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Research Article
Histological and Haematological Alterations in Female Mice after Exposure to Electromagnetic Fields

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Abstract

Background and Objective: As use of modern technology devices has increased, social concerns about the possible harmful effects of electromagnetic fields (EMFs) that emitted by these devices on human health have increased. The research designed to study the effect of EMFs in X-band frequency on the heart histopathological and haematological effects in female mice. **Materials and Methods:** A total of 30 female albino mice were assigned for this study and divided into 3 groups: Group 1 (control group), group 2 (2 weeks old) and group 3 (8 weeks old) were both exposed to a signal of 9.0 GHz frequency and 40 mW power for 4 h/day for 2 months. **Results:** The findings showed histological and haematological changes in both exposed groups compared with control. These alterations included a distortion in the architecture of cardiac muscular with wider interfibrous spaces, foci of haemorrhage with extravasation of red blood cells, congested and dilated blood vessels and a mild degree of degeneration. The haematological analysis showed a reduction of in granulocytes, red blood cell, haemoglobin, the mean platelets volume and plateletcrit. **Conclusion:** The exposure to EMFs caused histological and haematological alterations in female mice. Some of these alterations were more prominent in 2 weeks old group compared with eight weeks old group.

Key words: Electromagnetic field, X-band radar frequency, histology, haematology, haemorrhage, granulocytes, haemoglobin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Electromagnetic (EM) waves with frequencies ranging from 0.3-300 GHz, have been widely used in the environment[1]. Wireless devices such as cell phones, radar, cellular antennas and towers generate electromagnetic fields (EMFs) that can penetrate human tissue and cause harmful effects[2]. Although a number of researches are focusing on the biological effects of EMFs, the precise understanding of the X-band effect remains uncertain. The X-band radar frequency operates on a wavelength of 2.5-4.0 cm and a frequency between 8-12 GHz[3]. This band is widely used in radar applications such as military radars, marine radars, air traffic control radars and weather forecasting[4]. The EMF emitted by radar system is one of the most important risk factors that have negative effects on the biological systems[1]. People who live or work near radars have concerns about long-term detrimental effects of radio frequency (RF) on human health. The biological effects of RF radiation are divided into 2 types: Thermal effects which are prominently associated with the high-frequency and high-power and non-thermal effects which are predominantly associated with low-power of EMFs[1]. The tissues of an organism absorb RF fields below 10 GHz to 1 MHz and convert it to heat and cause many damage risks. Generally, there are several factors affect the energy absorption and EMF distribution in the tissue organism. These factors include the frequency and the intensity of EMF, shape, distance from the source and time of exposure[5].

In vivo and *in vitro* studies have been widely used to investigate the effects of EMF at behavioural, cellular or molecular levels. They observed that the exposure to EMFs increased tumour incidence and showed a risk for cardiovascular morbidity and mortality[6], caused cataracts incidence and reproductive malfunction[3,4,7]. However, the understanding of their effect on heart tissue and blood parameters remains ambiguous. The heart is a contractile organ consisting of a special type of striated muscle called cardiac muscle, it contracts to pump blood throughout body tissues. The heart can affect by external stimulants including EMFs exposure due to its stimulation properties[8,9]. In previous study, the researchers observed that the long-term exposure of EMF decrease the heart rate on workers at electricity delivery centres fed by 50 Hz, 400-500 kV high-voltage lines. A reduction of the heart rate causes certain cardiovascular disorders such as acute myocardial infarcts and cardiac arrhythmia[8]. However, other investigators found that the heart rate did not show any changes on workers exposed to 50 Hz, 400-500 kV lines for more than 5 years[8,9]. Also research found to an increase in heart rate on employees of electrical trains with 26 kV m^{-1} electrical field exposure[10]. Moreover, Australian Radiation Protection report founds that the user's head absorbed 70% of the waves that released by mobile phones and this leads to increase blood pressure, decrease in the haemoglobin and increased the incidence of leukaemia in children[11-13]. The histological characterizations of the heart tissues and haematological variations have not been documented after exposure to EMFs emitted by radar system. Therefore, this study performed to investigate EMF effects on heart tissue and blood parameters among 2 and 8 weeks old of female mice and to identify whether their effects are related to the age of animals.

