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RESEARCH ARTICLE

THE EFFECT OF CHRONIC EXPOSURE TO 900 MHZ RADIOFREQUENCY RADIATION ON CELLULAR COMPOSITION OF THE BONE MARROW IN THE MATURE RATS.

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Abstract

The daily exposure to radiofrequency radiation (RFR) from the wireless communication devices has been a subject of debate for the past decade, many studies have been carried out to detect possible effects of the radiofrequency radiation on human and their consequences on human health. The goal of our study was to assess to whether the chronic whole-body exposure to non-thermal radiofrequency (RF) radiation from cellular phones could affect the bone marrow cells in mature rats.

Twenty-three Wistar rats weighing 116 and 184 g were randomly divided into 4 groups according to time of exposure: control and exposed groups, animals in treatment groups were exposed to 900-MHz RF (217-Hz pulse rate, 2-Wmaximum peak power, SAR 0.873-0.352 w/kg) for: 0h (control), 1h, 2h and 3h daily, 7 days a week for up 15 weeks..

After the period of exposure, bone marrow aspirations were obtained from the femur of all rats. The cell response was assessed by number and type of the bone marrow nuclear cells using our standard laboratory methods.

The results revealed that the number of polychromatophilic and acidophilic erythroblast cells in mature rats increased significantly compared with their control group and number of lymphocytes in mature rats decreased significantly compared with its control group and this effect is less remarkable with increasing time of exposure.

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Introduction: -

The growing use of wireless communication devices and many new technologies in our environment induced an electromagnetic pollution in the form of radiofrequency (RF) and microwave (MW) radiation during the recent years, and especially rapid growth of mobile phone telecommunications has increased the scientific interest on biological effects of radiofrequency (RF) radiation emitted from cellular phones for human health.

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The close proximity of portable device and base station antenna to the human body has raised diverse concerns about the biological interactions with RF and MW radiation, the biological effects may be depended on the intensity and frequency of the radiation and exposure duration and other parameters, It is well known that this electromagnetic radiation is considered as a type of non-ionizing radiation which have an energy not strong enough to break the bonds of the cell tissues of living organisms.

The cell phones operates nearly at 300 MHz-2 GHz and their antenna transmits and receives electromagnetic radiofrequency (RF) radiations,

The radiofrequency and microwave radiation emitted by cellular phones generally is non-thermal in nature and absorbed by skin and other organism tissue, causing in insignificant rise of temperature in the head or other organs of the body.

In this context, the specific absorption rate (SAR) considered as a main parameter assesses the transfer energy from electromagnetic radiofrequency (RF) radiation to organism tissue and is directly related to thermal effects, it expressed as with units of W/kg.

There are many reports which indicate that RF/MW radiation may elicit a biological effect in target cells or tissues, however, it is not clear how these radiation interacts with living organisms, several studies assessing the genotoxicity of RF/MW radiation have been conducted in humans and animals at various frequencies and the conclusions are controversial.

Hematopoiesis is an ongoing process; there is continuous progression of cells from blasts to mature cells, which is balanced in the steady-state condition.

As the bone marrow is the most radiosensitive and proliferative tissue in the body, the effect of RF/MW radiation on the haematopoietic system which is susceptible to be damaged, the high mitotic activity of the bone marrow make it the most vulnerable organ system to the effect of radiation RF/MW.

Many studies on effect of radiofrequency and microwave exposure on hematopoietic cells has been investigated using animal models, rodent and human cell lines and the cell response was assessed by number (increases, decreases or no change in bone marrow cell) and type of the bone marrow nuclear cells and peripheral blood white cells.

Jelodar et al.(2011) reveal that the exposure to 900 MHz radiofrequency radiation generated by mobile base stations on hematological parameters and cellular composition of bone marrow in mature and immature rats, lead to a deleterious effect on hematological parameters and bone marrow composition; this effect was more severe in immature animals.

However, Kumar et al (2011) revealed no effect on the hematopoietic system in rats after the exposure to 900 MHz RRF at SAR: 2 W/ kg.

The aim of the current study is to investigate the potential effects of chronic exposure to 900 MHz radiofrequency radiation on cellular composition of bone marrow in mature rats exposed for: 0h (control), 1h, 2 and 3h a day 7 days a week for up 15 weeks.

Materials and Methods:-

Animals

All procedures were performed in compliance according to international laws and regulations on animal welfare. Twenty-three Wistar rats involved in this study were purchased from Chouaib Doukkali University animal facility, the animals were approximately 3 months old and weighted 116 and 184 g(males and females) at the beginning of the experiment, These rats were housed in polycarbonate, maintained under a 12-h light/12-h dark cycle in a temperature (23 ± 1 °C), humidity was ($40 \pm 10\%$) with free access to water and food.

Then they are divided into four groups as follows: the control group (n = 8), the 1h exposed group (n = 5), the 2h exposed group (n = 5) and the 3h exposed group (n = 5), respectively, irradiated (0h, 1h, 2h and 3h) daily for 15 weeks and weighted every weekend during the treatment.

