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Effects of pre and postnatal 2450 MHz continuous wave (CW) radiofrequency radiation on thymus: Four generation exposure

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ABSTRACT

This study aims to investigate the effects of pre- and postnatal 2450 MHz continuous wave (CW) radiofrequency radiation (RFR) on the thymus of rats spanning four generations. Four groups; sham, irradiated female, irradiated male, irradiated male and female, each consisting of four rats (one male and three females), were created. During the experiment, rats in the exposure groups were whole-body exposed to 2450 MHz CW-RFR for 12 h/day. Irradiation started one month before the fertilization in the experimental group. When the offspring were two months old, four rats, one male and three female, were allocated for the second-generation study. The remaining offspring were sacrificed under general anesthesia, and their thymuses were removed. The same procedure was applied to the next generation. Two months after the second generation gave birth, third-generation rats were decapitated, and their thymuses were removed. In all groups, cortex, medulla and resident cells could be clearly distinguished in the second and third generations. No differences were observed between the control and two experimental groups, defined as irradiated female and irradiated male. In contrast, vascularization was observed in the thymus of the fourth-generation offspring of the group where both males and females were irradiated. The number of offspring and mass of all rats decreased in the third-generation group. Pre- and postnatal 2450 MHz continuous wave radiofrequency radiation exposure may potentially affect the thymus of future generations.

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KEYWORDS

2450 MHz RF; pre- and postnatal exposure; continuous wave; thymus; four generations

Introduction

The classification of radio frequencies (RF) as 2B agents by the International Agency for Research on Cancer (IARC) and the common use of wireless communication (Wi-Fi) devices in data transmission have raised health fears associated with electromagnetic fields (EMF) exposure. Developing tissues and organs are susceptible to harmful agents. The World Health Organization (WHO) has led research on the effect of early-life exposure to microwave (MW) as high precedence for 2006 and 2010 research on EMF because of the possible vulnerability of children (Laudisi et al. 2012).

Long-term pre- and postnatal RF radiation (RFR) exposure and the delayed effects are not known sufficiently. As the effects of RFR are still uncertain, the Council of Europe recommends that restrictions be placed on internet access and usage of mobile phones in all schools to preserve juveniles from the potential harm of radiation (Watson 2011). There are many reports about the effect of mobile phone exposure on

health, but research on the effect of Wi-Fi 2.4 GHz RFR is considerably less than mobile phones (Dasdag et al. 1999, 2000, 2009; Dasdag et al. 2015a). However, Dasdag et al. investigated the effect of pulsed 2.4 GHz RFR on the brain and found it can cause adverse effects (Dasdag et al. 2015b). Although many organs, especially the brain, have been the focus of these studies, the thymus is often overlooked and has not been considered as being affected by RFR.

The thymus is a central organ of the immune system and the lymphatic system, which differentiate into antigen-recognizing cells and lymphocytes produced by the bone marrow in mammalian cells (Dominguez-Gerpe and Rey-Menéndez 2003; Miller 2020; Rezzani et al. 2014). During early embryogenesis, with the onset of hematopoiesis begins development of the immune system and then throughout prenatal life continues with successive hematopoietic cell production, cell migration, and differentiation (Laudisi et al. 2012). The thymus is vulnerable to physiological alters such as pregnancy,

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aging, and external factors. In recent decades, researchers have found that ionizing and non-ionizing radiation achieve an external stimulus that changes thymus functions and modulates immune responses (Chen et al. 2000; Hekmat et al. 2013; Quaglini et al. 2004).

It was reported that CD8 + T and mature CD4+ cells migrated from the thymus into peripheral blood, initiating immune surveillance. The thymus was highly radio-sensitive because of the rapid proliferation and turnover of thymocytes (Wu et al. 2020). Wu et al. also stated that “Radiation damage was found to be characterized by important tissue-specific restoration and complexity patterns, especially in the thymus.” Sambucci et al. investigated the thymocytes maturation effects of Wi-Fi signals. The early life exposure to 2.45 GHz Wi-Fi 33-like signals showed no differences in total cell number however it showed differences between the thymuses of exposed mice. Therefore, they reported that their results did not exhibit on thymocyte development any effects of early life exposure to Wi-Fi signals (Sambucci et al. 2011). Furthermore, Laudisi et al. investigated the thymocyte development and T cell effects of prenatal Wi-Fi signals. They stated that prenatal Wi-Fi exposure did not cause detrimental effects on T cell development (Laudisi et al. 2012). Most of the studies discussed above examined the effects of prenatal irradiation on the fetus. Therefore, studies in which prenatal irradiations were performed mainly were based on whether the babies born were affected or not. Furthermore, limited studies have addressed the effects of pulsed RFRs, and the effects of the continuous (CW) RFR are unknown. The effects of irradiation spanning several generations have not been examined to date. The purpose of this study is to investigate the effects of pre-and postnatal 2450 MHz CWRFR on the thymus of consecutive generation rats.

