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Effect of Electromagnetic Field on Red Blood Cells of Adult Male *Swiss albino* Mice

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ABSTRACT: The harmful effect of electromagnetic radiations emitted from VDU (video display unit) on red blood cells of Swiss albino mice, 20 cm away, at power density of 0.295 µw/cm^2 was investigated, at the interval of 7, 14, 28, 42 and 56days. The result showed altered blood smear, morphology and scanning electron micrograph of RBCs. red blood cell count, hemoglobin concentration was reduced up to day 42 of irradiations. The exposed mice consume less feed and water and have reduced body weight. All the changes normalized in late effect group. Collectively these findings indicate that EMF issuing from VDU is harmful and adversely affects biological system.

Key words: EMF, VDU, RBC, Swiss Albino Mice, Scanning Electron Micrograph and Hemoglobin.

I. INTRODUCTION

In the last few decades, various studies pertaining to biological effect of electromagnetic field exposure have been in progress. Most of studies carried out on mice, rat and human showed that electromagnetic fields induced changes in hematological parameters in these organisms [22, 3, 34, 21]. Technology is the lifeline of modern day society. We are exposed to the electromagnetic fields as a result of progresses in technology and science. Every electronic equipment used by us in day to day life produce electromagnetic fields [32]. Despite of benefits electromagnetic fields can affect living organisms [23]. Not only animals, the electromagnetic irradiation can also affect growth of plants [17] and microorganisms [37]. It was reported that, electromagnetic field emitted by color TV screen may cause alteration in humoural immunity and reduction in growth and body weight [42]. In the present study swiss albino mice were exposed to electromagnetic field of CRT monitor (VDU screen) to note the alterations in the red blood cells of exposed mice.Continuous exposure to extremely lew frequency electromagnetic field may affect the haemopoitic organs [6, 43]. Results of present study points to harmful effects of continuous long term exposure of computer monitors on *Balb/C* mice.

II. MATERIAL AND METHODS

Swiss albino mice *Mus musculus* (*Balb/C* strain) of male sex, 6-8 weeks old, weighing about 16-22 g were used as experimental model for the present study. These were obtained from the central animal house, Punjab University, Chandigarh and kept for one week in the experimental room for acclimatization and after one week animals were used for experimentation. Animals were fed a standard pellet diet and water. Control and exposed mice were exposed to the same environment barring the exposure field. The temperature of the room was maintained at 25-28°C. Relative humidity was 50-70% and dark/light schedule of 12/12 hours was maintained.

The mice of various groups were given exposure to electromagnetic radiations 8-10 hours/day by placing 20 cm away from video display unit (VDU) of computer monitor in the especially designed plastic cage. Exposure source was Samsung–syncmaster 753s (17" digital color monitor). Power density (0.295 µw/cm^2) was measured with 'RF Field Strength Meter' at 20 cm in front of monitor. The control and exposed group were kept apart so that there is no interference of exposed field.

III. EXPERIMENTAL DESIGN

Two groups having twenty five mice respectively were used for the present study. The experimental mice were given exposure of computer monitor (VDU) for 8-10 hours daily for different time intervals.

GI: This group will consist of twenty five normal control mice which will not be exposed to any source of radiations.

GII: This group will consist of twenty five normal exposed to VDU (8-10h/day) for different time intervals i.e. one week (7 days),two weeks (14 days), four weeks (28 days), Six weeks (42 days) respectively.

After six weeks (42 days) the left out mice will not be given any exposure for two weeks in order to examine, weather the changes be reversed after exposure be removed. These mice are therefore called late effect group (56 days).

Daily record of feed consumption, water intake and weekly record of change in body weight in both the groups was maintained. Total red blood cell count (RBC Count) and hemoglobin concentration (Hb) of was recorded on day 0and after one week (7 days),two weeks (14 days), four weeks (28 days), Six weeks (42 days) and eight weeks (56 days). Statistical Analysis of the data was presented as the mean ± SD for each correlation was calculated using Microsoft Excel. The data were analyzed using SPSS program (statistical package for social sciences Inc. Chicago, Illinois).

