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Biological Effect of Electromagnetic Field of VDU on Immune cells of *Balbl* C Mice

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ABSTRACT: The present study investigates the harmful effect of electromagnetic radiations emitted from VDU (video display unit) of CRT monitoron white blood cells of male Balb/C mice, 20 cm away, at power density of 0.295 µw/cm^2. The white blood cells were evaluated for morphology, physical nature of blood smear, Differential leukocyte count (DLC), total leukocyte count (TLC), mortality and scanning electron microscopic studies at the interval of 7, 14, 28 and 42 days of irradiations. The result showed altered blood smear, morphology, apoptosis rate andscanning electron micrograph of WBCs. Lymphocytes (L) and TLCin exposed groups were increased while monocytes (M) and neutrophils (N) reduced at various interval of experiment. Collectively these findings indicate that EMF issuing from VDU adversely affects immune system.

Keywords: Balb/c mice, Immune cells, VDU, Electromagnetic field and SEM.

INTRODUCTION

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 produces electromagnetic fields (Ongel et al, 2009). Despite of benefits electromagnetic fields can affect living organisms (Hood, 2004). These fields interfere with body's own electromagnetic energy system and thus causing a considerable stress, which are manifested in various health problems including lowered immune system (Hashish *et al.*, 2008; Simko and Mattson, 2012). Electromagnetic fields of certain frequency make alterations in T- lymphocytes and other immune system leading to immunosupression, partially through induced calcium ion efflux (Cherry, 1997). Continuous exposure to ELF-EMF may induce testicular germ apoptosis in mice (Lee *et al.*, 2004) and affect lymphetic organs such as liver, spleen and lymph nodes (Attia and Yehia, 2002; Zaghloul,2011). Electromagnetic field emitted by VDU may cause alteration in humoural immunity and reduction in growth and body weight (Youbicier-Simo *et al.*, 1997). In the present study Balb/c mice were exposed to EMF of VDU screen of CRT monitor to note the alterations in the immune cells of exposed mice.

MATERIAL AND METHODS

White Swiss 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, Panjab 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.

Experimental Design: Two groups having five mice and twenty 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 consisted of five normal control mice which were not exposed to any source of radiations.

GII: Twenty mice of this group were exposed to VDU (8-10h/day) for different time intervals i.e. for 7 days, 14 days, 28 days and 42 days respectively.

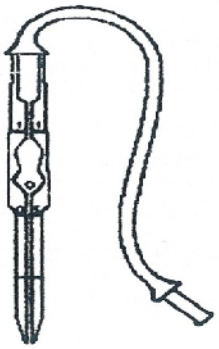
Differential leukocyte count (DLC), total leukocyte count (TLC) of both groups control (GI) and exposed (GII) was recorded after 7, 14, 28 and 42 days. Statistical Analysis of the data was presented as the mean ± SD for each correlation was calculated using Microsoft Excel. The variations observed in the values of various parameters under study in VDU exposed group were compared with control (GI) at different intervals of experiment. The data were analyzed using SPSS program (statistical package for social sciences Inc. Chicago, Illinois).

Mice of GII were sacrificed by jugular vein incision after anesthetizing with diethyl ether on day 7, 14, 28 and 42. Control mice were sacrificed on day 42of experiment. 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).

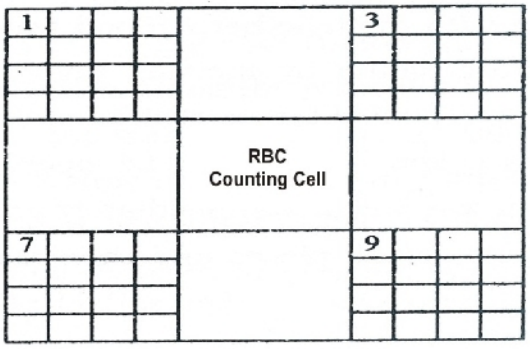
Differential Leukocyte Count (DLC):
For DLC thin blood smears of were made from tail snips and stained in Giemsa stain (3% Giemsa stock in 0.95 % (w/v) sodium phosphate dibasic, 0.91% (w/v) potassium phosphate monobasic) for half an hour and observed under microscope (100x) using immersion oil. The percentage of mononuclear (MN) cells i.e. lymphocytes (L) and monocytes (M) and polymorphonuclear (PMN) cells *i.e.* neutrophils (N) was calculated (Fatayer, 2006).

$$\frac{\text{Number of M/L/N}}{\text{Total number of WBCs}} \times 100$$

Calculation of Total Leukocyte Count (TLC):
Using Neuber's Hemocytometer kit, TLC of all the mice of both groups (GI and GII) was done using Turk's fluid (10% (w/v)) gentian violet and 1.5% glacial acetic acid in distilled water). The number of WBCs will is counted in 4 squares (1, 3, 7 & 9) of the slide under microscope at 10x or 40x as per convenience.



