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RESEARCH ARTICLE

ASSESSMENT OF CYTOGENETIC EFFECTS IN LYMPHOCYTE CELLS OF IMMATURE RATS EXPOSED IN UTERO TO 900 MHZ RADIOFREQUENCY RADIATION.

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Abstract

The widespread use of electromagnetic devices such as mobile phones (GSM) raises the fear about possible health hazards from exposure to radiofrequency (RF) radiation, especially during the stage of pregnancy. Since the early life (embryo, fetus) is at a higher risk of environmental toxicants and it is considered more vulnerable than adults because their immune system is still developing.

The goal of our study was to evaluate whether whole-body exposure in utero to non-thermal radiofrequency (RF) radiation from cellular phones could induce cytogenetic effects on peripheral blood lymphocytes of immature rats.

Twenty Wistar immature rats were exposed only in utero to 900 MHz at SAR of 0.873-0.352 W/kg, for 0h (control), 1h, 2h and 3h daily, 7 days a week. All rats were visually checked daily, body weight and length were measured weekly starting the 2 weeks after their birth.

Nine weeks after the delivery, blood samples were collected from all groups and peripheral blood cultures were performed using standard laboratory methods for the extent of genotoxicity, assessed by the cytokinesis-block micronucleus assay. No differences in micronucleated (MN) cells and proliferation index in immature rat lymphocyte cells were found among the study groups. The body weight and length gains were also insignificantly changed in all groups exposed comparing to unexposed rats.

This study shows that the exposure only in utero to 900 MHz radiofrequency radiation from cell phones following by 9-week recovery period may not induce cytogenetic alterations in immature rat lymphocytes.

Introduction:-

The prenatal exposure to radiofrequency radiation emitted by the wireless devices and other emitters has increased the scientific interest on possible adverse effects and their consequences on human health especially for embryo/fetus health. Many fears were expressed about the potential vulnerability of such categories to

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radiofrequency electromagnetic radiation because the damage induced may be irreversible compared to adults; fetuses are even more vulnerable than children (Morgan et al., 2014),

In this context, several studies have studied the effects of prenatal exposure to radiofrequency radiation.Tenorio et al.(2011), Bas et al.(2009) reported that these radiations may affect embryo and fetus development and compromise the normal development of vital organs, Ferreira et al.(2006) have showed that the exposure to same radiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring.

In contrast, other studies found no effect after the exposure to radiofrequency radiation. Vijayalaxmi et al.(2003) did not find gentoxic effect in bone marrow cells after prenatal and postnatal exposure by Iridium 1.6 GHz signals, Huuskonen et al.(1989) have also reported that the exposure radiofrequency radiation is not teratogenic or embryotoxic.

The objective of this study is to investigate the potential cytogenetic effects of exposure to 900 MHz radiofrequency radiation on peripheral blood lymphocytes of immature rats exposed only in utero during the pregnancy until birth for 1h, 2h and 3h a day, 7 days a week using the micronucleus test and the cellular proliferation index.

Materials and Methods:-

Animals

All procedures were performed in compliance according to international laws and regulations on animal welfare. Twenty Wistar immature rats involved in this study were obtained from the parents exposed respectively to 0h,1h, 2h and 3h, 7J /7J for 15 weeks,. After their birth, we stoped the exposure of pups and put them in cages far from any radiations, whereas their mothers continued the daily treatment, and join them at the end of each exposure session. The immature rats were housed with their mothers in polycarbonate, maintained under a 12-h light/12-h dark cycle in a temperature (23 ± 1 °C), humidity was (40 ± 10%.) with free access to water and food.

When the pups reached 2 weeks of age, their body weight and length were measured every weekend for 7 weeks without any treatment. Then they are divided into four groups as follows: the control group (n = 5), the1h 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 only in utero.

Exposure system

The exposure system (Figure.1) consisted of two cell phones and a plastic cage 30x40x40cm (WxLxH), all experimental animals were suited in the same conditions with daylight, in room without near sources of RF radiation. 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), mobile phones were activated by calling each other, all study groups were exposed to radiofrequency radiation at 10:00 a.m daily. 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. The temperature inside the cage was monitored during the experiments; it was almost unchanged (23 ± 1 °C).