MATERIALS AND METHODS

Animal handling: A Total of 30 albino female mice were used in this experiment, they were obtained from the Animal Centre in College of Veterinary/University of Basrah. The mice were maintained used on standard laboratory rodent diet pellets and housed in humidity and temperature-controlled ventilated cages equipped with wood-shaving bedding on a (12 h light/12 h dark cycle). Food and water were given to the mice without any restriction throughout the study period. All animal were approved by the Animal Research Ethical Committee of Basrah University. The study was carried out at Department of Physics, College of Science, University of Basrah from April, 2019-June, 2019.

EMFs exposure system: The animals were assigned into 3 groups with 10 animals in each group.

Group 1 (control group, this group was not exposed to EMFs). Group 2 (2 weeks old group) and group 3 (8 weeks old group) were both exposed to EMFs at frequency of 9.0 GHz and 40 mW power for 4 h/day for 2 months. All mice of group 2 and 3 were kept in ventilated cages during EMFs exposure. On the other hand, mice of group 1 was kept in ventilated cages in the other room without exposure to EMFs. In this study, EMFs exposure system with horn antenna was set up to produce X-band radar frequency at 9.0 GHz frequency with 40 mW output power. To supply an equal distribution of the EMFs to mice, the antenna was oriented to the middle of the ventilated cage and the height of the antenna from the ground of exposure system was about 5 cm (Fig. 1a-b). At the end of the experimental period, the mice in all groups were anesthetized by a piece of cotton soaked in chloroform in a plastic container in the package and were sacrificed. Tissue samples from heart and blood samples collection were prepared for histopathological and haematological studies, respectively.

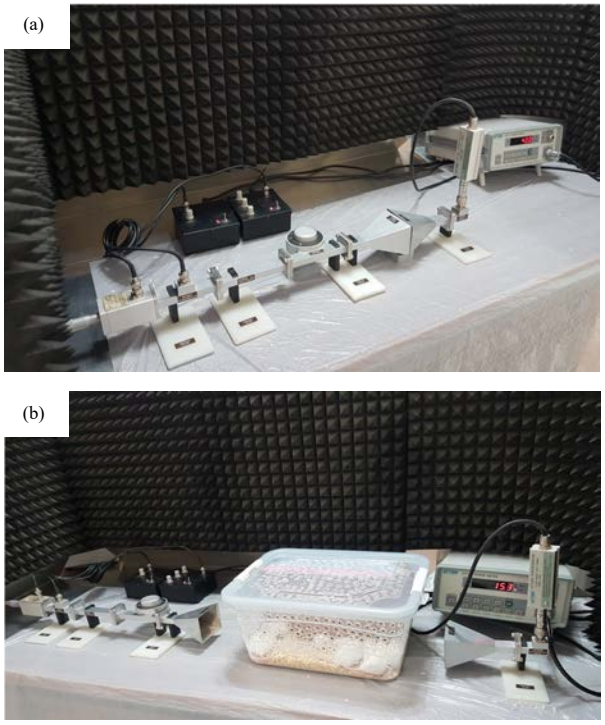

Fig. 1(a-b): Electromagnetic field exposure system, (a) Before exposure time and (b) During exposure time

Histopathological studies: Fresh portions of the heart from each mouse were cut rapidly after anaesthesia for histological examinations. The hearts were fixed in room temperature, 10% neutral buffered formalin solution then processed for routine paraffin embedding. After processing, the tissues were sectioned at approximately 5 µm and mounted onto slides, deparaffinised with xylene, rehydrated through graded alcohol solutions, stained with haematoxylin and eosin (H and E). Stained sections of control and exposed groups were examined for alterations in the heart tissues using light microscopic.

Haematological studies

Collection of blood samples: At the end of experiment, animals were anesthetized for blood collection. Blood samples were taken into a lavender top collection tube containing ethylenediaminetetraacetic acid (EDTA) for automated complete blood cell counts.

