ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effects of 10 GHz MW exposure on hematological changes in Swiss albino mice and their modulation by Prunus domestica fruit extract

[1]Faiza Rifat,[2]Virender Kumar Saxena [1]Preeti srivastava, [3]Archana Sharma [3]Rashmi Sisodia*

1. Department of zoology, Mahatama Jyoti Rao Phooley University, Jaipur, Rajastha, Inida.
2. Department of Physics University of Rajasthan, Jaipur, India.
3. Neurobiology Laboratory, Department of Zoology, University of Rajasthan, Jaipur, India.

Manuscript Info

Manuscript History:

Received: 15 December 2013
Final Accepted: 29 January 2014
Published Online: February 2014

Key words
Blood, Microwaves, Histopathology, Prunus domestica, LPO, GSH, serum, blood indices, antioxidants.

Corresponding Author

Rashmi Sisodia

Abstract

Purpose: To study the effects of 10 GHz microwave (MW) exposure on hematological changes in Swiss albino mice and possible modulatory role of *Prunus domestica* fruit extract (PDE). **Materials and Methods:** Mice were exposed to 10 GHz (Giga Hertz) microwaves with the power density of 0.25 mW/cm^2 (milliwatt per centimeter square) with average whole body specific absorption rate (SAR) 0.1790 W/kg(watt per kilogram) daily for 2 hours per day (h/day) for 30 days. For this purpose mice (6 – 8 weeks old) selected from an inbred colony were divided into three groups: Group I: (Sham exposed) Mice of this group served as control they were placed in plexi glass cage without energizing the system for 2h/day for 30 consecutive days. Group II: (Microwave exposed) Mice of this group were exposed with microwaves 10 GHz for 2 h /day for 30 consecutive days. Group III: Mice of this group received 500mg/kg/b.wt (milligram per kilogram body weight) of PDE orally once daily 1 hour before exposure to10 GHz (2 h/day) for 30 consecutive days. After exposure mice blood samples were collected from mice of all the groups and analysis of the blood samples was carried out. **Results:** MW exposure resulted in significant decrease ($P \leq 0.001$) in hemoglobin, monocytes, packed cell volume, red blood cells, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration whereas, white blood cells, lymphocytes, erythrocyte sedimentation rate and mean corpuscular volume increased significantly ($P \leq 0.001$) in MW exposed mice compared to the sham exposed. Cholesterol, alkaline phosphatase and lipid peroxidation also increased significantly ($P \leq 0.001$) after MW exposure compared to sham exposed mice. Depletion was noted in blood sugar, total protein, acid phosphatase and glutathione level after microwave exposure compared to sham exposed mice. Histopathological alterations in blood cells were also noted. Signs of improvements in the hematological and histopathological parameters were recorded in Group III where PDE was supplemented prior to exposure. **Conclusion**: MW exposure has influence on the hematological parameters which could be ameliorated by supplementation of PDE.

INTRODUCTION

Electromagnetic fields (EMF) are an integral part of our existence. 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, 2001). Not only animals, the electromagnetic irradiation can also affect growth of

plants and microorganisms (Tadevosian et al, 2007). Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. MW at X- band lies in frequency ranging from 8-10 GHz (Gigahertz) and is widely used in communication systems for civil and military application devices such as aircraft, weather forecast system and various types of radars. The 10 GHz band is the easiest microwave bands to get on primarily because of its proximity to frequencies heavily used by different radars and the resulting equipment availability. Its increased usage in occupational environment has caused potential threat to human health, resulting in growing public concern. This has attracted a great deal of attention as evident by increased number of research publications. (Jauchem 2008, Ballardin et al. 2011, Shakya et al. 2011, Kumar et al. 2012, Sharma et al. 2013, Sisodia et al. 2013).

EMF has been reported to affect a wide range of other basic cellular functions. These effects include cell proliferation, the cell cycle (Zhang et al 2013), protein synthesis (Gerner et al 2010), gene transcription and expression (Zhao et al 2007), neurite outgrowth (McFarlane et al, 2000) and tissue damage in different organs of the experimental animals (Zare et al. 2007, Khayyat and Abou-zaid 2009). Gagnon et al 2000, observed gender specific immune response, histological changes, and hematology gender differences in experimental mice. The effect of microwave radiations on biological systems is primarily identified as due to an increase in temperature i.e. thermal (Stuchley 1988) though non thermal effects have also been identified (Paulraj and Behari 2004). Experiments have pointed enhancement of the presence of free radicals after electromagnetic field exposure (Yoshikawa et al. 2000; Kumar et al. 2010).

Free radicals generated during pathological processes may cause acute or chronic tissue damage (Salvemini and Cuzzocrea 2002). Excessive production of free radicals specifically reactive oxygen species (ROS), have also been reported in wide variety of clinical disorders and environmental stress (Galli et al. 2005, Houten et al. 2006). The balance between production and neutralization of ROS levels can increase dramatically, which may cause damage to cell structures leading to behavioral, histopathological and biochemical alterations (Desai et al. 2009).

Researches have confirmed that an adequate intake of antioxidant rich diet helps to prevent these degenerative disorders because food rich in antioxidants play an essential role in the prevention of many diseases viz cardiovascular diseases cancers and neurodegenerative diseases.

