 0.5 ml of each sample was mixed with 1 ml of a solution containing 100 mM Tris-HCl (pH 8.2), 1% sodium dodecyl sulfate and 2 mM EDTA. The mixture was incubated for 5 min at 25 °C and centrifuged to remove any precipitant. 5,5-dithiobis (2-nitrobenzoic acid)/ DTNB) 0.3 mM was then added to each reaction volume and incubated for 15 min at 37 °C. The absorbance of each sample was determined at 412 nm. The GSH levels were calculated assuming a molar extinction coefficient of 13.000 at 412 nm.

Statistical analysis

The data are presented as the mean±SD. The statistical analyses were conducted using Statistical Package for Social Sciences 13.0 software. Differences between the groups for each parameter were compared using a one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Differences (Tukey's HSD) post-hoc test. Statistical significance was defined as $p<0.05$.

RESULTS

Effect on blood hematocrit and viscosity

Blood hematocrit levels were not different in the experimental groups compared to the Control. In the RFR+H group the blood hematocrit is higher than the Hypertension group ($p<0.05$). In the RFR group the blood hematocrit is higher than the Hypertension group ($p<0.01$). Blood hematocrit increased in RFR+H group compared to the Hypertension group ($p<0.05$). The RFR+H group displayed a significant increase in blood viscosity compared to the Control ($p<0.05$). Blood viscosity increased in RFR+H group compared to the RFR group ($p<0.01$). There was no significant difference in plasma viscosity level among the experimental groups table 1.

Table 1. Effects on blood hematocrit and viscosity.

Groups	Blood hematocrit (%)	Blood viscosity(sn^{-1}) strain=0.2	Plasma viscosity (sn^{-1}) strain=0.2
C	42.16 ± 2.04	5.37 ± 0.8	1.08 ± 0.11
H	38.16 ± 2.4	6.1 ± 1.2	1.16 ± 0.04
R	46.66 ± 3.4	4.7 ± 1.5	1.14 ± 0.05
R+H	44.66 ± 5.2 *	7.8 ± 1.2 # **	1.04 ± 0.09

The values are means±SD; n = 6. # $p < 0.05$ versus Control ; * $p < 0.05$ versus Hypertension ; ** $p < 0.05$ versus RFRC.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension.

Effect on cardiac and plasma MDA, NOx and GSH/RSH levels

The cardiac and plasma MDA levels increased in the RFR and Hypertension group, but the highest increase was in the RFR+H group ($p<0.01$) figure 1 and 2.

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The RFR and RFR+H group displayed a significant increase in cardiac NOx content compared to the Control ($p<0.01$). But the increase in the RFR+H group was more ($p<0.01$). There was a significant increase in the plasma NOx levels in the RFR group compared to the Control ($p<0.01$). There was a significant reduction in plasma NOx levels in the Hypertension and RFR+H groups compared to the Control ($p<0.01$) figure 3 and 4.

A significant reduction of cardiac and plasma RSH levels was observed in the RFR and RFR+H groups ($p<0.01$). The Cardiac GSH levels decreased in Hypertension groups ($p<0.05$) figure 5 and 6.

Effects on body and ventricular weight

No statistical difference was found in body weight of the experimental groups. The heart weight-to-body weight ratio was significantly increased in Hypertension and RFR+H groups ($p<0.05$) figure 7.

Hematoxylin-Eosin Staining Results of aorta and ventricular heart tissues

Tunica intima, media and adventitia layers were seen in normal histological structures through aortic sections of Control. Interrupted, duplicated or thickened elastic lamellaes were seen in some regions of Tunica media, and these are the most significiant findings for Hypertension group. The formations of elastic lamellaes were similar to Hypertension group in RFR group. But also in RFR group, hypertrophic and hyperplasic smooth muscle cells and intercellular edema were distiguished through Tunica media. In RFR+Hypertension group, the findings were similar but more intense when compared to Hypertension group and RFR group figure 8.

Normal histological structures were observed for ventricular heart tissue of Control. In Hypertension group and RFR group, hypertrophic heart muscle cells were distinguished and also some of the collateral branches were seen less obviously. Also disorganisation was observed in some myofibrils of heart muscle cells through this group. Disrupted cross striations were observed in these type of cells. In RFR+H group, the findings were similar to Hypertension group and RFR group but also increased amount of connective tissue were distinguished between muscle fibers. This finding was associated with typical fibrosis figure 9.

