

EFFECT OF MOBIL PHONE ELECTROMAGNETIC WAVES ON THE HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN LABORATORY MICE (*MUS MUSCULUS*)

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ABSTRACT.

In present study, some hematological and biochemical parameters were accomplished to assess the effects of mobile phone electromagnetic waves (EMW) in mice. Two groups of sixteen (16) male mice each were used; the first group is a control group and the second one is an exposure group. The mice of the second group were exposed to (1200 MHz) (EMW) for 45 days as 6 hours daily. The hematological results showed a significant increase in red blood cells count, total leukocytes count, lymphocytes, monocytes and acidophils of the exposure group as compared with those of the control group at ($P \leq 0.05$). While there was a significant decrease in hemoglobin, packed cells volume, and neutrophils of the exposure group as compared with those of the control group.

The biochemical results showed a significant increase in blood calcium and a significant decrease in total cholesterol, blood glucose, AST, and ALT enzymes against those of the control group at ($P \leq 0.05$).

INTRODUCTION

Electromagnetic (EM) radiation is associated with numerous industrial, military, consumers and medical uses (1). All electromagnetic energy falls somewhere on the electromagnetic spectrum, ranging from extremely low frequency (ELF) radiation to microwaves, x-rays and gamma rays (2). ELF fields include household appliances and overhead power lines. Scientists agree that ELF fields are hazardous to human health. It's considered 'possibly carcinogenic', and has been linked to cases of childhood leukemia (3). Moreover, an increasing number of reports indicate that magnetic field is involved in cancer induction as

co-carcinogenic factors able to enhance the effects of other mutagenic substances (4, 5). Non-ionizing radiation (NIR) is widespread in human environment. The most frequent sources of NIR are mobile phones which work at (800 – 2200 MHz) and cell towers which emit microwave radiation (MWR). They emit powerful electromagnetic field (EMF) (6). The exposures to magnetic fields (MF) affect mammalian health include liver function (7), hematopoietic parameters (8), lipid and glucose metabolism (9), and leukemia (10).

The aim of this study was to clarify the effects of the mobile phone electromagnetic waves on different physiological parameters in mice.

MATERIALS AND METHODS.

● Experimental animals.

The experiment was conducted at the laboratory of researches of the College of Medicine-University of Al-Muthanna, where 32 males mice (*Mus musculus*), 12 weeks old, and of 20 – 25 grams weights were used. The experiment conditions were unified for all animals, where the room temperature was set between 20 – 25 C° by the use of air conditioners, and the humidity rate was about 50 %. Food and water were provided daily (*ad libitum*).

● Experimental design.

The animals of the experiment were divided randomly into two equal groups of 16 male mice each. The hematological and biochemical tests were done after 45 days of exposure. The groups were:

☐ Control group. In this group, 16 male mice were used and euthanized after 45 days for the necessary tests.

☐ Exposure group. In this group, 16 male mice were exposed to (1200 MHz) (EMW) for 6 hours daily and euthanized after 45 days for the necessary tests. The (EMW) – electromagnetic waves – were obtained by the use of Frequency Generator (Type D 14, No. 36943. Made in Dan bridge-Denmark).

● Specimens collection

Once the mice were anaesthetized by the use of clorophorm, blood samples were collected directly by cardiac puncture by the use of disposable syringes of 1 cc capacity at the end of the exposure period, and the blood samples were divided into:

◆ 0.4 ml was poured into ethylene diamine tetra acetic acid (EDTA) containing tubes as an anticoagulant to accomplish the blood parameters.

◆ 0.6 ml was poured into test tubes free from anticoagulant to isolate blood serum to estimate the biochemical parameters.

● **Study parameters.**

◆ ***Hematological parameters.***

A. Red blood cells count (R.B.C.) (cell/mm³).

The red blood cells count was obtained by the use of haematocytometer (Neubauer improved double) and (Hayme's solution) and a special pipette for dilution (11).

B. Hemoglobin concentration (Hb) (g/dl).

Hb concentration was estimated by the use of a special kit (CYPRESS DIAGNOSTICS, Code HB011, Langdorp – Belgium) (12, 13), and a spectrophotometer (BIOTECH ENGINEERING MANAGEMENT CO.LTD. UK, VIS-7220G).