Studies undertaken in our laboratory have shown that the fruits *viz. Grewia asiatica*, *Prunus avium* and *Prunus domestica* particularly rich in anthocyanin and other antioxidants possess the radio protective efficacy against gamma rays (Sharma and Sisodia 2010; Sisodia et al, 2011, Sharma and Sisodia 2012). Other studies (Aweda et al, 2011; Aziz et al, 2012) have also evaluated the role of oxidative stress and ameliorative effect of antioxidant vitamins in microwave induced oxidative damage, but there is still paucity of information on the modulatory effect of herbal antioxidant against MW radiations. Therefore, the study was undertaken to evaluate the modulatory role of antioxidant rich fruit *Prunus domestica* (Family Rosaceae) commonly known as Alu bukhara, against EMF which has been used as a traditional medicinal food in humans to enhance immunity against infectious agents. Plums are fruits rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than e.g. oranges, apples or strawberries (Kayano et al. 2002, Leong and Shui 2002).The fruit contain anthocyanins (type cyanidin-3- glucoside and cyanidine-3-rutinoside), flavanols (catechin) (Los et al. 2000) and 3-caffeoylquinic acid (Jaiswal et al, 2013). According to nutrient database, 100 grams(g) of edible portion of fruits *of Prunus domestica* has protein 0.7 g, fat 0.5 g, carbohydrate 11.1 g, minerals and fibre 0.4 g, calcium 10 milligram (mg), phosphorus 12 mg, iron 0.6 mg, magnesium 147 mg, sodium 0.8 g, potassium 247 g, copper 0.13 g, sulphur 33 mg, carotene (Vitamin A) 166 micro gram (µg), thiamine (Vitamin B1) 0.04 mg, riboflavin (Vitamin B) 0.1 mg, niacin 0.3 mg, vitamin C 5 mg and oxalic acid 1 mg (Goplan et al. 2000). Therefore, the present investigation has been undertaken to study the effects of 10 GHz microwave exposure in hematological and serum biochemical parameters of Swiss albino mice and possible modulatory role of PDE against these 10 GHz induced damages.

MATERIALS AND METHODS

Experimental animals

Adult male Swiss albino mice, 6-8 weeks old and weighing 25±2 grams were used for the present study. Initially the mice were procured from Central Drug Research Institute (CDRI), Lucknow, India and maintained in the animal house as an inbred colony as per the norms established by Institutional Animal Ethical Committee (IAEC). The animals were housed in clean polypropylene cages and maintained under controlled conditions of temperature (25 ±1.5ºC) and light (12 hours light: 12 hours dark). They were maintained on standard normal diet obtained from Hindustan Lever, Delhi, India and water *ad libitum*.

10 GHz exposure system, exposure conditions and dosimetry

Mice were divided into three groups consisting of 10 mice in each group. Two mice were housed at a time in a rectangular partitioned cage made of plexiglass which was well ventilated with holes of 1 centimeter (cm) diameter. The dimensions of the cage (4.5×9×9cm) were such that animals were comfortably placed, though they could not move. The horn antenna was kept in H (Magnetic field) plane configuration. Therefore electric field was perpendicular to the ground surface. Field was almost uniform because the dimension of the cage is of the order of wavelength. At near field distance from the horn antenna, power density measured was 0.25 mW/cm^2 (milliwatt per centimeter square) which was maximum. Every day, the cage with mice was placed in the same position facing the horn antenna. The mice were exposed with 10 GHz MW radiation source through the antenna for 2 h/ day (hours/ day) for 30 consecutive days as shown in Figure 1. The whole microwave exposure system was procured from Wavetech, Faridabad, Haryana, India.

The emitted power of microwaves was measured by a power meter which is a peak sensitive device (RF power sensors 6900 series and infra red (IFR) 6960 B RF power meter; made of Aeroflex Inc., Wichita, Kansas, USA). A similar experiment was performed with sham exposed animals without energizing the system. The power density at the cage location was 0.25 mW/cm^2 and the SAR was calculated as 0.1790 W/Kg (watt / kilogram) based on one animal.

Plant material and extraction procedure

Fresh fruits of *Prunus domestica* were washed, shade dried, and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 48 hours (4 x 12) at 40°C. The extract thus obtained was vacuum evaporated so as to get it in powdered form. The extract was re dissolved in double-distilled water (DDW) just before the oral administration. For the various concentrations, a known amount of PDE was dissolved in DDW. The mice were given 500 mg/kg body weight of PDE by oral gavage.

Experimental design

Mice were dived into three groups:

Group I: Sham exposed (Control) Mice of this group which served as control were kept in a plexi glass cage and placed symmetrically along the pyramidal horn antenna aperture connected with klystron power supply without energizing the system for 2 hours/day for 30 consecutive days.

Group II: Microwaves exposed

Mice of this group were exposed with microwaves 10 GHz for 2 hours/day for 30 consecutive days.

Group III: (PDE treated +MW exposed)

Mice of this group received 500mg/kg/b.wt. of *Prunus domestica* extract (PDE) once daily 1 hour before exposure to 10 GHz pulsed density (2hr/day) for 30 consecutive days.

Collection, preparation of smear and quantification of blood samples

At the end of experiment blood samples of mice of all the groups were collected making cut on the vein of tail. For the hematological analysis, blood sample was received at a tube containing dipotassium ethylene diaminetetra acetate (EDTA) to stop it from clotting as recommended by Dacie and Lewis, 2006. Hemoglobin (Hb) content (gm %) of each animal was estimated by Sahli's hemoglobinometer. Hematological parameters like total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, were estimated on fully automated fluorescence flow cytometry 5-part different analyzers (Sysmex XS800i, Japan). PCV % was measured by the use of micro hematocrit method and erythrocyte sedimentation rate (ESR) was obtained using Wintrobe's method. In addition, for biochemical analysis blood sample was collected into a centrifuge tube without any anticoagulant and centrifuged at 25000 rpm for 20 minutes. Clear serum samples were separated in glass tubes and then they were subjected to different biochemical assays. All the biochemical assays were carried out on fresh serum samples. Blood sugar level was determined by GOD-PAP Tinder method, measurement of total protein in serum was done by Biuret method, estimation of total cholesterol was done by CHOD-PAP method using, determination of alkaline phosphatase activity was carried out by kinetic method using p-nitrophenyl phosphate (p-NPP) and estimation of acid phosphatase activity was carried out by kinetic method using α- naphthylphosphate, estimation of of LPO and GSH was done using method of Singh et al. (2007).Blood smears were made and smeared slides were then left for drying and were stained with Giemsa standard solution for microscopic examinations.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed using Student's't' test.