Sample Preparation For Scanning Electron Micrography: Mice of GI and GII were sacrificed by jugular vein incision after anesthetizing with diethyl ether on day 7, 14, 28, 42and 56. Blood was aspirated in citrate saline (0.85% (w/v) sodium chloride; 3.8% (w/v) sodium citrate). Pooled blood of same group was subjected to density gradient centrifugation using histopaque-1119 (sigma). Red blood cells are separated using procedure as per Czuprynski and Brown [14]. Red blood cells separated by histopaque of each group were subjected to SEM studies to note changes in surface of these cells after electromagnetic field exposure. A slightly modified method of Clarence *et al.* [12] was used for preparing samples. The Stubb was viewed in SEM (JEOL JSM-6100 scanning microscope.

Hemoglobin Estimation And Total Red Blood Cell Count: Hemoglobin estimation was performed using hemoglobinometer while, Total red blood cells (RBCs) count was done using Neuber's Haemocytometer kit with Hayem's fluid, (0.5 % (w/v) sodium chloride; 0.25 % (w/v) sodium sulphate; 0.25 % (w/v) mercuric chloride).

IV. RESULTS AND DISCUSSION

The present study was carried to assess the effect of electromagnetic field emitted from VDU on RBC count, Hemoglobin estimation, and physical structure of blood smear, morphology of RBCs and alteration in scanning electron micrograph of RBCs as a result of electromagnetic field exposure from these screens. Beside, studies were also carried to note the effect of EMR on feeding habits, body weight of male *Balb/C* mice. Several studies have reported reduction in body weight of animals exposed to electromagnetic field. Most of the studies pertain to the effect of electromagnetic fields from power lines, microwave, color TV screens etc. [31, 18, 7, 5]. But little work so far has been reported on the harms of electromagnetic field of computer monitors. Computers are extensively used in every sphere of life. During on phage the screen of CRT monitor can emit electromagnetic field ranging from X-rays to extremely low frequency (ELF) and very low frequency fields (VLF) [25, 28]. A reduction in the body weight, increased fetal loss in embryos and young chicks exposed to electromagnetic fields of color TV screen during embryonic phases [42].

Reduction in body weight of exposed mice (GII) has been observed in present investigation as compared to control (Table.1). It is reported to increase with increase in exposure time [33]. Maximum reduction up to 33% in body weight of GII mice has been observed after 42 days of exposure, as compared to GI. The reduction in body weight can be directly related with change in feeding habits of exposed mice. The exposed mice groups were observed to consume less feed and water as compared to control mice (Table 1). Related observations were made by others [33, 29]. Persinger *et al.* studied that the rats exposed to 1–30 G at 0.5 Hz for 10-26 days showed progressive changes in the total body weight, thyroid weight and water consumption. Similarly, Marino *et al.* have reported that mice exposed to low electric fields (150 V/cm) at 60 Hz for one month have reduced body weight and decreased water consumption.

In GII maximum decrease in the amount of feed consumption and water intake was observed after 42 days and 28 days of exposure respectively, compared to GI (Table1). It was observed that the exposed mice showed irritating behavior, aggressiveness and hyperactivity [38].

The reduction in feeding can leads to reduced growth and body weight. Earlier it was reported that, electromagnetic field exposure can leads to alteration in mitochondria, as the later are most sensitive to electromagnetic field. The rapid change in structure and physiological responses of mitochondria leads to different kinds of stress generating factors [19] and could disturb the homeostasis [39].

Table 1. Change of values in percentage and Mean ± SD of various parameters at different intervals of experiment in Control (GI) and Exposed groups of mice (GII).

Parameters	Groups	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
Feed(mg) consumption	GI	8.46±0.46	8.59±0.43 +1.53%	8.79±0.3 +3.9%	8.96±0.23 +5.9%	8.99±0.32 +6.3%	9.02±0.28 6.6%
	GII	8.26±0.42	7.87±0.28 -4.7%	7.45±0.32 -9.8%	7.64±0.39 -7.5%	7.62±0.28 -7.7%	8.02±0.41 -3%
Water intake (ml)	GI	4.67±0.5	4.72±0.53 +1.1%	7.76±0.64 +1.9%	4.81±0.62 +3%	4.81±0.54 +3%	4.86±0.57 +4%
	GII	4.53±0.46	4.16±0.5 -8.2%	3.79±0.43 -16.3%	3.93±0.45 -13.2%	3.99±0.41 -12%	4.42±0.59 -2.4%
Body weight (g)	GI	16.6±1.6	19.2±1.43 +15.7%	21.2±2.12 +27.7%	24.2±2.95 +45.8%	28.6±2.92 +72.3%	30.5±3.15 +83.7%
	GII	16.5±1.5	18.2±2.4 +10.3%	20.2±2.18 +22.4%	21.6±2.38 +30.9%	23.1±2.64 +40%	25.6±2.92 +55.2%
RBC Count (10^7/mm^3)	GI	0.95±0.1	0.96±0.16 +1%	0.96±0.18 +1%	0.98±0.2 +3.2%	0.97±0.2 +2.1%	0.98±0.09 +3.2%
	GII	0.93±0.22	0.91±0.28 -2.2%	0.89±0.32 -4.3%	0.88±0.23 -5.4%	0.86±0.26 -7.5%	0.89±0.13 +3%
Hb (g/dl)	GI	13±1.14	13.2±1.6 +1.5%	13±1.68 00	13.6±2 +4.6%	13.65±2.5 +5%	13.8±1.83 +6.2%
	GII	13.2±1.05	12.6±1.5 -4.5%	12.4±1.56 -6%	12±1.84 -9%	11.8±2.12 -10.5%	13.6±2.38 +3%

