The Egyptian Journal of Hospital Medicine Vol., 16 : 29 –38 September 2004
I.S.S.N: 12084
1687-2002

# Effect Of Exposure To Electromagnetic Field On Tumour Growth And Some Serum Parameters In Mice Implanted With Ehrlich Tumour.

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## Abstract

**Objective:** To investigate the bio-effects of electromagnetic field (EMF) exposure on blood elements, blood glucose, liver and kidney functions of mice implanted with Ehrlich tumour. **Methods:** female BALB/c mice inoculated subcutaneously with cell suspension isolated from Ehrlich ascitis carcinomas were exoposed to sinusoidal 50 Hz, 0.2 Tm for 21 days. **Results:** exposure to low frequency EMF resulted in a pronounced decrease in tumour growth. The increase in total leucocytic count, liver enzymes, bilirubin, blood glucose and creatinine observed in mice with implaned tumours, was significantly reduced by low frequency EMF exposure. **Conclusions:** these results suggest that exposure to low frequency EMF of 50 Hz inhibits malignant growth in mice with Ehrlich tumour. It is suggested that low frequency EMF may find utility as a method in the treatment of tumours, either alone or in conjunction with chemotherapeutic agents.

**Key Words**: Magnetic field, Ehrlich tumour, Mice, kidney, Liver, Blood picture

## Introduction

There has been considerable interest in the potential biological effects of electromagnetic flied (EMF) exposure over the past few years. Previous studies suggested that EMF may influence the physiological processes in biological systems. Particular interest has been given by many researchers to the effects of low frequency EMF (Pasquinelli *et al*., 1993; Petrini *et al*., 1990). For example, Pasquinelli *et al*. (1993) suggested that exposure to low frequency EMF of 75 HZ and an intensity of 20 Gauss caused an increase in mouse life span. More recently, Chater *et al* (2004) provided data suggesting that sub-acute exposure to EMF (128 mT 1hour/day for 10 consecutive days) stimulates plasma corticosterone and liver metallothionein activities in rats. Epidemiological studies have suggested that EMF may increase the risk of various types of cancer, including leukemia, brain and breast tumours (Juutilainen *et al*., 1990; Wrensch *et al*., 1993; Loomis *et al*., 1993; Savitz and Loomis, 1995). One major problem with the epidemiological studies was that information on exposure was scarce. Exposure to EMF is so "universal and unavoidable", so that true controls in the sense of zero exposure to technofields are not available. Hence, all of these studies compare higher exposure to lower exposure. (Havas, 2000).

On the other hand, many attempts have been made to explain the anti-tumour effectiveness of electrotherapy alone or of its combined use with other established therapeutic treatments (Ito and Hashimoto, 1989; Nilsson *et al*., 2000). Gamaley et. al. (1995) showed that macrophage cells are sensitive to electromagnetic fileds in the frequency range 15-99 HZ with maximum sensitivity at 50 HZ. They concluded that their data may have some significance for the effect of therapy using high frequency therapeutic equipment.

The present study was therefore designed to examine the effect of low frequency EMF exposure on the tumour growth, blood elements and other serum parameters including liver enzymes, creatinine and glucose in mice implanted with Ehrlich tumour.

## Materials and methods

### Animals

Female BALB/c mice in the weight range of 17-20 g maintained at the National Cancer Institute (NCI), Cairo University were used. Groups of 20 mice were inoculated subcutanously with 1x10 single cell ml suspension islolated from Ehrlich ascites carcinomas. Injections of 0.2 ml were made into the left flank. Ten days after inoculation the animals were randomly divided into groups. Each group contained at least 10 mice. Group A: was the control group which was not inoculated (control normal), group B: was inoculated with Ehrlich tumour, but not exposed to electromagnetic field and group C: contained animals inoculated with Ehrlich tumour and exposed to 50 HZ, 0.2 mT for 21 days. Measurement of tumour diameter was performed daily to estimate the growth rate of the malignancy.