Results

Hematological data

MW exposure (Group III) resulted in significant decrease in different parameters of blood viz Hb, monocytes, PCV, RBC, MCH and MCHC compared to sham exposed mice. There was no significant change recorded in hemoglobin percentage between the microwave exposed group (group II) and PDE+ MW exposed group (group III). However, PDE was supplementation prior to microwave exposure resulted in statistically significant increases ($p \leq 0.001$) in monocytes, PCV, RBC, MCH and MCHC. WBC, platelets, lymphocytes, ESR and MCV were found to be increased significantly ($P \leq 0.001$) after microwave exposure (group II) compared to sham (group I) but supplementation of PDE prior to exposure (group III) was not able to improve the parameters i.e. the presence of PDE was unable to restore the damage caused by microwave exposure. However, lymphocyte percentage was significantly decreased with PDE supplementation.

Table 1: Variations in the different hematological parameters in the blood of Swiss albino mice in the presence /absence of *Prunus domestica* extract (PDE).

Parameters	**Sham Exposed N=10 Group I**	**MW Exposed N=10 Group II**	**PDE+MW Exposed N=10 Group III**
Hb % gm/dl	11.06±0.03	10.08±0.01*	10.15±0.04***
WBC th/mm^3	5.51±0.06	6.25±0.01*	6.46±0.10***
Platelet lakh/mm3	0.94±0.01	1.48±0.09*	1.45±0.0.7***
Lymphocytes %	35.3±0.01	39.31±0.03*	34.95±0.16*
Monocyte %	3.57±0.06	2.41±0.08*	3.14±0.06*
PCV %	45.47±0.06	40.13±0.01*	41.83±0.60*
TRBC mil/cu-mm	5.7±0.06	3.48±0.01*	5.65±0.05*
ESR %	36.56±0.03	38.20±0.03*	38.78±0.07***
MCV (pg)	47.18±0.05	48.16±0.06*	48.56±0.16***
MCH (pg)	16.13±0.01	15.44±0.08*	16.09±0.13*
MCHC (pg)	34.27±0.13	33.66±0.08*	34.1±0.04*

All values are expressed as mean ± S.E.M
Significance levels: *= highly significant differences at p<0.001.
**=significant differences at p<0.01
***= non significant differences at p < 0.05
Statistical comparison: Sham exposed Vs. MW exposed. MW exposed Vs PDE+ MW exposed.

Biochemical assays

MW exposure resulted in significant decrease ($p< 0.001$) in blood sugar, total protein, acid phosphatase and GSH levels compared to sham exposed mice. PDE supplementation prior MW exposure resulted in significant elevations ($p< 0.001$) in the levels blood sugar and total protein compared to exposed mice. Microwave exposure resulted in significant changes in levels of acid phosphatase and GSH which could not be modulated by PDE supplementation.

Significant increase in levels of cholesterol, ALP and LPO were noticed in MW exposed mice compared to sham exposed mice. Supplementation of PDE prior MW exposure (group III) decreased the elevated levels of cholesterol, ALP and LPO.

Table 2: Variations in the different serum biochemical parameters in the blood of Swiss albino mice in the presence /absence of Prunus domestica extract (PDE).

Parameters	**Sham Exposed N=10**	**MW Exposed N=10**	**PDE+MW Exposed N=10**
Blood sugar mg/dl	123.7±0.5	111.4±0.42 *	119.31±0.05*
Total Protein mg/gm of tissue	8.45±0.07	6.45±0.01*	7.58±0.19*
Cholestrol mg/dl	130.4±0.63	140.1±0.37 1*	116±0.57*
Acid Phosphatase IU/L	4.34±0.04	1.2 ±0.06*	1.29±0.06***
ALP IU/L	88.9±0.52	94.9±0.84*	92±0.54*
LPO (nano mole MDA/ml of protein)	111.4±0.42	123.7±0.5*	102.66±0.02*
GSH (nano mole /ml)	8.43±0.07	7.38±0.01*	7.94±0.02***

All values are expressed as mean ± S.E.M.

Significance levels: *=highly significant differences at $p< 0.001$

**=significant differences at $p<0.01$

***= non significant differences at $p < 0.05$

Statistical comparison: Sham exposed Vs. MW exposed. MW exposed Vs PDE+ MW exposed.

Histopathological observations

Observation of the blood smear of sham exposed group (group I), revealed normal RBCs viz. format disc shaped having regular amount of hemoglobin (Fig 2). The blood smear of MW exposed group (group II) showed poikilocytosis in the erythrocytes, erythrocytes became distorted in shape some of the cells were found to be staked next to each other (Fig 3). Blood smear of PDE+ MW exposed group (group III) in the present investigation showed normal shape of the erythrocytes but with hyper pigmentation (Fig 4).