Complete blood count: The blood samples were placed in tubes containing EDTA. Automatic blood cell analyser was used for a complete blood count (CBC). The CBC test includes the determination of white blood cells (WBC) count and other WBC formula including granulocytes (GRA), lymphocyte and monocyte counts, red blood cells (RBC) count and various RBC parameters including haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT) parameters including mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW).

Statistical analysis: All data from the effects of EMFs on female mice were analysed using Prism statistical analysis software (GraphPad) and were presented as mean±standard error of the mean (SEM). The $p<0.05$ was chosen as the level of statistical significance. The differences between groups were analysed by one-way ANOVA with Bonferroni's multiple comparison tests (MCT) as appropriate.

RESULTS

Effect of EMFs on the histopathology of heart: The histological study of the heart in control group exhibited regularly arranged cardiac muscle fibres, the myocardium, consists of cross-striated muscle cells, cardiomyocytes, with one centrally placed nucleus (Fig. 2a). The heart histological examination of group 2 showed irregularly arranged cardiac muscle fibres with central nuclei, congestion of blood vessels with a few scattered lymphocytic infiltrations and extravasation of RBC combined with an increase of cytoplasmic vacuolization and degeneration (Fig. 2b, c). Also, group 3 showed congested blood vessels and wider interfibrous spaces (Fig. 2d).

MF effects on haematological parameters of the exposed mice: Haematological study was assessed to investigate the adverse effects of *in vivo* exposure to EMFs on haematological parameters in female mice. Results of the CBC analysis showed a significant reduction in GRA (Fig. 3a), RBC (Fig. 3b), HGB (Fig. 3c), HCT (Fig. 3d), MPV (Fig. 3e) and PCT (Fig. 3f) levels in exposed group compared to control group. Interestingly, the present findings found that levels in group 2 were significantly lower than in group 3 (Fig. 3a-f). However, the results did not showed a significant differences in the WBC (Fig. 4a), lymphocyte (Fig. 4b), monocyte (Fig. 4c), MCV (Fig. 4d), MCH (Fig. 4e), MCHC (Fig. 4f), RDW (Fig. 4g) and PLT (Fig. 4h) levels. Although total leukocytes count (Fig. 4a) and monocytes (Fig. 4c) did not reach statistical significance, there were tendencies to reduce their levels in both exposed groups compared to control group.

