Progress In Electromagnetics Research B, Vol. 36, 69–87, 2012

MICROWAVE EFFECT OF 0.9 GHZ AND 1.8 GHZ CW FREQUENCIES EXPOSED TO UNRESTRAINED SWISS ALBINO MICE

A. D. Usman[1, *], W. F. Wan Ahmad[1], M. Z. A. Ab Kadir[1], M. Mokhtar[2], and R. Ariffin[3]

[1]Department of Electrical and Electronic Engineering, Faculty of Engineering, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of Computer & Communication Engineering, Faculty of Engineering, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Faculty of Electrical Engineering, Universiti Teknologi Mara (UiTM) Pulau Pinang, 13500 Permatang Pauh, Pulau Pinang, Malaysia

Abstract—Long term radio frequency electromagnetic field (RF EMF) exposures due to Global System for Mobile communication (GSM) frequencies were investigated in this study. 158 Swiss Albino mice in unrestrained conditions were used as surrogate and divided into four groups. The average peak field strength generated and measured inside the cages placed at a far field from the antennas is 0.6×10^{-3} mW/cm^2, and the specific absorption rate at 0.9 GHz and 1.8 GHz is 2.33×10^{-3} W/kg and 1.97×10^{-4} W/kg, respectively. Three samples of the mice chosen at random each from sham and exposed groups in week 4 and subsequently biweekly basis were taken for haematology and histopathology tests. The complete blood count result shows that haematological parameters of both the sham exposed and exposed mice were within the normal range of mice in the control group. A statistical analysis was conducted to determine whether differences observed between the experimental groups were significant. The histopathology examination on some internal organs shows that spleen and bone marrow of the mice were normal for all the three experimental groups, while a sign of tissue degeneration and inflammations were observed after 8 weeks of exposure on the brain,

Received 27 July 2011, Accepted 18 October 2011, Scheduled 3 November 2011

* Corresponding author: A. D. Usman (aliyu_d_usman@yahoo.com).

liver and lungs of the mice in the exposed groups. These signs increase in severity with prolonged exposure.

1. INTRODUCTION

The increased usage of electromagnetic (EM) principles for domestic and industrial purposes proves that EMF plays an important role in our daily life. The emergence of telecommunication services using EMF principles greatly enhance the ability of individuals and groups to communicate with each other. Nowadays, phones are not only used for making and receiving calls but also for many other applications, such as banking transactions and web browsing. The world economic boom also benefitted from these technologies, the current worldwide economic crises have little or no significance to the mobile industries. According to International Telecommunication Union (ITU), the number of mobile phones subscribers in the world was estimated to about 4.6 billion in 2009, and it was expected to reach 5 billion by 2010 [1]. This means that around one out of every two individuals in this world carries a mobile phone. The increased usage and growing popularity of wireless technologies in RF EMF range represent one of the fast growing environmental influences. This usage is not without a lot of controversy and public concern on the possible adverse health effect associated with the energies emitted by these technologies [2–4].

Though regulations were provided as in [5, 6] which are aimed at safeguarding the general public from dangers associated with the usage of these facilities, people still nurture fears due to the fact that more and more technologies emitting EMF are now in our midst. This emitted EMF is capable of altering the biological function of the body. The cells of living organisms naturally maintain an electrical charge across their membranes that are essential for normal functioning of human tissues. This is extremely sensitive to very week EMF because RF EMF can rearrange and damage molecules and alter metabolism. A process of chain like reaction will firstly alter the organisms stability and affect cell polarisation. This resulting disharmony may eventually lead to changes in cell activity, affect the synthesis of genetic material and alter with the flow of substances in and out of cells [7].

A lot of cutting edge studies were performed aimed at building public confidence on the usage of these technologies. Researches on health effects of RF EMF are quite numerous and diverse. It cuts across many disciplines of engineering, physics, biology and medicine. This emerging technology to date has witnessed a lot of research papers with many contradictions. Most significant researches were done using

mice exposed to either GSM frequencies or frequencies around that of GSM as in [8–17]. Research on haematological parameters in [18] reported that thermal effects of RF EMF was capable of increasing neutrophils and decreased the levels of lymphocytes. An increase in WBC with prolongs exposure in addition to significant difference observed between 970 MHz continuous wave and sham exposed group in monocyte was reported by [19]. Some of these researches uses animal in restrained position where animals do not have access to water or food during exposure. This restrained position is reported causing stress related changes in [17, 20] and imposes constraints on behavior and brain development of the animals [21]. The study of long term effect in this condition is also not appropriate as no exposures per day and per week will be identical leading to serious comparative difficulties as reported in [16, 22, 23].

Though a lot of studies were conducted most especially on the way towards having a clear understanding of the effect or no effect of RF EM exposure, the exposure period per day for the long term investigations were short, and the results have been contradictory. This work, therefore, investigates the long term microwave exposure effect due to 0.9 GHz and 1.8 GHz frequencies on haematological parameters using unrestrained Swiss Albino mice as surrogate.