Figure 1: Sketch of the experimental arrangement of 10 GHz microwave source

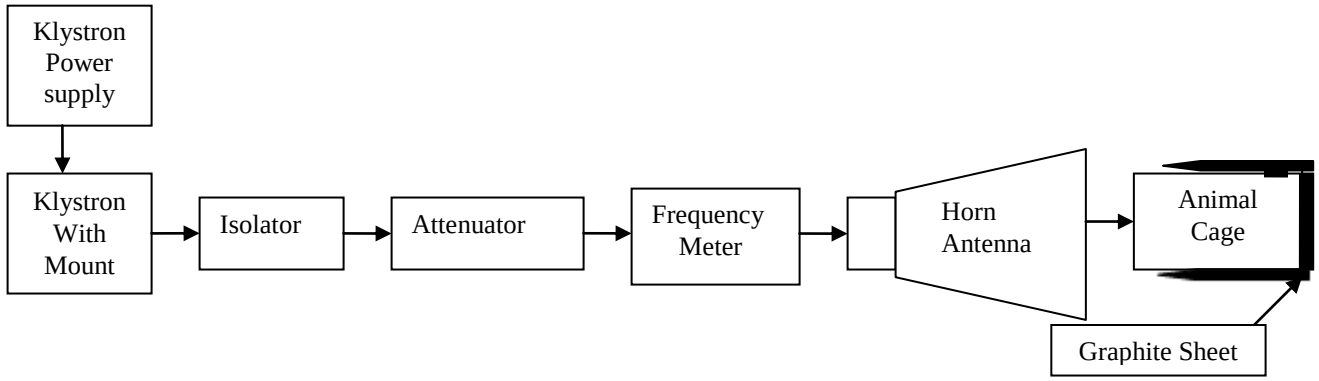
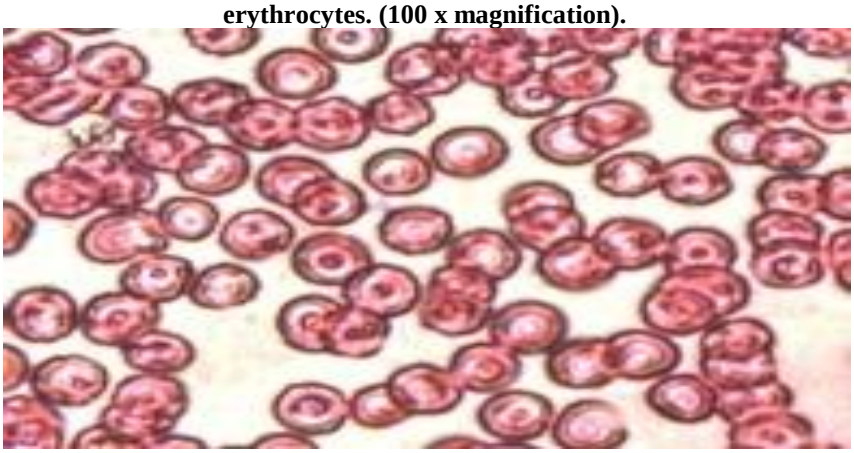


Figure 2: Geimsa stained blood smear of mice of sham exposed mice, showing normal appearance of erythrocytes. (100 x magnification).



Firgure 3: Geimsa stained blood smear of MW exposed mice showing hemolysed (H) and distorted (D) erythrocytes with abnormal blood smear (100 x magnification).

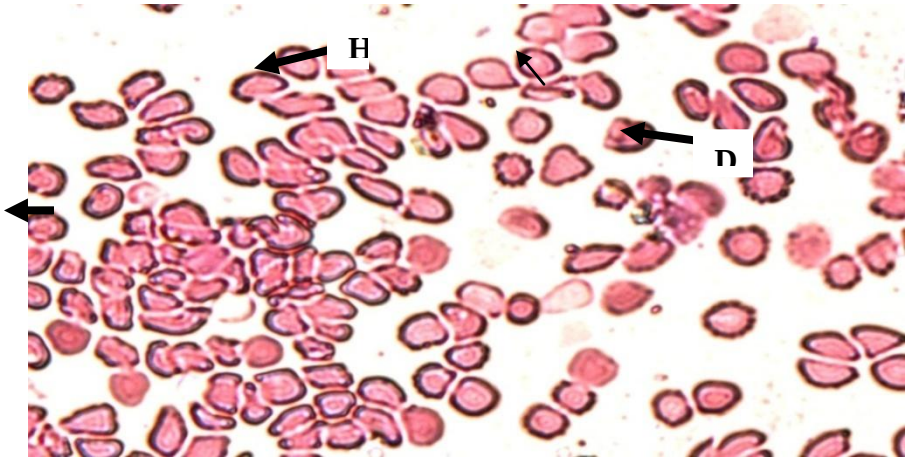
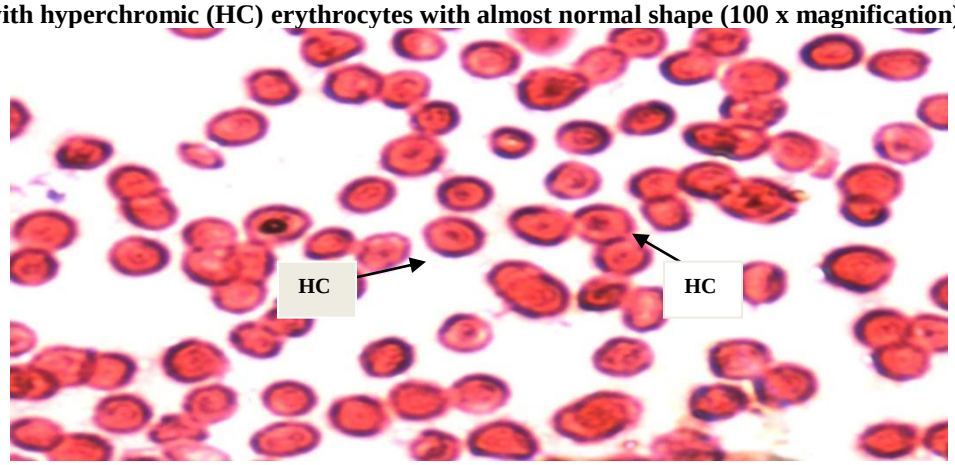


Figure 4: Geimsa stained blood smear of MW exposed mice showing overall better picture of blood smear with hyperchromic (HC) erythrocytes with almost normal shape (100 x magnification).



Discussion

Measurements of blood parameters are the most important means to determine the health status of experimental animals. Blood and blood parameters are believed to be one of the primary particles that come in contact with RF /electromagnetic field (EMF). Blood, comprised of ions are likely to react with induced EMF generated by EMF charges.