Materials and methods

The experimental animals

All provisional protocols applied to rats were carried out in accordance with the Principles of Laboratory Animal Care and the rules of the Scientific and Ethics Committee of Experimental and Clinical Research Center (DEKAM) of Erciyes University. This research has been approved by the Local Ethics Committee of Animal Experiments of Erciyes University (Decision No: 16/111). Twelve female and four male Wistar Albino rats were used in this study at the beginning. All rats were acquired from Erciyes University Experimental Research and Application Center (Kayseri, Turkey). Rats were housed under standard conditions (12 h light/12 h dark cycle and the temperature kept at

$23 \pm 2.5^{\circ}\text{C}$). Rats were fed ad libitum, and tap water was provided as drinking water. Water and food were available from the center.

The rats were divided into sham control and three exposure groups, with one male and three female rats in each group (Figure 1). During the experiment, rats in the exposure groups were whole-body exposed to 2450 MHz CW-RFR for 12 h/day. In the first exposure group, only male rats were exposed. Both female and male rats were exposed in the second exposure group, and in the third group, only female rats were exposed. The same process was applied to rats in the sham control group by turning off the generator.

Radiofrequency radiation exposure

An RFR generator (220 V; 2370–2450 MHz, 0–4 Watt; Nar Technology, Kayseri, Turkey) with 2450 MHz CW utility was used in the study. The study was performed in a Faraday cage to eliminate environmental exposures from other RF devices such as mobile phones, base stations, radio-TV transmitting stations. A cylindrical cage with wire grid top and bottom covers enables free-air access to the rats during exposure measurements. Wire-grid spacing was selected as 5 mm, which was much smaller than free-space wavelength λ_0 , at 2.45 GHz. Experimental and simulation setups are illustrated in Figure 2. Before RFR exposure, ambient RF level was measured with a wideband antenna (Log-periodic Antenna, HF59B, FF6E, Gigahertz Solutions, Langenzenn, Germany) in the inner and outer surface of the cylindrical cage. The power of the RF generator was set to 1 Watt. RF field levels inside and outside the cylindrical cage was remeasured with the generator turned on. The electromagnetic power density was recorded as $8.5 \pm 0.65 \text{ W/m}^2$ inside each section of the cylindrical cage. Rats could freely move in the cage. The entire study was conducted at the Erciyes University Research Center in a room (Temperature: $25 \pm 2^{\circ}\text{C}$, Humidity: 40%-50%, 12 h constant light-dark cycle) specifically allocated for this research. Animals were fed ad libitum, and tap water was provided as drinking water. All the animals were exposed to RFR between 08:00 and 20:00 during the study.

RF exposure started one month before the fertilization, and the fertilization took place at the end of this period (first-generation). The pregnancy was determined by a vaginal smear test. When the offspring were two months old (second-generation), four rats, one male and three female, were allocated for the next generation study. The remaining offspring were sacrificed under general anesthesia, and their thymuses were removed. After the second generation gave birth, the

