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Effects of 2.45 GHz Microwave Radiation Exposure on Rat Erythrocytes and Leukocytes

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ABSTRACT

Exposure to microwave (MW) radiation causes a variety of changes in biological systems, including the hematopoietic and immune systems. This study was conducted to evaluate the effects of low-frequency MW radiation on erythrocytes and leukocytes in Sprague Dawley rats. Twelve male Sprague Dawley rats with an average body mass of 250 g were divided into control group (n=6) and exposed group (n=6). Rats in the exposed group were irradiated to 2.45 GHz MW radiation for eight weeks (five days a week, eight hours a day). Blood profile was then analyzed following completion of exposure regime. Findings of this study demonstrated that MW radiation exposure caused a significant increase in the total erythrocyte count, total leukocyte count and total differential count of lymphocyte, neutrophil, monocyte and eosinophil compared to the non-exposed group. In contrast, the basophil count was significantly decreased in comparison to control group. However, the mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were not significantly affected by MW radiation exposure. For blood morphology, neutrophils exhibited an alteration in the cell where degradation of cytoplasm was observed, while lymphocyte, monocyte, basophil and eosinophil were found to be normal after exposure. In summary, exposure to MW radiation affects several haematological parameters and morphological changes which may pose deleterious negative health impacts.

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INTRODUCTION

The world population is now more vulnerable towards microwave (MW) radiation because they are present or emitted in many instruments and devices frequently used in daily routines such as mobile phones and electrical appliances. According to the Department of Statistics Malaysia (2016), the usage of mobile phones the country had increased to 97.5% in the year 2015 from 94.2% in 2013. Apart from mobile phones, other daily usages involving MW radiation are wireless networking technology (Wi-fi) and medical applications such as MW tumour ablation and cancer management.

Exposure to MW radiation can pose serious problems towards human being because they could affect several organs and haematological system. Even at low or high doses, non-ionizing MW radiation exposure can impact metabolic reactions and biological function of cells in the human body (Ghanbari et al., 2016). For example, generation of

heating effects from radiation may cause an alteration in chemical reactions that damage components of white blood cells (Ghanbari et al., 2016). An animal study has also shown that MW radiation exposure cause alteration of some haematological and endocrine parameters in experimental male mice (Aziz et al., 2010).

Existing in vivo studies indicate that exposure to electromagnetic (EM) radiation pose injurious effects on haematological parameters and bone marrow composition. A study by Jelodar et al. (2011) demonstrated a decrease of hematocrit, total leukocyte, erythrocyte and platelet count after irradiation, and these effects are more hazardous in immature animals. Free radicals formed due to exposure of MW radiation can also be hazardous as they cause blood cell membrane modification by lipid peroxidation, causing damage in amino acid residue and forming lipid-protein crosslinks. Several studies have proven that 2.45 GHz MW radiation have an adverse effect on the erythrocyte membrane by causing changes in the erythrocyte osmotic fragility which increases in the degree of hemolysis and also increases the mean corpuscular fragility (Hassan et al., 2010).

A study by Abdolmaleki et al. (2012) indicated that low-frequency EM radiation significantly increases erythrocyte and platelet count, while leukocyte and haemoglobin decrease in comparison with the normal range. This was supported by Aziz et al. (2010) which shows that haematological components are sensitive towards MW radiation and cause degeneration of total lymphocytes. Due to the serious damage, it may cause to human, and the present study aimed to further determine the influence of low-frequency microwave radiation on full blood profile following two months exposure.

MATERIALS AND METHODS

Animal Acclimatization and Irradiation

Prior to animal experimentation, the study has obtained ethical approval from Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH) Animal Ethics Committee (FYP/AEC/MESTECH-UNIKL/2017/014/JULY-2017-SEPT-2017). Twelve adult male of Sprague Dawley rats weighing between 180g to 200g were equally divided into two groups as control and exposed groups respectively. They were acclimatized under standard controlled environment and received standard food pellet and water *ad libitum*, with alternating 12 hours light and dark cycles throughout the experimental period. The irradiation processes were carried out in the Non-Ionizing Radiation Department at Malaysian Nuclear Agency. A Gigahertz Transverse Electromagnetic (GTEM) chamber was used to generate a continuous wave operation that produced 2.45 GHz of MW radiation. The rats were placed in the GTEM chamber and exposed to MW radiation for seven hours a day, five days a week, throughout eight weeks duration.