The present study reported that MW exposure results in a significant decrease ($p \leq 0.001$) in the hematological constituents of blood viz. Hb, monocytes, PCV, TRBCs, MCH and MCHC) compared to sham exposed group. The depletion in the values of hematological parameters following MW radiation exposure may be attributed to (a) direct damage caused by lethal dose of radiation (b) due to overproduction of ROS by MW interactions (Sisodia et al, 2013). Changes in hematological parameters may be attributed to inhibition by free radicals produced by MW interactions, while the antioxidants present in PDE have suppressed these harmful effects by scavenging some of the free radicals. Higher values may be possibly due to accelerated hematopoietic regeneration.

In the present investigation the increase in lymphocytes may be due to the harmful action of MW exposure that stimulates the hematopoietic system to release more lymphocytes causing an increase in their number in the blood stream. Aziz et al, 2010 also have reported an increase in lymphocytes in cases of anemia, specifically macrocytic anemia, which arise under the influence of exposure to radiation, increased temperature, and increased resistance to the body's immune system. Significant increase in MCV in the present investigation corroborates the results of (Abdolmaleki et al, 2013, Sisodia et al, 2013) and rejects the results of Yousefi, 1996.

Results of the present research are contradictory with the results of Zsolt et al 2006, who noted increase in RBC and PCV values after mice exposure for 2 weeks for 2 hours per day. Mean corpuscular hemoglobin (MCH) has medical importance in the diagnosis of some types of anemia, which indicates the decrease in the value of MCH on the disease of anemia caused by iron deficiency (Althbyta, 2002). Our findings agree with previous studies done by Forgács et al, 2006 who reported that several hematological variables are sensitive to RF/MW exposure. According to these authors, changes in the above parameters repeatedly occurred after either short- or long-term MW/RF exposures, regardless of the power density of the electromagnetic field. Rotkovska et al, 1993 reported no differences in the total erythrocyte count between irradiated and non irradiated mice, although she reported a statistically significant decrease in total leukocytes in the exposed animals. Supplementation of PDE before exposure could modulate these parameters. Some of the parameters investigated by Aziz et al, 2010 showed a significant increase in some blood parameters of WBC, MCV, blood platelets and a significant decrease in RBC, HB, MCH and MCHC in EMF exposed mice compared to control mice they also reported that supplementation of vitamin C and vitamin E also modulated the effects of electromagnetic radiations in blood of Swiss albino mice. Similarly, the effect of broadband EMF exposure on mice reported by Gagnon et al, 2002 also shows effects on hematological parameters. Our results are in agreement with the findings of Hassan, 2011 who reported that MW exposure of female rats caused a significant decrease in total serum protein, total RBC count, PCV% and Hb concentration as well as significant increase in total cholesterol and total WBC count. Although these results contradict those found by Amara et al, 2006 who reported significant increase in the above parameters viz. RBC, WBC, PCV, MCV, MCHC they hypothesized that action of SMF on the geometrical conformation of hemoglobin was reinforced by the fact that static magnetic field (SMF) induced a prominent effect on hemoglobin structure.

Serum biochemical parameters viz Cholesterol, ALP and LPO were found to be significantly increased ($p \leq 0.001$) in MW exposed mice compared to sham exposed. MDA a product of lipid peroxidation was found to increase during 10 GHz induced oxidative stress. High levels of serum MDA in 10 GHz exposed mice indicate that MW enhances LPO and produces oxidative stress by producing free radicals as reported earlier (Sisodia et al, 2013; Faiza et al, 2013). Kula et al 2002 also reported significant increase in malondialdehyde (MDA) level and lowered GSH-Px activity after exposure to electromagnetic field. In the present study, it was observed that PDE treatment significantly lowered the microwave radiation-induced elevated levels of LPO in terms of malonaldehyde. The inhibition of LPO in biomembranes can be caused by antioxidants. Earlier studies also showed that whole-body radiation significantly increased LPO contents of mice spleen (Manda and Bhatiya, 2003) and in blood serum (Singh et al. 2007; Sisodia et al. 2008) which can be modulated by supplementation of exogenous substances. Free radicals damage various tissue components and the question of whether oxidative stress is a major cause of hematological damage remains equivocal.

The depletion in GSH content after exposure to MW radiation noted in the present study in blood serum may be due to the reaction of GSH with free radicals resulting in the formation of thiyl radicals that associate to produce glutathione disulfide (GSSG) (Ballatori et al 2009). Moreover, the availability of GSH can also be limited by deficiency in synthesis, enhanced efflux, or inefficient reduction of GSSG (Chatterjee, 2013). In the normal condition, the cells are intact and healthy and GSH is restored by synthesis but in the irradiated animals, normal synthesis and/or repair is disrupted due to damage to DNA and membranes.

Decrease in serum transaminases alkaline phosphatase (ALP) in microwave treated mice compared to the sham exposed group is in agreement with the results of Moussa, 2009. The reason behind increased activity of serum alkaline phosphatase remains unknown.

Increased cholesterol levels, in our study after MW exposure are contradictory to the results found by Sedghi et al. 2006 in guinea pigs. In our study, PDE-treated mice showed a marked decline in increased level of cholesterol compared to the exposed group which may be due to free radical scavenging activity of the antioxidants present in PDE.

Decrease in total protein concentration and increase in cholesterol in the present investigation get support from the findings of Kula et al. 2002 who also reported a significant decrease in total serum protein and cholesterol level. This decrease in MW exposed group, may be probably due to lysis or inhibition of protein synthesis, or may be by the depression of enzymes involved in the activation; this could be due to excessive damage to the genetic machinery. Increased protein concentration in the present study after PDE supplementation may be due to improved ribosomal activities, which enhance protein synthesis. Several studies (Finn et al, 2006; Vangdal and Slimestad, 2006) reported that purified anthocyanins increase low-density lipoprotein and plasma cholesterol. Study indicated that the mechanism by which anthocyanin promotes cholesterol efflux from macrophages is relevant to the regulation of PPAR-LXR-ABCA1 activation. However, more work is needed to demonstrate whether anthocyanin directly or indirectly induced activation of the PPAR-LXR-ABCA1 pathway (Weiet al, 2005). The present study shows a sharp decrease in amount of blood sugar following MW exposure. Higher blood sugar level in PDE-treated group compared to sham exposed group shows that it protects the enzymatic mechanism in the liver. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid per oxidation.