Exposure system

The exposure system (Figure.1) consisted of two cell phones and a plastic cage (WxLxH) 30x40x40cm, all experimental animals were suited in the same conditions with daylight, in room without near sources of RFR. Each cell phone was positioned and fixed above the side ceiling of plastic cage about 2-3 cm from the body of the rats. The cell phones were placed as closely as possible to the whole body of the rats for uniform field distribution of electromagnetic field (EMF). During wave exposure, the cage was constantly aerated and all rats are able to move around freely. The experimental groups were continually exposed to radiofrequency radiation from two cell phones (900MHz GSM, electromagnetic field pulsed at 217 Hz), the peak specific absorption rate (SAR) of the head was 0.873W/kg and the average SAR of the whole body was 0.352W/kg, mobile phones were activated by calling each other, all study groups were exposed to radiofrequency radiation at 10:00 a.m daily. The temperature inside the cage was monitored during the experiments; it was almost unchanged (23 ± 1 °C).



Figure.1 : Exposure Device

Bone marrow smears collection and examination

At the end of the exposure period (15 weeks), all rats were anesthetized using the ether and the femurs were isolated using a needle and femurs of all rats were dissected from each animal, bone marrow smears were prepared and stained using Giemsa stain (Jain et al. 1986), at least three air-dried wedge slides of bone marrow smears were prepared from each rat and were counted using an optical microscope (Nikon, Japan) at 1000X magnification.

Differential count included cells from the erythroid series (rubriblasts, prorubricytes, basophilic rubricytes, polychromatophilic rubricytes and metarubricytes) ,the myeloid series (myeloblasts, promyelocytes, myelocytes, metamelocytes, bands cells, segmented neutrophils) and lymphocytes and were made on 1000 cells in mature rats , the bone marrow aspirate smears was evaluated the cellularity and particle distribution and to classify the erythroid ,myeloid precursors and lymphocytes (Jain 1993; Latimer et al. 2003)

Statistical analysis

Data were expressed as means \pm SEM, statistical analysis were carried out by analysis of variance (ANOVA) \pm SEM followed by appropriate post-hoc tests including multiple comparison (LSD), differences were considered significant at 0.05 level.

Results and Discussion:-

Results

Table1:-bone marrow cellular composition in different groups of rats (control and exposed groups).

haematopoietic elements	The control group (n=8)	The1h exposed group (n=5)	The2h exposed group (n=5)	The3h exposed group (n=5)
proerythroblasts	5,5 \pm 0,84	5,6 \pm 1,06	6,2 \pm 1,06	7,4 \pm 1,06
Basophilic erythroblasts	74,62 \pm 3,72	82,62 \pm 4,71	79, 2 \pm 4,71	81,6 \pm 4,71
Polychromatophilic erythroblasts	137,62 \pm 4,57	192 \pm 5,78*	205,6 \pm 5,78*	208,8 \pm 5,78*
orthochromatic erythroblasts	138,12 \pm 3,98	174 \pm 5,03*	184,4 \pm 5,03*	185,4 \pm 5,03*
Myeloblasts	6,37 \pm 0,93	5,2 \pm 1,18	4,8 \pm 1,18	5,6 \pm 1,18
Promyelocytes	14,37 \pm 1,63	14,8 \pm 2,07	10,4 \pm 2,07	11 \pm 2,07
Myelocytes	68,5 \pm 3,85	67,4 \pm 4,86	65 \pm 4,86	61,4 \pm 4,86
Metamyelocytes	94,25 \pm 2,70	91,4 \pm 3,42	88,6 \pm 3,42	90,4 \pm 3,42
Band cells	116,87 \pm 3,99	107,8 \pm 5,05	104,8 \pm 5,05	107,00 \pm 5,05

Segmented neutrophils	121,87±4,08	109±5,17	110,8±5,17	111,6±5,17
Lymphocytes	221,87±6,83	150,4±8,64 *	140,2±8,64 *	129,6±8,64 *

Values are given as the mean ± SEM and differences were considered significant at *P < 0.05

Means ±SEM of cellular composition of bone marrow in different groups of rats (control group, The1h exposed group, The2h exposed group The3h exposed group,) are shown in table 1.

In statistical comparison of bone marrow cells, the number of polychromatophilic and orthochromatic erythroblast in the exposed groups increased significantly compared to the control group.

So, the number of polychromatophilic erythroblast among all exposed groups (192±5,78, 205,6±5,78 et 208,8±5,78 respectively for the1h ,2h and the3h exposed groups) exceeds that observed in the control group, these differences were significant when compared each exposed group (respectively P1h; P2h; P3h <0.001) or all exposed groups to control group (D=4,94 ;P<0.001).