Figure 1:-Exposure Device

Cellular culture

After the recovery period (9 weeks), blood samples were collected into the glass tubes (with heparin as anticoagulants) by cardiac puncture under the ether anaesthesia, the rats were then decapitated according to the technical conditions employed in our laboratory, in fact, 0.5 ml of the whole blood were incubated in glass tubes

containing 5 ml RPIM 1640 growth medium supplemented with 15% fetal calf serum, 1% phytohemagglutinine and 1% antibiotics (penicillin/streptomycin).

Culture tubes were incubated at 37 °C for 72 h. After 44 h of cultivation, 0.1ml of cytochalasin B was added to stop the cytoplasm division without inhibiting nuclear division.

After 72 h incubation at 37 ◦C, total blood cultures were centrifuged at1000 rpm for 10 minutes and then exposed to a light hypotonic shock with 0.075 M KCl and fixed with a 1/3 acetic-acid/methanol solution, then spreaded over microscope slides, which were air-dried and stained with 5 % Giemsa in phosphate buffer (pH = 6.8) and coded.

Micronuclei (MN) analysis
The micronuclei were scored in 500 binucleated lymphocytes per animal, and were counted only in binucleated cells.

The criteria for identifying MN are as follows: nuclear entities are independent of the main nucleus and have same color than principal structure, the size must be between the sixteenth and the third smallest of the main nucleus (Fenech, 1993)

Proliferation index
Cell proliferation index (PI) is an indirect measurement of cell cycle duration, and is calculated according to the following formula (Titenko-Holland et al., 1997):

$$PI = \frac{(1\times N1 + 2\times N2 + 3\times N3 + 4\times N4)}{1000\ analyzed\ cells}$$

Where N1 is the number of cells in the first division, N2 the number of cells in the second division, N3 the number of cells in the third division and N4 the number of cells in the fourth division.

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

Results And Discussion:-

The weekly body weight gain study
We define the weekly body weight gain as: WBWG = the body weight difference of immature rat after one week.

Table1:-weekly body weight gain of all four groups during the recovery period

WBWG(g) Week	The group control (n=5)	The1h exposed group (n=5)	The2h exposed group(n=5)	The3h exposed group(n=5)
1	4,60	1,14	1,30	4,94
2	9,70	5,84	12,40	8,48
3	9,30	13,32	11,94	12,04
4	7,96	11,70	10,20	5,22
5	18,59	8,92	12,64	12,28
6	6,90	15,70	6,76	10,74

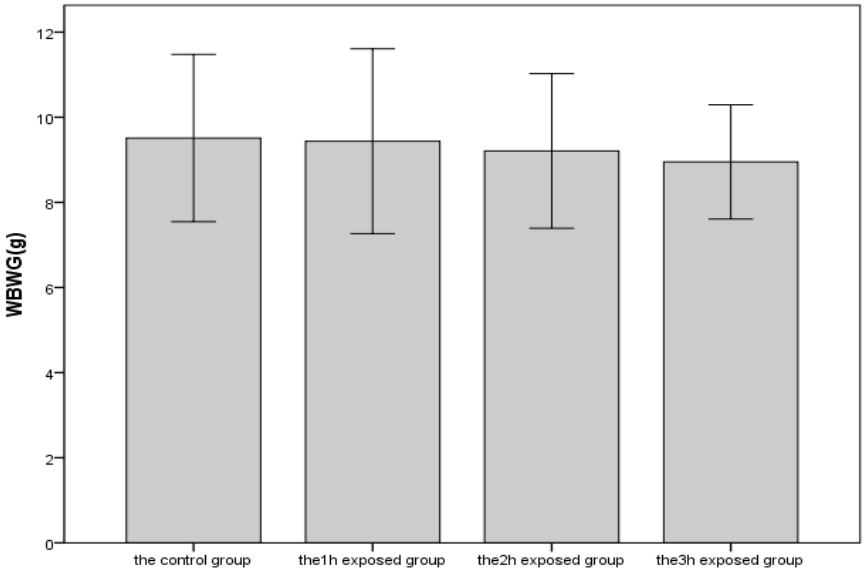

Figure 2:-The values of weekly body weight gain of the study groups.