Collagen types I and III Immunstaining Results of aorta and ventricular heart tissues

Weak Collagen Type-I immunreactivity was seen in Tunica adventitia of aortic sections in Control. More intense immunreactivity was distinguished in Hypertension group through collagen fibers but also fibroblasts of Tunica adventitia. Also some of the smooth muscle cells of Tunica media showed positive immunreactivity in this group. Collagen Type-I immunreactivity was similar to Hypertension group in RFR group and RFR+Hgroup, but these groups also showed positive immunreactivity in Tunica intima figure 8.

Collagen fibers and fibroblasts showed moderate to strong Collagen Type-I immunreactivity in epicardium of ventricular heart tissue through Control and RFR groups. Also strong immunreactivity was seen in Hypertension group depending on thickened epicardium. Findings were similar to Hypertension group in RFR+H group but due to increased amount of intramyocardial connective tissue, positive immunreactivity was observed in myocardium in this group figure 9.

Weak Collagen Type-III immunreactivity was seen in Tunica media and adventitia, strong immunreactivity was observed in Tunica intima of aortic sections in Control and Hypertension groups. Similar immunreactivity was distinguished in RFR group but in this group, immunreactivity was more intense through Tunica media. The strongest immunreactivity was seen in RFR+H group for all layers figure 8.

Positive immunreactivity was observed in intramyocardial connective tissue of ventricular heart tissue in Control and Hypertension groups. Also the strongest immunreactivity was seen in RFR group and RFR+H group figure 9.

Wall thickness of Aortic vessel and Left ventricular muscle cell count

The wall thickness of the aortic vessel

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increased in the RFR and RFR+H groups ($p<0.05$).

A significant increase in left ventricular muscle cell count was observed in the Hypertension and RFR+H groups ($p<0.01$) table 2.

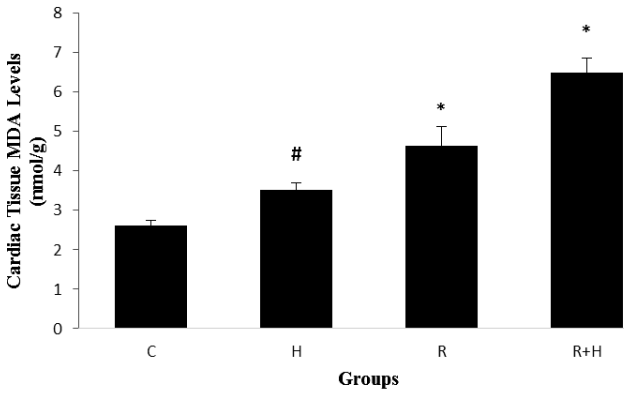


Figure 1. Cardiac Tissue MDA Levels (nmol/g). The values are means±SD; n = 6. * $p < 0.01$ versus Control, # $p < 0.05$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

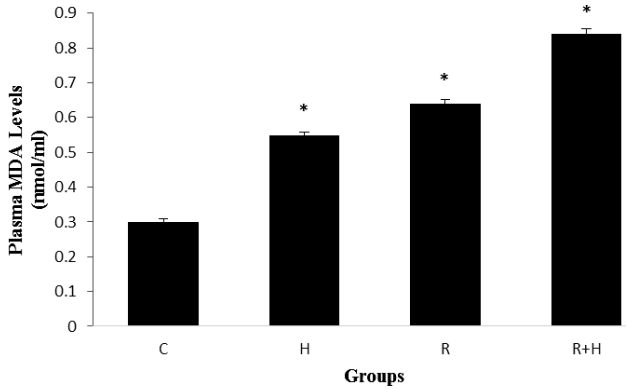


Figure 2. Plasma MDA Levels (nmol/ml). The values are means±SD; n = 6. * $p < 0.01$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension.