2. MATERIAL AND METHOD

The study was designed to investigate long term RF EMF exposure effect due to GSM frequencies using unrestrained Swiss Albino mice. Animal care and handling was carried out according to Malaysian animal handling code of conduct adopted from National Research Council guide for the care and use of laboratory animals [24]. The RF EMF design and exposure set-up were done following European electronic communication commission (EEC) protocol [25]. Male Swiss Albino Mice (Mus Musculus) aged about 4 weeks on arrival and weighing between 34 g and 45 g were obtained from Veterinary Research Institute (VRI) in Ipoh, Perak, Malaysia. A total of 158 mice were used for this study where the mice were divided into 4 groups of control, sham, and 0.9 GHz and 1.8 GHz groups. 50 mice were used for the control group and were housed in two cages of 25 each and kept at VRI for the determination of reference values. The remaining 108 mice were randomly selected and divided into 3 experimental groups of 36 mice each. The mice in each group were further selected randomly and housed in 2 cages of 18 mice each and were allowed to acclimatize for a week before the exposure started according to the suggestion by [26–28]. The cages were made of polyethylene, and the dimensions

were 0.6 m × 0.42 m × 0.24 m. The floor is a removable type covered with wooden chips bedding (Living World Shavings, Rolf C. Hagen, Holand). This cage is capable of accommodating 25 unrestrained mice according to [24]. Hence, with only 18 mice in each cage of the experimental groups, the mice were able to move around freely and to prevent stress related changes as observed in [17]. Both the sham exposure and the RF exposure groups' ambient temperature and humidity throughout the experiment were maintained at $27 \pm 2°$C and $65\% \pm 5\%$, respectively. The set-ups were placed in an environment shielded from EMF sources where ferrite tile absorbers were used to prevent ground reflection and interference from other sources. The water bottle and food container were made of plastics and assorted mixed food (Habitrail, Rolf C. Hagen Inc., Canada) ,and water was available to the mice ad libitum. Wooden chips beddings were used to supply comfort and absorb mice waste. The beddings were changed regularly to avoid infection. Both the food and the beddings were obtained from Toby pet shop at 272, JLN Air Itam, 11400 Penang — Malaysia.

GSM like frequencies of 0.9 GHz and 1.8 GHz were used for the two exposed groups, while the sham exposed group has similar set up with the exposure turn off. Two signal generators (R & S SMB1000A) with frequency range of 9 KHz to 2.05 GHz and a resolution of 1 Hz were used and connected to a directional antennas (Panel antenna of 7.5 dBi and beam width of 65° for 0.9 GHz, and 14 dBi gain and a beam width of 35° for 1.8 GHz all from Huber + Suhner) to provide the required exposure signal for 0.9 GHz and 1.8 GHz groups. FSL spectrum analyzer (R & S FSL6) with frequency range of 9 kHz to 6 GHz was used to measure the received signal strength at 20 different positions inside the cage and the average of the peak signal found. This was repeated after 4 weeks of exposure and subsequently biweekly after taking out some samples from the cage for examination. The cage were place at far field distance using far field equation parameters shown in Equation (1), where R is the distance from the source to the far field, D the largest dimension of the source antenna, and λ the wavelength of the transmitted signal.

$$R = \frac{2D^2}{\lambda} \tag{1}$$

The far field approximate distances were found to begin at 0.7 m and 1.4 m for 0.9 GHz and 1.8 GHz, respectively. The average peak field strength generated and measured inside the cages with the spectrum analyzer is 0.6×10^{-3} mW/cm^2. This however, corresponds to the typical field strength that we obtained during the GSM base stations site surveys conducted at far field distances in Malaysia.

An empirical formula for calculating the average SAR of an irradiated object over broad range for prolate spheroidal models of human and animals developed by [29] using a combination of antenna theory, circuit theory and curve fitting was used.

The average SAR for incident power density of 1 mW/cm^2 and E polarization with semi major axis a and semi minor axis b, in meters is shown in Equation (2), where A_1, A_2, A_3, and A_4 are functions of a and b, A_5 is a function of ε, the permittivity of muscle and ε_{20} is the permittivity at 20 GHz.

$$\begin{aligned}&\text{SAR(W/kg)}\\&= \frac{A_1 f^2/f_0^2\left[1+A_3(f/f_0)u(f-f_{01})+A_4A_5(f^2/f_0^2)u(f-f_{02})\right]}{10^3 f^2/f_0^2 + A_2(f^2/f_0^2-1)^2}\end{aligned} \quad (2)$$

The unit function $u(f-f_{01})$ is defined by

$$u(f-f_{01}) = \begin{cases} 0, & f<0 \\ 1, & f_{01}\geq 0 \end{cases}, \quad u(f-f_{02}) \text{ is similarly defined.}$$

The average length is $2a$, and b is defined in Equation (3), where V is the volume.

$$b = \sqrt{\frac{3V}{4\pi a}} \quad (3)$$

The resonant frequencies f_0, f_{01}, f_{02} and A_1, A_2, A_3, A_4 and A_5 are defined in Equations (5.2) to (5.9) in [29]. This equation allowed computation of the frequency dependant normalized SAR of mouse appropriate for estimating the average SAR for free moving mouse. Resonant frequencies f_0, f_{01}, and f_{02} were found to be 683 MHz, 1661 MHz and 103 MHz, while a and b were calculated to be 0.095 m and 0.00984 m, respectively. Also, A_1, A_2, A_3, A_4 and A_5 were calculated to be 5562.23, 1743.45, -0.278, 0.05 and 0.90, respectively.

The average SAR for an incident power density of 0.6×10^{-3} mW/cm^2 at 900 MHz and 1800 MHz, using Equation (2), is 2.33×10^{-3} W/kg and 1.97×10^{-4} W/kg, respectively.