Values are given as the mean ± SEM and differences were considered significant at *P < 0.05
The analysis of variance (ANOVA) revealed no significant difference in weekly gain of body weight between all exposed groups and control group (F=0 19 ; P= 0,99).

Figure.2 reveals that the weekly gain of body weight decreases slightly for the exposed groups compared to the control group, the WBWG of the1h exposed group is 9,44±1,85g . The WBWG of the2h exposed group is 9,21±1,85g and the WBWG of the3h exposed group is 8,95±1,85g while the WBWG of the control group is 9,51±1,85g. However, there were no statistically differences when compared each exposed group (1h,2h,3h) respectively to the control group (P1= 0,98 ; P2=0,91 ; P3 = 0,83), all of tem are greather than 0,05.

The weekly body length gain study
We define the weekly body length gain as: WBLG= the body length difference of immature rat after one week.

Table2:-weekly body length gain of all four groups during the recovery period

WBLG(cm) Week	The group control (n=5)	The1h exposed group (n=5)	The2h exposed group(n=5)	The3h exposed group(n=5)
1	1,06	1,82	1,66	1,48
2	3,24	3,76	2,70	1,94
3	2,68	2,44	3,00	3,54
4	1,38	1,60	1,44	-0,14
5	3,90	4,54	3,92	2,42
6	2,86	0,76	1,14	2,62

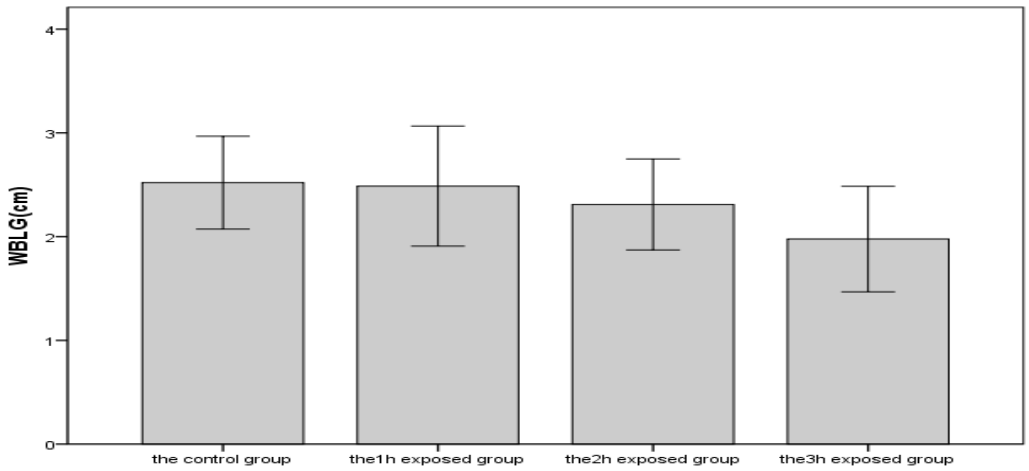

Figure 3:-The values of weekly body length gain of the study groups.

Values are given as the mean ± SEM and differences were considered significant at *P < 0.05
The analysis of variance (ANOVA) revealed no significant difference in weekly gain of body length between all exposed groups and control group (F=0, 25; P= 0, 86).

Figure.3 shows that the weekly gain of body length decreases marginally for the exposed groups compared to the control group, the WBLG of the1h exposed group is 2,49±0,50cm, the WBLG of the2h exposed group 2,31±0,50cm and the WBLG of the3h exposed group is 1,98±0,50cm while the WBLG of the control group is 2,52±0,5cm. However, there were no statistically differences comparing each exposed group (1h,2h,3h) respectively to the control group (P1= 0,96 ;P2=0,77 ;P3 = 0,45).

Discussion:-

Body weight and length are indicators of health status of living organisms to assess the growth and development, body weight and length loss in early life is associated with certain diseases or health outcome (Chandra, 2002).

Our results demonstrated that the body weight and length gains of the immature rats exposed in utero decreased slightly over the exposure time, but there was no significant difference in the body weight and length gains between the exposed groups and control group.