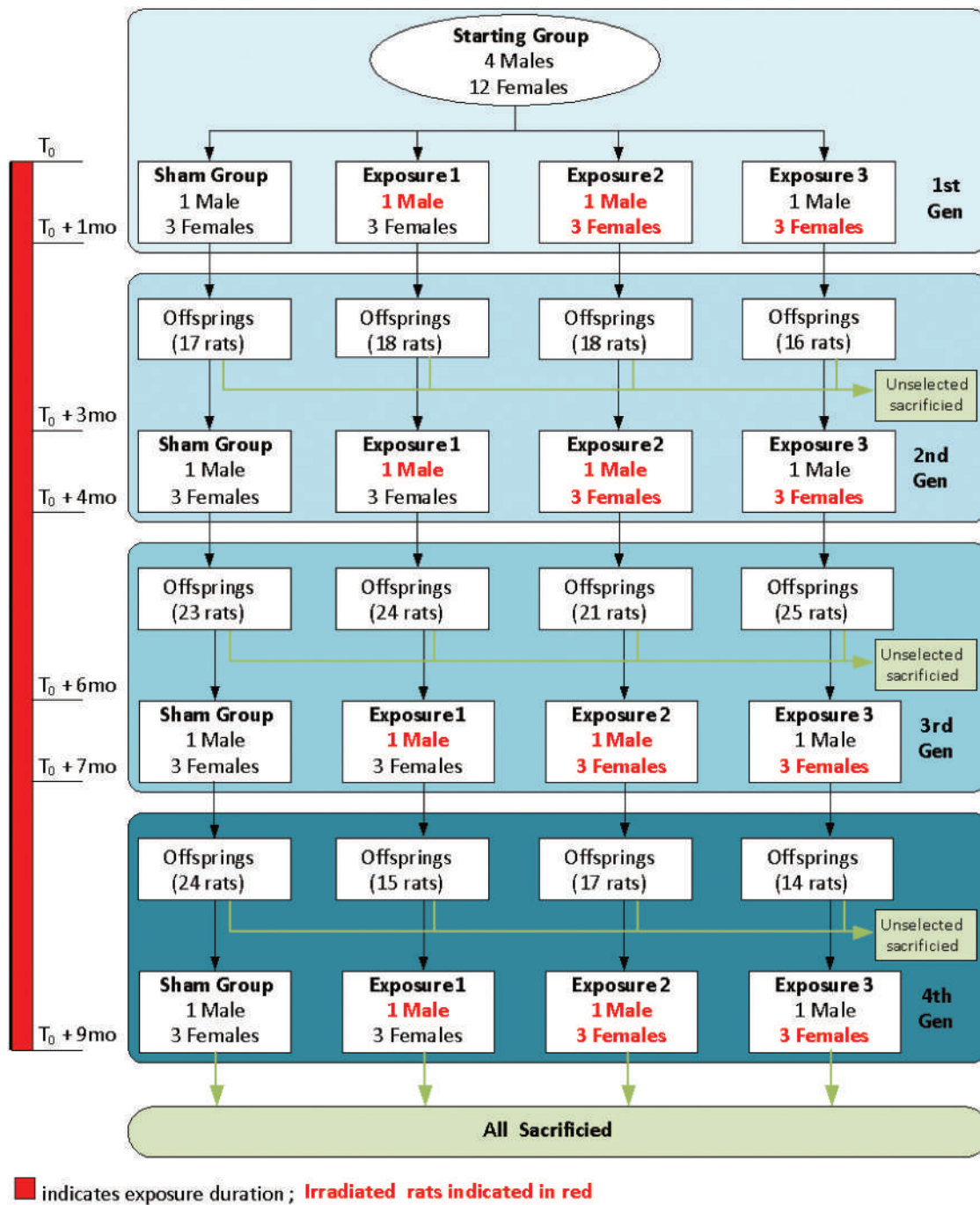


Figure 1. Rats in respective groups and generations.

offspring (third-generation) were grown for two months, and then, all rats were decapitated under general anesthesia to remove their thymus. After the third generation gave birth, the offspring (fourth-generation) again were let grow for two months, and then, again, all rats were sacrificed under general anesthesia to extract their thymuses. In summary, the irradiation started one month before the pregnancy of the first generation and continued until the fourth-generation animals were

sacrificed. However, successive generation animals were also designed and experienced the same processes as the first-generation rats (Figure 1).

Specific absorption rate (SAR)

The exposure setup with the antenna, the Faraday cage, and the rats was modeled in a 3D electromagnetic field solver using CST (CST AG, Darmstadt,

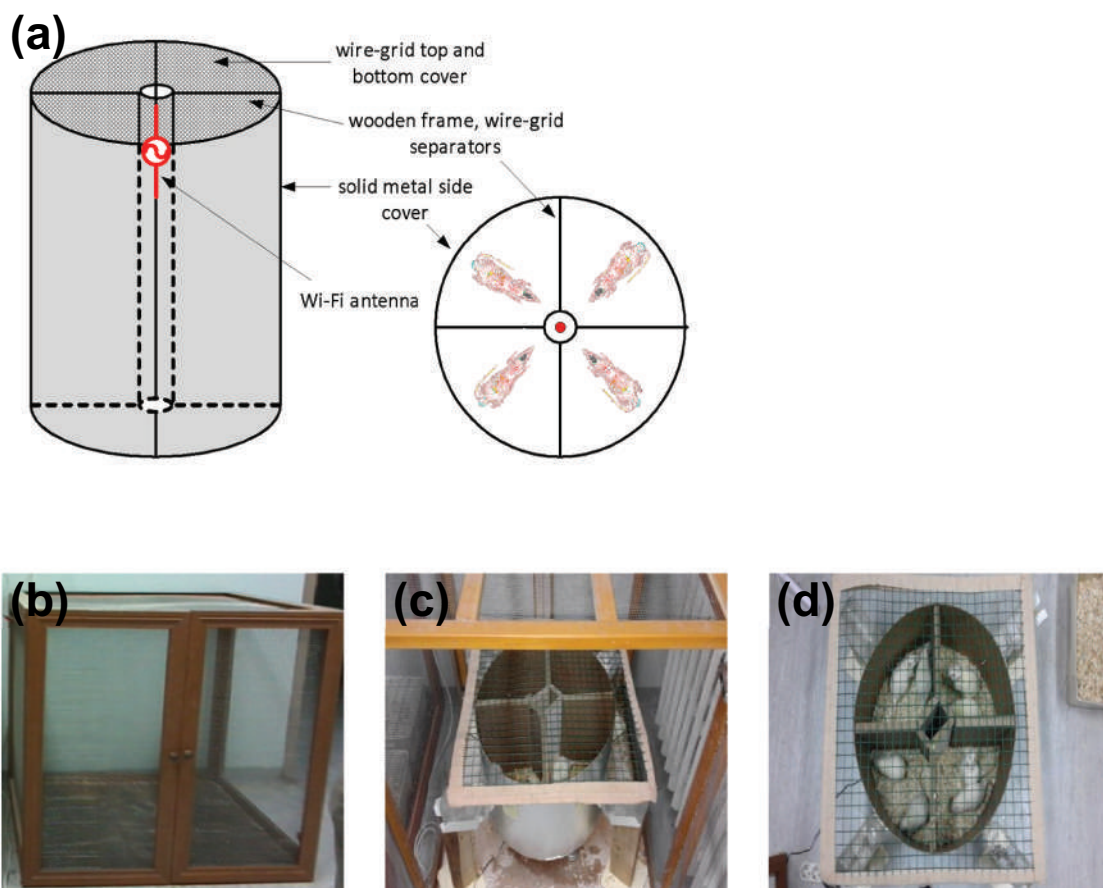


Figure 2. Simulation and experimental setups. (a) Cylindrical cage for RF irradiation, (b) large cylindrical cage to house rats, (c) The cylindrical cage inside the rectangular Faraday cage, (d) top view of the Faraday cage.