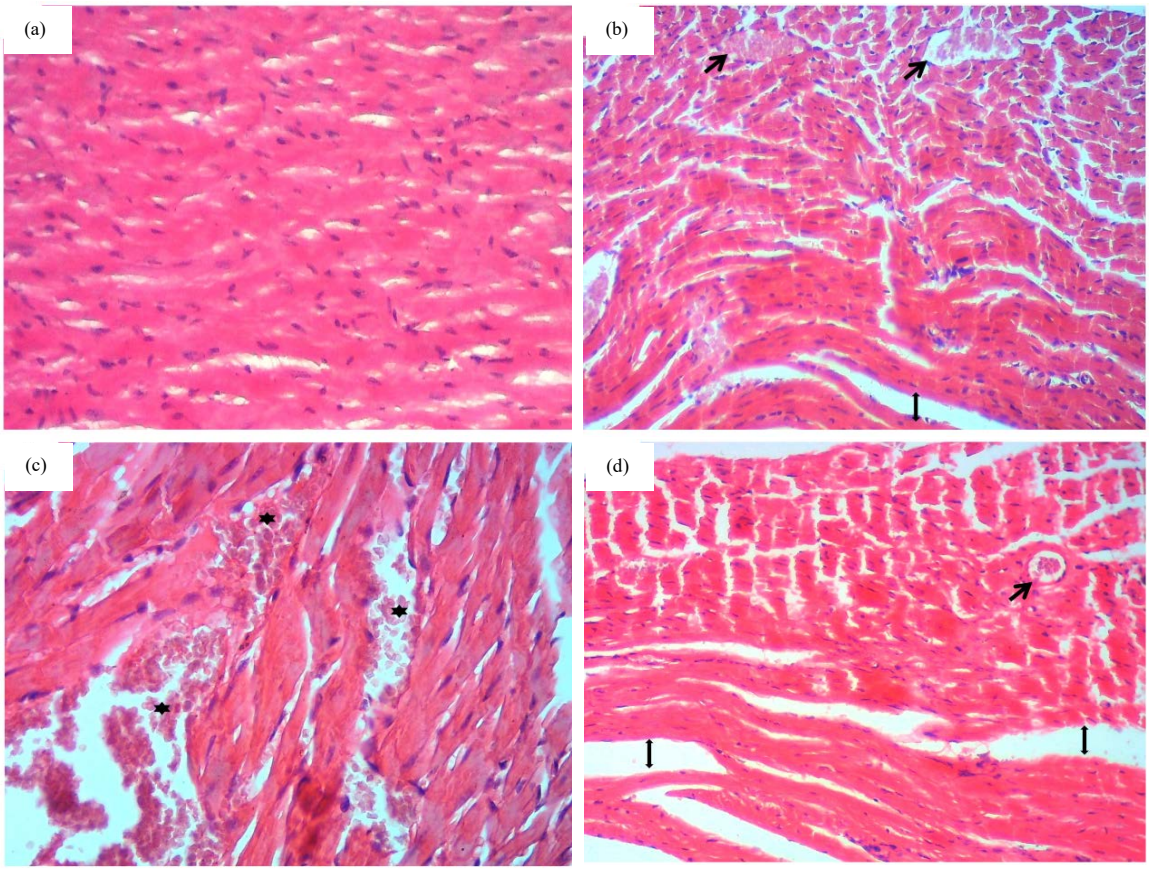

Fig. 2(a-d): Histological sections of exposed mice groups cardiac muscle, (a) Photomicrograph of group 1 showing normal heart architecture, (b) Photomicrograph of group 2 showing irregularly arranged cardiac muscle fibres with central nuclei and congestion of blood vessels (arrow), (c) Photomicrograph of group 2 showing extravasation of RBC (asterisk) and (d) Photomicrograph of group 3 showing congestion of blood vessels (arrow) and wide inter-fibre space between cardiac fibres (block arrow)
Sections were stained with H and E, ×200

DISCUSSION

The intensive development of various EMFs devices that widely used in the transportation, telecommunications, agriculture, medical and military fields, workers at these fields are always exposed to intensive EM radiation. Many studies report concerning some biological effects of EMFs including the nervous, immune, reproductive and hematopoietic systems[14–16]. However, little is known about the effects of X-band radar frequency on the heart female mice. Therefore, this study was designed to determine the effects of EMFs emitted by radar system on the heart tissue and on blood parameters among two different ages of animals. The histological analysis of the heart tissue in exposed group 2 showed irregularly arranged cardiac muscle fibres, congestion of blood vessels and extravasation of RBC combined with wider interfibrous spaces, inflammation and degeneration. While, exposed group 3 showed less alterations such as congestion of blood vessels and wider interfibrous spaces. These findings are in line with Kerimoglu *et al.*[17], who studied the EMF effects of postnatal day 21 of male rats exposed to EMFs. They observed vacuolization and structural impairment of heart tissue, pyknotic nuclei in the muscle fibres and capillary congestion[17]. The histological alterations of heart tissue may suggest the effects of EMFs exposure on the antioxidant defence mechanism, leading to reactive oxygen species (ROS) production which, in turn, may imitate these abnormal responses. A number of studies showed that the exposure to EMFs induced damage in the heart tissue. It is shown that the EMFs generate ROS in biological system[18] which are implicated in many diseases such as neurodegenerative diseases, cardiovascular diseases and inflammation[19,20]. Furthermore, the haematological analysis demonstrated that the exposure to EMF induced a reduction