Hence, the exposition of immature rats only in utero respectively for 0h,1h, 2h and 3h a day, 7 days a week to 900 MHz radiofrequency (RF) radiation at an average special absorption rate (SAR) of 0.873- 0.352 W/kg) followed by 9-week recovery period did not induce a significant effect on the two parameters of growth ($F = 0.19$, $P = 0.99$ (weight), $F = 0.25$, $P = 0.86$ (length)).

This study is in agreement with Lee et al.(2009) who exposed pregnancy groups of mice to 848.5 MHz (CDMA code division multiple access) and 1950 MHz (WCDMA wideband code division multiple access) radio frequency signals or both simultaneously at SAR of 2 W/kg until 17 days of gestation for two sessions of 45 minutes. In their study, no differences in weight, length, malformations or other parameters were observed between the exposed groups and control.

Similarly, Poulletier de Gannes et al.(2012) were concluded that the exposure of pregnant rats and their pups to a 2450 MHz Wi-Fi signal at SAR of 0.08, 0.4, and 4 W/kg for 2 h/day, 6 days/week for 18 days, did not induce any effect on the pre- and postnatal development of the pups .

Ait Aissa et al.(2010) did not record any changes in body weight after the exposure of prenatal (2 weeks of gestation) and Postnatal (5 weeks after birth) rats for 2 hours/ day (5 days / week) by a Wi-Fi signal 2.45 GHz radiofrequency (RF) radiation at SAR =0, 0.08, 0.4 and 4 W / kg.

In contrast, Amer FI et al (2013) found a reduction of body length after the exposure of pregnant mice to 950–1,800 MHz for 2 h/day for 8 consecutive days from day 7 to 14 of gestation at SAR of 1-1.6 W/kg.

Cytogenetic study:-

Table3:-Cytogenetic results for the all groups

The exposure time	MN (total)	Cells with 1 MN	Cells with 2 MN	Cells with 3 MN	PI
1h	2	2	0	0	1,552
1h	1	1	0	0	1,611
1h	3	3	0	0	1,553
1h	2	2	0	0	1,542
1h	4	0	2	0	1,582
2h	2	2	0	0	1,542
2h	1	1	0	0	1,672
2h	4	4	0	0	1,457
2h	2	2	0	0	1,602
2h	3	1	1	0	1,513
3h	4	4	0	0	1,526
3h	2	2	0	0	1,432
3h	1	1	0	0	1,427
3h	3	3	0	0	1,721
3h	1	1	0	0	1,390
0h (control)	2	2	0	0	1,521
0h	3	3	0	0	1,455
0h	2	2	0	0	1,685
0h	1	1	0	0	1,668
0h	2	2	0	0	1,612

Micronucleus (MN) test

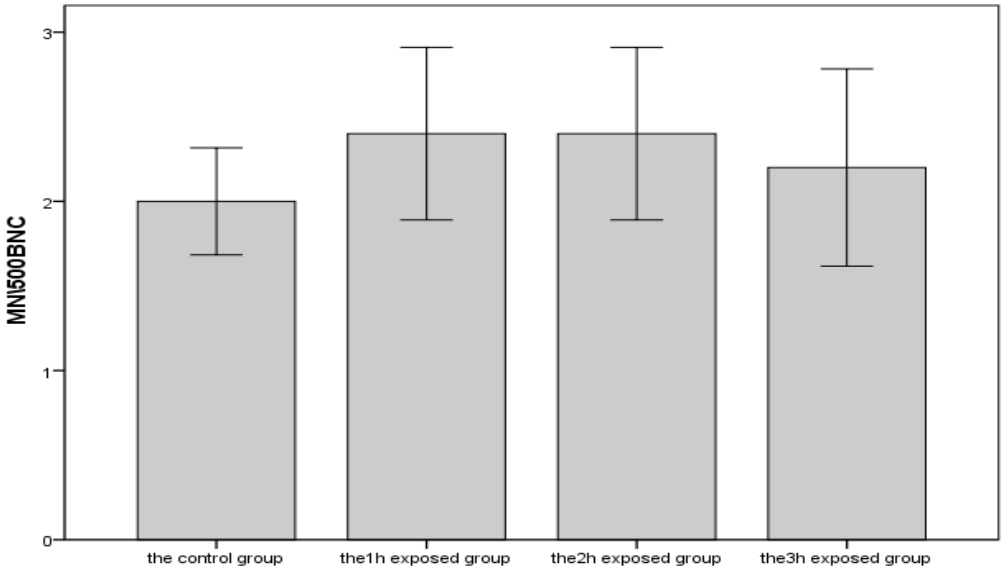

Figure 4:-The values of MN in the peripheral blood of all study groups.