WBC Pipette



Neuber's Slide

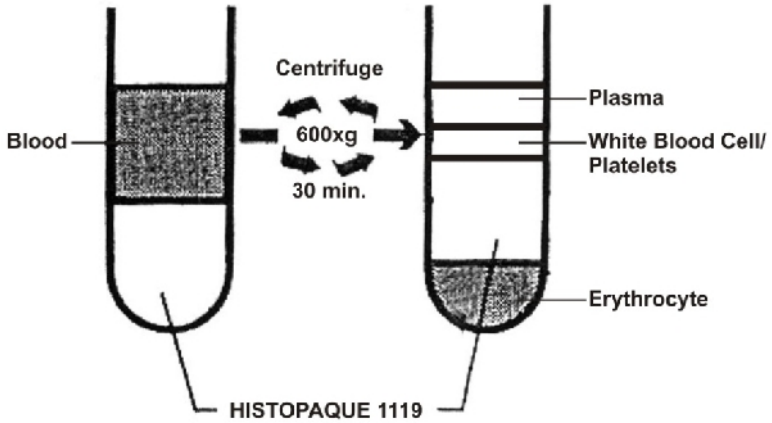
Using the number of WBCs in 4 squares TLC will be calculated according to following calculations:

Say number of WBCs in square 1,3,7,9 is
a + b + c + d = y
Area of each square = 1 mm^2, height = 1/10 mm
Therefore, volume = 1/10 x 1 = 1/10 mm^3
1/10 mm^3 contains = y/4 WBCs
1 mm^3 contain = y/4 x 10 WBCs
As the solution is diluted 20 times
Therefore, 1 mm^3 contains =

$$\frac{Y \times 10 \times 20}{4} = Z \text{ WBC's}$$

Separation of WBCs by Density Gradient Centrifugation:

Blood of mice was aspirated using Pasteur's pipette in citrate saline by incising jugular vein. Pooled blood of each group of mice was subjected to density gradient centrifugation using histopaque-1119 to separate WBCs using following procedure (Czuprynski and Brown, 1998).



Separation of white blood cells.

Fluorescent Staining Using Acridine Orange (AO) / Ethidium Bromide (EB):

WBCs aspirated by density centrifugation of blood were subjected to fluorescent staining to study apoptosis. WBCs suspension was incubated with 20µl of AO/EB solution (1 part 100µg/ml AO in PBS; 1part of 100µg/ml EB in PBS). The suspension was mixed gently and each sample was mixed just prior to its examination by fluorescent microscope (Leica, Germany) and its quantification, there after (Kasibhatla, 1998).

Added 1-2 drops of this suspension on a microscopic slide and covered with a glass cover slip. Cells were examined under fluorescent microscope using fluorescent filter. 500 cells were counted in each group to calculate the percentage of live and dead cells by the following formula:

$$\frac{\text{Number of live/apoptotic/dead cells}}{\text{Total number of white blood cells}} \times 100$$

Live cells stained fluorescent green, whereas, dead cells appeared orange under ultra violet light.

RESULTS AND DISSCUSSION

Most of the studies pertain to the effect of electromagnetic fields from power lines, microwave, color TV screens and self designed instruments. But little work so far has been reported on the harms of EMF of computermonitors.Computers are extensively used in every sphere of life. During on phage the screen of CRT monitor can emit EMF ranging from X-rays to extremely low frequency (ELF) and very low frequency fields (VLF) (Kavet and Tel,1991; Luketina,1975). In the last few decades various studies carried out on mice, rat and human showed that electromagnetic fields induced changes in hematological parameters in these organisms (High *et al.*, 2000; Ali *et al.*, 2003; Sihem *et al.*, 2006; Hassan and Abdelkawi, 2010).

The present study was carried to assess the effect of EMF emitted from VDU of computer monitor on cells of immune of male Balb/c mice. Results of present study points to harmful effects of continuous long term exposure of computer monitors on *Balb/C* mice. Percentage of lymphocytes in the exposed group of mice increased while that of N and M decreased with the rise in period of exposure (Fig. 1-3). In exposed group L showed maximum increase (27.9%) after 42 day irradiation while maximum decrease in M (59.9%) and N (29.3%) was reported after 42 days of exposure compared to control (Table 1). Number of lymphocytes is normally enhanced due to external antigen stimulus or diseased state under normal conditions. The increase in the lymphocytes may be because of reduction in the value of monocytes and neutrophils. Declined number of phagocytic cells (N and M) points to depressed level of immunity in exposed mice to pathogens. Similarly Total Leukocyte Count (TLC) in exposed mice group was more when compared with the control, which indicate activation of the immune system as a result of exposure to electromagnetic radiations (Table 1). In exposed group maximum increase in the TLC was observed after 42 days of exposure (24.7%), when compared with GI at respective time intervals (Fig. 4). Morphological assessment of apoptosis in WBCs was done by AO/EB staining. Live cells stained fluorescent green, whereas, dead cells appeared orange under ultra violet light (Fig. 7A, B, C). Maximum percentage of live WBCs was seen in normal mice i.e. 89.16% (Table 1). The percentage of dead white blood cells increased with increase in exposure time in GII. It was 15.8%, 19% and 21.4% and 25.54% after days 7, 15, 30 and 45 of exposure respectively (Fig. 5).