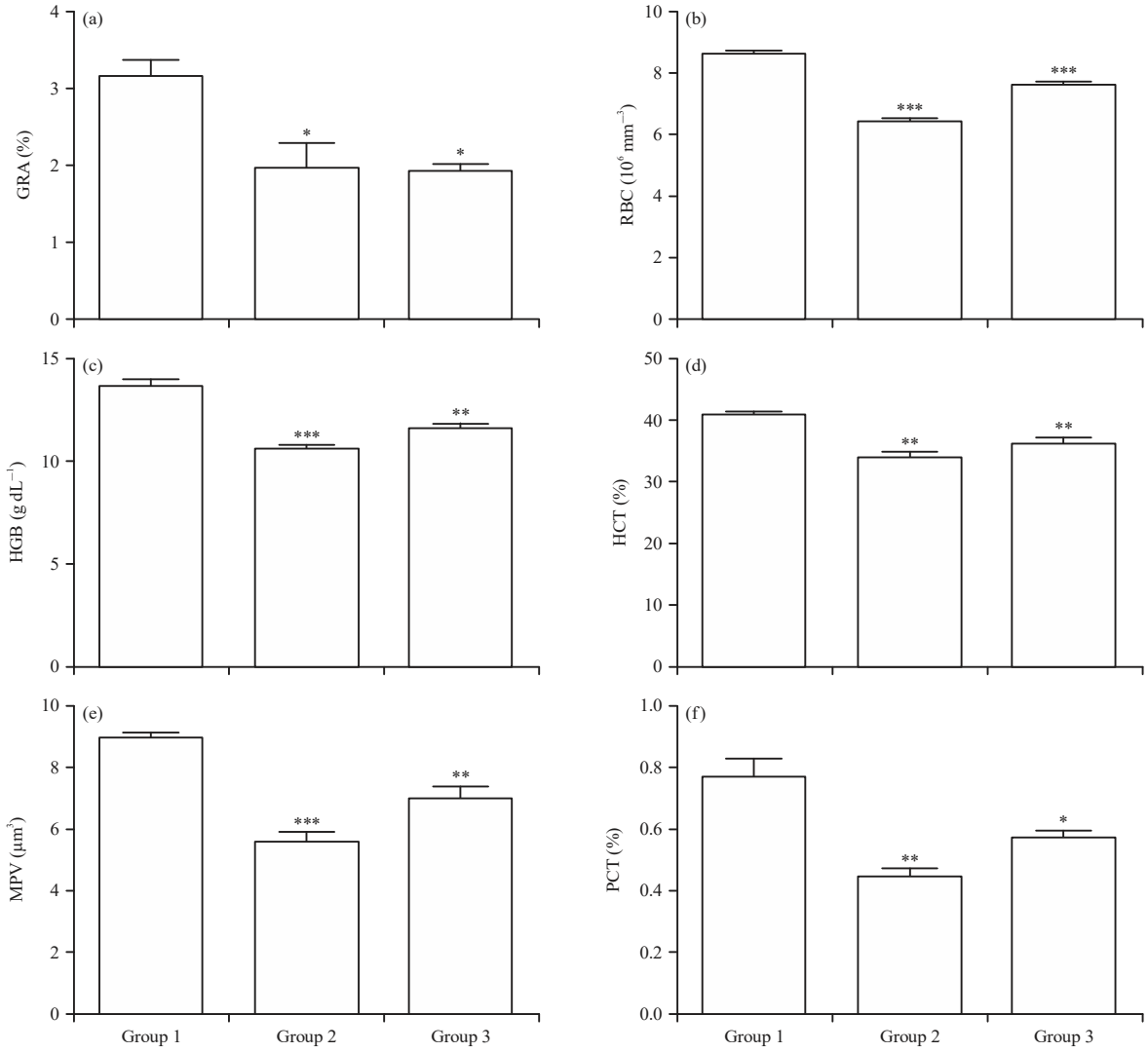

Fig. 3(a-f): Significant effects of EMFs on some haematological parameters in female mice. The graphs show the, (a) Percentage of granulocytes (GRA), (b) Counts of red blood cells (RBC), (c) Concentration of haemoglobin (HGB), (d) Percentage of haematocrit (HCT), (e) Mean platelet volume (MPV) and (f) Percentage of plateletcrit (PCT)