The present study reported that, the Total red blood cell count and hemoglobin content of exposed mice (GII) has been observed to decrease as compared to normal mice. In GII maximum decrease (10.9%) in the RBC count and hemoglobin concentration (16%) was observed after 42 days of exposure when compared to GI (Table 1).

Moreover in late effect groups, the RBC count and hemoglobin concentration reached to their normal values as a result of removal of exposure (Table 1). Thus we can say that the electromagnetic field from these screens do not have long lasting effect on blood cells ,as the cells résumés their normal shape and number when kept away from the exposure source. Above all, the alteration in the various hematological parameters clearly indicates that the hemopoeitic system was adversely affected because of these radiations.

The red blood smear of the exposed group (GII) showed that, the RBCs have distorted shape and stake next to each other to form rouleaux (Fig. 3). The red blood cells have increased ability to stick together due to increased viscosity of blood as a result of exposure to electromagnetic field. Most of the RBCs appear pale in color because of lack of concentration of hemoglobin (hypochromia). The structure of membrane was found altered. Hemolysis of red blood cells were also observed as the period of exposure increased (Fig. 2,3).

Scanning electron micrograph of normal red blood cells appeared biconcave and elliptical in shape. The size was 4.25 ± 0.3 μm and depression size 0.9 ± 0.1μm (Fig. 4).It was reported that the size of RBC was reduced and depression enlarged in VDU exposed group as compared to control group.

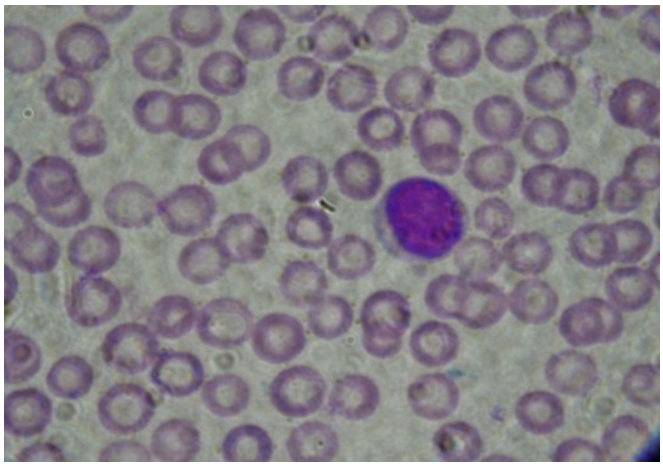

Fig. 1. Giemsa's stained blood smear of control mice showing normal red blood cells.

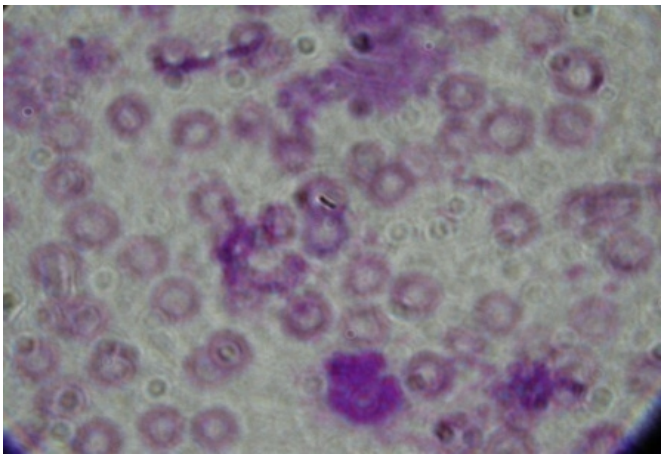

Fig: 2. Giemsa's stained blood smear of exposed mice showing hypochromic red blood cells with abnormal blood smear. The structure of membrane was found altered.