### Exposure facility

Animals were exposed to a homogenous magnetic field generator in which animals can be housed and exposed as shown in figure (1). There was no measurable difference in temperature between the room and the chamber. The animals were kept in special plastic cages that permit normal ventilation and daylight. The cage with the animals was fixed on supports inside the irradiation chamber. Food and water were allowed *ad libitum* and kept in special open containers fixed on the walls of the cages. The magnetic field exposure was locally manufactured.

### Biochemical assessment

Animals from different groups were anaesthetized with ether and blood samples were obtained from the retro-orbital vein plexsus. Laboratory investigations included complete blood picture, serum glucose, bilirubin, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and creatinine level. Blood samples were collected for hematological and biochemical parameters. EDTA, an anticoagulant, was added at the collection of blood for hematological parameters, while clotted blood samples for biochemical parameters were centrifuged for 10 min at 5000 rpm and supernatant sera were immediately separated for analysis. Hemoglobin concentration was determined according to Van-Kampen & Zulstra (1995), and haematocrit values (Hct) were estimated using the technique of Rodak (1995). The biochemical analysis was carried out on the blood sera. Glucose determination was based on the enzymatic method described by Siest&Schielf (1981). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were measured according to Reitman-Frankel colorimetric method (Crowley, 1967), using commercially available kits (BioMérieux, France). Creatinine and bilirubin in serum was determined using standard laboratory methods (Bauer, 1982).

### Statistical analysis

All results are expressed as means ± SE. A two-tailed Student's t test was used. A probability value less than 0.05 was considered statistically significant (Sokalt & Hahit, 1981).

## Results

### Tumour growth

By the $10^{th}$ day after inoculation of Ehrlich tumour into the animals, the growth measured a mean of 1.5 cm in diameter. In contrast to non-exposed mice, those subjected to EMF exhibited slower rate of tumour growth that reached stationary state during the last week of the experimental period. By the end of the study, the tumour measured a mean of 1.7 cm in diameter in mice subjected to EMF as compared to 2.2 cm in their corresponding controls (Fig. 2).

**Blood picture**

It was noticed that in mice with Ehrlich tumour, total leucocytic count was significantly raised by 71.6% over normal control rats. On the other hand, no significant change was observed as regards red blood cell count, haemoglobin level, haematocrite values or mean cell volume. The rise in leucocytic count was reduced markdely by exposure to low frequency EMF (Table 1, Fig. 3).

**Biochemical changes**

In mice inoculated with Ehrlich carcinoma cells, the levels of AST, ALT and bilirubin were markedly raised in plasma indicating the severity of hepatic injury. The serum ALT and AST levels were significantly elevated from 61.6 ± 6.2 and 67.8 ± 5.1 (saline control) to 153.3 ± 4.8 and 151.8 ± 3.9 IU/L, respectively ($P < 0.01$). This represented 148.8 and 123.8% increases over control values, respectively. Exposure to low frequency EMF for 21 days resulted in significant reduction in the levels of the serum enzymes by 27.1 and 24.2%, respectively (Fig. 4). Bilirubin was raised by 136.3% over control values; from 0.42 ± 0.1 to 0.97 ± 0.12 mg/dl. After exposure to EMF, bilirubin declined to normal level (Fig. 5).

Serum glucose was markedly increased by 51.5% in mice inoculated with Ehrlich carcinoma cells, suggesting impaired glucose homeostasis. The rise in serum glucose was ameliorated in mice exposed to EMF (Fig. 6).

Serum creatinine levels were also raised by 107.5% in mice inoculated with Ehrlich carcinoma cells compared with their saline treated normal counterparts (0.04 ± 0.01 vs 0.083 ± 0.01; $P<0.02$). Serum creatinine levels declined to almost normal levels in mice exposed to EMF (Fig. 7 ).