Table 1. Mean± SD of various parameters at different intervals of experiment in GI and GII.

Parameters		**Day 0**	**Day 7**	**Day 14**	**Day 28**	**Day 42**
L (%)	**Control**	63.8±6.67	62.1±6.98	63±9.42	61.8±8.4	61.6±6.9
	Exposed	53±9.78	57.3±10.76	58.6±11.33	61.47±9.42	66±12.45
M (%)	**Control**	3.69±0.6	3.72±0.37	4.02±1	3.78±1.2	3.82±0.68
	Exposed	6.69±1.2	5.84±1.15	4.73±0.9	3.34±0.62	2.92±0
N (%)	**Control**	32.5±8.33	34.2±7.46	33±8	34.4±9.2	34.6±6.48
	Exposed	40.3±11.4	36.9±10.1	36.7±9.69	35.2±8.53	31.08±11.11
TLC/mm^3	**Control**	6420±1220	6850±1403	7200±1469	7766±1463	9080±2651
	Exposed	6540±1296	6990±1340	7400±1992	7686±1090	7890±1340
Mortality of WBCs (%)	**Live**	89.16	84.2	81	78.6	74.46
	Dead	10.84	15.8	19	21.4	25.54

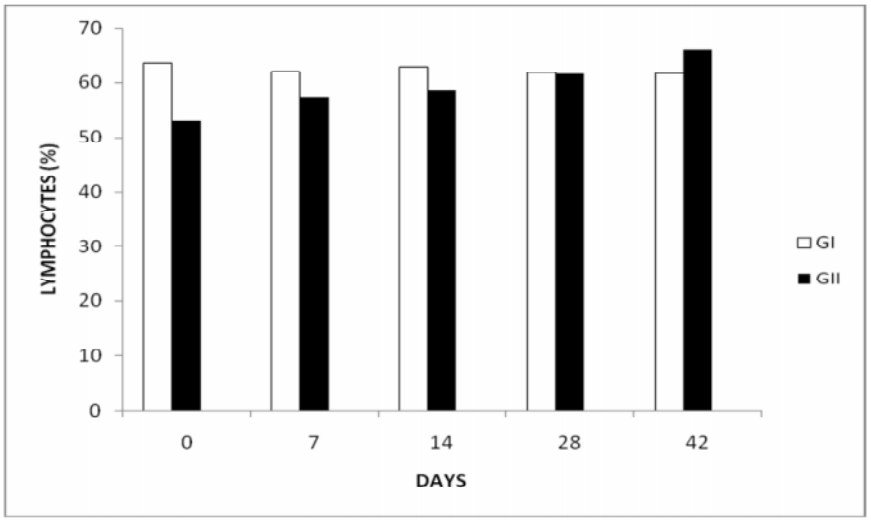


Fig. 1. Diagram showing percentage of lymphocytes (mean ± S.D.) in GI and GII mice.

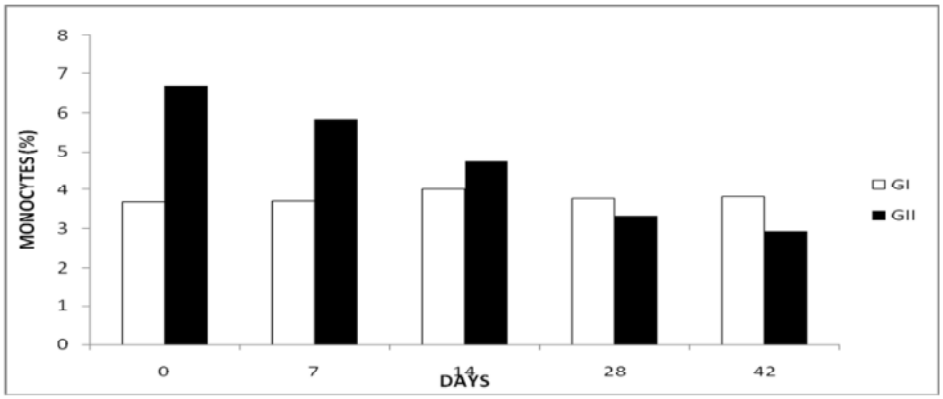


Fig. 2. Diagram showing percentage of monocytes (mean ± S.D.) in GI and GII mice.