Germany). CST is based on the finite-integration technique, which employs discretization in the integral form of electromagnetic fields rather than their derivatives. Whole-body SAR's were calculated as 0.186 W/kg for 1 g average and 0.093 W/kg for 10 g average using IEEE/IEC 62704–1 method. SAR at thymus was calculated as 0.051 W/kg and 0.068 W/kg for 10 g and 1 g averaging, respectively. Rat voxel model and sagittal plane SAR distribution for 1 g averaging are displayed in Figure 3(a, b), respectively. Since the antenna and the rats were symmetrically located in the simulation setup, no variation of SAR from one chamber to another was observed. However, dosimetry levels are expected to change slightly due to size differences between the animals. This variation is expected to be small as the mass of rats does not differ more than 5% of the mean mass at exposure groups. Another 3D electromagnetic field simulator, Sim4Life (ZMT Zurich MedTech AG, Zurich, Switzerland), was used to simulate SAR for offspring because only Sim4Life had a 7-day old infant rat voxel model (Figure 3c),

which could be used for SAR simulations. Sim4Life utilizes finite difference time domain technique as 3D electromagnetic fields solver. The same exposure setup was replicated in Sim4Life, and SAR at thymus was calculated as 0.116 W/kg for 1 g averaging. Whole-body SAR for offspring was calculated as 0.287 W/kg. For offspring, whole-body SAR and at the thymus were 1.54 times and almost twice more than adults, respectively.

Histopathological evaluation

The thymus morphology was evaluated hematoxylin-eosin staining in two-month-old rats. After, thymus tissues were excised from each animal, and the tissues were cut longitudinally. The tissues were fixed in 10% formaldehyde for 24 h. Specimens were dehydrated through graded concentrations of ethanol, cleared with xylol, and embedded in paraffin wax. 5 μ m thick slices were cut from each paraffin block and stained with hematoxylin and eosin to show histological changes.