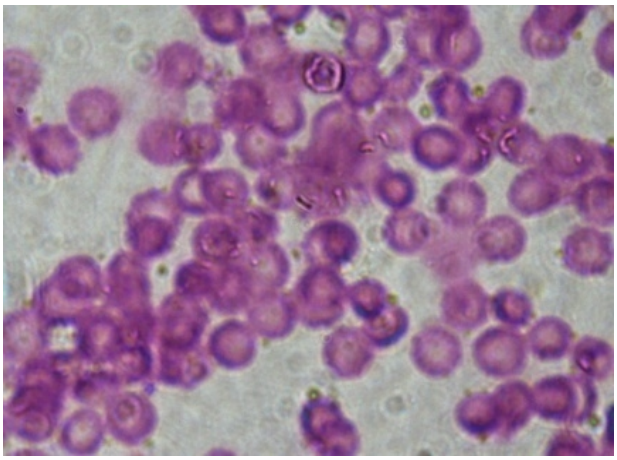

Fig. 3. Giemsa's stained blood smear of exposed mice showing hemolysed and distorted red blood cells. The red blood cells stake next to each other to form rouleaux.

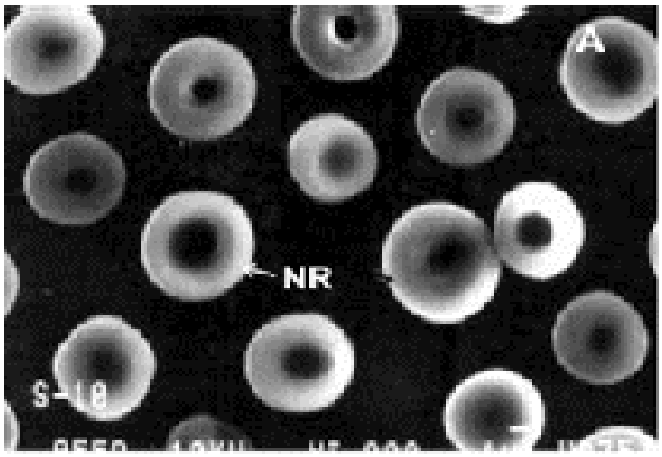

Fig. 4. Scanning Electron micrograph of RBC's of exposed mice obtained after separation of blood cells by density gradient centrifugation of normal control mice showing normal red blood cells.

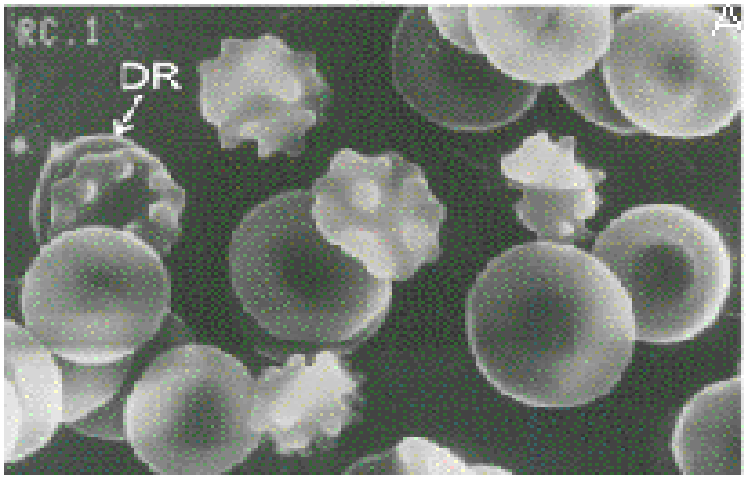

Fig. 5. Scanning Electron micrograph of RBC's of exposed mice obtained after separation of blood cells by density gradient centrifugation of exposed mice showing defected red blood cells.