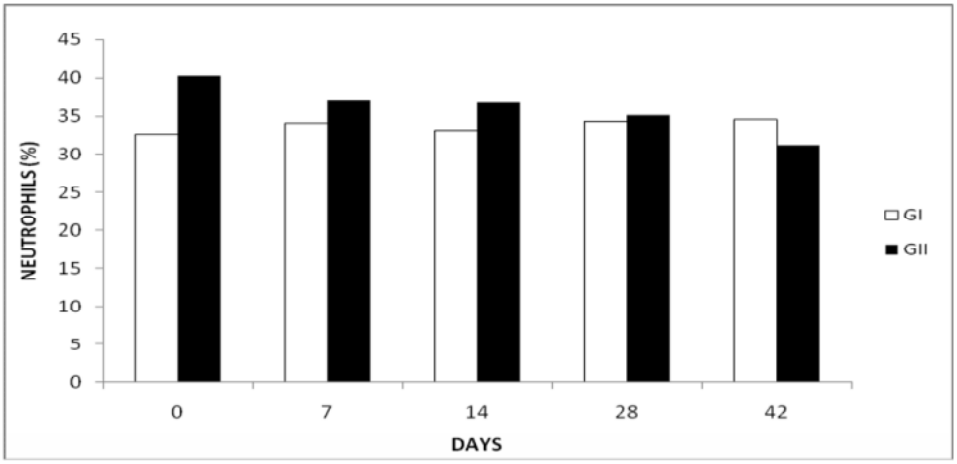


Fig. 3. Diagram showing percentage of neutrophils (mean ± S.D.) in GI and GII mice.

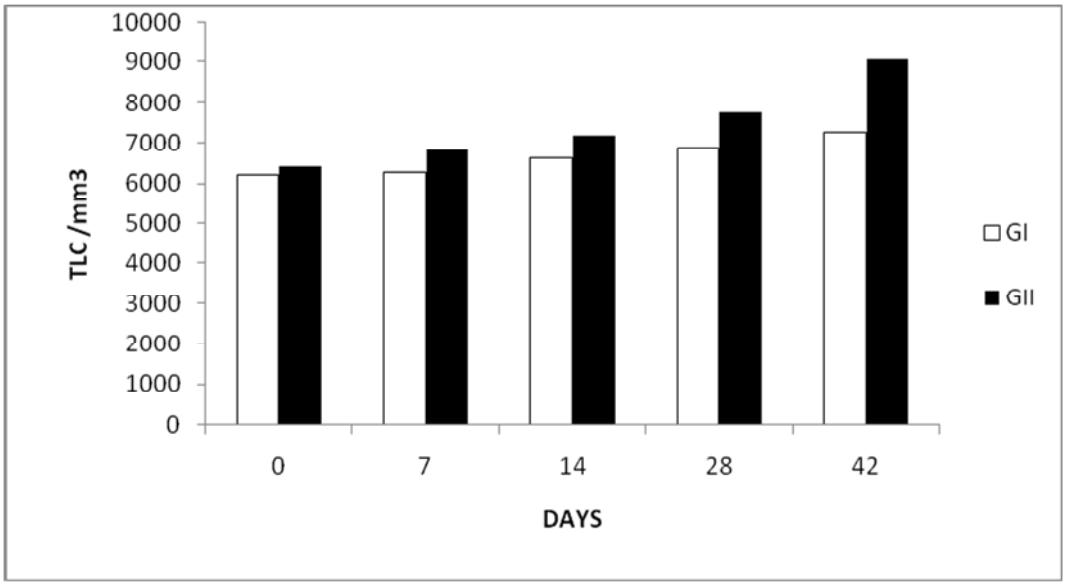


Fig. 4. Diagram showing total leukocyte count (mean ± S.D.) in GI and GII mice.

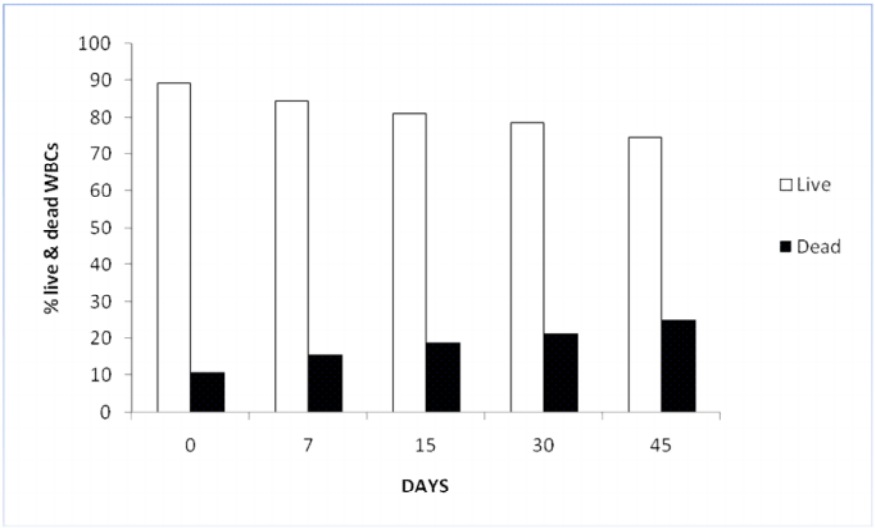


Fig. 5. Diagram showing the status of live and dead cells obtained by density gradient Centrifugation of pooled peripheral blood of GI and GII mice.