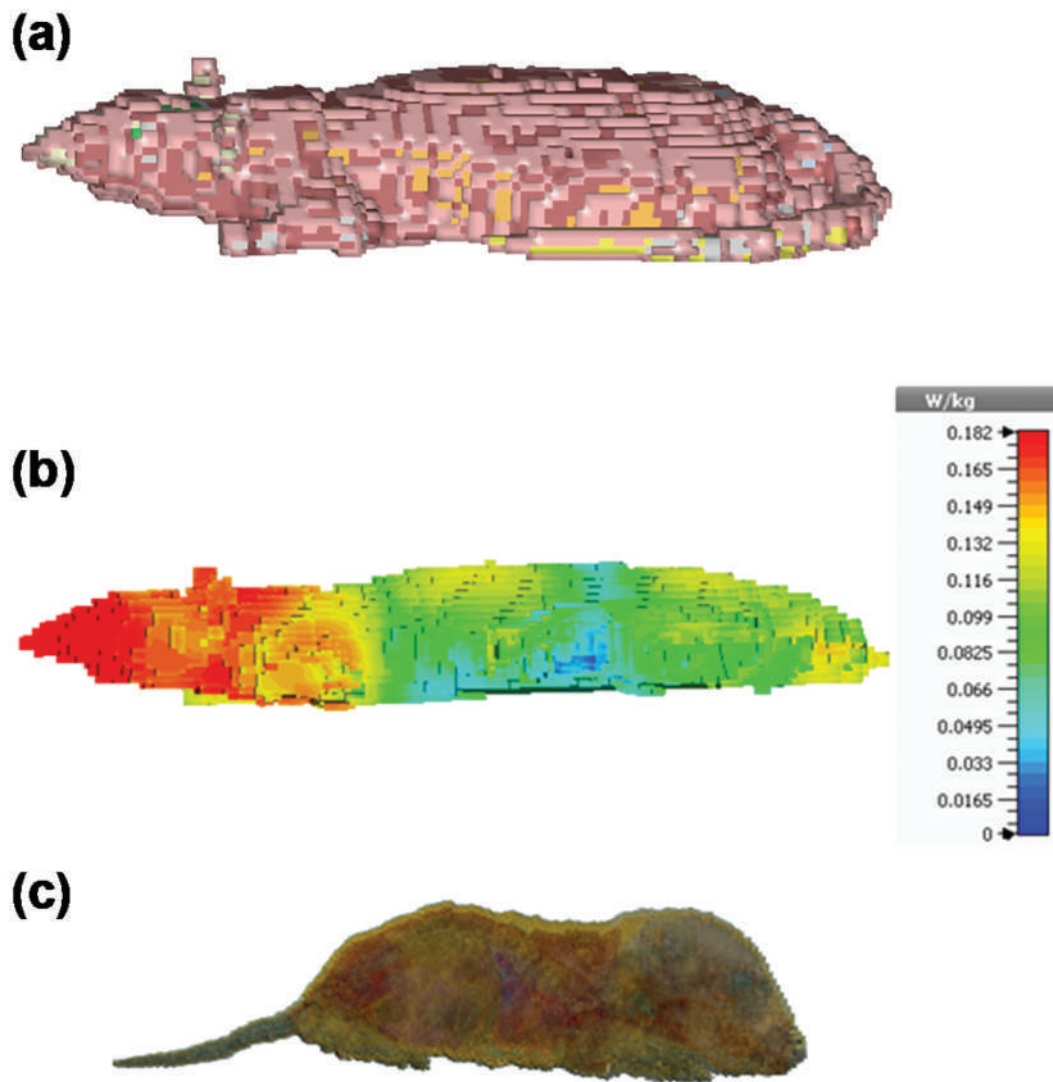


Figure 3. SAR simulations at 2450 MHz. (a) Adult rat voxel model, (b) adult rat sagittal cut whole body SAR distribution for 1 g average, (c) one week old baby rat model.

After staining, the slices were examined using a light microscope (Olympus BX51, Tokyo, Japan) and images were captured.

Results

Differences were not recorded in the gross anatomy of all generation rats. However, visibly pronounced thymus hypertrophy was observed in the exposed animals of the third and fourth generations. On the contrary, this was not encountered in the second-generation rats. Offspring's numbers were especially decreased fourth-generation according to second-generation (Figure 1). In addition, the average body masses of both female and male offspring in all generations were traced for five weeks. The average body masses of offspring were decreased in the fourth-generation (Figure 4).

Histopathological findings

In the slices stained with hematoxylin and eosin, the thymus is surrounded by a thin capsule consisting of connective tissue. Trabeculae extended from the capsule to the parenchyma of the organ. The thymus formed lobules that had apparent cortex and medulla. The organ cortex was more basophilic due to developing T cells, while the medulla was stained lighter. In each lobe, the thymus cortex was formed multiple lymphocytes and reticuloepithelial cells. The medulla of the organ consisted of numerous mature lymphocytes, reticuloepithelial cells, and Hassall corpuscles. In all groups, both second and third generation, cortex, medulla and the residing cells were noticeable (Figures 5 and 6). Although morphological differences did not appear among the groups, vascular structures were remarkable