The exposure was conducted for 7 hours/day, 7 days/week and for 12 weeks. Three mice samples chosen at random from each of the 2 exposed and the sham exposed groups were taken to VRI after 4 weeks of exposure and subsequently on a biweekly basis. Tests were conducted in haematology and histopathology laboratories. On arrival, the mice were anesthetized and exsanguinated. Blood samples were collected in a test tube containing anticoagulant (EDTA) tubes and sent for haematology test. A statistical analysis was conducted aimed at comparing the sham exposed and exposed group's haematological parameters as well as to determine if the observed deference is

significant or not with prolonged exposure. The comparisons were done each time the blood was collected from the sample mice. Analysis of variance (ANOVA) test was carried out using Statistical package for social science (SPSS) version 18. The null hypothesis was rejected at $p < 0.05$.

A complete blood count (CBC) that gives information about the cells in the mice blood was conducted. A CBC consists of the total red blood cells (RBC $\times 10^{-6}$ µl), haemoglobin (Hg g/dl), hematocrit or packed cell volume (PCV%), white blood cells (WBC $\times 10^{-3}$ µl) and RBC indices, i.e., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also investigated.

The blood collected from the mice in EDTA samples was put on a soft rolling machine to obtained homogeneous mixture of the blood. Blood parameters were identified using both VET ABC Machine and smear method. The smeared slide is put on a dryer for 5 minutes. A methanol is then dropped on the smeared glass to ensure that the cells were attached firmly on the slide. Then, 8% giemsa and 92% buffer solutions were used for staining the slides. The slides were soaked in the mixed chemicals for 45 minutes, and then rinse using a tap water and dried. Microscopic examinations were then performed on the dried slides.

The sample mice after exsanguinations and collecting of the blood were subjected to surgery, and 5 organs were removed for histopathology examination. The organs of interest in this study were the brain, liver, spleen, lungs, and bone marrow. The removed organs from the mice are placed in a fixative of formaldehyde. The organs were later taken out from the formaldehyde and put under running tap water to remove the formalin, while the bone marrow was passed into formic acid for decalcification. The organs were then cut into small pieces and put in cassettes and cassettes rack ready for tissue processing. The tissues were then embedded in paraffin, and sectioned using microtome and then stained with hematoxylin and eosin. The stained slides were covered with thin piece plastics and passed through the reverse process so that it went through from paraffin section to water and observation using the microscope.

3. HAEMATOLOGY RESULTS AND DISCUSSION

Figure 1 shows the mean PCV results obtained from the 3 experimental groups after 12 weeks of exposure. PCV normal range of Swiss albino mice obtained from the control group is 15.3 to 56, and the average is 48. From the graph, the sham exposed mean PCV in week 4 is 39.4,

and at the end of week 12 it increases to 43.6. The PCV value of 0.9 GHz group in week 4 is 35.1, and this is increased to 41.1 in week 12. The mean PCV of 1.8 GHz group was determined as 35.2 in week 4 and increased to 37.6 in week 12. The PCV results are found to be lower than that of the sham exposed group. It is also observed that there is slight increase of PCV with prolonged exposures.

The normal range for WBC values was 1.2 to 10.5, and the average value was 5.6. Figure 2 shows the WBC results obtained for the 2 exposed groups and the sham exposed group. From the results the sham exposed WBC mean values is 4.6 in week 4 and 2.9 in week 12. WBC for 0.9 GHz group in week 4 is 7.3 and decreased to 3.5 in week

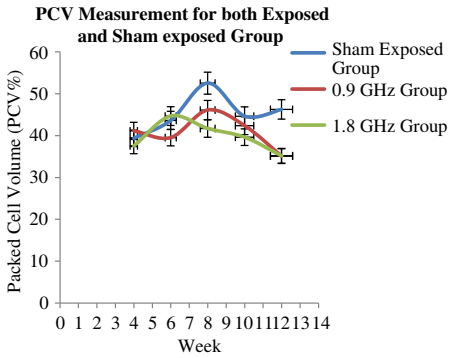

Figure 1. Mean PCV values for exposed and sham exposed groups.

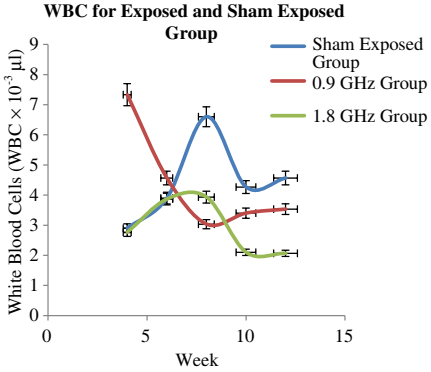

Figure 2. Mean WBC values for exposed and sham exposed groups.

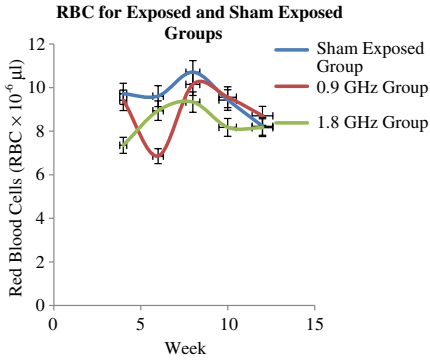

Figure 3. Mean RBC values for exposed and sham exposed groups.

12. The mean value of WBC for 1.8 GHz group is 2.8 in week 4 and decreased to 2.1 in week 12. The graph of 0.9 GHz and 1.8 GHz groups shows a decrease in WBC after prolonged exposures.

Figure 3 shows the results obtained from RBC count. The normal range from the control group is 2.8 to 10.93, and the average is 9.3. From the results, sham exposed mean RBC values measured in weeks 4 and 12 are 8.2 and 9.7, respectively. The RBC values measured in weeks 4 and 12 for 0.9 GHz exposed group were 9.4 and 8.7, respectively. The 1.8 GHz mean RBC values in weeks 4 and 12 are 7.4 and 8.2, respectively. There is an increase of RBC with prolongs exposure in sham and 1.8 GHz exposed groups, while 0.9 GHz exposed group is on the contrary.