Lymphocytes in exposed mice (GII and GIII) have been observed to have less basophilic nucleus, large size and cytoplasm clearly visible (Fig. 6D, G, J, M). Horse shoe shaped nucleus of monocytes has also been observed to swell up in exposed groups (Fig. 6E, H, K, N). Similarly multi-lobed nucleus of neutrophil has become hyper segmentation. The neutrophil took little stain like that of lymphocytes and monocytes (Fig. 6F, I, L, O). Our results confirm the findings of Gagnon *et al.* (2003) and Usman, A.D. (2012).

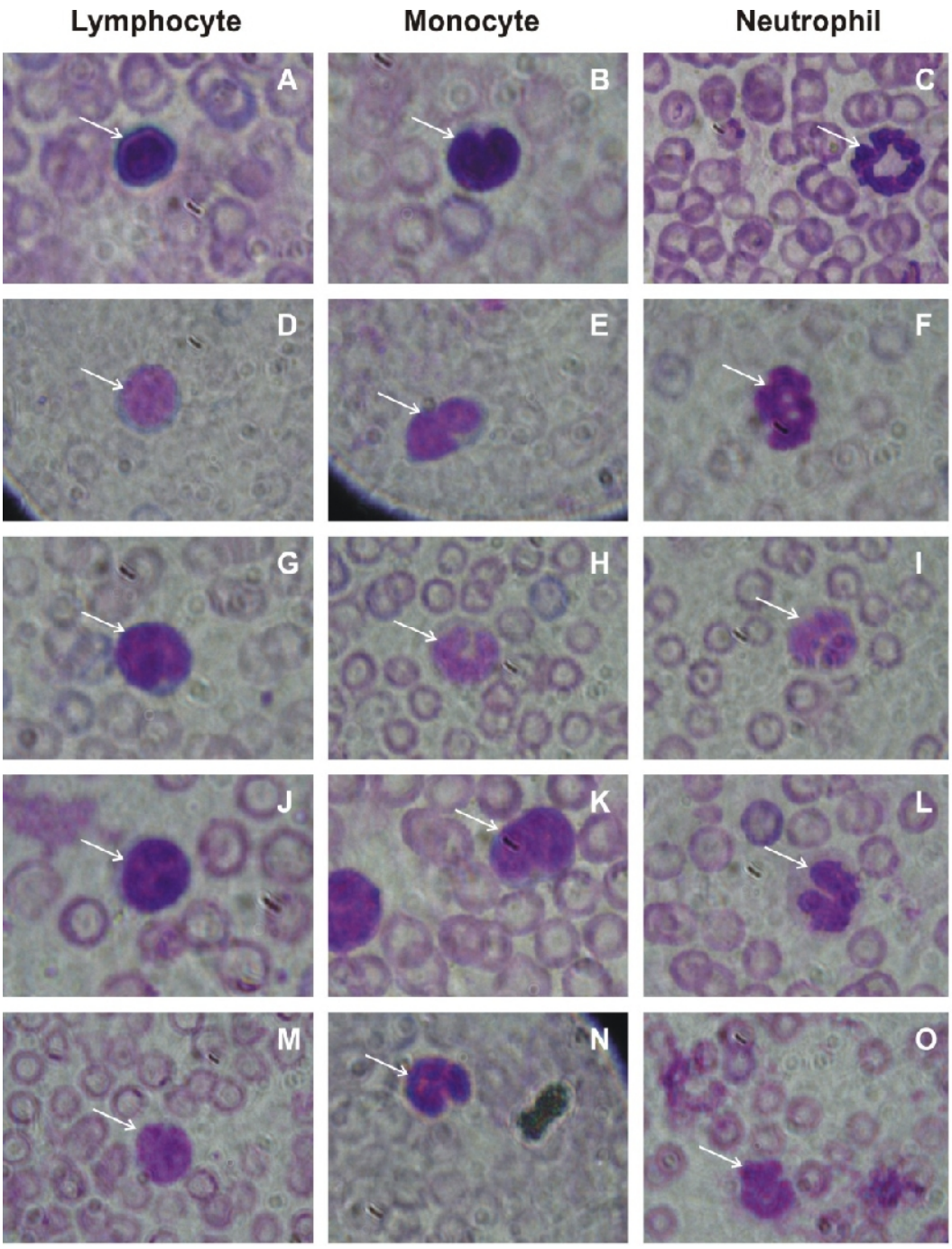


Fig. 6. Giemsa's stained blood smear showing different types of leukocytes (1000x) in control (A,B,C) and exposed mice for various intervals i.e. 7 days (D,E,F) , 14 days (G,H,I), 28 days (J,K,L), 42 days (M,N,O).