Blood Sample Collection and Analyses

Following completion of the irradiation process, the rats were anaesthetized using sodium pentobarbital and the blood samples were collected by cardiac puncture technique for haematological analysis. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total leukocyte count were determined by using the Medonic CA530-Hematology Analyzer. Leukocytes differential count was performed according to the standard manual protocol.

To assess morphological changes in leukocytes, blood smears were prepared manually and stained with Wright-Giemsa stain. The stained blood smear slides were observed and examined under 100x magnification of light microscopy.

Statistical Analysis

Analyses were computed using the Statistical Package for the Social Sciences (SPSS) for Windows software, Version 20.0. Independent t-test was used to determine the differences between groups, where p-value <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Total Erythrocyte and Leukocyte Count

The effects of MW radiation on total erythrocyte and leukocyte counts for each group were presented in Figure 1. The results showed that the total erythrocyte count 7.51±0.32 (10^{12}/L) and leukocyte counts 8.65±0.54 (10^{12}/L) were significantly higher ($p<0.05$) in the exposed group compared to control group; 6.71±0.71 (10^{12}/L) and 5.90±1.71 (10^{12}/L) for erythrocyte and leukocyte counts respectively.

The result for leukocyte count is in agreement with previous studies by Rifat et al. (2014) and Aziz et al. (2010) which shows a significant increase in total leukocyte and lymphocytes of irradiated animals (10 GHz and 900 MHz respectively) compared to control group.

The immune system of animals exposed to MW irradiation will show a response that causes a rise in leukocyte count. In contrast, Aweda et al. (2010) found that neutrophil value did not vary significantly as the effect of MW exposure merely reduced the value from the control. Our results also differ to findings of Rifat et al. (2014) and

Aziz et al. (2010) in total erythrocyte count whereby the total number recorded in this study are increased in the exposed group compared to control. These varied results found in the literature were probably due to the differences in the frequency and duration of radiation exposure.