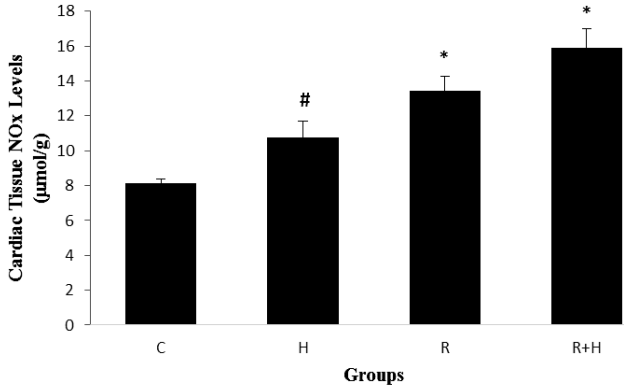


Figure 3. Cardiac Tissue NOx Levels (mmol/g). The values are means±SD; n = 6. * $p < 0.01$ versus Control, # $p < 0.05$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

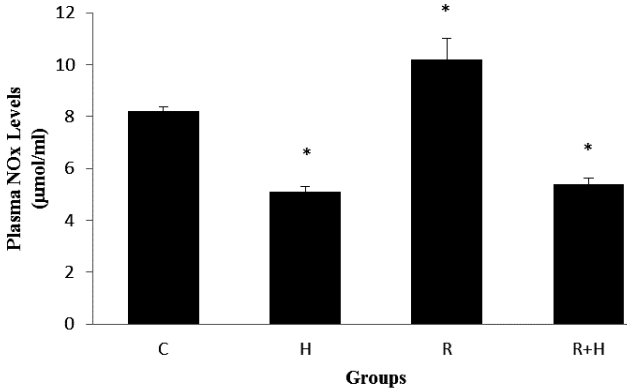


Figure 4. Plasma NOx Levels (mmol/ml). The values are means±SD; n = 6. * $p < 0.01$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

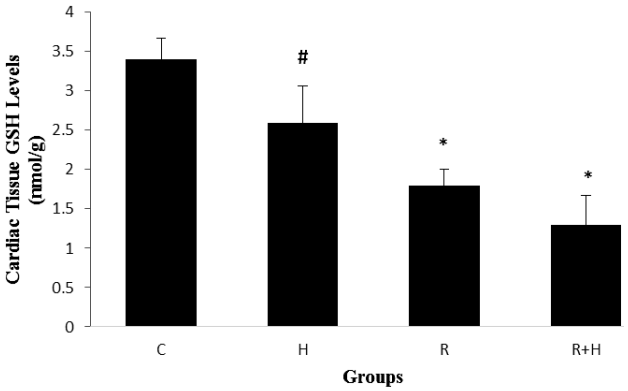


Figure 5. Cardiac Tissue GSH Levels (nmol/g). The values are means±SD; n = 6. * $p < 0.01$ versus Control, # $p < 0.05$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

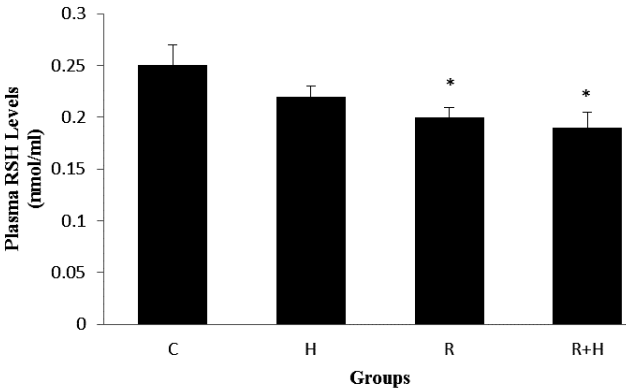


Figure 6. Plasma RSH Levels (nmol/ml). The values are means±SD; n = 6. * $p < 0.01$ versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

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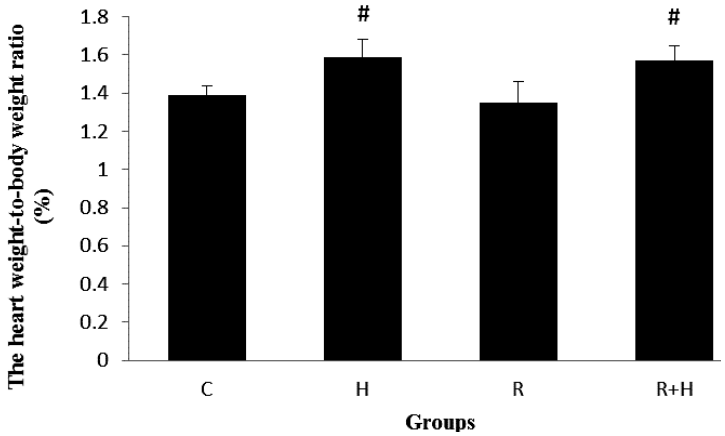