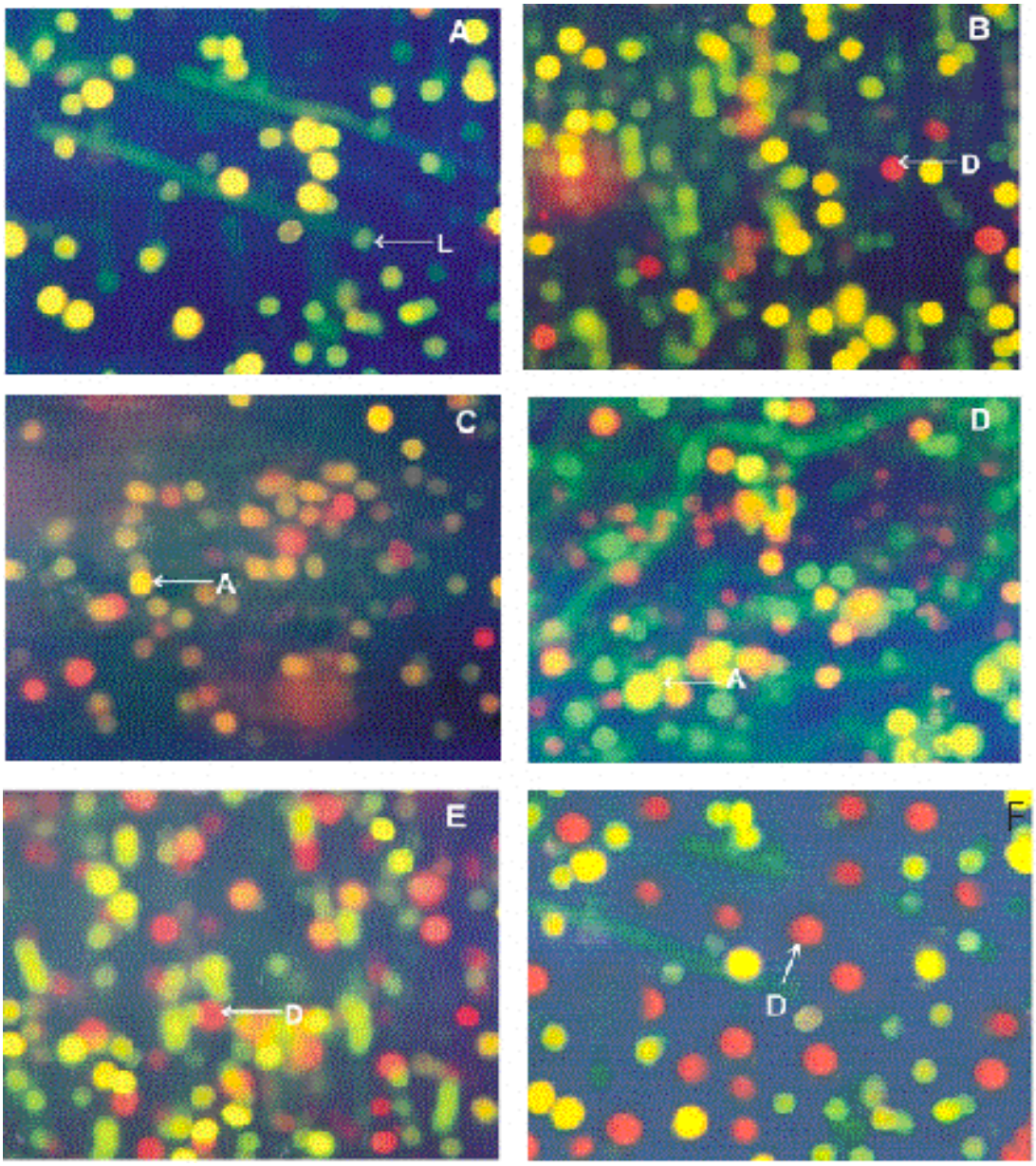


Fig.7. AO/EB staining of WBCs obtained by density gradient centrifugation using histopaque-1119 from normal (A, B) and mice exposed for 7,14,28,42,days(C,D,E,F respectively). Abbreviations: L-Live; D-Dead; A –Apoptotic.

SEM studies of WBCs in exposed mice reveals that the MN cells were sticking together, and size was increased as compared to GI (Fig. 8, 9). PMN cells also showed a variety of shapes. Some of the PMN cells were having finger like projections and some had small blebs. In exposed groups PMN cells were decreased in size and the projections were more prominent All these in exposed mice were reported to become more severe with increase in exposure period. This will clearly indicate that electromagnetic fields emitted from these screens have great influence on the immune cells (Veyret *et al.,* 1991; Novoslova *et al.* ,1999).