The Hb normal values range from 5.3 to 49.1, and the average is 15.5. Figure 4 shows the results obtained of Hb for exposed and the sham exposed groups. The sham exposed group mean Hb measured in weeks 4 and 12 were 13.5 and 13.8, respectively. The mean Hb for 0.9 GHz exposed group measured in weeks 4 and 12 were 12.1 and 12.8, respectively. Mean values of Hb for 1.8 GHz group measured in weeks 4 and 12 are 12.4 and 11.4, respectively. There is a slight increase of Hb values in sham exposed and 0.9 GHz groups while 1.8 GHz is on the contrary.

Figure 5 shows the results obtained for MCV. The reference values range from 15.4 to 59.8, and the average is 52. Sham exposed mean MCV in weeks 4 and 12 are 48.0 and 45.3, respectively. Mean MCV of 0.9 GHz in weeks 4 and 12 were found to be 41.4 and 47.2, respectively. 1.8 GHz group mean MCV found in weeks 4 and 12 are 48.0 and

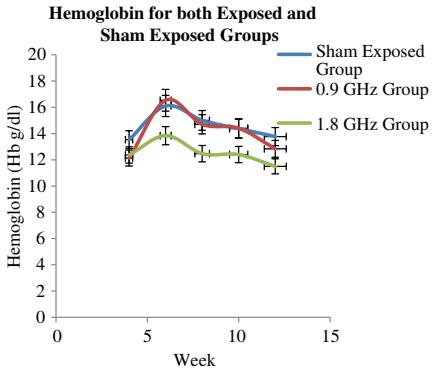

Figure 4. Mean Hb values for exposed and sham exposed groups.

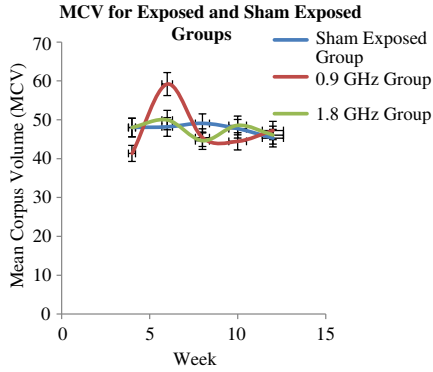

Figure 5. Mean MCV values for exposed and sham exposed groups.

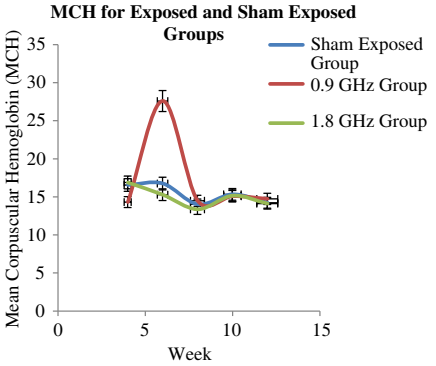

Figure 6. Mean MCH values for exposed and sham exposed groups.

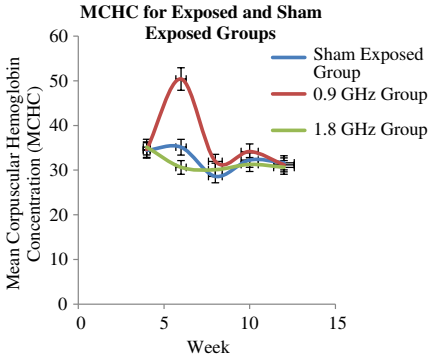

Figure 7. Mean MCHC values for exposed and sham exposed groups.

46.0, respectively. 0.9 GHz group shows an increase in MCV with prolonged exposure, while 1.8 GHz and sham exposed groups were on the contrary.

The MCH normal values range from 13.4 to 49.4 and the average being 16.8. Figure 6 shows the exposed and the sham exposed results of MCH. Mean MCH of sham exposed group in weeks 4 and 12 were found to be 16.6 and 14.2, respectively. The mean MCH for 0.9 GHz group in weeks 4 and 12 are 14.3 and 14.7, respectively. 1.8 GHz group mean MCH measured in weeks 4 and 12 are 16.9 and 14.1, respectively. The sham exposed and 1.8 GHz group show a decrease in MCH with prolonged exposure while 0.9 GHz group is on the contrary.

Figure 7 shows the MCHC results obtained for both exposed and sham exposed group. The MCHC normal values range from 28.4 to 320, and the average is 37. The sham exposed mean MCHC measured on week 4 and week 12 were 34.4 and 31.6, respectively. Mean MCHC for 0.9 GHz group measured in weeks 4 and 12 are 34.6 and 31.2, respectively. The mean MCHC values found in weeks 4 and 12 for 1.8 GHz group were 35.2 and 30.6, respectively. The MCHC values of both sham and exposed groups decrease with prolonged exposure.

Though, there exist some variations in the results obtained from the exposed groups as compared either to sham exposed or normal values. In general, the results obtained in complete blood count for both the sham and the exposed groups were found to be within the normal range of the Swiss Albino mice normal values.

Blood and blood parameters are believed to be one of the primary particles that come in contact with RF EMF. Blood being ions are likely to react with induced EMF generated by EMF charges.

Researches on interactive ability of field generated by GSM frequencies with blood cells were also contradictory. While some reports indicated that RF EMF might have effects on some blood parameters and immune systems of mammals depending on power densities, others are on the contrary.

