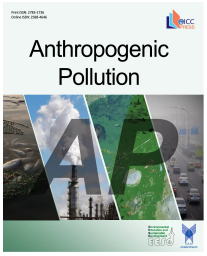

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Evaluation of blood parameters changes of mice exposed to long-term Wi-Fi waves as a major environmental pollutant

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Abstract:

As the world entered the industrial era, the nature of environmental threats and dangers posed by technology also have gone through fundamental changes. One of these environmental pollutants is the propagation of waves with different wavelengths in the environment. In this study, we aimed to investigate the effect of 2.45 GHz microwaves on blood biomarkers of mice. In this study, 80 immature male BALB/c mice were used. According to the inclusion criteria, 72 mice were included in the study. Mice were divided into two groups (control group = 24 mice, exposed group = 48 mice). Exposed groups were divided into two subgroups of 24 (groups A and B). Subgroup A was exposed to a simple modem without antenna and subgroup B was exposed to a modem with two antennas. In the first phase of the study, mice were exposed daily for 60 minutes for 90 days and in the second phase for 8 hours daily for 90 days. Blood samples were taken on days 90 and 180. Data analysis was done by SPSS software version 25 (P value$<$0.05). This study shows that blood cells are affected by long-term exposure to Wi-Fi waves by decrease in number and volume. Furthermore, no significant difference was observed between the blood parameters of the two groups exposed to different modems which vary in the number of antennas. The results showed that the highest effect of Wi-Fi waves was on the following blood factors, respectively PLT, RBC, HCT, HGB, and WBC.

Keywords: Wi-Fi; Mice; Blood biomarker; 2.45 GHz; long exposure; ELF waves

1. Introduction

Public concern about the potential health consequences of wireless technologies such as Wireless Fidelity (Wi-Fi) communication devices using radiofrequency (RF) is growing due to increased environmental electromagnetic field (EMF) and its related health risks. The biological hazards of EMF emitted from mobile phones and wireless devices such as Wi-Fi have raised concerns, although the health effects of such devices are still unclear (Obajuluwa et al., 2017). Understanding the relationship between electromagnetic fields and health's diseases is crucial for the general public, who frequently use wireless Internet throughout their lives (Karipidis et al., 2007). Safety boundaries are defined to protect the community from RF exposure. However, the World Health Organization has encouraged research in this area.

A number of animal studies have been performed on rat to investigate the effects of Wi-Fi signals on health parameters and stress indicators (Atasoy et al., 2013; Sambucci et al., 2010).

The environmental wireless 802.11.g device (Wi-Fi or WIAD) has a higher frequency range and longer exposure time in comparison wireless phones (Viel et al., 2009).Therefore, the level of health risks associated with Wi-Fi devices may be higher than that of mobile phones (MB). Even Wi-Fi devices typically is capable of exposing the entire body to RF, unlike MB, which typically is capable of irradiating the certain parts of the body (Atasoy et al., 2013).

The studies have evaluated a group of vertebrates (i.e., small rodents [rats, and rabbits]), then birds (embryos or eggs), insects, and plants in laboratory setting while significant effects from RF-EMF were mostly found to be linked to birds, insects, (i.e., honey bees and fruit flies) and plants. As reported by a review study, the type of endpoints was different in the studied groups.

Fertility was the commonly assessed endpointend for birds. Growth was among the factors affected in all experiments performed on plants and other living organisms, while growth factor was affected in about 25% of studies in other vertebrates. This impact rate for birds has been seen in 40% of studies. Effects of RF-EMF on behavior in thirteen of twenty studies on other vertebrates and about 85% of studies on insects.

All in all, a growing body of evidence reveals that the RF-EMF is ecologically capable of effecting about 50% of the animal investigations and about 75% of the plant investigations at high and low dosages (Cucurachi et al., 2013). Different tissue changes may occur as a result of animals being exposed to RF-EMF, depending on the wireless exposure features, animal species, and histological methods applied to detect the effects (Westerman and Hocking, 2004; Atasoy et al., 2013). Decreased number and fertility of rat sperm have been observed after RF exposure (Cleary et al., 1989).