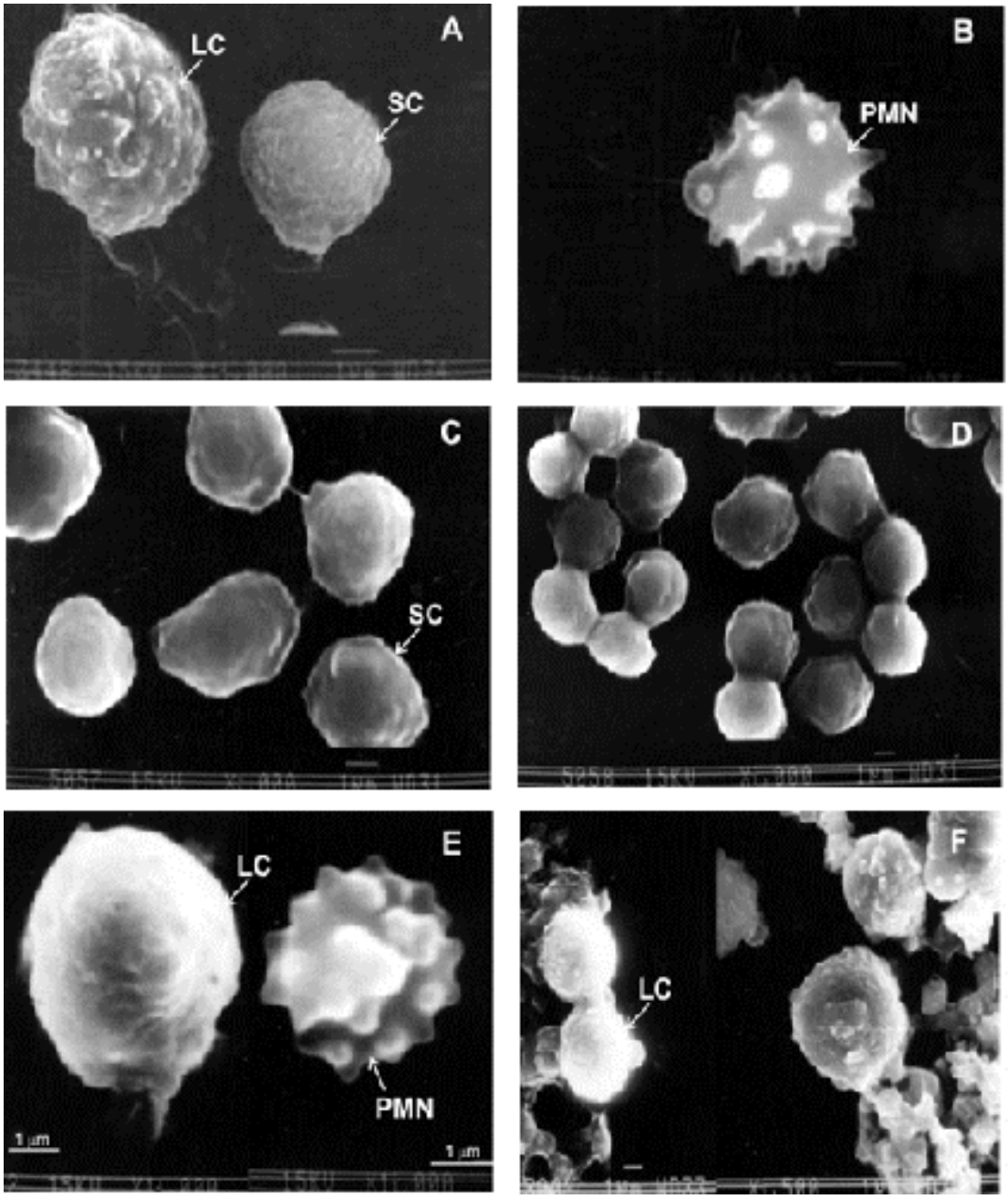


Fig. 8. Scanning Electron micrograph of WBC's of Balb/c mice obtained after separation of blood cells by density gradient centrifugation. (A and B: MN and PMN cells of normal control mice, C and D: WBC's of mice exposed for 7 days, E and F: WBC's of mice exposed for 14 days) ABBREVIATIONS: LC- layered surface cells; SC-smooth surface cells PMN- Polymorphonuclear cells; MN-Morphonuclear Cells.

But our results did not confirm the findings of Hashish *et al.*, 2007.He reported a significant decrease in the count of monocytes, peripheral lymphocytes as well as spleen total T and B lymphocyte values in mice subjected to static magnetic field and ELF-MF exposure. White blood cells constitute the defense system of body and any alteration in their value has been associated with various types of infections. But in present case only radiations are source of stimulation of WBC which shows variation in number, morphology and mortality.