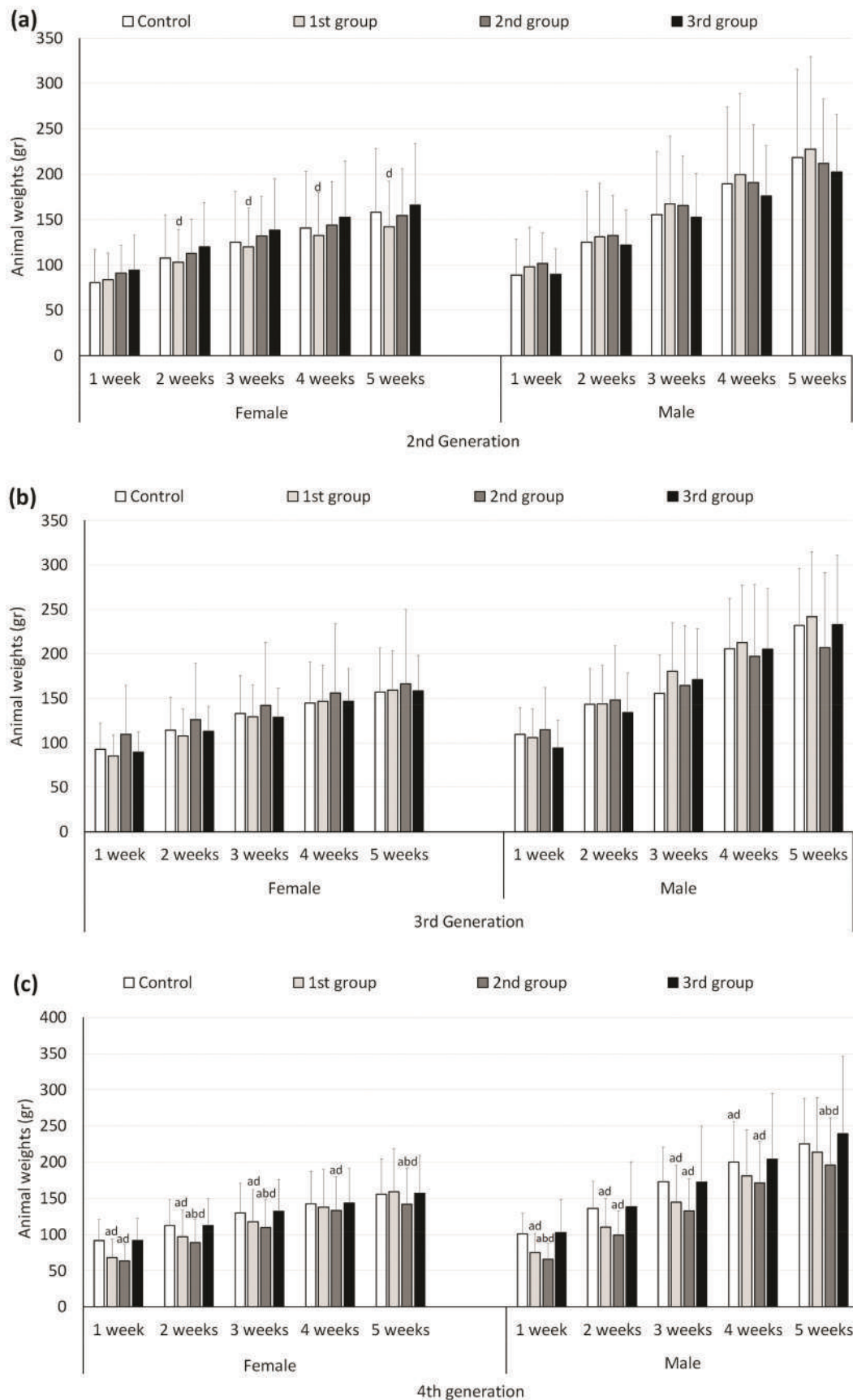


Figure 4. The average body masses of both female and male offspring in (a) second, (b) third and (c) fourth generations were traced for five weeks. The average body masses of offspring were decreased in the fourth-generation. Note that there was a significant difference between the groups ($p = .006$) in 2nd and 4th generation. a indicates significant difference compared to control group. b indicates significant difference compared to 1st group. d indicates significant difference compared to 3rd group.

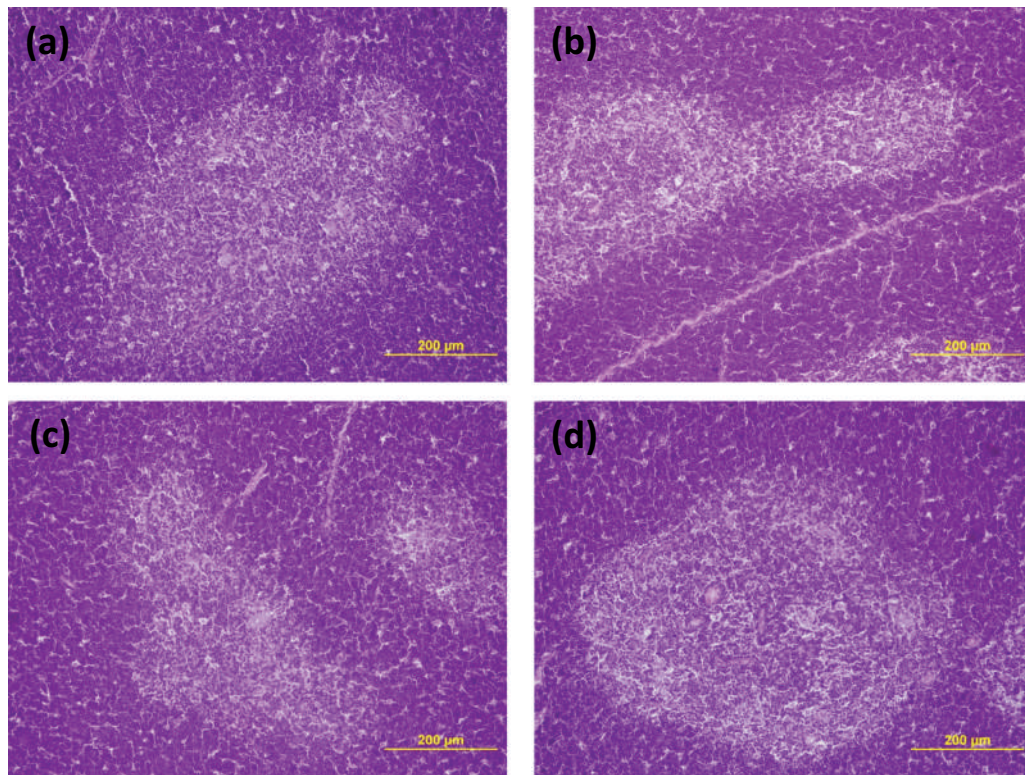


Figure 5. Histopathological changes in the thymus belonging to second generation rats exposed to EMF. In all groups of second generation; cortex, medulla and the residing cells were noticeable. The medulla of the organ consisted of numerous mature lymphocytes, reticuloepithelial cells and Hassall corpuscles. (a) Control group, (b) first group, (c) second group, (d) third group (H&E staining x400, Olympus, BX51, Tokyo, Japan).

when the second group of the fourth-generation was compared with the other experimental groups (Figure 7).

Discussion

The thymus is a fundamental lymphoid organ responsible for the maturation and selection of T cells. Thymus size and function are believed to decline with age (Gui et al. 2012). The thymus reaches its peak size during puberty, after which it gradually atrophies. If the thymus hypertrophy is not dependent on the patient's age, further investigation should be done (Khan and Anjum 2020). Thymic epithelial tumors manifest lobulated, contour, mediastinal fat invasion, hypertrophy, and vascular structure (Nishino et al. 2006).

This study investigated the effects of pre and postnatal 2450 MHz CWRFR on rats' thymus spanning four generations. Our study found morphological changes in the thymus tissue. Our most critical histopathological findings were visibly pronounced thymus hypertrophy in the exposed animals of the third and fourth generation and

the striking vascular structures in the second group of the fourth generation compared to the other experimental groups.