Values are given as the mean ± SEM and differences were considered significant *P < 0.05
The analysis of variance (ANOVA) revealed no significant difference in micronucleus frequency (F=0,15 ; P= 0,93) between all exposed groups and control group.

As shown in the Figure.4, the MN formation increases slightly for all exposed groups compared to the control group. Indeed, the number of MN in binucleated cells (BNC) among all exposed groups (2,4 ±0,49 ; 2,4 ±0,49; 2,2 ±0,49

respectively for the1h exposed group , the2h exposed group and the3h exposed group) exceeds marginally that observed in control group (MN=2,00 ±0,49), these differences were not significant comparing each exposed group (1h,2h,3h) respectively to the control group (respectively P1= 0,57; P2=0,57; P3 = 0,78).

Proliferation indices (PI) test

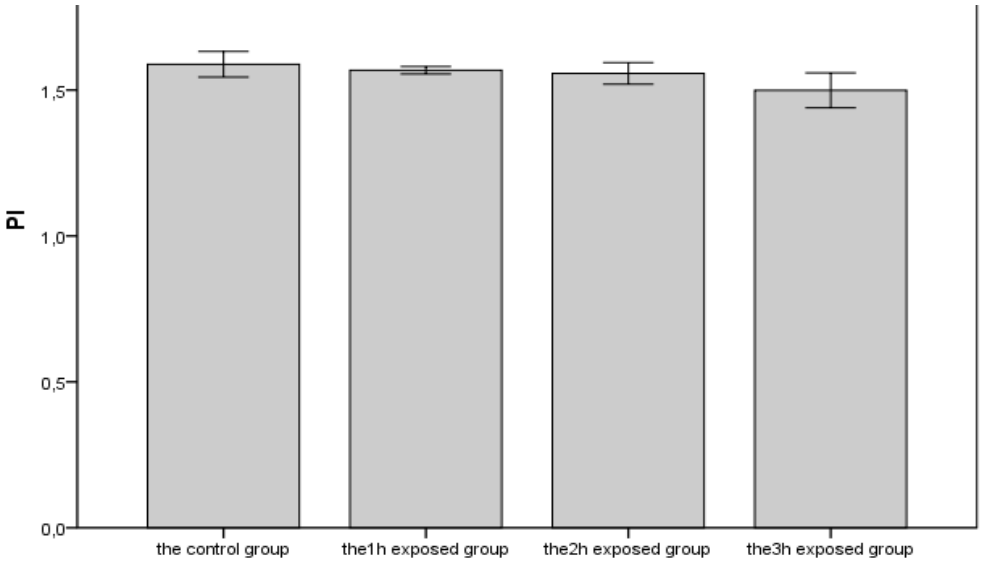

Figure 5:-The values of PI in all groups.

Values are given as the mean ±SEM and differences were considered significant at *P < 0.05.
The analysis of variance (ANOVA) revealed no significant difference in Proliferation indices (PI) between all exposed groups and unexposed group (control) (F = 0,83;p =0,5).

As shown in Figure.5, the PI among all exposed groups was slightly reduced, the values of PI in the exposed groups (PI=1,568± 0,042; PI =1,557 ± 0,042; PI =1,499 ± 0,042 respectively for the1h exposed group, the2h exposed group and the3h exposed group) is marginally lower compared to that observed in the control (PI = 1,588 ± 0,042), these differences were not significant comparing each exposed group (1h,2h,3h) to the control group (respectively P1= 0,738; P2=0,608; P3 =0,153).