One-way ANOVA, Bonferroni's post-test with error bars representing SEM, *p<.05, **p<0.01, ***p<0.001 vs. unexposed group

in the levels of GRA and RBC. Similarly, Cakir *et al.*[21] found a reduction in eosinophil counts following 50 days and 100 days of EMF exposure. Seto *et al.*[22] demonstrated that a high intensity (80 kV m^{-1}) 60 Hz electric field reduced WBC and eosinophil counts without any effects on RBC and their parameters. Also, The findings showed that the concentrations of HGB significantly decreased in both exposed groups following EMFs exposure and this finding is in agreement with previous study[23]. Furthermore, The results demonstrated that the HCT level decreased after EMFs exposure in both exposed groups compared to the unexposed group and this result is in line with Cabrales *et al.*[24]. They observed that HCT, HGB and neutrophil levels significantly reduced in rats following EMF exposure. The reason for the observed variation in haematological parameters after EMF exposure was due to spleen hyperfunction which increases the rate of destruction of WBC, RBC and platelets[25,26]. In contrast, another study found that HGB levels increased after subacute exposure to EMF[27]. Moreover, in the present study, PCT level also reduced after EMFs exposure in both the exposed groups, PCT reflects alterations in PLT level. Also, the present study investigated that exposure to EMFs reduced MPV in both exposed groups compared to control. The MPV measure the platelet size therefore, it is useful marker to reflect platelet activity and it is associated with different prothrombotic and proinflammatory diseases[28]. For this reason, a reduction in MPV leads to