C. Packed cells volume (P.C.V.) (%).

The microhematocrit method is used to calculate the percentage of P.C.V. by the use of capillary tubes which contain heparin, where one end of which was closed by an elastoplast after being filled to 3/4 of its length with blood, and it's put in micro centrifuge (HAEMATOKRIT 210, HETTICH ZENTRIFUGEN D-78532, TYPE: 2104, GERMANY) on a velocity 1200 rotation/minute for five minutes, then the hematocrit value is obtained by service device (14).

D. Total white blood cells count (W.B.C.) (cell/mm³).

The W.B.C. was obtained by the use of Haemocytometer (Neubauer improved double) and Thoma's solution and special pipette for dilution (15).

E. Differential W.B.C. count.

After the blood smear is done, the slide is stained with Leishman's stain for 10 minutes then it is washed with water to eliminate the over stain, and left to dry, then examined under oil immersion power to count the percentage of each type of W.B.C. (14).

◆ **Biochemical parameters.**

A. Blood glucose (gm/dl)

The blood glucose level was estimated by the use of a special device (ACCU-CHEK ACTIVE and SOFTCLIX. ROCHE DIAGNOSTCS GmbH, D-68298 Mannheim, Germany).

B. Total cholesterol (gm/dl)

The total cholesterol was estimated by the use of a special kit (Biomaghreb, CHOD-PAP) (17), and a spectrophotometer (BIOTECH ENGINEERING MANAGEMENT CO.LTD. UK, VIS-7220G).

C. Blood Calcium level (mg/dl)

Blood Calcium level was estimated by the use of a special kit (Biomaghreb) (18), and a spectrophotometer (BIOTECH ENGINEERING MANAGEMENT CO.LTD. UK, VIS-7220G).

D. Serum transaminases activity determination (Unit/ml)

ALT and AST enzymes were determined by the use of a special kit (BIOMERIEUX, LYON-FRANCE) (19), and a spectrophotometer (BIOTECH ENGINEERING MANAGEMENT CO.LTD. UK, VIS-7220G).

*** Statistical Analysis.**

Data were analyzed by SPSS software using independent samples t-test, Version 17.

RESULTS AND DISCUSSION

Hematological Parameters.

The hematological results showed a significant increase in red blood cells count (polycythemia), and a significant decrease in packed cells volume (P.C.V.), and hemoglobin (Hb) of the exposed group as compared with those of the control group at ($P \leq 0.05$) as it's shown in table (1).

Table (1). Effect of mobile phone EMW on blood parameters of mice

<i>Parameters</i> <i>Groups</i>	R.B.C. (cell/mm³) x 10⁶	P.C.V. (%)	Hemoglobin (gm/dl)
CONTROL	8 ± 0.1 b	53.7 ± 1.2 a	17.5 ± 0.5 a
EXPOSED	40 ± 0.1 a	37.2 ± 0.7 b	12 ± 0.1 b

The different letters refer to significant difference between groups. The numbers represent the mean ± standard deviation. Independent samples test (t =38.2, df =22, p<0.001)

The significant increase of R.B.C. count in the exposed group can be explained by installation of an hypoxia-like status following exposure to (EMW) which in turn associates with increased oxidative stress (20), and this result agrees also with those of (21) and (22). On other hand, the significant decrease in (Hb) of the exposed group is due to the effect of the (EMW) on the permeability of the red blood cells membranes leading to lysis of hemoglobin and leakage of it outwards the R.B.C. (23), and this was also found by (24). For the (P.C.V.) - packed cells volume - , there was a significant decrease as compared with the control group and this result agreed with those of (1) and (23), which can be explained as due to the high significant increase of (R.B.C.) but with a significant decrease in (Hb), this would lead to decrease in the size of (R.B.C.) and hence a final decrease in (P.C.V.).

The hematological results showed also a significant increase in total leukocytes count, lymphocytes, monocytes, and acidophils with a significant decrease in neutrophils of the exposed group as compared with those of the control group as it's shown in table (2).