**Table 1: The variation of CBC in control, Ehrlich tumour and Ehrlich tumour of EMF exposure rats.**

| Item | **Saline treated normal mice** | **Mice inoculated with Ehrlich tumour** | **Mice inoculated with Ehrlich tumour + EMF exposure** |
|---|---|---|---|
| TLC | 10.2<br>±2.357 | 17.5<br>±3.109 | 13.5<br>±1 |
| RBCs 10 mm | 4.6666<br>±0.1527 | 4.825<br>±0.28722 | 4.3<br>±0.24494 |
| Hb g/dL | 13.0666<br>±0.474377 | 12.935<br>±5686 | 11.445<br>±0.9079 |
| Hct% | 39.1667<br>±1.37961 | 40.225<br>±2.4864 | 33.25<br>±1.6217 |
| MCV (FL) | 83.78667<br>±0.60285 | 82.807<br>±0.702 | 83.74<br>±1.025 |

**N.B.: Data are given as mean values + S.D**
**TLC ( Total leucocytic count ).**

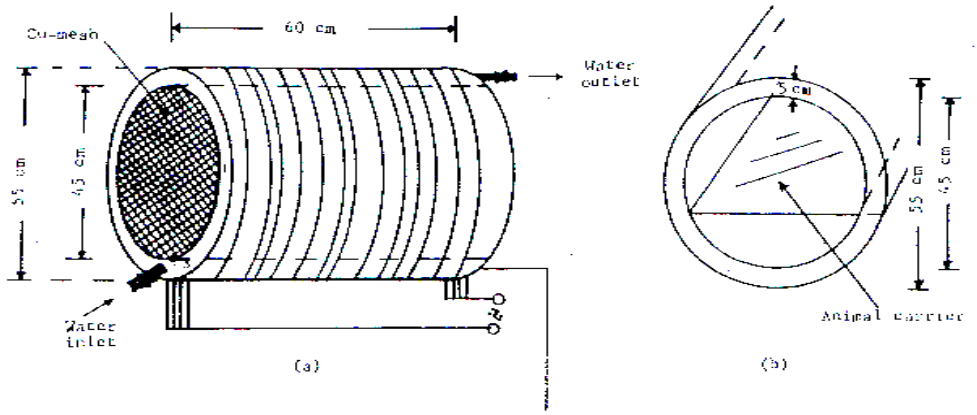
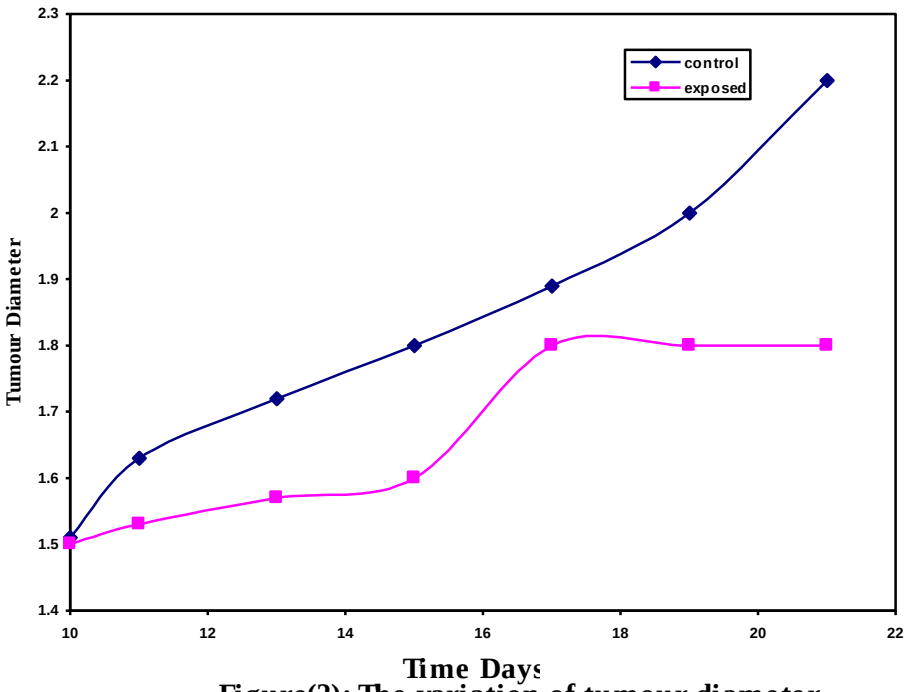