Figure 7. The heart weight-to-body weight ratio (%) The values are means±SD; n = 6. # p < 0.05 versus Control C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

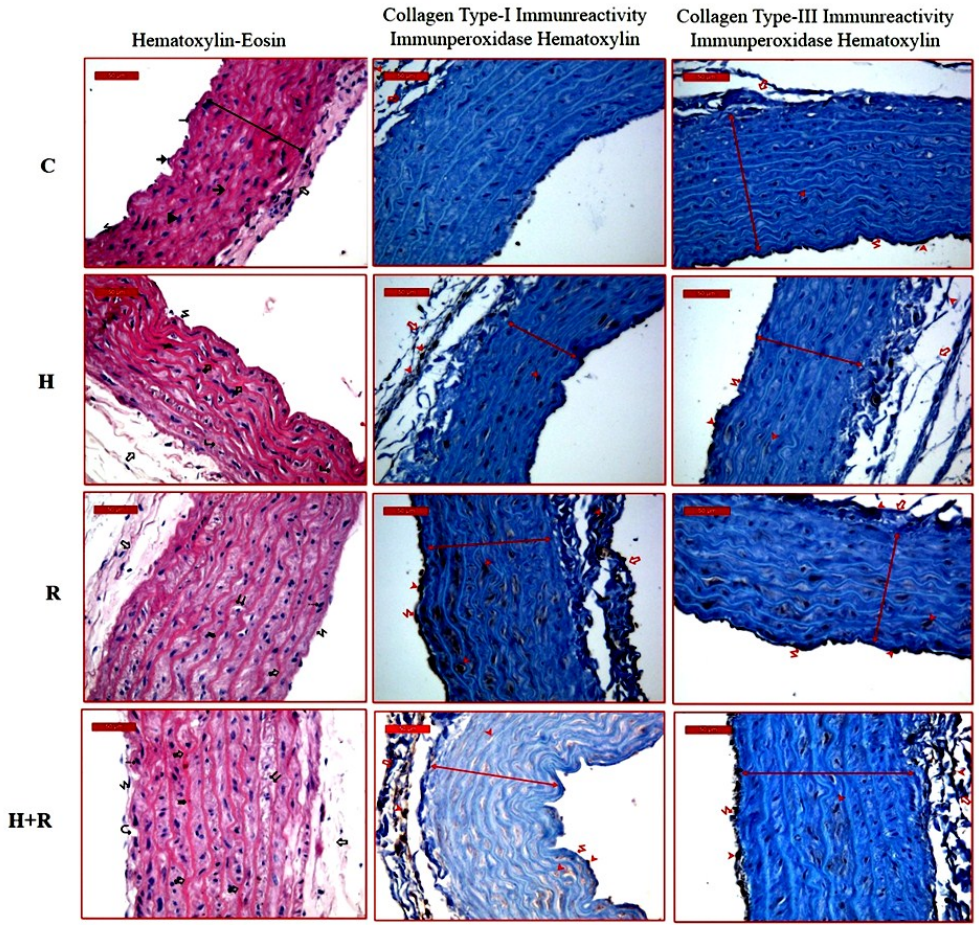


Figure 8. In aort sections; Tunica intima (⇳), endothelium cells (➔), subendothelial layer (→), Tunica media (⇔), elastic lamellaes (➔), smooth muscle cells (▶), Tunica adventitia (⇨), thickened elastic lamellaes (➡), interrupted elastic lamellaes (⮡), duplicated elastic lamellaes (⇪), edema in smooth muscle cells (⇉), heterochromatic nucleus of smooth muscle cells (⇢) and edema in Tunica intima (⮎) were seen in all groups (Hematoxylin-Eosin x400). Tunica adventitia (⇨), Collagen immunreactivity (➤), Tunica media (⇔) and Tunica intima (⇳) were distinguished in all groups (Immunperoxidase Hematoxylin x400). C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension

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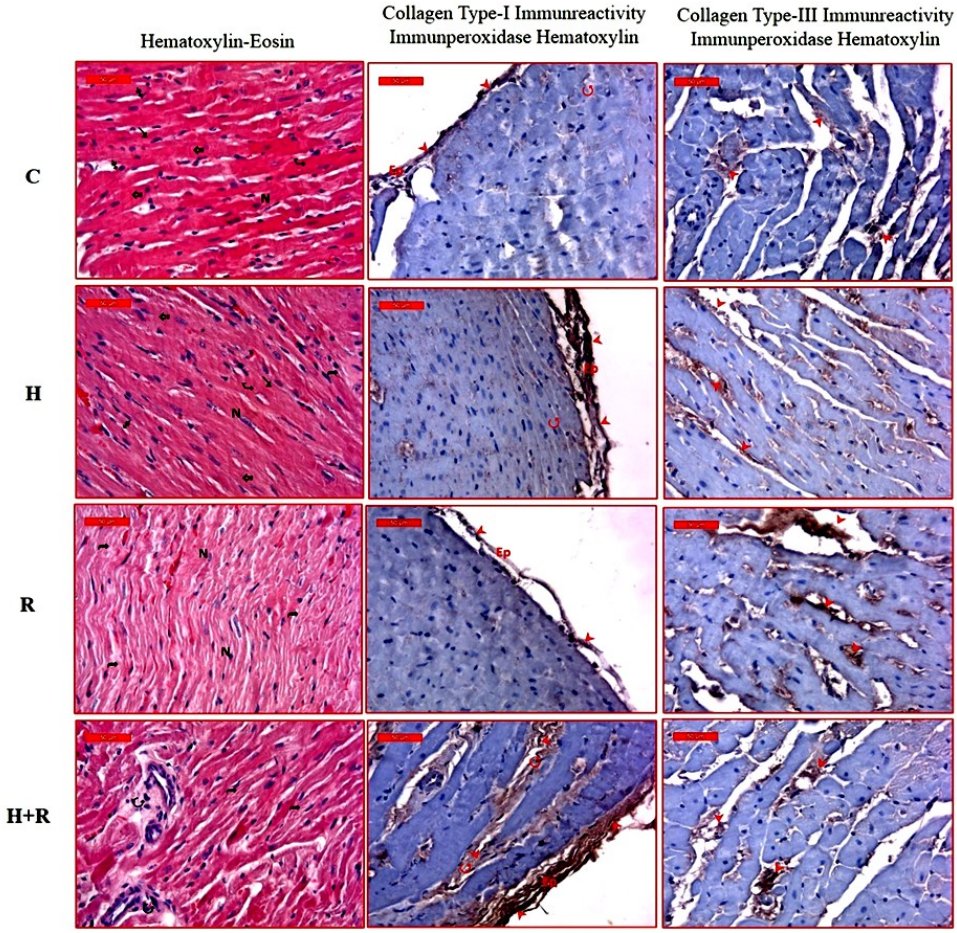


Figure 9. In ventricular heart sections; heart muscle cells (↘), nucleus (↳), collateral branches (↟), cross striations (↪), intercalated disc (⇪), disorganisated myofibrils (↬) and connective tissue between cardiac muscle fibers (↺) were seen in all groups (Hematoxylin-Eosin x400). Connective tissue between cardiac muscle fibers (↺), epicardium (↓↑▲), Collagen immunreactivity (➤) were observed in all groups (Immunperoxidase Hematoxylin x400). C.Control, H.Hypertension, R.RFR, R+H.RFR+Hypertension.

Table 2. Effects on Wall thickness of Aortic vessel and Left ventricular muscle cell count.

Groups	Wall thickness of Aortic vessel (µm)	Left ventricular muscle cell count number)
C	196.7±10.63	19.5±0.6
H	195.5±18.07	31.4±0.9 #
R	216.8±15.7 #	20.3±0.8
R+H	210.1±14.01 #	29.3±0.7 #

DISCUSSION

The objective of our study is to investigate whether rats with L-NAME induced hypertension are more negatively affected by exposure to radiation. For the induction of hypertension, we employed L-NAME, a NO synthase inhibitor, at a dose of 60 mg/kg. inhibitor.

Similar to previous studies, in the present study, L-NAME hypertensive animal models showed a significant increase in hypertension in terms of elevation of SBP and DBP, and a significant decrease in serum NO. Dillenburg DR *et al.* and Khan SA *et al.* reported that L-NAME at a dose of 20-60 mg/kg per day caused hypertension with high vascular resistance [14, 22]. Duplicated or thickened elastic lamellaes

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were seen in some regions of Tunica media through aortic sections. Hypertrophic heart muscle cells were distinguished and a significant increase in collagen I and III in myocardium was observed. The data of the present study are in accordance with the findings of Sadek *et al.* [23] and Nasiri *et al.* [24].