The haematology results obtained due to long term exposure of Swiss Albino mice to RF EMF in this study causes an increase in PCV, RBC and Hb values with prolonged exposure in all the exposed groups. However, MCHC and WBC show a decrease with prolonged exposure in all the exposed groups. Also, comparison between the groups shows that the results obtained in Hb values of mice in 0.9 GHz exposed groups increase with prolonged exposure, while mice in 1.8 GHz exposed group is on the contrary. WBC values of 0.9 GHz and 1.8 GHz exposed groups were found to decrease with prolonged exposure. MCH values of mice in 1.8 GHz exposed group decreases with prolonged exposure while mice in mice in 0.9 GHz exposed group increases. MCV values of mice in 0.9 GHz exposed group increased with prolonged exposure while mice in the 1.8 GHz exposed group are on the contrary. These results are in agreement with previous studies done by [16] which reported that long-term intermittent exposure to EMF can enhance the probability that mice carrying a lymphomagenic oncogene will develop lymphomas. Significant decrease was also recorded related to RBCs, HB, and HT, MCV and MCH compared to the control group. The works of Mcree [30] and Roberts et al. [31] reported that several hematological parameters are sensitive to RF/MW exposure, not only in animals, but also in humans. A study conducted by [32] where mice were exposed to 2.45 GHz CW microwave frequency at 2 hrs/day for 30 days reported alteration in blood pictures among others.

Some of the parameters investigated in [33] also show a significant increase in some blood parameters like WBC, MCHC and blood platelets compared to control group and a significant decrease in RBC, HB, MCV and MCHC. Also exposure of 0.65 GHz microwave radiation on white mice done for 7 months reported in [12] observed decrease in RBC after the fifth month of exposure. These observations are in line with findings of this work where 0.9 GHz exposed group RBC decreases with prolongs exposure. An increase in RBC and PCV values reported in [14] after mice exposure for 2 weeks on work days only for the duration of 2 hours per day also support the findings of this investigation where RBC of 1.8 GHz exposed group, and PCV values of all the exposed groups were found to increase with prolonged exposure.

3.1. Statistical Analysis

ANOVA test was used to find out if the deviation of the haematological parameters of exposed groups is significant when compared to the sham exposed group. Also to find out if differences between 0.9 GHz and 1.8 GHz exposed groups are significant at 0.05 level of significance. Post hoc analysis were performed for ANOVA test results that show significant difference.

The mean PCV values of the three experimental groups were not significant in weeks 4, 6, 8 and 10. However, a significant difference in PCV values of 0.9 GHz and 1.8 GHz exposed groups was found in week 12.

There is no significant difference observed in weeks 4 and 6 among the three experimental groups in WBC values. However, a significant difference were obtained between the WBC values of sham and 0.9 GHz, sham and 1.8 GHz, and between 0.9 GHz and 1.8 GHz exposed groups in weeks 8, 10 and 12, respectively.

The differences observed among the three experimental groups for RBC values in weeks 4, 6, 10 and 12 were not significant. However, significant difference was found between the RBC values of sham and 1.8 GHz group exposed group.

For Hb values, no significant difference was obtained in weeks 4 and 6. However, a significant difference was found in weeks 8, 10 and 12 between sham and 1.8, and between 0.9 GHz and 1.8 GHz exposed groups. No significant differences were found for MCV, MCH and MCHC values of the three experimental groups. From the statistical results, it is shown that some of the differences observed within the experimental groups were significant with prolonged exposure. This shows that though haematological parameters were within the normal values, and there is the possibility that with prolonged exposure the deviation of these parameters may be well below or above the normal values and can constitute serious hazard.

4. HISTOPATHOLOGY RESULTS AND DISCUSSION

The mice samples of both the sham and exposed groups after exsanguinations were subjected to surgery where five organs of the mice were removed for histopathological examination. The organs of the sham exposed group were found to be normal throughout the period of exposure. The exposed groups histopathology examinations of the 5 organs were also normal within the first 8 weeks of exposure. However, three signs begin to emanate after week 8 of exposure in both 0.9 GHz and 1.8 GHz RF exposed groups. The signs were that of cell degeneration, inflammation and abscessation of lung, liver and

the brain tissues while the spleen and the bone marrow were normal. Figures 8 to 12 shows the normal spleen, bone marrow, brain tissue, lung and liver obtained from histopathology of sham exposed group. The cell degeneration of brain, liver and lungs tissues was shown in Figures 13 to 15.

The changes observed may be due to the fact that RF EMF exposure is capable of raising the temperature of a body which will in turn results in the formation of free radicals. These free radicals are capable of attacking ions in the body thereby changing their nature and breaking the protein bonds, i.e., causing cells damage. The cells degeneration observed in this experiment is similar to the findings in [34] where it is shown that histopathological studies of low frequency EMF affect liver, testis and kidney of guinea pig. The finding in [35], corroborated our results where it was reported that increased level

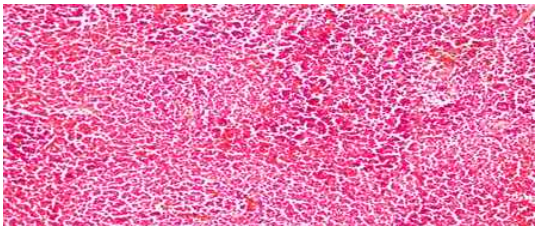

Figure 8. Normal spleen tissues of mice in sham exposed group.