**Figure (3-1)** Magnetic field generator




**Figure(2): The variation of tumour diameter exposed and nonexposed mice**

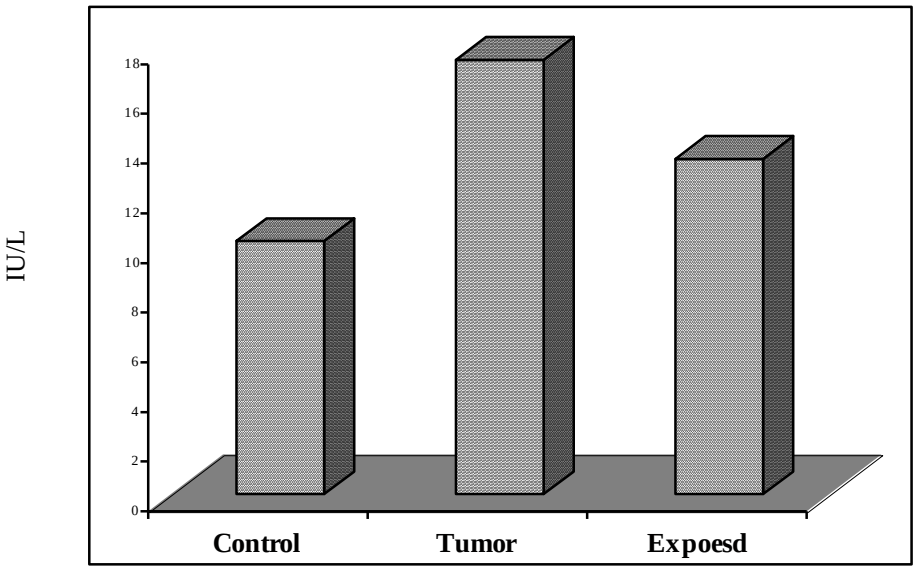
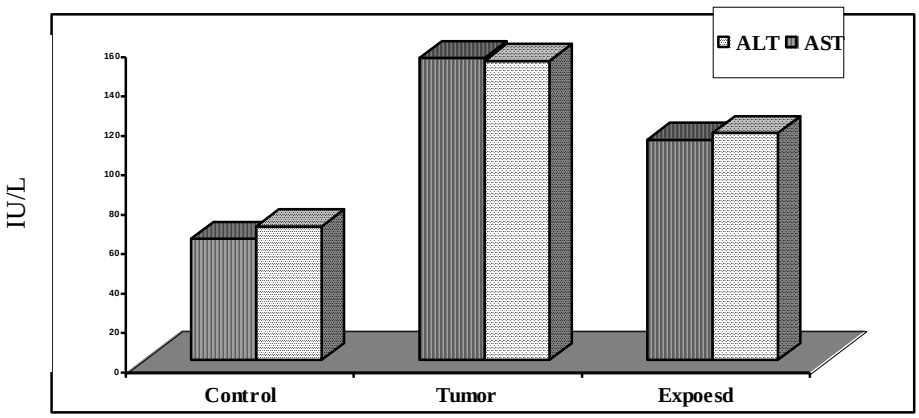
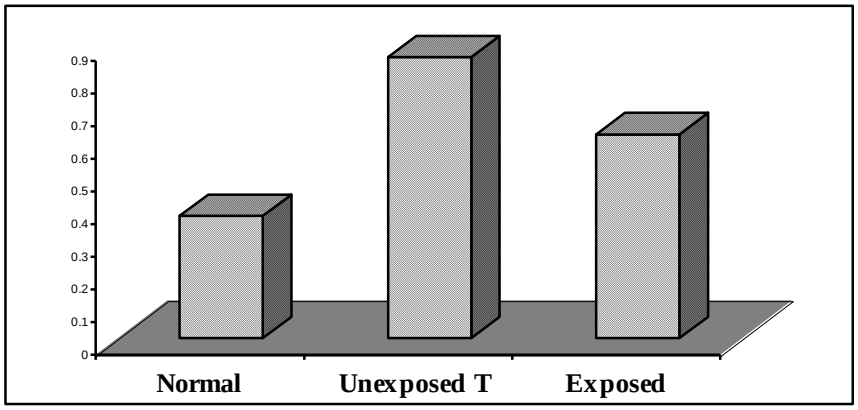