Similarly, So, the number of orthochromatic erythroblast among all exposed groups(174±5,03 ;184,4± 5,03 ;185,4± 5,03 respectively for the1h ,2h and the3h exposed group) exceeds that observed in the control group (138,12±3,98), these differences were significant when compared each exposed group (respectively P1h; P2h; P3h <0.001) or all exposed groups to control group (D=26,64 ;P<0.001)

However, the number of polychromatophilic and orthochromatic erythroblast among the2h and 3h exposed groups exceeds marginally that observed in the1h exposed group , these differences were not significant (respectively for of polychromatophilic and orthochromatic erythroblast (P2=0,17;P3=0,70);(P'2=0,16 ; P'3=0,25)).

In contrast, the number of lymphocytes in the exposed groups (150,4±8,64 ,140,2±8,64 and 129,6±8,64) is lower compared to that observed in the control group (221,87±6,83), these differences were significant when compared each exposed group (respectively P1h; P2h; P3h <0.001) or all exposed groups to control group (D=32,12 ;P<0.001).\

However, the number of lymphocytes among the2h,3h exposed groups(140,2±8,64 and 129,6±8,64) is lower that observed in the1h exposed group (159,4±8,18), these differences were not significant (respectively P2=0,41 ; ;P3=0,10).

the number of proerythroblast and basophilic erythroblasts showed no significant increase in the exposed groups (1h,2h,3h) compared to the control group (p>0.05).

the number of myeloblasts, promyelocytes ,myelocyte, metamyelocytes, band cells and Segmented neutrophils decreased in the the exposed groups (1h,2h,3h) compared to the control group, but the difference was not statistically significant (p>0.05; Table 1).

Discussion:-

The bone marrow examination is one of the numerous parameters assessed to determine the potential toxicity of a compound (Irons, 1991), its high mitotic activity and strong radiosensitivity make it the most vulnerable part of the body.

In this context, the present study aimed to evaluate the effect of radiofrequency radiation on cellular composition of the bone marrow in the mature rats.

So, the study revealed that a chronic exposure to 900 MHz of rats respectively for :0h,1h, 2h and 3h at a maximum SAR (0.873- 0.352W / kg) for up 15 weeks induce effect on the hematopoietic cells ,in statistical comparison of bone marrow cells ,the number of polychromatophilic and acidophilic erythroblast cells increased significantly in the exposed groups compared to control group (P<0.001) and the number of lymphocytes in decreased significantly compared with its control group in mature rats ,these effects did not depend on duration of exposure. However, the number of metamyelocytes, neutrophils, band neutrophils, eosinophils, and basophils cells decreased insignificantly

in the exposed groups compared to the control group ($p > 0.05$). In fact, this is probably related to cytotoxic and genotoxic effect of radiofrequency radiation (Jelodar et al., 2011). Indeed erythropoiesis is considered as a continuous process of cell progression from blasts to mature cells, is balanced in the steady-state condition, Stimuli which could influence balance of maturation or proliferation of cells activate a known feedback mechanism of homeostatic control mechanism (Guyton, 2000).

In another study Jelodar et al. (2011) who showed that the exposure of mature rats to 900 MHz radiofrequency radiation for 5h during 70 days induce a significant increase in polychromatophilic erythroblasts for both exposed groups, but there was no reduction in myeloid counts was observed in exposed rats.

Trosic et al. (2004) found a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow and the sporadic appearance of micronucleated immature bone marrow red cells after the exposure Wistar rats to a 2.45 GHz continuous RF/MW field for 2 h daily, 7 days a week, at SARs (specific absorption rate) :1.25 +/- 0.36 (SE) W/kg and power density of 5-10 mW/cm.

Similarly Trosic and Pavicic (2009) revealed a increase in frequency of immature erythrocytes in groups exposed to radiofrequency radiation and so, the proliferation and maturation of erythropoietic cells were affected by the applied irradiation.

Significant decrease in lymphoblast count was obtained at 15 and 30th experimental day after the exposure of wistar rat for 2, 8, 15 or 30 days at whole-body average specific absorption rate (SAR) of 1-2W/kg and power density of 5-10mW/cm (Trosic et al., 2004).

In contrast to our results, an in vitro study was conducted by Kumar et al. (2010) how demonstrated that the exposure of the bones (femur and tibia) placed in a Petri dish by a 900-MHz continuous-wave (CW) radiofrequency radiation at SAR of 2 W/kg did not cause any significant change in the proliferation rate of bone marrow cells and lymphocytes, erythrocyte maturation rate and DNA damage of lymphocytes.

Galvin et al (1982) reported that the percentages of lymphocytes and neutrophils for the exposed groups were similar to those in the control groups after the exposure of the male rats to 2450-MHz cw microwave radiation for 8 h with SAR of 0.44-2.2 mW / g at incident power densities of 0 (sham), 2, or 10 mW/ cm².

Conclusion:-

the exposure to 900 MHz of mature rats respectively for: 0h, 1h, 2h and 3h during 15 weeks at a maximum SAR 0.873- 0.352W / kg induce a effect on hematopoietic cells by either stimulation or inhibition of cell division and so that it could be represented sometimes as decrease and sometimes as increase in the number of cells (Javad et al 2014) ,this effect is less remarkable with increasing time of exposure.

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