Another effect of RF-EMR is its carcinogenic induction (Krewski et al., 2001). Numerous researches have been done on the consequences of these waves and various complications have been ascribed to them. Therefore, the inevitable destructive effects of strong electromagnetic waves cannot be ruled out. Thus they are classified in group B as carcinogens. However, this phenomenon was later called "magnetic field sensitivity", which also included symptoms of the people who had been involved in magnetic field-related jobs complained about. These symptoms, as mentioned, which subjectively but not objectively have been reported were complaints such as headache, nausea, dizziness, etc. This set of symptoms that cannot be cited and followed objectively have been seen in some people. Some researchers describe these concerns as signs of "autosuggestion". Of course, there is no reason to reject that some people may face difficulties due to these powerful fields (Hardell, 2017).

Many experimental studies on rat, mouse (*Mus musculus*; *Balb/c* and *Balb/cf*), rabbits (*White New Zealand Rabbit*) and rhesus monkey (*Macaca mulatta*) have examined endpoints such as fertility, growth, behavior, mortality or mutation in a laboratory setting (Cucurachi et al., 2013). Rats and rabbits exposed to RF-EMF exhibited contradictory findings in terms of behavior changes (Cucurachi et al., 2013; Daniels et al., 2009). Furthermore, no significant findings of an effect of RF-EMF under the physical and experimental examinations applied by a value of MW CW exposures (from 0.6×10^{-6} to 20 mW/cm^2), (Cucurachi et al., 2013; Daniels et al., 2009; Lee et al., 2009; Imai et al., 2011; Collins and Smith, 2001; Poulletier et al., 2012; Jiang et al., 2012).

Studies have also been performed to elucidate the direct biological effects of radiation on cells and whole organisms (Goodman et al., 1995; Kwee and Raskmark, 1998; Velizarov et al., 1999; Adebayo et al., 2015). A range of cellular responses to RF-EMR has been previously reported by *in vivo* and *in vitro* experiments including gene expression, differentiation, proliferation, apoptosis, changes in Ionic homeostasis, free radical production, modulation of membrane receptor function, as well as histological, hematological and histochemical changes (Goodman et al., 2009; Schwartz et al., 2008; Foletti et al., 2009; Iorio et al., 2011; Di-Loreto et al., 2009; Adebayo et al., 2019; Usikalu et al., 2010). Although we are seeing an increase in surveys, the results are somewhat controversial (Usikalu et al., 2010; Behari and Rajamani, 2012; SCENIHR, 2015) and the resulting changes have been attributed to a variety of factors (Lerchl and Wilhelm, 2010).

Due to the rapid growth of Wi-Fi technology and the large number of its users around the world, as well as the fact that waves of these devices are everywhere and are now known as a form of invisible pollution; in this study, we aimed to investigate the effect of 2.45 GHz microwaves on blood biomarkers of mice.

2. Materials and methods

The goal of this study is to investigate the effect of Wi-Fi waves (2.45 GHz) on the blood biomarkers of mice. These biomarkers include red blood cells (RBC), white blood cells (WBC), hematocrits (HCT), hemoglobin (Hb), and platelets (plt). The study site is an animal house. Before initiating the trial, the animal house where the mice were kept was examined with a radiation detector to make sure it was clear of 2.45 GHz waves, and no such waves were found around the place like low-frequency waves. The sampling method is non-probability and availability sampling. The inclusion criteria of the study includes male newborn mice, with standard blood tests, without any underlying diseases or any disorders in their blood system. Exclusion criteria of the study contains any disorders in blood biomarkers, diseases, and not being approved by the hematologist. The rest of the conditions of the mice were also controlled by the veterinarian. In this study, all the ethical guidelines for working with animal laboratory have been considered. In this study, 80 immature male BALB/c mice with a weight of almost 10 grams and an age of almost two weeks were used. Animal cages were made of polycarbonate with a steel mesh roof and the dimensions of 30*50*30 cm. (The laboratory mice used in this study are Mus musculus species and BALB/c

breed. Mice were obtained from Almas Company.) The floor of the cages were covered with sawdust and wood chips. The cages' floor were being changed every two days, and the cages were being washed with soap and water and disinfected with a 70% solution of ethyl alcohol once a week. During the study, all the mice were kept in the animal house with a constant 24-hour cycle (12 hours night and 12 hours day), the temperature of 24±2 degrees Celsius and 30% humidity, to prevent the environmental effects on the experiment. In this study, according to the veterinarian, the diet of the mice was approximately 15 grams of industrial food (Javaneh Company) per 100 grams body weight daily, and there was enough water supply for them during the experiment. After moving the mice to the animal house, they had two weeks to adjust to the new environment.