**Figure (3): Effect of 50 Hz EMF on TLC in mice with Ehrlich tumour .**




**Figure(4): Effect of 50 Hz EMF on serum AST and ALT activities in mice with ehrlich tumour**




**Figure(5):The effect of EMF exposure in serum bilirubin level in with Ehrlich tumour**

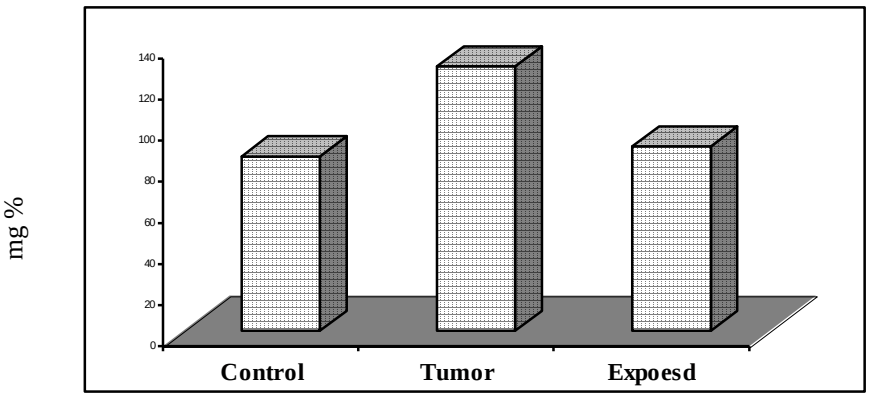



**Figure (6): The effect of EMF exposure in serum glucose level in mice wi Ehrlich tumour**

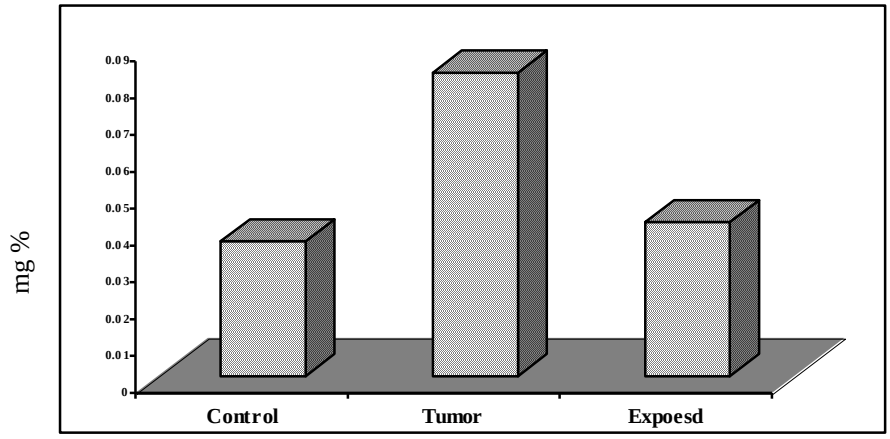



**Figure (7): The effect of50 Hz EMF on creatinine in mice with ehrlich tumour.**

## Discussion

The effects of extremely low-frequency electromagnetic fields (< 100 Hz) on the biological functions of man is a focus of intertest. Studies suggested that EMF may influence the physiological processes in biological systems. Choi *et al* (2003) found that exposure to extremely low frequency magnetic field affects the normal diurnal rhythm of the pain threshold in mice. Other workers suggested that EMF exposure might impair mammalian female reproductive potentiality (Cecconi *et al*., 2000). One important issue is the effect of EMF on different types of cancer in man. Epidemiological data suggest,that there is an association between EMF exposure and the increased incidence of certain types of tumor,particularly leukemia and brain cancer (Juutilainen *et al*., 1990; Wrensch *et al*., 1993; Loomis *et al*., 1993; Savitz and Loomis, 1995).