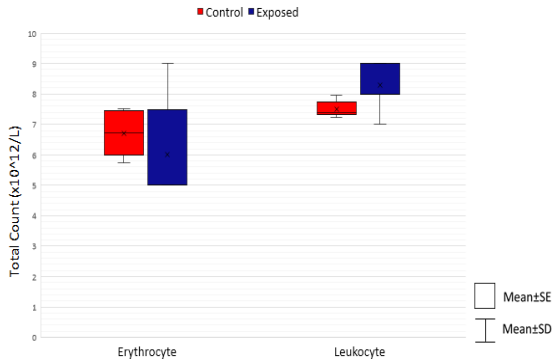

Figure 1: Total erythrocyte and leukocyte count in control and exposed groups

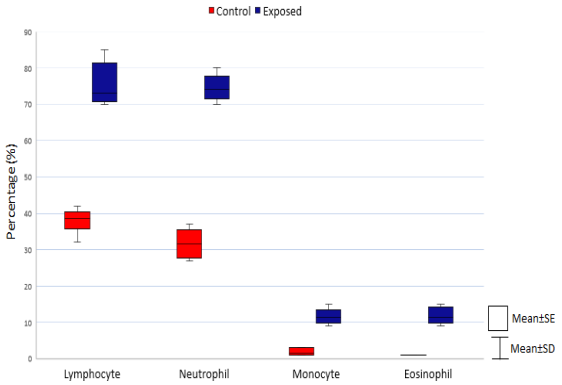

Figure 2: Total leukocyte differential count in control and exposed groups

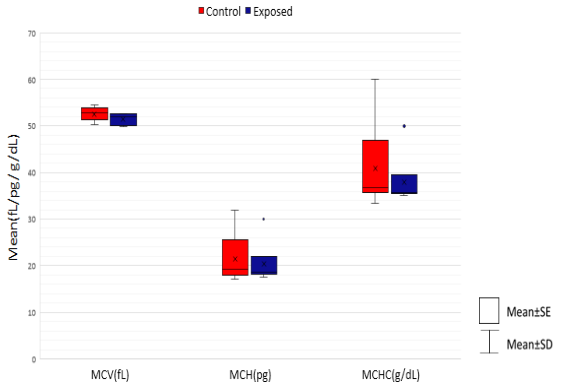

Figure 3: MCV, MCH and MCHC in control and exposed groups

MCV, MCH and MCHC

Results shown in Figure 2 revealed that there were no significant differences in MCV, MCH and MCHC between exposed and control group. These findings were in contrast with the study conducted by Rifat et al. (2014) that showed MCV and MCH were significantly decreased while MCHC were generally significantly increased in response to electromagnetic field exposure as compared to the control group. The contrasting results by Rifat et al. (2014) could be due to the higher frequency and shorter exposure time of MW radiation.

Total Differential Count

MW radiation exposure caused significant increases ($p<0.05$) in total differential count of neutrophil (75.50±4.20), eosinophil (11.50±2.46), lymphocyte (77.75±6.07) and monocyte (11.50±2.46) compared to the control group; neutrophil (31.67±3.98), eosinophil (1.00±0.00), lymphocyte (38.00±3.40) and monocyte (1.83±0.98) as shown in Figure 3. Basophil count showed no significant difference ($p>0.05$) between both irradiated and non-irradiated rats with a value of (0.50±0.57) and (1.50±0.55), respectively.

Findings of this study correspond with the results obtained by Rifat et al. (2014) that demonstrated a rise in the amount of lymphocytes due to MW exposure which initiated the hematopoietic system to discharge more lymphocyte in the bloodstream. The study by Aziz et al. (2010) also revealed a similar outcome where the increase of lymphocyte count happened as a consequence of radiation exposure along with the intensification of temperature and counteraction of body immune system.

Koyama et al. (2015) demonstrated elevation of neutrophil after human HL-60 cells were exposed to radiofrequency as opposed to the group without radiofrequency exposure. This presents a clear indication that MW radiation caused an inflammatory response in cells as well elevation in neutrophil and lymphocyte count to fight against harmful effects of MW radiation.

Monocyte count is also found to be elevated in response to MW radiation. According to David et al. (2008) monocyte assembles slower but continuous elevated compared to neutrophil with the presence of Fc receptor which only reacts as inflammatory stimuli. Contrary to total basophil count and morphology revealed unaffected due to the absence of helminth and allergic response. According to Mukai and Galli (2013), basophil was accountable for agitating reactions during immune response also in the development of acute and chronic allergic diseases.

Morphological Changes

Neutrophil morphology (Figure 4) observed in the control group showed the presence of a normal

nucleus which consists of two to five lobes joined together with well-formed cytoplasm. In contrast, degradation in colour of cytoplasm was demonstrated in the neutrophil of exposed group. This outcome corresponds with a study by Dabo and Songden (2014) that demonstrated degradation of cytoplasm with unclouded granules after sixty days of irradiation on Sprague-Dawley rats. It is hypothesized that constant MW exposure caused loss of sensing signal along with dysfunction of the surface receptor in neutrophil that led to an elevation in neutrophil count. Both groups also exhibited normal morphology for lymphocyte (Figure 5) where large nucleus was stained with dark purple and spherical to slightly kidney-shaped, as well as for monocyte (Figure 6) where the nucleus was slightly indented in shape. This is however in contrast to the research done by Chandra et al. (2011) which revealed that exposure to electromagnetic radiation can initiate lymphocyte to become lymphoblast. This might be because the absorbed energy by cells is inadequate to cause interference to the morphology of lymphocytes in this study. The morphology of monocyte also revealed normality although the monocyte count of irradiated rats was altered. A study by David et al. (2008) claimed the difference in granules associated protein, as well as lagging during accumulation of monocyte, enlighten the apprehension of undamaged monocyte in comparison to neutrophil. Moreover, the characteristic and beneficial properties (less metabolic burst) of monocyte differentiation itself make it less susceptible to alteration by MW exposure. Figure 7 illustrates the normal morphology of basophils observed in both groups showing dark blue or purple granules overlying the nucleus. This might be due to the insignificant amount of basophil among white blood cells which are mainly involved in the allergic reaction and parasite infection thus unaffected by microwave radiation. Finally, eosinophil (Figure 8) showed round-oval in shape with two-lobed nuclei consistent with normal morphology.