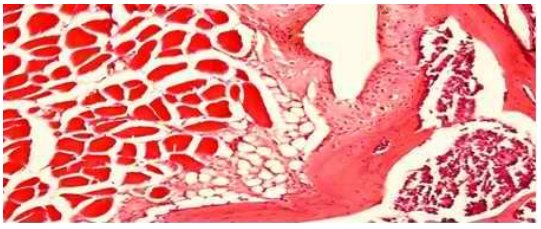

Figure 9. Normal bone marrow tissues of mice in sham exposed group.

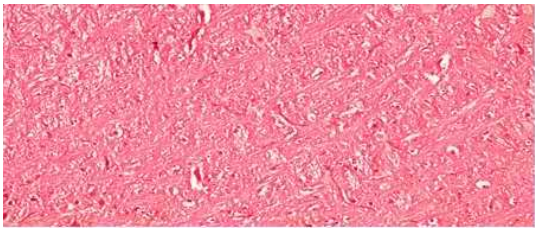

Figure 10. Normal brain tissues of mice in sham exposed group.

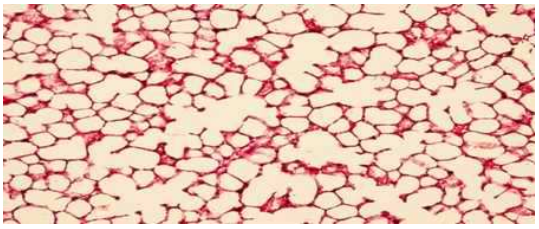

Figure 11. Normal lung tissues of mice in sham exposed group.

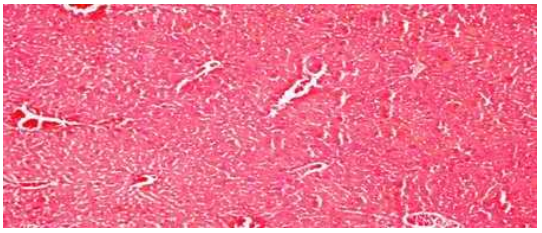

Figure 12. Normal liver tissues of mice in sham exposed group.

of lipid peroxidation and protein oxidative modification as a result of exposure of mice to GSM frequency leads to significant disorders of function and structure of brain and liver cell in mice. The normal tissue observed in bone marrow throughout this study agrees with the findings of [36] where it is shown that there is no effect on exposure to 0.9 GHz GSM modulated RF EMF to mice at SAR of 2 W/kg, 2 h/day, 5 days a week and for 4 weeks. A report in [13] on the effect of electromagnetic radiation from a mobile phone on mice putting into consideration the answering mode and the standby mode is also in good agreement with the findings presented in this work. In the report, though mice exposure was only for 1 hour and 12 hours a day and for 10 days only, the inflammatory cellular infiltration in one of the group was detected. Also, liver section of the other group exposed to 10 hours showed more intense inflammatory response around the central vein. The kidney in the 1 hour exposed group were vacuolated while in the 10 hours exposed groups three renal tissue sections appeared with some inflamed areas in between the kidney tubules. Contrary to the finding of this work, the spleen tissues revealed enlarge white pulp with increased sinusoidal spaces. Similar tissue changes using low frequencies were reported earlier in [15] where mice were exposed to 50 Hz and a flux density of 100 μT for 10, 20 and 50 days. The results show that long term exposure affected greatly the destruction of liver and spleen tissues as compared to short time.

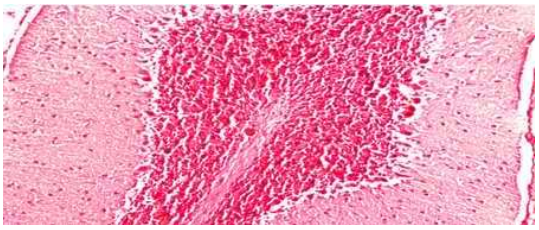

Figure 13. Degenerated brain tissues exposed to GSM frequencies.

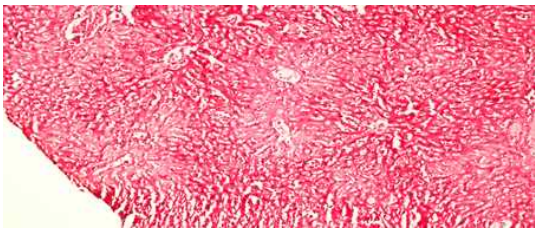

Figure 14. Degenerated liver tissues exposed to GSM frequencies.

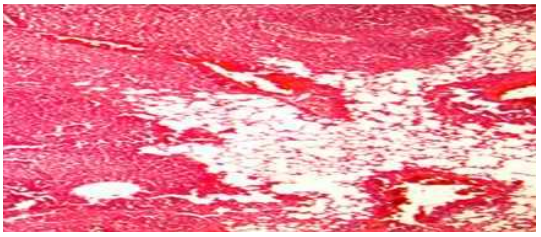

Figure 15. Degenerated lung tissues exposed to GSM frequencies.

The result of this finding is also similar to several biological responses reported at SAR far below any imaginable heat transfer. The work of [37] reported changes in cell proliferation at SAR of 21 μW/kg to 2.1 mW/kg. Magras and Xenos [38] found a decrease in reproductive functions in mice exposed to RF intensities of 160 to 1053 nW/cm^2.

5. PHYSICAL EXAMINATION

Observations of individual and collective behavior of the mice in each group during the period of exposure were made. The most important observations were changing of basic behavior models and expression of aggressive or panic behavior. The mice in the exposed group showed

some manifestation in the form of aggressiveness, hyperactivity and redness of the eyes. In contrast, these signs were not so apparent in the mice in sham exposed group. Collective defense behavior was different for mice in the exposed groups compared to sham exposed group. The eating habit were also monitored were it was observed that eating habit in the exposed groups are slow and less compared to that in sham exposed group.