Other investigators failed to demonstrate significant association between EMF exposure and promotion of cancer. In their study, Imaida *et al* (1998) found that local body exposure to a 929.2-MHz field, modulated in a PDC waveform, has no significant effect on chemically-initiated rat liver carcinogenesis in the rat. More recently, Chater *et al* (2004) provided data suggesting that sub-acute exposure to EMF (128 mT 1hour/day for 10 consecutive days) stimulates plasma corticosterone and liver metallothionein activities in rats. Induction of metallothionein (low molecular weight proteins; 6-7 kDa) is considered to be an important adaptive mechanism which protects against the toxicity of heavy metals such as cadmium (Goering *et al.*, 1995), acts as free radical scavenger protecting against oxidative damage (Sato and Bremne, 1993), and protects against toxicity of alkylating anti-cancer drugs (Lazo and Pitt, 1995) . Further work by Khanduja and, Syal (2003) suggested that 50Hz sinusoidal electromagnetic field of 1mT can prevent bone calcium loss due to aging in animals. Interestingly, Oda and Koike (2004) provided data suggesting that exposure to a rotating(50 Hz) ELF for 5 days saved rat immature cerebellar granule neurons from apoptosis and promoted survival at the flux density of 300 mT, whereas virtually no neuronal survival was observed without exposure.

The present study provided the first evidence that exposure of mice with Ehrlich tumour to low frequency EMF of 50 HZ

was associated with regression of the tumour size concomitant with prevention of hepatic and renal injury associated with this malignancy. Humans are commonly exposed to EMF including low frequency magnetic field, which is generally produced by power lines and many kinds of electric appliances.

In our study, exposure to 50 Hz EMF markedly reduced the increase in total leucocytic count associated with Ehrlich tumour, prevented the decline in renal function and the leakage of hepatocellular enzymes into plasma. It is likely that the observed biochemical changes are a direct consequence of tumour regression. Other mechanisms are also possible, because low frequency EMF has been associated with alterations in cell function and hormonal changes. Exposure to 50 Hz EMF appears to alter both stress responses and energy metabolism in stressed rats. Restraint induced increase of plasma ACTH and glucose levels tended to be suppressed by exposure to the EMF (Harakawa *et al*., 2004). This could explain the finding in the present study where exposure to 50 Hz EMF also normalized glucose levels in mice with Ehrlich tumour. In accordance with findings in the present work are the obervations by Fedorowski *et al* (2004) who stated that low-frequency electromagnetic stimulation may lead to regression of Morris hepatoma in buffalo rats in vivo. In light of the above, it is suggested that low frequency EMF may find utility in the treatment of tumours, either alone or in conjunction with chemotherapeutic agents. Further studies must be done to clarify the effect of EMF on other physiological parameters.