The results of this study were also consistent with earlier findings. Lee et al. investigated the effects of RF-EMF on AKR/J mice used for animal models of lymphoma. The mice were exposed to RF-EMF for 45 min/day, 5 days/week, over a total of 42 weeks. The thymus showed significant enlargement in most mice, and the thymoma was abnormal tissue (Lee et al. 2011). Misa-Agustino et al. exposed different levels of microwave radiation on rats. They reported histopathological changes induced 2450 MHz EMF in rat thymus. The researchers found extravascular erythrocytes in thymic tissue of animals exposed to EMF as in our study. Furthermore, they also reported an increased number of blood vessels in the hemorrhagic reticulo-epithelial cells and medulla (Misa-Agustino et al. 2015). It is evident from our results and the findings of Misa-Agustino et al. that EMF can cause changes in the morphology of thymic tissue.

Hanci et al. reported that light microscopy and transmission electron microscopy demonstrated pathological changes in splenic and thymic tissues of 900 MHz EMF

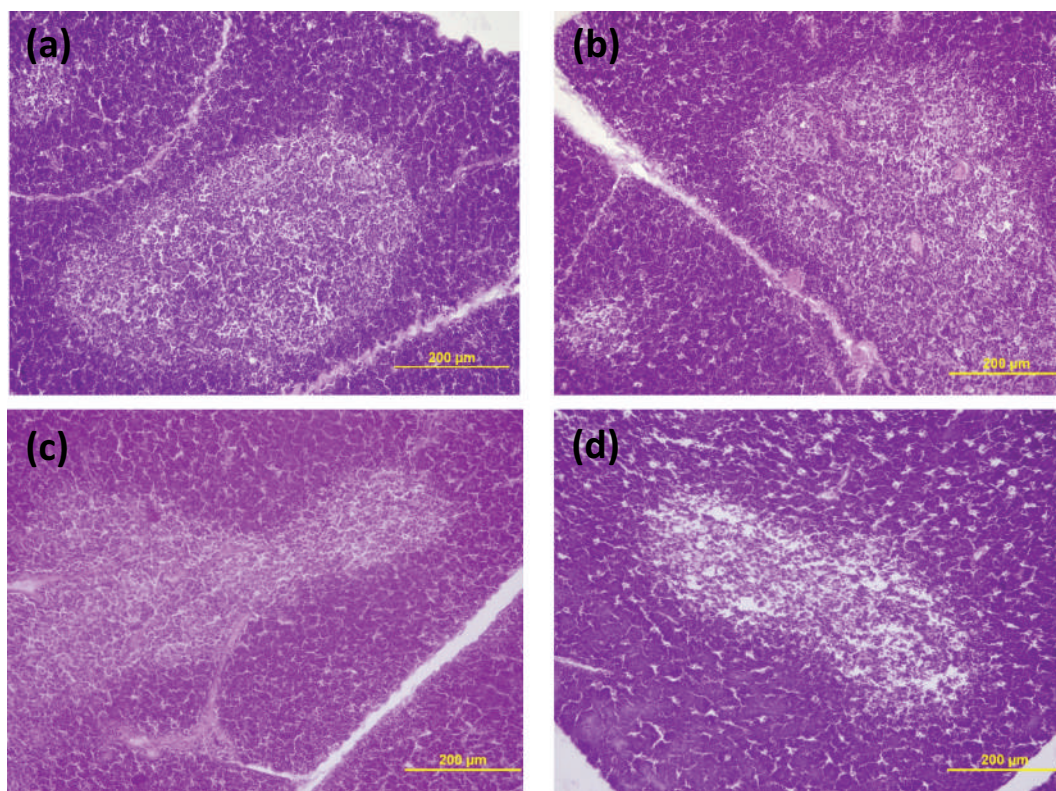


Figure 6. Histopathological changes in the thymus belonging to third generation rats exposed to EMF. In all groups of third generation; cortex, medulla and the residing cells were noticeable. The medulla of the organ consisted of numerous mature lymphocytes, reticuloepithelial cells and Hassall corpuscles. (a) Control group, (b) first group, (c) second group, (c) third group (H&E staining x400, Olympus, BX51, Tokyo, Japan).

exposed on rat offspring during the prenatal period. It was found that exposure during the prenatal period altered the antioxidant defense system and caused oxidative stress by increasing lipid oxidation. These researchers also reported mitochondrial degeneration, vacuolization, necrotic thymic lymphocytes, and epithelial cells in slices of EMF-exposed offspring (Hanci et al. 2015). Hashish et al. reported the effects of continuous whole-body exposure to both fields for 30 days on several blood and liver parameters in mice. They applied two magnetic fields: an extremely low-frequency electromagnetic field (ELF-EMF) and a static magnetic field (SMF). A significant reduction in the number of platelets, peripheral lymphocytes, monocytes, splenic total, T, and B lymphocyte levels was evaluated in the groups exposed to SMF and ELF-EMF (Hashisha et al. 2008). Kulaber et al. investigated changes in the thymic tissue of male rats exposed to continuous 900 MHz EMF for 1 h/day on postnatal days 22–59. They reported that a continuous 900 MHz EMF can change histopathology and increase the tissue MDA in male rat thymic tissue (Kulaber et al. 2017). Hekmat et al. applied 940 MHz RF-EMF on calf thymus DNA and stated that 940 MHz RF-EMF could alter the structure of DNA (Hekmat et al.