At the beginning, an initial blood sample was taken from the mice, and according to the inclusion criteria and approval of the hematologist, 72 out of 80 mice were involved in the study. The mice were divided into two groups, the first one with 24 mice was the control group, and the second one with 48 mice was exposed to the waves. The second group was divided into two subgroups of 24 (group A and B). Then two Wi-Fi modems were provided, subgroup A was exposed to a simple modem with internal antenna without external antenna and 2.45 GHz frequency bandwidth, and subgroup B was exposed to the second type of modem with two external antennas and 2.45 GHz frequency bandwidth. The specifications of the modems used in this study are given in Table 1. The modems differ only in the number of antennas and both modems emit 2.45 GHz. The photos of the study can be seen below (figure 1).

The modems were installed and launched in the animal house and 50 cm away from the mice cages. In the first phase of study, the mice were exposed daily for 60 minutes for 90 days. In the second phase, they were exposed for 8 hours daily for 90 days. The control group was never exposed to Wi-Fi waves these 180 days. In this study, mice were placed in a real situation exactly like what we are exposed to at work or home. Blood sampling performed on days 90 and 180. Also, after the first trimester, the study site was examined again with a radiation detector to make sure it was clear of 2.45 GHz waves and other waves. Also, the location of the control group was examined in several stages with a radiation detector and it was found that there are no waves in this location. We used a measuring device to ensure that the mice were exposed to the waves. In this study, we removed the confounding factors of the rest of the waves through the control group.

In this study, descriptive statistics (mean, standard deviation, frequency, and percent) and inferential statistics (Chi-square test, independent t-test, Mann-Whitney U test and Generalized Estimating Equation (GEE)) in addition LSD post hoc test were used to describe and analyze data, respectively. SPSS software (version 25) and $P<0.05$ were used for sta-

Table 1. The specifications of the modems used in this study.

Feature	Modem group A	Modem group B
Wi Fi systems	Wi Fi (802.11b/g/n)	Wi Fi (802.11b/g/n)
Primary Application	Wireless LAN	Wireless LAN
Frequency Band	2.45 GHz ISM	2.45 GHz ISM
Channel Bandwidth	25 MHz	25 MHz
Radio Technology	Direct Sequence Spread Spectrum	Direct Sequence Spread Spectrum
Bandwidth	<=0.44 bps/Hz	<=0.44 bps/Hz
Modulation	QPSK(Quadrature Phase Shift Keying)	QPSK(Quadrature Phase Shift Keying)
Access Protocol	CSMA/CA (Carrier Sense Multiple Access with Collision Avoidance)	CSMA/CA (Carrier Sense Multiple Access with Collision Avoidance)
Internal antenna	Yes/ 1 antenna	No
External antenna	No	Yes/ 2 antenna
Output Power (Amplitude)	100 mW (20 dBm)	100 mW (20 dBm)
Model of modem	HUAWEI (EchoLife HG521)	D-Link (DSL-124)

Figure 1. Pictures were taken during the study.

tistical analysis and statistical significance, respectively.

3. Results

In this study, after applying the selection criteria, 72 mice were included, 5 of which died (2 mice in the control group, two mice in group A, and one mouse in group B). Based on the Chi-square test, the three groups were similar in terms of mortality rate (P = 0.807).

In Table 2, the mean and standard deviation (SD) of 5 biomarkers (WBC, RBC, HCT, HGB, and PLT) are reported divided by time and group type. According to the results reported in this table, in the beginning of the study, the three groups were similar in terms of 5 biomarkers (WBC, RBC, HCT, HGB, and PLT), however other results showed that these variables were significantly different in the three groups on days 90 and 180.

Further results also illustrated that the variables (WBC, RBC, HCT, HGB, and PLT) increased significantly in the control group during the study, but they decreased significantly in the two intervention groups (groups A and B) (see figure 2 and Table 3).

Results also show no significant differences between the blood parameters of the two groups that have different modems (see Table 3).

4. Discussion

In this study, we investigated the effect of Wi-Fi waves (2.45 GHz) on the blood biomarkers of mice. According to the results, the variables of WBC (P<0.001), RBC (P<0.001), HCT (P<0.001), HgB (P<0.001), and PLT (P<0.001) increased significantly in the control group during the study, while they decreased significantly in the two intervention groups.

A study examined live blood in a an electromagnetically clean environment and the same person's blood for 10 min spoke on a cordless phone and after using a wired computer for 70 min. After exposure, red blood cells showed rouleau due to an increase in the concentration of fibrinogen or other changes in plasma proteins, as well as a decrease in electrical potential in the cell membrane, leading to a weakening of the repellent forces between cells (Havas, 2013).