## References


1. **Bauer JD. (1982).** Clinical Laboratory Methods, 9th ed. ST.Louis, Toronto, London: The C.V. Mosby Company.
2. **Cecconi S , Gualtieri G, Bartolomeo AD , Troiani G , Cifone MG and Canipari R (2000).** Evaluation of the effects of extremely low frequency electromagnetic fields on mammalian follicle development. Human Reproduction 15 (11); 2319–2325.
3. **Chater S, Abdelmelek H , Sakly M & Rhouma K.(2004).** Effects of sub-acute exposure to magnetic field on synthesis of plasma corticosterone and liver metallothionein levels in female rats. Pak. J. Med, Sci 20 (3) 219-223.
4. **Choi YM, Jeong JH, Kim JS, Lee B-C, Je HD, Sohn UD. (2003).** Extremely Low Frequency Magnetic Field Exposure Modulates the Diurnal Rhythm of the Pain Threshold in Mice. Bioelectromagnetics 24:206 –210.
5. **Crowley LV (1967).** The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. *Clin. Chem.* **13:** 482-7.
6. **Fedorowski A, Steciwko A, Rabczynski J. (2004).** Low-frequency electromagnetic stimulation may lead to regression of Morris hepatoma in buffalo rats. J Altern Complement Med. 10(2):251-60.
7. **Feychting, M., and Ahlborm, A. (1993).** Magnetic fields and cancer in children residing near Swedish high-voltage power lines. Am. J. Epidemiol. **13**8: 467–481.
8. **Gamaley, I.; Augesten, K.; Berg, H. (1995).** Electrostimulation of macrophage NADPH oxidase by modulated high-frequency electromagnetic fields. Bio-electrochem. Bio-energ. 38, 415- 418.
9. **Goering PL, Waalkes MP, Klaassen CD. Toxicology of cadmium. In:** Goyer RA, Cherian MG (Eds), pp 189-213. Toxicology of metals. Biochemical Aspects Vol 115, Springer Verlag York 1995.
10. **Harakawa S, Takahashi I, Doge F, Martin DE. (2004).** Effect of a 50 Hz electric field on plasma ACTH, glucose, lactate, and pyruvate levels in stressed rats. Bioelectromagnetics. 25(5):346-51.
11. **Havas M. (2000).** Biological effects of non-ionizing electromagnetic energy: A critical review of the reports by the US National Research Council and the US National Institute of Environmental Health Sciences as they relate to the broad realm of EMF bioeffects. Environ. Rev. 8: 173–253.
12. **Juutilainen J, Laara E, Pukkala E. (1990).** Incidence of leukemia and brain tumors in finnish workers exposed to ELF magnetic fields. Int Arch Occup Environ Health; 62: 289-93.
13. **K Imaida, M Taki, T Yamaguchi, T Ito, S Watanabe, K Wake, A Aimoto, Y Kamimura, N Ito and T Shirai**. (1998). Lack of promoting effects of the electromagnetic near-field used for cellular phones (929.2 MHz) on rat liver

carcinogenesis in a medium- term liver bioassay. Carcinogenesis, 19: 311-314.