The smallest size of RBC (3.87 ± 0.5) and largest depression in RBC (1.6 ± 0.3 μm) was observed after 7 days of exposure (Fig. 5). RBCs showed distorted shapes. About 5% of RBCs showed bulging and protuberances in the membrane. About 40% of RBCs have enlarged depression in their centre (Fig. 5). After exposure of 14 days, RBCs were observed to have oblong shape. Bulging and protuberances on surface also observed, but less as compared to GI (20-25%). About 25-30% RBCs were defected. 5-10% of RBCs show enlarged depression in their centre, which was measured to be 1.5 ± 0.2μm. The size of RBCs is comparatively smaller (3.9 ± 0.2μm) as compared to GI (Fig. 5). After exposure of 28days, 15-20% RBCs have distorted and oblong shapes. 5-8% of RBCs exhibits bulging and protuberances in their membranes. The size of RBC was measured to be 4 ± 0.2 μm. 20-25% of RBCs shows enlarged depression in their centre which measured to be 1.3 ± 0.4 μm (Fig. 5).After exposure of 42 days, 30-35% RBCs have been observed to be distorted and exhibit oblong shapes as compared to GI. Although the bulging and protuberances in the membrane are uncommon. 15-20% of RBCs shows enlarged depression (1.3 ± 0.1 μm) in the centre.

The size of RBC was measured to be 4 ± 0.2 µm (Fig. 5). Our observations confirm the findings made by Ali *et al.*, 2003; Ali, 2006; Dudek *et al.*, 2000, up to certain extent. It was reported that the exposure of animals to 50 Hz, 0.2 mT magnetic fields resulted in decrease of RBC membrane elasticity and permeability and changes the structure of Hb and no sign of repair in the newly generated RBC structure and the ECG after removing the animal from the magnetic field, which indicates that the blood generating system is severely injured in exposed mice [3]. Similar effect of VDU monitor radiations might have been generated on RBC of Balb/c mice in present investigation. A change in the composition and shape of the membrane, can alter membrane's natural cycle, which in turn leads to the emergence of some serious diseases [15]. The electromagnetic waves exposure to human from mobile phones lead to damage and a clear influence on the cell walls, especially walls of red blood cells and cause an imbalance in blood enzymes [4].

All these morphological alterations were found to increase with increase in the period of exposure and were more prominent in group exposed to VDU (Table.1). Moreover in late effect group the various changes were observed to normalize and maintained recovery towards normal as a result of removal of exposure source. This may be because of recovery of spleen and other hemopoitic tissues. Zagloul, reported that the spleen tissue appeared almost normal and attained tendency towards recovery after thirty days following electromagnetic field exposure [43].

Measurement of blood parameters is one of the most impartment way by which we can determine the health status of experimental animals [36]. Indices of red blood cells like average size of red blood cells can gives a clear picture of performance and efficiency of RBCs, hemoglobin concentration and any variation in volume of red blood cells [1]. The decrease in the concentration of hemoglobin could be attributed to the interaction between iron of haeme and electromagnetic field, by which magnetic field enters the body and acts on ions in all the vital organs such as spleen, bone marrow, kidney and liver etc. It alters the cell membrane potential and distribution of ions [26, 8].

Moreover, regular exposure to electromagnetic field can increase plasma volume, which leads to decrease in the concentration of hemoglobin and red blood cells in blood [5, 11]. We have reported, decrease in the hemoglobin concentration red blood cell count was reported in exposed groups compared to control suggesting an anemia like stage. The red blood cell production is decreased in the rabbit exposed to electromagnetic field [13]. This anemia was confirmed by marked decrease in the hemoglobin concentration and red blood cell count. Our results are in agreement with those previously reported by Bonhomme-Faivre, *et al.* [9]. Present study points towards harmful effect of electromagnetic field on scanning electron micrograph of red blood cell. The iron / foliate deficiency as a result of electromagnetic field exposure can leads to abnormality in erythrocytes [10]. A change in the composition of hemoglobin inside red blood cells membrane was observed which affects the physiological function of and capacity to transport oxygen, as observed during decrease in hemoglobin concentration after exposure to electromagnetic waves [41]. Destruction in the splenic tissues was reported [6, 2, 43] which confirms the harmful effect of these radiations on hemopoitic tissue. Spleen is a lymphatic organ which store blood corpuscles. Spleen hyper function increased the rate of destruction of RBCs, WBCs and platelets which could ultimately leads to decrease in hemoglobin concentration [27]. Any abnormality in spleen as a result of electromagnetic field can leads to destruction or hemolysis of RBCs within the spleen [43]. It is proved by the production of hemosiderin granules in macrophages as Electromagnetic field exposure results in increased phagocytic activity of red blood cells [35]. Dead cells and dying cells in the living tissues were reported as a result of exposure of electromagnetic field [40]. At power density of $1uw/cm^2$, the cytotosic activity of natural killer cells in rat's spleen increased by 130 -150 %. These values persist within 24 hours after end of the treatment [16].

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