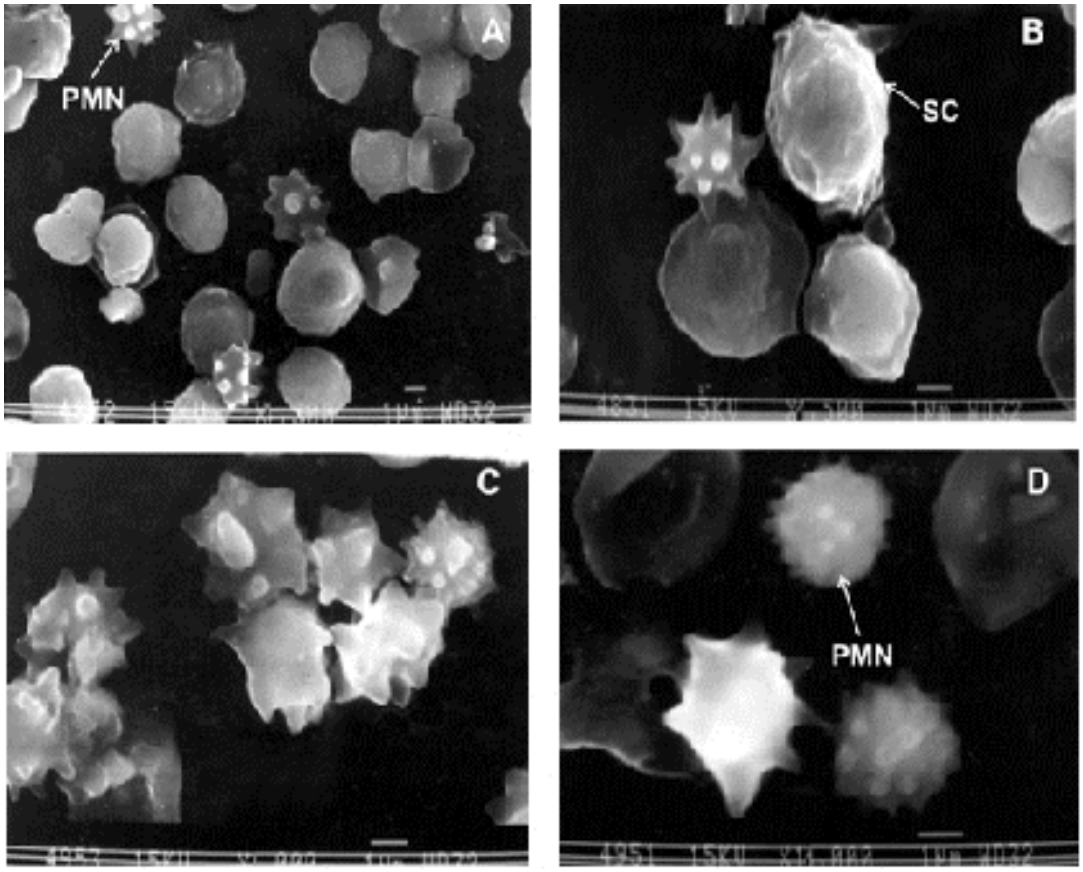


Fig. 9. Scanning Electron micrograph of WBC's of Balb/c mice obtained after separation of blood cells by density gradient centrifugation. (A and B: WBC's of mice exposed for 28 days, C and D: WBC's of mice exposed for 42 days) ABBREVIATIONS: LC- layered surface cells; SC-smooth surface cells PMN- Polymorphonuclear cells; MN-Morphonuclear Cells.

Increase in the lymphocytes and TLC points towards stimulation of immune system of mice after exposure to EMF as observed in infection with pathogens (Shandala and Vinogradov, 1978; Alghamdi and El- Ehazaly, 2012). The increase in lymphocytes may be due to the harmful action of electromagnetic fields exposure that stimulates the hemopoitic system to release more lymphocytes causing the increase in their number in the blood. Lymphocytes are associated with antibody production and antibody mediated immunity (B-cell and T-cell) in the body. Continuous rise in lymphocytes, when there is no any infection in the body can leads to many diseases (Bonhomme-Faivre *et al.*, 2004). The blood smear was found altered because of exposure as observed by Alghamdi and El- Ehazaly 2012. The shapes of various leucocytes were distorted, which may cause lowered cell activity (Gagnon *et al.*, 2003; Usman, A.D. 2012).

Majority of WBCs seen in DLC are actually apoptotic/ dead cells. It emphasize that the immune system is affected because dead WBCs cannot take part in defense mechanism.The SEM of MN and PMN did not reveal its normal membrane structure and morphology may be because of damaging effect of EMF on cell membrane (Ali *et al.*, 2003).

There was decrease in the number of M (phagocytes) and N in both the exposed groups. This could also affect the immune system of mice. Thus this clearly indicate that exposure to VDU radiations even for short period of time affect the immune system adversely and make the body prone more infections.

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