Table (2). Effect of mobile phone EMW on total and differential leukocytes count

<i>Parameters</i> <i>Groups</i>	Total Leukocytes Count (cell / mm³) ×10³	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Acidophil (%)	Basophile (%)
CONTROL	4^b ± 0.09	56.3^a ± 0.87	37.8^b ± 0.87	3.7^b ± 0.49	1.8^b ± 0.45	0.6^a ± 0.51
EXPOSED	8^a ± 0.11	16.0^b ± 0.95	74.6^a ± 0.51	5.9^a ± 0.79	2.9^a ± 0.67	0.6^a ± 0.51

The different letters refer to significant difference between groups. The numbers represent the mean ± standard deviation. Independent samples test (t =108.2, df =22, p<0.001)

The significant increase in total W.B.C. count comes from the significant high increase in lymphocytes which is due to the harmful action of electromagnetic waves (EMW) exposure that stimulates the haemopoietic system to release more lymphocytes causing an increase in their number in the blood stream (1), and this conforms also what was mentioned by (25). The significant decrease in neutrophils of the exposed group conforms the results of (26), which might be due to the absolute increase in lymphocytes (lymphocytosis).

Biochemical Results.

As it's clear from table (3), the biochemical results reveal a significant increase in blood calcium level (Ca⁺⁺) with a significant decrease in total serum cholesterol (TSCH), blood glucose level (BG), and transaminase enzymes (AST), (ALT) of the exposed group as compared with those of the control one.

Table (3). Effect of mobile phone EMW on some biochemical parameters of mice

<i>Parameters</i> <i>Groups</i>	Calcium (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	AST (Unit/ml)	ALT (Unit/ml)
CONTROL	19.8 ^b ± 0.2	65.3 ^a ± 0.2	242.8 ^a ± 2.3	69.8 ^a ± 0.2	16.2 ^a ± 0.2
EXPOSED	29.9 ^a ± 0.2	49.8 ^b ± 0.2	131.4 ^b ± 1.2	40.2 ^b ± 0.2	7.7 ^b ± 0.3

The different letters refer to significant difference between groups. The numbers represent the mean ± standard deviation. Independent samples test (t =116.8, df =22, p<0.001)

Blood Calcium.

The increase in blood calcium is well elicited by (27) and (28) who mentioned that most biological membranes are negatively charged, which makes them attract and adsorb positive ions. However, these ions are not stuck permanently to the membrane but are in dynamic equilibrium with the free ions in the environment. The relative amounts of each kind of ion attached at any one time depends mainly on its availability in the surroundings, the number of positive charges it carries and its chemical affinity for the membrane. Calcium normally predominates since it has a double positive charge that binds it firmly to the negative membrane. Potassium is also important since, despite having only one charge, its sheer abundance ensures it a good representation (potassium is by far the most abundant positive ion in virtually all living cells and outnumbers calcium by about ten thousand to one in the cytosol). When an alternating electrical field from an eddy current hits a membrane, it will tug the bound positive ions away during the negative half-cycle and drive them back in the positive half-cycle. If the field is weak, strongly charged ions (such as calcium with its double charge) will be preferentially dislodged. Potassium (which has only one charge) will be less attracted by the field and mostly stay in position. Also, the less affected free potassium will

tend to replace the lost calcium. In this way, weak fields increase the proportion of potassium ions bound to the membrane, and release the surplus calcium into the surroundings.

Transaminases (ALT, AST).

As it is known, AST and ALT are synthesized by hepatocyte cells and they are sensitive and specific enzymes for liver disease (29). Although these enzymes are expressed at a highest level in liver, they are also found in other tissues such as kidney, muscle and heart (30, 31). In humans, AST and ALT levels rise during periods of chronic alcoholism, hepatocellular carcinoma and tissue injury (32).

The important fall in serum AST and ALT values could be related to the inhibitory effect of electromagnetic waves on enzyme activity. It was reported that radiation, directly or indirectly, causes damage in structure of hydroxyl, carboxyl and sulphhydryl groups in structure of organic compounds as protein and enzyme. It also causes inactivation of enzymes or alteration of functions (33). And this explanation elicits the decrease in AST, ALT in the present study which conforms also the results of (34).