Microscopic examination of blood smears revealed modifications in the shape and size of the erythrocytes representing morphologic abnormality. Poikilocytosis, spherocytosis, hemolysed and distorted erythrocytes seen in MW exposed mice (figure 3) may be due to the formation of active loci on the red blood cell membrane as a result of interaction with free radicals thereby resulting in the alteration of shape of erythrocytes. Marked increase in the LPO observed is also the result of altered erythrocyte morphology evident in group III normal increase in the number of discocytes was also observed. The protective effect of PDE could possibly be attributed to its anti per oxidative potential, whereby, it maintains the membrane lipid composition and thus the normalization of the erythrocyte morphology. Mariam et al 2012 also reported sticking of erythrocytes to each other due to increased viscosity of blood, when exposed to any stimuli. Microwave exposure resulted in variation in cell shapes (Poikilocytosis). Our results are in agreement with Dudek, 2000 who also found difference in abnormal forms of RBCs which could be due to anisocytosis.

Conclusion

From the present study, it can be concluded microwave induced damage in blood can be reduced by supplementation of PDE prior to MW exposure. Synergistic activity of the antioxidants present in PDE are responsible for the protective activity noticed in the present study.

References

1. Abdolmaleki A, Sanginabadi F, Rajabi A, Saberi R. The effect of electromagnetic waveexposure on blood parameters. IJHOSCR. 2013; 6:13-6.
2. Althbyta G. 2002. Introduction of Haematology and Blood Diseases, Laboratory Diagnostic Methods: King Fahd National Library, Riyadh.
3. Amara S, Abdelmelek H, Salem MB, Abidi R, Sakly M. Effects of Static Magnetic Field Exposure on Hematological and Biochemical Parameters in Rats. Braz Arch Biol Techn. 2006. 49(6): 889-95
4. Aweda MA, Meindinyo ROK, Gbenebitse SO, Ibitoye AZ. 2011. Microwave radiation exposures affect cardiovascular system and antioxidants modify the effects. Advances in Applied Science Research 2: 246-251.
5. Aziz A, El- Khozondar HJ, Shabat M, Elwasife K, Mohamed-Osman A. 2010. Effect of electromagnetic field on body weight and blood indices in albino rats and the therapeutic action of vitamin C or E. Romanian Journal of Biophysics 20: 235-244.
6. Aziz A, El- Khozondar HJ, Shabat M, Elwasife K, Mohamed-Osman A. 2010. Effect of electromagnetic field on body weight and blood indices in albino rats and the therapeutic action of vitamin C or E. Romanian Journal of Biophysics 20: 235-244.
7. Ballardin M, Tusa I, Fontana N, Monorchio A, Pelletti C, Rogovich A, Barale R, Scarpato R. 2011. Non-thermal effects of 2.45 GHz microwaves on spindle assembly, mitotic cells and viability of Chinese hamster V-79 cells. Mutation Research 716: 1– 9.
8. Blackman, C.F.; Benane, S.G.; House, D.E. Evidence for direct effect of magnetic fields on neurite outgrowth. The FASEB J. 1993, 7, 801–806
9. Blank, M.; Khorkova, O.; Goodman, R. Changes in polypeptide distribution stimulated by different levels of electromagnetic and thermal stress. Bioelectrochem. Bioenerget. 1994, 5, 34–51.
10. Dudek RW. High-Yield Histology. 2nd Edition, Lip-pincott Williams & Willkins, Philadelphia, 2000.
11. Durney CH, Iskander MF, Massoudi H, Johnson CC.1984. An empirical formula for broad band SAR calculations of prolate spheroidal models of humans and animal. In: Osepchuk JM, editor. Biological Effects of Electromagnetic Radiation. New York: IEEE Press. pp 85–90.
12. Elizabeth H McFarlane, Gavin S Dawe, Maureen Marks, Iain C Campbell, 2000. Changes in neurite outgrowth but not in cell division induced by low EMF exposure: influence of field strength and culture conditions on responses in rat PC12 pheochromocytoma cells. Bioelectrochemistry. Volume 52, Issue 1, September 2000, Pages 23–28.
13. Faiza Rifat, Archana Sharma, Preeti Srivastava, Shikha Patni, Rashmi Sisodia. 2013. Protective Effects of Prunus Avium Extract on 10 GHz Induced Damages in the Spleen of Swiss Albino Mice.International journal of applied research. Volume: 3, Issue: 8, pp 680-864.
14. Finn RD, Mistry J, Schuster-Böckler B, Griffiths-Jones S, Hollich V, Lassmann T, Moxon S, Marshall M, Khanna A, Durbin R, Sean R, Eddy, Erik L, Sonnhammer L, Bateman A. Pfam: clans, web tools and services. Nucleic Acids Res. 2006; 34: 247–51.
15. Forgács Z, Somosyb Z, Kubinyib G, Bakosb J, Hudákc A, Surjána A, Thuróczyb G. Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reprod Toxicol. 2006; 22: 111–7.
16. Gagnon ZE, Fenendez VA, Conetta JA. The effect of broadband electromagnetic field exposure on mice (Mus Musculus). J. Environ. Sci. Health. 2002; 35(3): 299-311.
17. Gagnon, Z.E.; Fernandez, V.A.; Conetta, J.A. The effects of broad-band electromagnetic field exposure on mice (Mus musculus). J. Environ. Sci. Health 2000, A209, 299–311.
18. Galli F, Piroddi M, Annetti C, Aisa C, Floridi E, Floridi A. 2005. Oxidative stress and reactive oxygen species. Contributions to Nephrology 149: 240-260.
19. Gerner C, Haudek V, Schandl U, Gundacker N, Hutter HP, Mosgoeller W. Increased protein synthesis by cells exposed to a 1,800-MHz radio-frequency mobile phone electromagnetic field, detected by proteome profiling. 2010. Int Arch Occup Environ Health. 83(6):691-702.
20. Goldsmith JR. Epidemiologic evidence relevant to radar (microwave) effects. Environ HealthPersp. 1997;105: 1579–87.
21. Goodman, R.; Henderson, A.S. Exposure of salivary gland cells to lowfrequency electromagnetic fields alters polypeptide synthesis. Proc. Natl. Acad. Sci. 1988, 85, 3928–3932.
22. Gopalan C, Sastri BVR, Balasubramanyam SC.2002. Nutritive value of Indian foods. Hyderabad, India pp-156.