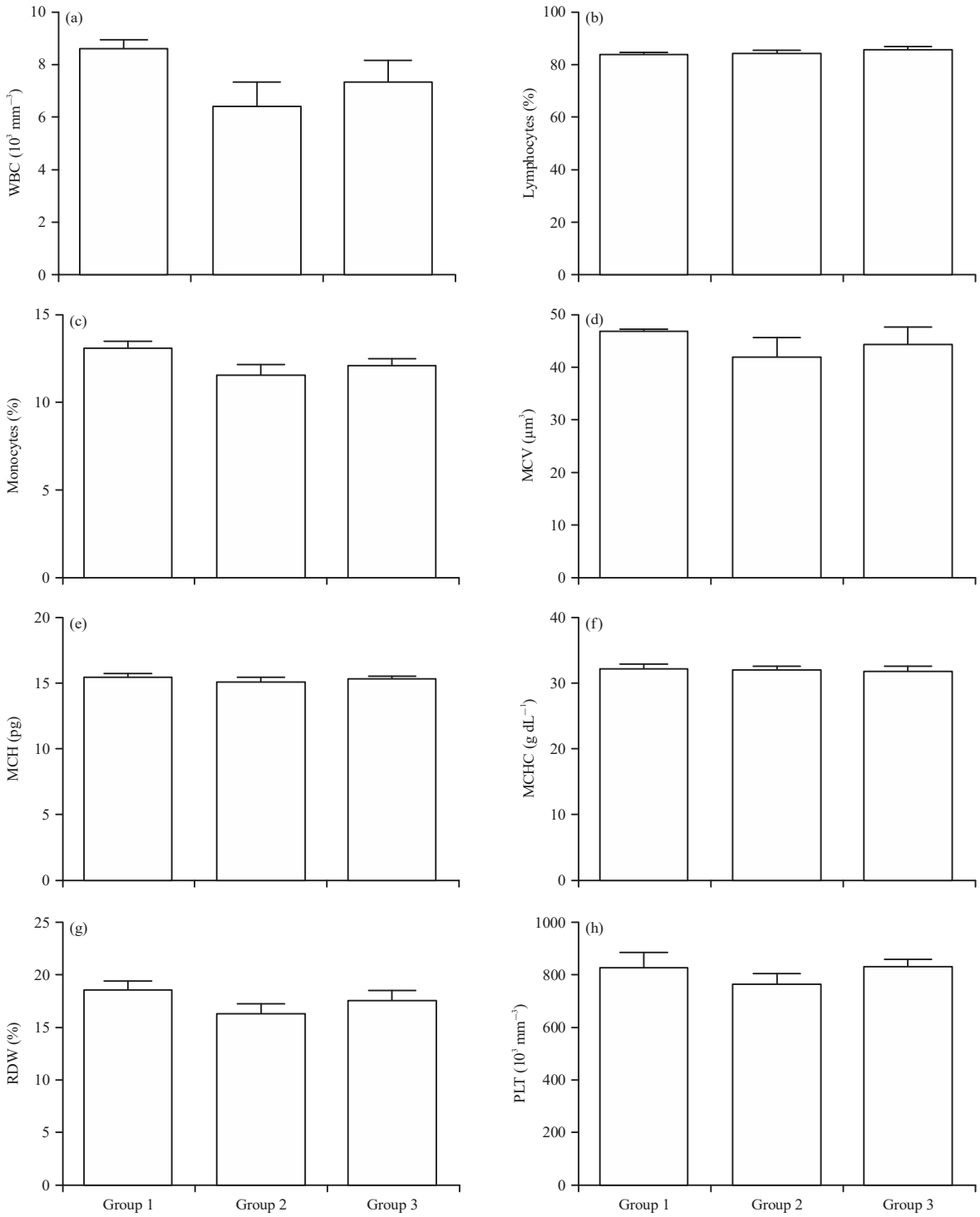

Fig. 4(a-h): EMFs have no significant effects on some haematological parameters in female mice. The graphs show the, (a) Counts of white blood cells (WBC), (b) Percentage of lymphocytes, (c) Percentage of monocytes, (d) Mean corpuscular volume (MCV), (e) Mean corpuscular haemoglobin (MCH), (f) Mean corpuscular haemoglobin concentration (MCHC), (g) Percentage of red cell distribution width (RDW) and (h) Counts of platelets (PLT)

No significance, one-way ANOVA, Bonferroni's post-test with error bars representing SEM

microthrombocyte states in the peripheral blood and thrombocytopenia of bone marrow insufficiency[29]. Additionally, the findings showed that there was a tendency to reduce WBC level in both exposed groups but this did not reach statistical significance. Another research investigated that EMF effects reduce the number of leukocytes[30]. Whereas, Li *et al.*[31] found that WBC and lymphocytes were significantly increased in exposed groups compared with unexposed group but they did not observed any alterations in RBC, HGB and PLT after EMFs exposure.

Interestingly, the histopathological and haematological effects of EMFs were more prominent in group 2 compared to group 3. These effects seem to be consistent with other experimental studies. They found that the pathological effects of EMFs are greater in children than those in adults[17]. Children absorb EMF exposure greater than adults. Also, during the adolescent stage, some organs have not yet reached the anatomical structures of adulthood[32,33]. Looking at the above-mentioned studies, some studies observed alterations in some haematological parameters after exposure to EMFs, whereas others did not observe any changes. The reason for these discrepancies may be due to the variations in exposure setups, duration time of EMF, different frequency and intensities and different investigation targets. The present study demonstrated that EMFs induced heart histopathological and haematological changes in female mice. However, the mechanisms behind the adverse effects of EMFs are not fully understood. Therefore, further molecular studies are needed to investigate the mechanisms underlying the EMFs adverse effects.

CONCLUSION

The present findings show that exposure to EMF causes pathological changes in heart of both exposed groups female mice. Also, EMF exposure induces a reduction in some haematological parameters in both exposed groups. The pathological and haematological changes were more prominent in group 2 compared with group 3.

SIGNIFICANCE STATEMENT

This study determines the possible adverse effect of EMFs on heart histopathological and haematological alterations in female mice. The present study will help the researchers to uncover the precise understanding of EMFs cellular effects. Also, obtained findings, encourage more research in this field to investigate safe usage of electrical devices such as a mobile phone and radar systems that emitted EMFs and the mechanism behind these effects.

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