An increase in urine secretion, clustering of the mice in one place and non free movement of the mice in exposed groups were observed while this is contrary to the mice in sham exposed group. This result is quite comparable to the study conducted in [39], where gradual body loss was recorded on mice exposure at 1.4 mT for 30 days.

6. CONCLUSION

Long term RF EMF exposures due to GSM window frequencies were investigated. Swiss albino mice in unrestrained conditions were used as surrogate. Three samples of the exposed mice chosen at random at the end of exposure after 4 weeks and subsequently on biweekly basis were taken to VRI. On arrival, blood samples of the mice were taken for haematology analysis and the internal organs extracted for histopathology analysis as well. The complete blood count result shows that haematological parameters of the exposed mice were within the normal range of the mice in the control group. The histopathological examination on 5 internal organs shows that the spleen and bone marrow were normal throughout the period of exposure, while a sign of tissue degeneration and inflammations were observed after 8 weeks of exposure on the brain, liver and lung of 0.9 GHz and 1.8 GHz exposed groups. These signs increase in severity the most with prolonged exposure with 1.8 GHz exposed group. This therefore demonstrates that prolonged exposure due to GSM frequencies is capable of causing health hazards.

ACKNOWLEDGMENT

The work was funded by UPM RUG grant, Malaysia. The authors acknowledge the support and expert advice of VRI Ipoh veterinary Doctors and technicians of haematology and histopathology laboratories. Also the support of staffs of microwave laboratory UiTM Penang is hereby appreciated.

REFERENCES

1. Hamadoun, T., "Strong global mobile cellular growth across all regions," *International Telecommunication Union Mobile World Congress*, Bercelona, 2010.
2. http://www.who.int/peh-emf/project/EMF_Project, Aug. 2010.
3. Repacholi, M. H., "EMF concerns and WHO's international EMF project," *International Seminar on EMF Risk Perception and Communication*, 29–49, Ottawa, Ontario, Canada, 1999.
4. Schwan, H. P. and K. R. Foster, "RF-field interaction with biological systems: Electrical properties and biophysical mechanisms," *Proceedings of the IEEE*, 104–113, 1980.
5. International Commission on Non-Ionizing Radiation Protection, (ICNIRP), "Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic field (up to 300 GHz)," *Health Physics*, Vol. 74, 494–522, 1998.
6. Institute of Electrical and Electronics Engineering (IEEE), "Standard for safety levels with respect to human exposure to radio frequency electromagnetic fields, 3 kHz to 300 GHz," *IEEE Electromagnetic Compatibility Society*, New York, NY, 2005.
7. Vorst, A. V., A. Rosen, and Y. Kotsuka, *RF/Microwave Interaction with Biological Tissues*, 63–95, John Wiley & Sons, Inc., New Jersey, 2006.
8. Prausnitz, S. and C. Susskind, "Effect of microwave irradiation on mice," *IRE Transaction on Bio-Medical Electronic*, 104–108, 1961.
9. Eraslan, G., M. Akdogan, E. Yarsan, D. Essiz, F. Sahindokuyucu, and L. Altintas, "Effect of short-term exposure to pulsed electromagnetic field on some biochemical parameters in mice," *Indian Journal of Biochemistry & Biophysics*, Vol. 41, 57–59, 2004.
10. Kim, T.-H., T.-Q. Huang, J.-J. Jang, M. H. Kim, H.-J. Kim, J.-S. Lee, J. K. Pack, J.-S. Seo, and W.-Y. Park, "Local exposure of 849 MHz and 1763 MHz radiofrequency radiation to mouse heads does not induce cell death or cell proliferation in brain," *Exp. Mol. Med.*, Vol. 40, 440–447, 2008.
11. Prisco, M. G., F. Nasta, M. M. Rosado, G. A. Lovisolo, C. Marino, and C. Pioli, "Effect of GSM-modulated radiofrequency electromagnetic fields on mouse bone marrow cells," *Radiation Research*, Vol. 170, 803–810, 2008.
12. Rusnani, A., M. N. Norhayati, S. S. Noraini, and M. Marina, "Microwave radiation effect-A test on white mice," *International*

RF and Microwave Conference, 513–616, Kuala Lumpur, 2008.