Another hematological study reported a significant reduction in white blood cells counts in mice exposed to radiofrequency radiations from global satellite mobile (GSM) base station in comparison with group, but no difference was found between groups in terms of blood cells (RBC) counts (Kehinde et al., 2016). Exposure to electromagnetic fields has been reported to be associated with the variations of some hematological parameters in rats including anemia in short term exposure and pathological changes in red blood cells in long term exposure (Alghamdi and El-Ghazaly, 2012), which such findings is more or less in line with decreased RBC in the present study.

The decreased level of RBC in the exposed rat compared to the control group could be the response of stress in the animal system, the trend in red blood cell count in the present study are consistent with the study of Yousefi (n.d.) and Adebayo et al. (2019), The decreased in white blood cells and hemoglobin of the exposed is in agreement with the findings of Abdolmaleki et al. (2012), where the found the amount of hemoglobin, lymphocytes and WBC reduced in threated groups, while opposite results were reported by mentioned study for the amount of RBC, MCV and platelet (a significate increase). Decreased WBC in the present study negates the report of increased WBC of the exposed albino by Adebayo et al. (2019).

Our finding is also in line with the findings of Singh et

Table 2. The Mean and SD of study variables according to the time and groups.

Variable		Time						P-value	
		1st day		90th day		180th day			
	Group	Mean	SD	Mean	SD	Mean	SD	Within	Between
RBC	Control	9.16	0.36	9.41	0.25	10.77	0.35	<0.001	P<0.001 Partial η^2=0.366
	A	7.24	2.30	5.92	2.35	2.76	2.47	<0.001	
	B	7.95	2.56	6.26	2.65	2.52	2.45	<0.001	
		P-value	0.099	P-value	<0.001	P-value	<0.001		
WBC	Control	5.91	3.64	6.56	3.53	7.66	3.64	<0.001	P<0.001 Partial η^2=0.226
	A	5.13	2.6	4.53	2.65	3.44	2.77	<0.001	
	B	4.41	1.75	3.78	1.76	2.21	1.81	<0.001	
	P-value	P-value	0.287	P-value	<0.001	P-value	<0.001		
Hgb	Control	13.62	1.52	14.61	1.63	15.37	1.66	<0.001	P<0.001 Partial η^2=0.285
	A	13.81	1.85	11.63	1.77	5.08	1.68	<0.001	
	B	13.23	2.07	10.39	1.97	4.12	2.14	<0.001	
	P-value	P-value	0.294	P-value	<0.001	P-value	<0.001		
HCT	Control	43.23	2.38	44.55	2.35	45.1	2.5	<0.001	P<0.001 Partial η^2=0.303
	A	43.55	1.77	31.35	2.97	14.37	1.99	<0.001	
	B	44.06	3.14	30.73	2.76	15.43	3.41	<0.001	
	P-value	P-value	0.337	P-value	<0.001	P-value	<0.001		
Plt	Control	689.63	111.35	848.43	108.86	982.86	121.56	<0.001	P<0.001 Partial η^2=0.401
	A	680.58	121.97	575.65	124.14	214.55	102.95	<0.001	
	B	660.58	76.66	562.79	80.92	261.13	77.94	<0.001	
	P-value	P-value	0.789	P-value	<0.001	P-value	<0.001		