14. **Ito, H.; Hashimoto, S. (1989).** Experimental study of the anti-tumor activity of direct current. An effective adjuvant therapy in radiation. Gan to Kafaku Ryoho, 16, 1405-1411.
15. **Juutilainen J, Laara E, Pukkala E. (1990).** Incidence of leukemia and brain tumors in finnish workers exposed to ELF magnetic fields. Int Arch Occup Environ Health; 62: 289-93.
16. **Khanduja KL, Syal N. (2003).** Sinusoidal electromagnetic field of 50 hz helps in retaining calcium in tibias of aged rats. Indian J Exp Biol; 41(3):201-4.
17. **Lazo JS, Pitt BR. (1995).** Metallothionein and cell death by anticancer drugs. Annu Rev Pharmacol Toxicol, 35: 635-53.
18. **Loomis DP, Savitz DA, Ananth CV. Breast** cancer mortality among female electrical workers in the United States. J. Nat. Cancer Inst. 86: 921-25.
19. **London, S.J., Thomas, D.C., Bowman, J.D., Sobel, E., Cheng, T.-C, and Peters, J.M. (1991):** Exposure to residential electric and magnetic fields and risk of childhood leukemia. Am. J. Epidemiol. **13**4: 923–937.
20. **Nilsson, E.; Euler, H.; Berendson, J.; Thorne, A.; Wersall, P.; Naslund, I.; Lagerstedt, A.S.; Narfstrom, K.; Olsson, T. (2000).** Electrochemical treatment of Tumors. Bio-electrochemistry, 51: 1-11.
21. **Oda T, Koike T. (2004).** Magnetic field exposure saves rat cerebellar granule neurons from apoptosis in vitro. Neurosci Lett. 365(2):83-6.
    **Pasquinelli, P.; Petrini, M.; Mattii, L.; Galimberts, S.; Saviozz, M.; Malvaldi, G. (1993).** Biological effects of PEMF (Pulsing electromagnetic field): An attempt to modify cell resistance to anticancer agents. J. Environ. Path. Toxical. Oncal. 12 (4), 193-197.
22. **Petrini, M; Mattii, L.; Sabbatini, A.; Carulli, G.; Grassi, B.; Cadossi, R.; Ronea, G.; Conte, A. (1990).** Multidrug resistance and electromagnetic fields. J. Bioelectr. 9: 209-212.
23. **Robak, F.P. (1995):** Routin laboratory evaluation of blood cells and bone marrow. In: Diagnostic hematology, p.p.: 125-129, W. B. Saunders Comp. Phild. London, Toronto, Montreal, Sydney, Tokyo.
24. **Sato M, Bremne I. (1993).** Oxygen free radicals & metallo-thionein. Free. Radiol Biol Med. 14: 325-37.
25. **Savitz DA, Loomis DP. (1995).** Magnetic field exposure in relation to leukemia and brain cancer mortality among electric utility workers. Am. J. Epidemiol. 141: 123-34.
26. **Savitz, D.A., Wachtel, H., Barnes, F.A., John, E.M., and Tvrdik, J.G. (1988).** Case-control study of childhood cancer and exposure to 60 Hz magnetic fields. Am. J. Epidemiol. **12**8: 21–38.
27. **Siest, G.&Schielf. M. J. (1981):** Interpretation des Examens de laboratoir, Karger el., pp.: 206-223.
28. **Sokal, R.R.&Rahif, F.J.(19981):** The principles and practice of statistics in Biological Researches. 2nd .Ed. Freeman, W.H. Company, San Fransisco.
29. **Van Kampen, E.J.&Zulstra, W.G. (1961):** Standerization of hemogl-obinometry: The hemoglobin cyanide method. Clin. Chem. Acta. (53): 538-540 b.
30. **Wrensch M, Bondy ML, Weinche J, Yost M. (1993).** Environmental risk factors for primary malignant Brain tumors: A review, J. Neurooncol. 17: 47-64.

# تأثير التعرض للمجال الكهرومغناطيسى منخفض الترددات على نمو الورم وعلي دلالات مختلفة بالدم في الجرذان التي حقنت بخلايا سرطانية من نوع Ehrich

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هدف هذا البحث هو بيان تأثير التعرض للمجال الكهرومغناطيسى منخفض الترددات على صورة الدم، وظائف الكبد والكلي ومستوي السكر بالدم لجرذان حقنت بخلايا سرطانية من نوع Ehrich . و قد اجريت هذه الدراسة علي اناث جرذان BALB/c تم حقنها بمعلق اخذ من خلايا سرطانية من نوع Ehrich . أظهرت النتائج ان التعرض للمجال الكهرومغناطيسى منخفض الترددات ادي الي انخفاض ملحوظ في معدل نمو الورم. أيضا فأن زيادة عدد كريات الدم البيضاء، انزيمات الكبد، البليروبن, البولينا والكرياتينين ومستوي الجلوكوز بالدم في الجرذان التي حقنت بخلايا سرطانية انخفضت بدلالة ملحوظة بعد التعرض للترددات المنخفضة للمجال الكهرومغناطيسى. وقد استنتج من هذه النتائج أن بعد التعرض للترددات المنخفضة للمجال الكهرومغناطيسى 50 Hz يقلل من نمو وتأثير ورم Ehrich مما يرجح ان يكون للمجال الكهرومغناطيسى منخفض الترددات دورا في علاج بعض الاورام في الانسان.