13. Al-Glaib, B., M. Al-Dardfi, A. Al-Tuhami, A. Elgenaidi, and M. Dkhil, "A technical report on the effect of electromagnetic radiation from a mobile phone on mice organs," *Libyan J. Med.*, 8–9, 2009.
14. Forgács, Z., Z. Somosy, G. Kubinyi, J. Bakos, A. Hudák, A. Surján, and G. Thuróczy, "Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice," *Reproductive Toxicology*, Vol. 22, 111–117, 2006.
15. Attia, A. A. and M. A. Yehia, "Histological, ultrastructural and immunohistochemical studies of the low frequency electromagnetic field effect on thymus, spleen and liver of albino Swiss mice," *Pak. J. Biol. Sci.*, Vol. 5, 931–937, 2002.
16. Repacholi, M. H., A. Basten, V. Gebski, D. Noonan, J. Finnie, and A. W. Harris, "Lymphomas in Eμ-Pim1 transgenic mice exposed to pulsed 900 MHz electromagnetic fields," *Radiat. Res.*, Vol. 147, 631–640, 1997.
17. Rademacher, D. J., S. E. Meier, L. Shi, W. S. Ho, A. Jarrahian, and C. J. Hillard, "Effects of acute and repeated restrain stress on endocannabinoid content in the amygdata, ventral striatum, and medial prefrontal cortex in mice neuropharmacology," *Neuropharmacology*, Vol. 54, 108–116, 2008.
18. Liburdi, R. P., "RF radiation alters the immune system. II. Modulation of in vivo lymphocyte circulation," *Radiat. Res.*, Vol. 83, 66–73, 1980.
19. Adang, D., C. Remacle, and V. V. Andrew, "Results of a long-term low-level microwave exposure of rat," *IEEE Trans. on Microwave and Techn.*, Vol. 57, 2488–2497, 2009.
20. Gärtner, K., "Stress response of rats to handling and experimental procedures," *Lab. Animals*, Vol. 14, 267–274, 1980.
21. Würbel, H., "Ideal homes? Housing effects on rodent brain behaviour," *Trends. Neurosci.*, Vol. 24, 207–211, Apr. 2001.
22. Oberto, G., K. Rolfo, P. Yu, M. Carbonatto, S. Peano, N. Kuster, et al., "Carcinogenicity study of 217 Hz pulsed 900 MHz electromagnetic fields in Pim1 transgenic mice," *Radiat. Res.*, Vol. 168, 316–326, 2007.
23. Utteridge, T. D., V. Gebski, J. W. Finnie, V. Roberts, and T. R. Kuchel, "Long-term exposure of Eμ-Pim1 transjenic mice to 898.4 MHz microwaves does not increase lymphoma incidence," *Radiat. Res.*, Vol. 158, 357–364, 2002.

24. National Research Council (NRC), "Guide for the care and use of laboratory animals," Institute of Laboratory Animal Resources, National Academy Press, Washington DC, 1996.
25. European Electronic Compatibility (EEC), "Measuring nonionizing electromagnetic radiation (9 kHz–300 GHz)," *Recommended Practice (02)04*, EEC, 2003.
26. Warn, P. A., M. W. Brampton, A. Sharp, G. Morrissey, N. Steel, D. W. Denning, and T. Priest, "Infrared body temperature measurement of mice as an early predictor of death in experimental fungal infections," *Laboratory Animal*, Vol. 37, 126–131, 2003.
27. Shahryar, H. A., A. Lotfi, M. B. Ghodsi, and A. R. K. Bonary, "Effects of 900 MHz electromagnetic fields emitted from cellular phone on the T3, T4, and cortisol levels in Syrian Hamsters," *Bull. Vet. Inst. in Pulawy*, Vol. 53, No. 2, 233–236, 2009.
28. Weinert, D. and J. Waterhouse, "Daily activity and body temperature rhythms do not change simultaneously with age in laboratory mice," *Physiology and Behaviour*, Vol. 66, 605–612, 1999.
29. Durney, C. H., H. Massoudi, and M. F. Iskander, *Radiofrequency Radiation Handbook*, 4th Edition, USAF School of Aerospace Medicine, Brooks Air Force Base, Salt Lake City, 1986.
30. Mcree, D. I., "Review of Soviet/Eastern European research on health aspects of microwave radiation," *Acad. Med.*, Vol. 55, 1133–1151, 1979.
31. Robert, Jr., N. J., S. M. Michaelson, and S. T. Lu, "The biological effects of radiofrequency radiation: A critical review and recommendations," *Int. J. Radiat. Biol.*, Vol. 50, 379–420, 1986.
32. Chaturvedi, C. M., V. P. Singh, P. Singh, P. Basu, M. Singaravel, R. K. Shukla, A. Dhawan, A. K. Pati, R. K. Gangwar, and S. P. Singh, "2.45 GHz (CW) microwave irradiation alters circadian organization, spatial memory, DNA structure in the brain cells and blood cell counts of male mice, mus musculus," *Progress In Electromagnetics Research B*, Vol. 29, 23–42, 2011.
33. Abdel Aziz, I., H. J. El-Khozondar, M. Shabat, K. Elwasife, and A. Mohamed-Osman, "Effect of electromagnetic field on body weight and blood indices in albino rats and the therapeutic action of vitamin C or E," *Romanian J. Biophys.*, Vol. 20, 235–244, 2010.
34. Zare, S., S. Alivandi, and A. G. Ebadi, "Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in Guinea pig," *World Applied Sciences Journal*, Vol. 2,

509–511, 2007.

35. Kristic, D. D., B. J. Dindic, D. T. Sokolovic, V. V. Markovic, D. M. Petkovic, and S. B. Radic, "The result of experimental exposition of mice by mobile telephones," *TELSIKS 2005 Conference*, Serbia, Montenegro, 2005.
36. Prisco, M. G., F. Nasta, M. M. Rosado, G. A. Lovisolo, C. Marino, and C. Pioli, "Effect of GSM modulated radiofrequency electromagnetic field on mouse bone marrow cells," *Radiation Research Society*, Vol. 170, 803–810, 2008.
37. Kwee, S. and P. Raskmark, "Radiofrequency electromagnetic fields and cell prolifiration," *Second World Congress for Electricity and Magnetism in Biology and Medicine*, Bologna, Italy, 1997.
38. Magras, I. N. and T. D. Xenos, "RF radiation-induced changes in the prenatal development of mice," *Bioelctromagnetics*, Vol. 18, 455–461, 1997.
39. Ray, S. and J. Behari, "Physiological changes in rats after exposure to low levels of microwaves," *Radiat. Res.*, Vol. 123, 199–202, 1990.