Discussion:-

The formation of MN is a result of either clastogenic or aneugenic side effects; it has been used in peripheral blood lymphocytes and in bone marrow cells of animals or humans.

In this study, we used the micronucleus test considered as indicator of genotoxic exposition, it's able to detect both chromosome breakage and chromosome loss and we assess the proliferation index which measures the cellular kinetics and also detect the toxic activity.

In our study, the exposure to 900 MHz of immature rats in utero respectively for four times (0,1, 2 and 3h) at a maximum SAR (0.873- 0.352W / kg) following a 9 weeks recovery period do not induce cytogenetic alterations in immature rat lymphocytes. The statistical analysis (ANOVA) confirms that the values of MN and PI in the exposed groups were not significantly affected (F=0,15; P= 0,93) even with increasing time of exposure.

It may be that the exposure to radiofrequency radiation leads directly to a cytogenetic damage, but the recovery period was sufficient for the improvement to genotoxic damage among the adult rats exposed (Şekeroğlu et al.,2012).

In this context, Trosic et al.2004 revealed a transient effect on proliferation and maturation of erythropoietc cells in the rat bone marrow and a sporadic appearance of micronucleated immature bone marrow red cells after the exposure of Wistar rats to a 2.45 GHz continuous RF/MW field for 2h daily, 7 days a week, at SAR of 1.25 ± 0.36 W/kg.

The micronucleated polychromatic erythrocytes are eliminated by the mononuclear phagocyte system (MPS), whose functionality is adapted to the higher number of immature and micronucleated cells

Franzellitti et al. 2010 found after 2 h in the absence of irradiation was sufficient for the improvement to genotoxic damage in human trophoblast cells exposed to 1800 MHz EMF at SAR = 2 W/kg for 16 and 24 h .

These results are in agreement with data published by Abramsson-Zetterberg L and Grawe J .(2001) showing that the exposure of adult and fetal mice (in utero) to 50 Hz magnetic field for 18 days and after 35 days did not induce any increase in micronuclei in mouse bone marrow.

Laudisi et al. 2012 revealed that exposure in utero of mice to 2450MHz RF (WiFi) at SAR of 4W/kg for 2h per day during pregnancy (5 days after mating and ending 1 day before the birth) leads to no significant differences in cell count, phenotype, and proliferation of thymocytes, spleen cell count, CD4/CD8 cell frequencies, T cell proliferation, and cytokine production.

Nikolova et al. 2005 reported a low and transient increase in DNA double strand break in mouse embryonic stem cells after acute exposure to 1.7- GHz field.

Tomruk et al. (2010) did not find a DNA and lipid damage of liver tissues in newly born rabbits exposed in utero to of 1800 MHz for 15 min/day for a week, Guler et al. (2010) had also reported similar results in brain tissues.

Vijayalaxmi et al. (2003) found no significant increase in formation micronucleus in the bone marrow after the pre-natal and early postnatal exposure of rats to 1.6 GHz radiofrequency radiation for 2 h / day From 19 days of gestation until 35 days after birth.

However, the conclusions of Ferreira et al. (2006) reported a micronucleus induction in the bone marrow of newborn rats exposed in utero to 834 MHz RF at SAR of 0.55-1.23 W/kg.

Conclusion:-

In this paper we showed that, under our experimental conditions the exposure of immature rats only in utero to radiation from mobile phones (GSM-900MHz), at SAR (0.37 W/kg and 0.49W/kg) for 0h,1h, 2h and 3h a day, 7 days a week followed by 9-weeks recovery period did not induce significant differences in micronucleus frequency, proliferation indice, body weight and length gains among the study groups even with increasing time of exposure. However, specific precautions must be taken to minimize the exposure of radiofrequency (RF) radiation during the pregnancy.