SD: Standard Deviation

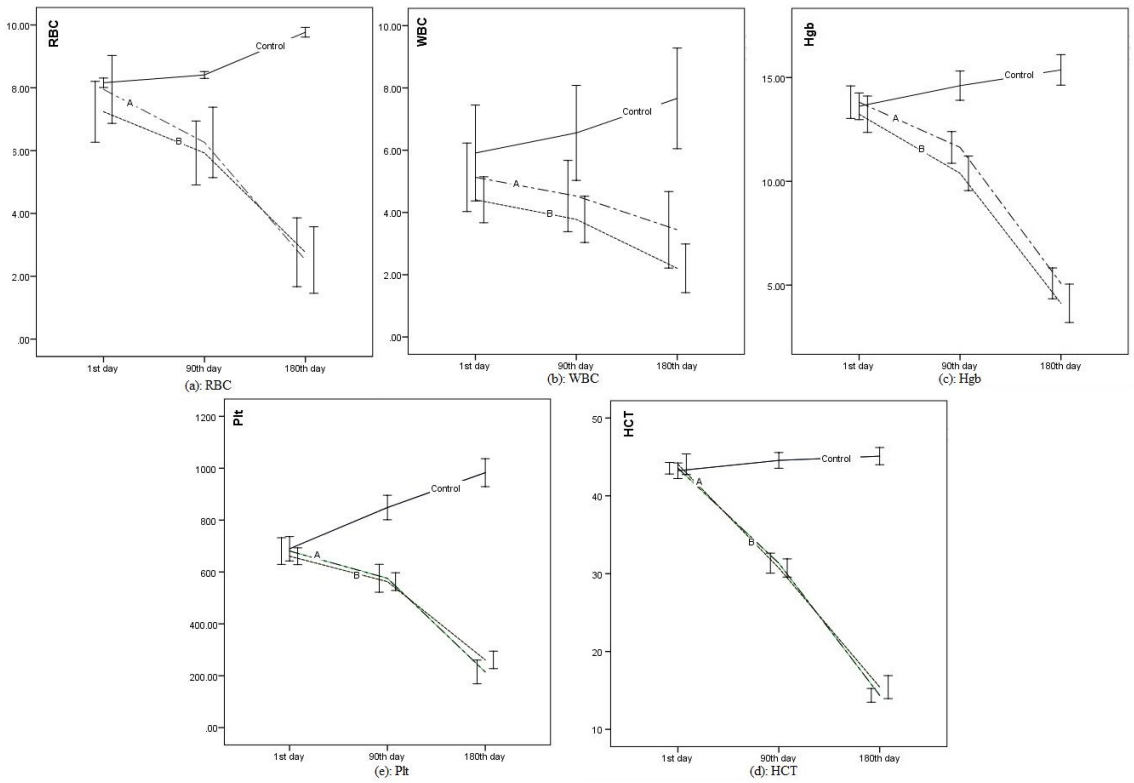

Figure 2. The trend of variables during follow up according to the three groups.

Table 3. The LSD post hoc test for comparison between 3 groups in two time points (90th and 180th day).

Variables	Group1 vs. Group2		P-value	
			90th day	180th day
RBC	Control	A	<0.001	<0.001
		B	<0.001	<0.001
	A	Control	<0.001	<0.001
		B	0.578	0.683
	B	Control	<0.001	<0.001
		A	0.578	0.683
WBC	Control	A	0.014	<0.001
		B	<0.001	<0.001
	A	Control	0.014	<0.001
		B	0.121	0.043
	B	Control	<0.001	<0.001
		A	0.121	0.043
Hgb	Control	A	<0.001	<0.001
		B	<0.001	<0.001
	A	Control	<0.001	<0.001
		B	0.020	0.086
	B	Control	<0.001	<0.001
		A	0.020	0.086
HCT	Control	A	<0.001	<0.001
		B	<0.001	<0.001
	A	Control	<0.001	<0.001
		B	0.436	0.196
	B	Control	<0.001	<0.001
		A	0.436	0.196
Plt	Control	A	<0.001	<0.001
		B	<0.001	<0.001
	A	Control	<0.001	<0.001
		B	0.678	0.131
	B	Control	<0.001	<0.001
		A	0.678	0.131

al. (2013), where they reported decreased total RBC count (10.9%) and hemoglobin content (10%) of exposed mice compared with normal mice after 42 days of exposure. Furthermore, the RBC count and hemoglobin concentration reached to their normal levels after removal of computer monitor (VDU) exposure (Singh et al., 2013). The change in the different hematological parameters suggest that the hemopoietic system was adversely affected by RF-EMR. The decrease in the concentration of hemoglobin could be the result of interaction between iron of haeme and electromagnetic field, by which magnetic field is capable of entering the body and affecting the ions in all the vital organs, e.g., spleen, bone marrow, kidney and liver etc., resulting in change of cell membrane potential and distribution of ions (Singh et al., 2013; Kula and Drozdz, 1996). In the rabbit to electromagnetic field, the RBC production was found to be decreased, resulting in reduction of hemoglobin concentration and red blood cell count.

Another study didn't find any effect of the cell phone waves (940 MHz) effect on the number of WBC, Hb, MCV, MCH, MCHC ($P>0.05$). While a decrease in the number of white pulp lymphocytes, and megakaryocytes in the experimental

Table 4. A summary of the most important related studies.