2013). Other researchers also reported the effects of ELF-EMF on the thymus and spleen tissues. Zhang et al. indicated that exposure to ELF-EMF in male mice decreased incidents of spleen and thymus tissues. Also, the exposure of ELF-EMF significantly decreased the NK-cell activity and proliferation of T and B lymphocytes (Zhang et al. 2016).

Contrary to our findings, some studies did not observe any change (Ohtani et al. 2015; Sommer et al. 2007, 2004). Sommer et al. (2004) exposed female AKR/J mice to 900 MHz EMF for 24 h/day, 7 days/week. Lymphoma incidence did not differ between the sham-exposed and exposed mice. Sommer et al. showed whether chronic exposure to the UMTS (Universal Mobile Telecommunication System) EMFs influenced the development of lymphoma in a lymphoma AKR/J mouse model. Exposure for 24 h/day, 7 days/week with an average whole-body SAR of 0.4 W/kg did not affect the lymphoma incidence and the malignancy of the lymphomas, which were genetically predisposed to this disease. Ohtani et al. reported the effects of continuous exposure to 2.14-GHz RF-EMF on T cell responses and its effects on the developmental immune systems. Their data indicated that long-term RF-EMF exposure had no

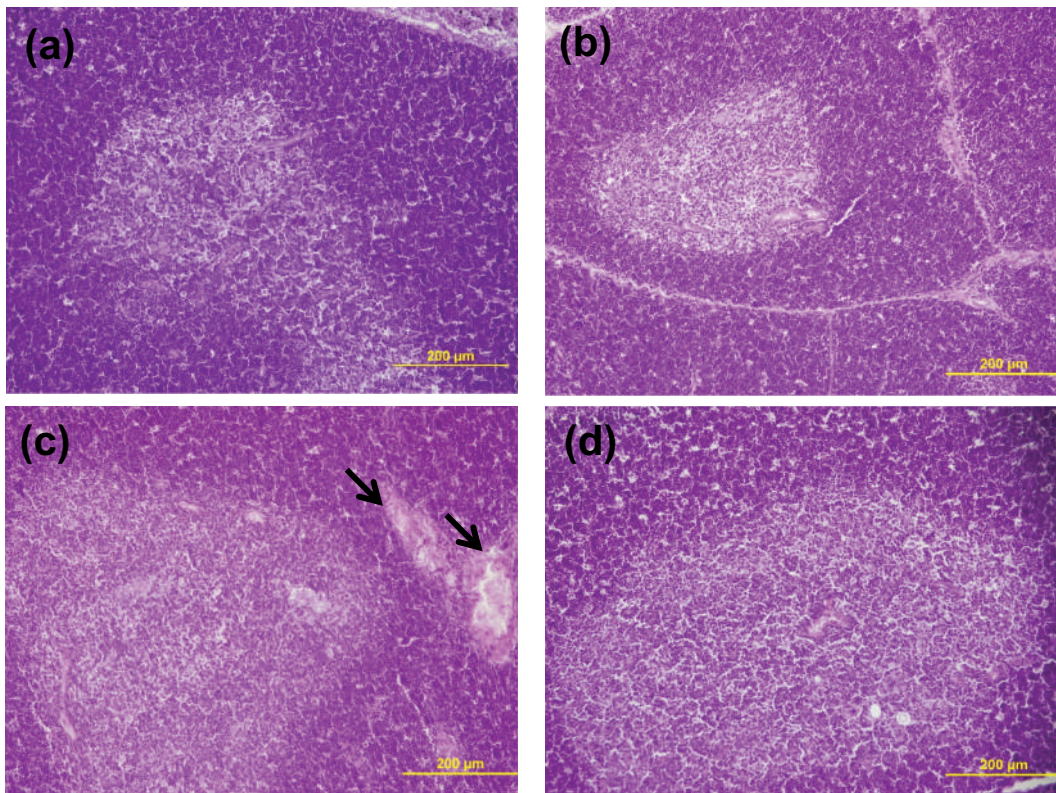


Figure 7. Histopathological changes in the thymus belonging to fourth generation rats exposed to EMF. The thymus cortex was formed multiple lymphocytes and reticuloepithelial cells. In first and third groups of fourth generation; cortex, medulla and the residing cells were noticeable. (a) Control group, (b) first group (c) in the second group, vascular structures were remarkable compared with other experimental groups, (d) third group (arrow; blood vessel, H&E staining x400, Olympus, BX51, Tokyo, Japan).

adverse impact on T cell activation, Th1/Th2 balance, and immune-like T cell populations in developing rats. However, important transcriptional effects were shown (Ohtani et al. 2015).

Conclusion

Our findings reveal that the effect of pre- and postnatal 2450 MHz CWRFR decreases the average body masses of rats. Pre- and postnatal 2450 MHz CW RFR exposure may potentially affect the thymus of future generations. Throughout three generations, increased vascularization and hypertrophy of the thymus can cause thymic epithelial tumors.

Disclosure statement

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

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Author contributions

F.C.T. and S.D. are responsible for the study design and writing of the article. B.Y. and A.H.Y. performed the microscopic analyses of slices. B.T. was responsible for the statistical analysis of the data. K.Y. was measured the SAR value.

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