In the present study, with added L-NAME, NO levels reduced in the plasma but increased in the cardiac tissues. Also, the MDA level increased in the heart and plasma. This finding was in line with that of Jaarin *et al.,* who reported that intraperitoneal L-NAME administration at a dose of 25 mg/kg for 8 weeks resulted in an increase in MDA levels in the heart and a decrease in NO levels in the plasma [25].

In a previous study by Thangarasu *et al.* animals were given L-NAME in drinking water at a dosage of 40 mg/kg body weight (b.w.) for 4 weeks. They demonstrated that L-NAME reduced GSH levels and increased MDA levels in the heart [26].

RF radiation has the potential of inducing oxidative stress in biological systems via free radicals by enhancing lipid peroxidation and re-ducing antioxidant levels. The results of our study showed that MDA and NOx increased and the levels of GSH decreased in heart and plasma due to RF ra-diation exposure. Especially in the hypertensive group exposed to RFR, the increase in MDA and NOx levels and the decrease in GSH levels was more severe. This finding was similar to previous studies, which reported that RFR exposure increased oxidative stress [27]. Özgüner *et al.* reported that MDA and NO levels increased while GSH-Px activities decreased at 900 MHz RFR exposure 30 min/ day, for 10 days in heart tissue [28]. In a previous study, rats were exposed to 900 MHz RFR 20 min/day for three weeks. In this study MDA and NOx levels increased significantly in heart tissues of the exposed group. Also GSH levels were significantly lower in exposed rat tissues [15].

In the present study, RFR exposure caused hypertrophic and hyperplasic smooth muscle cells in aortic sections and intercellular edema were distiguished through Tunica media. In the RFR group, increased amounts of connective tissue were distinguished between muscle fibers for ventricular heart tissue. This finding was associated with typical fibrosis but the results were similar to the other experimental groups.

In the RFR group Collagen Type I immune-reactivity was similar to all experimental groups in Tunica intima. Collagen Type III immunreactivity was more intense through Tunica media. In the intramyocardial connective tissue of ventricular heart tissue strong immunreactivity was seen in RFR group.

These findings are a first in literature in aortic sections and ventricular heart tissue. So we can not argue with similar or contrasting results. But notable edema can be a sign of damage in the RFR exposure group. In addition, increased immunoreactivity may be the result of adverse effects of RF exposure.

According to the results of our work, RFR exposure did not change plasma viscosity but in the hypertensive group with radiation exposure, blood hematocrit was increased compared to hypertensive group. Blood viscosity increased in the hypertensive group exposed to RFR. This finding contrasted with Abu Bakr *et al*. who reported that blood viscosity and plasma viscosity values decreased after exposure to mobile phone electromagnetic radiation. Also they said that Hemoglobin structure is affected due to exposure to mobile phone electromagnetic radiation. Also blood components of rats are significantly affected by exposure to RFR such as decreasing values of RBCs, WBCs, Platelets, and Hemoglobin molecules [13]. According to the study results of Levin *et al*. blood hematocrit did not change with microwave radiation with the range of 8 mm (80 mVt/cm2) for 5 hours a day for 3 days. But blood viscosity increased [29]. Fomenko *et al.* reported that blood viscosity increased due to ionizing radiation (100-1000 Gy) [30].

According to immunohistochemical findings, Collagen Type I and III immunreactivity was seen more intensely in RFR+H group for heart, strong Collagen Type III immunreactivity was observed in RFR+H group for aorta but Collagen Type-I immunreactivity was seen more intensely in RFR and RFR+H groups for aorta. Taken together it was concluded that the

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radiofrequency has shown its structural effects on aorta rather than on heart. Also it was seen that RFR played a more active role through tissues in the case of both pathogens applied together. According to immunohistochemical findings it was observed that fibrosis occured as a result of the Collagen Type III activation for both aortic and ventricular heart tissues.

In order to support our *in vivo* results obtained radiation induces numerous pathological changes in the cardiovascular system. Histological examination of the aorta and heart tissue show that hypertensives exposed to radiation are more affected. The most pronounced aortic degeneration and left ventricular fibrosis were detected in the hypertensive group with RFR exposure. Radiation exposure adversely affected heart and plasma oxidant stress, especially in hypertensives. In addition, radiation exposure can exacerbate the condition of hypertensives because it increases blood viscosity. These results show us that hypertensives may be more negatively affected by radiation than non-hypertensives.

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Conflicts of interest*: *Declared none.

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