23. Hassan BF. Subchronical effects of electromagnetic field exposure of adult female rats on some hormonal, biochemical and hematological parameters. Diyala agricultural sciences journal. 2011; 3: 47-53.
24. Hood, E., 2001. Electromagnetic field and DNA effects: Potential mechanism elucidated. Environmental Health perspectives, 112 (A): 368.
25. Houten BV, Woshner V Santos JH. 2006. Role of mitochondrial DNA in toxic responses to oxidative stress. DNA Repair (Amst) 5: 145- 152.
26. Jaiswal R, Karakose H, Ruhmann S, Goldner K, Neumueller M, Treutter D, Kuhnert N. 2013. Identification of phenolic compounds in plum fruits (Prunus salicina and Prunus domestica) by high-performance liquid chromatography/tandem mass spectrometry and characterization of varieties by quantitative phenolic fingerprints. Journal of agricultural food chemistry.
27. Jauchem JR. 2008. Effects of low-level radio-frequency (3 kHz to 300 GHz) energy on human cardiovascular, reproductive, immune, and other systems: a review of the recent literature. International Journal of Hygiene and Environmental Health 211: 1– 29.
28. Jones, D.P. (1985). The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic models. In: Sies H, editor. Oxidative stress. New York; DC; pp. 152–196.
29. Kayano S, Kikuzaki H, Fukutsaka N, Mitani T, Nakatani N. 2002. Antioxidant activity of prune (Prunus domestica L.) constituents and a new synergist. Journal of Agricultural and Food Chemistry 50: 3708-3712.
30. Khayyat LI, Abou-Zaid D. 2009. The effect of isothermal non-ionizing electromagnetic field on the liver of mice. Egyptian Journal of Experimental Biology (Zoology) 5: 93-99.
31. Kula B, Sobczak A, Kuska R. Effects of electromagnetic field on free-radical processes in steelworkers. part I: magnetic field influence on the antioxidant activity in red blood cells and plasma. J Occup Health. 2002; 44:226-229.
32. Kula B, Sobczak A, Kuska R. Effects of electromagnetic field on free-radical processes in steelworkers. part I: magnetic field influence on the antioxidant activity in red blood cells and plasma. J Occup Health. 2002; 44:226-229.
33. Kumar S, Behari J, Sisodia R. 2012. Impact of microwave at X-Band aetiology of male infertility. Electromagnetic Biology and Medicine 31: 223-232.
34. Kumar S, Kesari KK, Behari J. 2010. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian Journal of Experimental Biology 48: 586-592.
35. Kwee, S.; Rasmark, P. Changes in cell proliferation due to environmental nonionizing radiation. 1. ELF electromagnetic fields. Bioelectrochem. Bioenerget. 1995, 36, 109–114.
36. Leong LP, Shui G. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry 76: 69–75.
37. LEWIS, S.M., B.J. BAIN, I. BATES, Dacie and Lewis Practical Haematology, Churchill Livingstone, USA, 2006.
38. Liburdy, R.P. Calcium signaling in lymphocytes and ELF fields: evidence for electric fields metric and site of interaction involving the calcium ion channel. FEBS Lett. 1992, 301, 53–59.
39. Liburdy, R.P. Cellular studies and interaction mechanisms of extremely low frequency fields. Radio Sci. 1995, 30, 179–203.
40. Los J., Wilska-Jeszka J., Pawlak M. (2000) Polyphenolic compounds of plums (Prunus domestica). *Polish Journal of Food and Nutrition Sciences* **18507**:35-38
41. Manda, K. & Bhatia, A. L. (2003). Role of ß-carotene against acetaminophen-induced hepatotoxicity in mice. Nutrition Research. 23, 1097–1103.
42. Mariam SA, Nawal AE. 2012. Effects of exposure to electromagnetic field on some hematological parameters in mice. J Med Chem. 2012; 2: 30-42.
43. Moussa SA. Oxidative stress in rats exposed to microwave radiation. Romanian J Biophys. 2009; 19: 149–158.
44. Navarro, J., Obrador, E., Pellicer, J.A., Aseni, M., Vina, J., Estrela, M. (1997). Blood glutathione as an index of radiation induced oxidative stress in mice and humans. Free Radical Biology. 22, 1203–1207.
45. Ongel, K., Gumral, N., Ozguner, F., 2009. The potential effect of electromagnetic field: A review. Cell membrane and free radical research, 1(3): 85-89.
46. Rice-Evans C, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996; 20:933–56.
47. Roberts NJ, Michaelson SM, Lu SL. The biological effects of radiofrequency radiation: acritical review and recommendations. Int J Radiat Biol. 1986; 50: 379-420.