Subject / species	**Exposure Parameters / Biomarkers**	**Findings**	**References**
Human blood - 2.45 GHz	A study examined live blood in a an electromagnetically clean environment and the same person's blood for 10 min spoke on a cordless phone and after using a wired computer for 70 min.	After exposure, red blood cells showed rouleau due to an increase in the concentration of fibrinogen or other changes in plasma proteins, as well as a decrease in electrical potential in the cell membrane, leading to a weakening of the repellent forces between cells	Havas (2013)
Human blood - 0.9 GHz	this study reported a significant reduction in white blood cells counts in mice exposed to radiofrequency radiations from global satellite mobile (GSM) base station in comparison with group,	no difference was found between groups in terms of blood cells (RBC) counts	Kehinde et al. (2016)
Rat - Electromagnetic wave	Blood cells	Exposure to electromagnetic fields has been reported to be associated with the variations of some hematological parameters in rats including anemia in short term exposure and pathological changes in red blood cells in long term exposure..	Alghamdi and El-Ghazaly (2012)
Rat - Electromagnetic wave	Blood cells	The decreased level of RBC in the exposed rat	Yousefi (n.d.)
Human blood - Electromagnetic wave	Blood cells	The decreased in white blood cells and hemoglobin	Abdolmaleki et al. (2012)
Human blood - Electromagnetic wave	Blood cells	The results show that RBC, MCV and platelet levels have increased significantly	Adebayo et al. (2019)
Mice /rabbit - RF EMR	Blood cells	The total RBC count and hemoglobin content of exposed mice and rabbits were decreased compared to 42 days of exposure.	Singh et al. (2013) Kula and Drozdz (1996)
Human blood - 0.9 GHz	Blood cells	this study didn't find any effect of the cell phone waves (940MHz) effect on the number of WBC, Hb, MCV, MCH, MCHC ($P>0.05$).	Baharara et al. (2009)
Albino mice - 0.9 GHz	Blood cells	They reported increased levels of WBC count, PLT count and RBC count in albino mice exposed to radiation from GSM base stations	Otitoloju et al. (2012)
RF EMF	Blood cells	Blood cell exposed to RF-EMF indicated that no changes	Black and Heynick (2003)
RF EMF	Blood cells	Studies showed that radiofrequency EMF was capable of inducing tissue impairment in many organs of experimental animals liver and blood including brain, and testis, as well as changes in Ionic homeostasis, modulation of membrane receptor function, histological, hematological and histochemical changes leading to adverse effects	Hashem and El-Sharkawy (2009) Zare et al. (2007) Goodman et al. (2009) Usikalu et al. (2010)

samples were found when compared with the control and sham-exposed groups (Baharara et al., 2017).
The decrease in WBC, RBC and PLT of the exposed animals compared to control negates the findings of Otitoloju et al. (2012), where they reported increased levels of WBC count, PLT count and RBC count in albino mice exposed to radiation from GSM base stations (Otitoloju et al., 2012). Such a difference has been attributed to the type of samples, intensity difference, radiation methods and exposure period (Sarookhani et al., 2012; Tohidi et al., 2016). Blood cell exposed to RF-EMF indicated that no changes or damage occurred unless the cells heated as revealed by previous studies. The WBCs have been described as more sensitive than RBCs (erythrocytes), but the effects of WBCs are defined to be associated with normal physiological responses to systemic temperature fluctuations (Black and Heynick, 2003).
Studies showed that radiofrequency EMF was capable of inducing tissue impairment in many organs of experimental animals liver and blood including brain, and testis, as well as changes in Ionic homeostasis, modulation of membrane receptor function, histological, hematological and histochemical changes (Goodman et al., 2009; Schwartz et al., 2008; Foletti et al., 2009; Iorio et al., 2011; Di-Loreto et al., 2009; Adebayo et al., 2019; Usikalu et al., 2010; Hashem and El-Sharkawy, 2009; Khayyat, 2011; Kang et al., 1997; Zare et al., 2007), leading to adverse effects (Adebayo et al., 2019). See a summary of the most important related studies in Table 4.

5. Conclusion

This study shows that blood cells are affected by long-term exposure to Wi-Fi waves by decrease in number and volume. The results showed that the highest effect of Wi-Fi waves was on the following blood factors, respectively PLT, RBC, HCT, HGB, and WBC. Furthermore, no significant difference was observed between the blood parameters of the two groups exposed to different modems which vary in the number antennas. One of the pollutants that threatens the environment today is the spread of Wi-Fi waves in a wide range of societies. It seems that the use of these waves should be limited according to the results of this study.

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Ethical Statement

This study has been approved by the Ethics Committee of the Islamic Azad University, Science and Research Branch (Code: IR.IAU.SRB.REC.1398.200).

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Availability of Data and Materials

All data generated or analysed during this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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