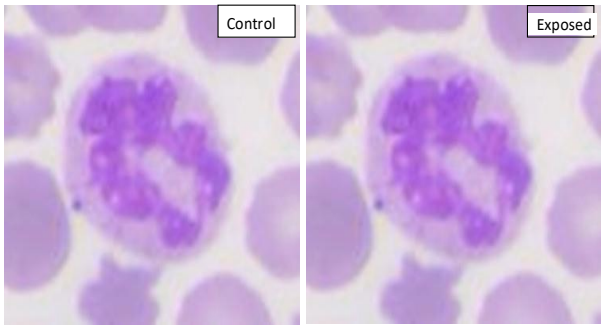

Figure 4: Neutrophil cell in control and exposed groups under 100x magnification of light microscopy

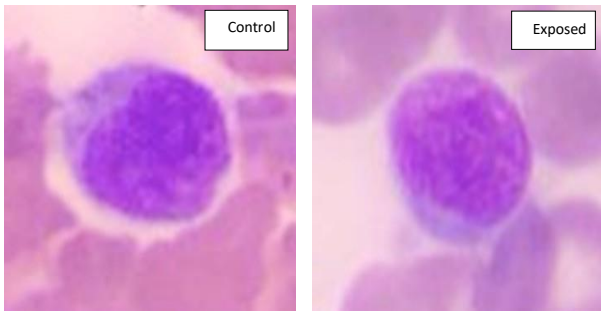

Figure 5: Lymphocyte cell in control and exposed groups under 100x magnification of light microscopy

Figure 6: Monocyte cell in control and exposed groups under 100x magnification of light microscopy

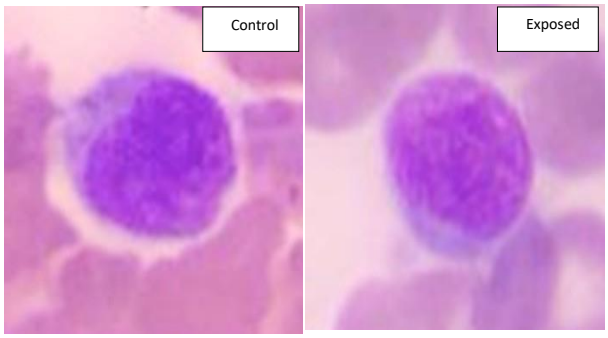

Figure 7: Basophil cell in control and exposed groups under 100x magnification of light microscopy

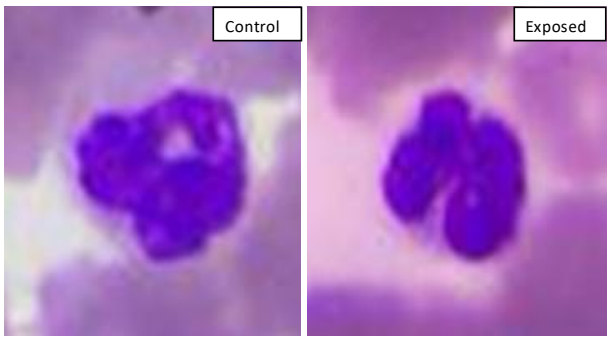

Figure 8: Eosinophil cell in control and exposed groups under 100x magnification of light microscopy

CONCLUSION

The results of this study demonstrated that 2.45 GHz MW exposure to rats throughout eight weeks experimental protocol affects the peripheral blood parameters (erythrocytes and leukocytes) and neutrophil morphology.

Acknowledgements

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