48. Rosenthal, I.M.; Obe, G. Effects of 50-Hertz electromagnetic fields on proliferation and on chromosomal alterations in human peripheral lymphocytes untreated or pretreated with chemical mutagens. Mutat. Res. 1989, 210, 329–335.
49. Rotkovska D, Moe J, Kautksa J, Bartonekova A, Keprtova J, Hofer M. Evaluation of the biological effects of police radar Ramer 7F. Environ. Health Persp. 1993;101: 134–36.
50. Salvemini D, Cuzzocrea S. Oxidative stress in septic shock and disseminated intravascular coagulation. Free Radic Biol Med. 2002;33(9):1173—85.
51. Sedghi H, Zare S, Hayatgeibi H, Alivand S, Ebadi AG. Biological effects of power frequency magnetic fields on serum biochemical parameters in guinea pigs. Pak J Bio Sci. 2006; 9:1083- 7.
52. Shakya AK, Sharma N, Saxena M, Shrivastava S, Shukla S. 2011. Evaluation of the antioxidant and hepatoprotective effect of Majoon-e-Dabeed-ul-ward against carbontetrachloride induced liver injury. Experimental and Toxicologic Pathology 64: 767-773.
53. Sharma A, Sisodia R, Bhatnagar D, Saxena VK. 2013. Spatial memory and learning performance and its relationship to protein synthesis of Swiss albino mice exposed to 10 GHz microwaves (doi: 10.3109/09553002.2013.835883). International Journal of Radiation Biology Pages 1-7.
54. Sharma G, Sisodia R. 2012. *Prunus domestica* protects against hepatic and intestinal damage induced by radiation in Swiss albino mice. International Journal of Pharma and Biosciences 6: 153- 165.
55. Sharma K V, Sisodia R. 2010. Hepatoprotective efficacy of *Grewia asiatica* fruit against oxidative stress in Swiss albino mice. Iranian Jounal of Radiation Research 8: 75-85.
56. Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. Mutat Res. 1996; 350:153–61.
57. Singh, S., Sharma, K.V., Sisodia, R. (2007). Radioprotective role of Grewia asiatica in Mice blood. Pharmacologyonline. 2, 32-43.
58. Sisodia R, Rifat F, Sharma A, Srivastava P, Sharma KV. 2013. Effects of 10 GHz on hematological parameters in Swiss albino mice and its modulation by *Prunus avium.* Journal of Environmental Pathology Toxicology and Oncology DOI: 10.1615. 32: 1-13.
59. Sisodia R, Rifat F, Sharma A, Srivastava P, Sharma KV. 2013. Effects of 10 GHz on hematological parameters in Swiss albino mice and its modulation by *Prunus avium.* Journal of Environmental Pathology Toxicology and Oncology DOI: 10.1615. 32: 1-13.
60. Sisodia R, Sharma G. 2011. Radio protective Potential of *Prunus Domestica* against Radiation Induced Oxidative Stress in Swiss Albino Mice with Special Reference To Spatial Learning. Pharmacologyonline 3: 1159-1174.
61. Sisodia R, Singh S, Mundotiya C, Meghnani E, Srivastava P. 2011. Radioprotection of Swiss albino mice by *Prunus avium* with special reference to the hematopoietic system. Journal of Environmental Pathology Toxicology and Oncology 30: 55-70.
62. Sisodia, R., Singh, S., Sharma, K.V., Ahaskar, M. (2008). Post treatment effect of Grewia asiatica against radiation induced biochemical alterations in Swiss albino mice. International Journal of Environmental Pathology Toxicology and Oncology. 27(2), 113–121
63. Smita Singh, K.V. Sharma and Rashmi Sisodia (2007): Radioprotective role of *Grewia asiatica* in Mice blood. Pharmacologyonline**.** Vol. 2: pp.32-43
64. Stuchley MA. 1988. Biological effects of radiofrequency fields. In M. H. Repacholi (Ed.), Nonionizing radiations, physical characterization, biological effects and health hazard assessment. Proceeding for the International Non-Ionizing Radiation Workshop, Melbourne, pp. 197–217.
65. Tadevosian, A., Kalantarian, V., &Trchunian, A., 2007. The effect of electromagnetic radiation of extremely high frequency and low intensity on the growth rate of bacteria Escherichia coli and the role of medium pH. Biofizika., 52(5): 893-898.
66. Vangdal E, Slimestad R. Methods to determine antioxidative capacity in fruit. J. Fruit Ornam. Plant Res. 2006; 14: 123.
67. Wei M, Guobin L, Binny J, Cherayil S, Kou C, Sunney-Xie X. Observation of a Power-Law memory Kernel for Fluctuations within a Single Protein Molecule. Phys Rev Lett. 2005; 94(19):198–202.
68. Wei, L.; Goodman, R.; Henderson, A. Changes in levels of c-myc and histone H2B following exposure of cells to low-frequency sinusoidal electromagnetic fields: evidence for a window effect. Bioelectromagnetics 1990, 11, 272–369.
69. Yoshikawa T, Tanigawa M, Tanigawa T, Imai A, Hongo H, Kondo M. 2000. Enhancement of nitric oxide generation by low frequency electromagnetic field. Pathophysiology 7: 131- 135.

70. Yousefi HA. Evaluation of relationship between occupational exposure to extremely low frequency (elf) electric and magnetic fields and risk of psychological symptoms and hematological changes among workers at high voltage [Dissertation]. Tehran (Iran): Tehran University of Medical Sciences; 1996. Persian
71. Zare S, Alivandi S, Ebadi AG. 2007. Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in guinea pig. World Applied Sciences Journal 2: 509-511.
72. Zhang M, Li X, Bai L, Uchida K, Bai W, Wu B, Xu W, Zhu H, Huang H. 2013. Effects of low frequency electromagnetic field on proliferation of human epidermal stem cells: An in vitro study. Bioelectromagnetics. 34(1): 74-80.
73. Zhao R, Zhang S, Xu Z, Ju Li, Lu D, Yao G. 2007. Studying gene expression profile of rat exposed to 1800 MHz radiofrequency electromagnetic fields with cDNA microassay. Bioelectromagnetics. \
74. Zsolt F, Somosy Z, Kubinyi G, Bakos J, Hudak A, Surjan A, Thuroczy G. Effect of wholebody 1800MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reprod Toxicol. 2006; 22: 111-7.