References:-

1. Abramsson-Zetterberg L, Grawé J.2001.Extended exposure of adult and fetal mice to 50 Hz magnetic field does not increase the incidence of micronuclei in erythrocytes. Bioelectromagnetics. Jul;22(5):351-7.
2. Aït-Aïssa, S., Billaudel, B., Poulletier De Gannes, F., Hurtier, A., Haro, E., Taxile, M., Ruffié, G., Athane, A.,Veyret, B., Lagroye, I., 2010. In situ detection of gliosis and apoptosis in the brains of young rats exposed in utero to a Wi-Fi signal. Comptes Rendus Physique 11, 592-601
3. Amer FI, El Shabaka HA, Zakaria I, Mohammed HA.2013.Effect Of Microwave Radiation On The Retina Of Mice Embryos Journal of Biology and Life Science ISSN 2157-6076, Vol. 4, No. 2.
4. Bas O, Odaci E, Mollaoglu H, Ucok K, Kaplan S.2009.Chronic prenatal exposure to the 900 megahertz electromagnetic field induces pyramidal cell loss in thehippocampus of newborn rats. Toxicol Ind Health; 25:377–84.
5. Chandra, R.K. 2002. Nutrition and the immune system from birth to old age. Eur. J. Clin. Nutr. 56 (S3), S73.
6. Fenech, M. 1993. The cytokinesis – block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in a human population. Mutat.Res., 285: 35-44.
7. Ferreira AR, Knakievicz T, Pasquali MA, Gelain DP, Dal-Pizzol F, Fernández CE, de Salles AA, Ferreira HB, Moreira JC.2006. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sciences, 80 : 43-50.
8. Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E.. 2010. "Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-

8/SVneo cell line evaluated with the alkaline comet assayassay." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 683(1–2): 35-42.
9. Huuskonen, H., Juutilainen, J., Julkunen, A., Mäki-Paakkanen, J. & Komulainen, H..1998b. Effects of low-frequency magnetic fields on fetal development in CBA/Ca mice. Bioelectromagnetics, 19, 477–485.
10. Laudisi F, Sambucci M, Nasta F, Pinto R, Lodato R, Altavista P, Lovisolo GA, Marino C, Pioli C .2012. Prenatal exposure to radiofrequencies: effects of WiFi signals on thymocyte development 531 and peripheral T cell compartment in an animal model. Bioelectromagnetics, 33(8):652-661.
11. Lee HJ1, Lee JS, Pack JK, Choi HD, Kim N, Kim SH, Lee YS.2009. Lack of teratogenicity after combined exposure of pregnant mice to CDMA and WCDMA radiofrequency electromagnetic fields. Radiat Res 172(5): 648-652.
12. Morgana Lloyd, Santosh Kesarib, Devra Lee Davisa .2014. Why children absorb more microwave radiation than adults: The consequences Journal of Microscopy and Ultrastructure 2(4):197-204.
13. Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. .2005.Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. FASEB J; 19:1686–1688.
14. Poulletier de Gannes F1, Haro E, Hurtier A, Taxile M, Athane A, Ait-Aissa S, Masuda H, Percherncier Y, Ruffié G, Billaudel B, Dufour P, Veyret B, Lagroye I..2012. Effect of in utero wi-fi exposure on the pre- and postnatal development of rats." Birth Defects Res B Dev Reprod Toxicol 95(2): 130-136.
15. Sekeroglu Vedat, Aysegul Akar, Zulal Atlı Sekeroglu.2012.Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicology and Environmental Safety 80 140–144.
16. Tenorio BM, Jimenez GC, Morais RN, Torres SM, Nogueira RA, Junior VAS.2011.Testicular development evaluation in rats exposed to 60 Hz and 1 mT electro-magnetic field. J Appl Toxicol; 31:223–30.
17. Titenko-Holland, N., Windhan, G., Kolachana, P., Reinish, F., Paravatham, S., Osorio, A.M. and Smith, M.T.1997. Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo : a study of malathion-exposed workers. Mut. Res., 388 : 85-95.
18. Tomruk A, Guler G, Dincel AS. 2010. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits." Cell Biochem Biophys 56(1): 39-47.
19. Trosic I, Busljeta I, Modlic B.2004b. Investigation of the genotoxic effects of microwave irradiation in rat bone marrow cells: in vivo exposure. Mutagenesis; 19:361–4.
20. Vijayalaxmi, L.B. Sasser, J.E. Morris, B.W. Wilson, L.E. Anderson.2003.Genotoxic potential of 1.6 GHz wireless communication signal: in vivo two-year bioassay, Radiat. Res. 159 (4) 558–564.