Total serum cholesterol (TSCH).

Different mechanisms proposed for EMF action on biological systems include exchange of electrons, ions and dipoles (35) increasing tissue temperature more than 1c° which can affect some immunological factors and hematopoietic system and subsequently, initiates the biochemical reactions, opening calcium canals and changing the electrochemical equivalency which results in initiation of signaling cascade inside the cells and affects the different metabolisms of cell such as lipid metabolism (36).

(37) reported that when calcium canals open, nitric oxide synthase converts Arginine to NO (nitric acid). The produced NO is non-polar and transports through a cell membrane to the other side easily. NO produces CGMP by activating the guanilil cyclase and transfers the message. When level of NO increases, TBARS (Thio Barbituric Acid Reaction Substances) acts as a biomarker of produced NO, for lipolytic actions at fatty cells. These cells (fatty cells), inhibit high degrees of lipolysis, with controlling the NO synthase. Different signals that actuate the lipolysis are completed by NO (38). (39) has reported the effect of exposing to electromagnetic fields on production of hipoxy (reduced oxygen) in body and tissues. Here, presence of lipolysis in tissues could be the results in hipoxy production. Exposition to electromagnetic fields causes changes in fields of the body and nerves. This problem results

in changing the voltage of gates and ion channels and affects their operation (39,40). Exposure to these fields causes sending signals into the body. During this process, signals for reducing cholesterol, triglyceride and VLDL in serum could be sent (40). Controlling the HMGCOA (3-hydroxy 3-methyl glutaryl CoA) reductase enzyme and initial effects of electromagnetic field may result in decreasing the cholesterol and LDL of the blood serum of adult female mice. LDL is the basic carrier of cholesterol which provides necessary cholesterol for nervous tissues, synthesis-steroids and cell membrane. After removing of LDL and cholesterol, sending messages to cells, causes the plasmatic cholesterol to decrease. Accordingly, cholesterol is used for production of biliary acids, mitralo-corticoids, androgens and cell membrane. After this stage, removal of cholesterol cloud is seen (41). And the decreased TSCH in the present study is similar also to that of (42).

Blood Glucose.

The hypoglycemia in EMW exposed group resembles the results of (43, 44) and can be explained as the EMW induce electric charge to cellular membrane and make dysfunctions in membranes lipid layers causing excessive glucose entrance to cells and the upshot is lower blood glucose level.

تأثير الموجات الكهرومغناطيسية للهاتف النقال على المعايير الدموية والكيموحيوية في الفئران المختبرية

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الخلاصة

في هذه الدراسة تم دراسة بعض المعايير الدموية والكيموحيوية لبيان تأثير الموجات الكهرومغناطيسية (EMW) للهاتف النقال في الفئران المختبرية. تم استخدام مجموعتين من ذكور الفئران بواقع ١٦ فأر لكل مجموعة، وكانت المجموعة الأولى هي مجموعة السيطرة والمجموعة الثانية هي مجموعة التعرض. لقد تم تعريض فئران المجموعة الثانية لـ (1200 MHz) (EMW) لمدة ٤٥ يوماً وبواقع ٦ ساعات يومياً. لقد أظهرت النتائج الدموية زيادة معنوية في عدد كريات الدم الحمراء، العدد الكلي لكريات الدم البيضاء، الخلايا اللمفية، الخلايا وحيدة النواة والخلايا أليفة الصبغة الحامضية لمجموعة التعرض مقارنة بتلك الخاصة بمجموعة السيطرة عند مستوى احتمال ($P \leq 0.05$). بينما كان هناك نقصان معنوي في الهيموغلوبين، حجم الخلايا المرصوصة، معدل حجم الكرية، معدل الهيموغلوبين في الخلايا. والخلايا العدلة لمجموعة التعرض مقارنة بتلك الخاصة بمجموعة السيطرة.

أظهرت النتائج الكيموحيوية زيادة معنوية في كالسيوم الدم ونقصان معنوي في الكولستيرول الكلي، كلوكوز الدم، الترانساميناسيز ALT, AST مقارنة بتلك الخاصة بمجموعة السيطرة عند مستوى احتمال ($P \leq 0.05$).

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