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Evaluation of Parenteral Antioxidant as a Protective Therapy Against Electromagnetic Radiation Produced by Cell Phones on Hematological and Biochemical Parameters in Rats

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ABSTRACT

Objectives: Radiofrequency of mobile phones may affect biological systems by increasing free radicals and by changing the antioxidant defense systems of tissues, thus leading to oxidative stress. L-cysteine, as a glutathione precursor, has an antioxidant property that may prevent oxidative damage to normal cellular processes. **Methods**: The effect of different concentrations of parentral L-CyS-HCl against harmful effects of EMR on rats' blood was studied namely: hematological parameters (osmotic fragility and blood picture), and biochemical parameters (reduced glutathione and malondialdehyde levels). **Results:** The results revealed that 20 mg parenteral L-CyS-HCl showed high protection for EMR radiated rats against hemolysis. In addition, EMR radiated rats received 20 mg parenteral L-CyS-HCl showed normal blood picture, GSH and Malondialdehyde levels, compared to radiated untreated rats. **Conclusion:** Parenteral L-CyS-HCl can be used for protection against mobile phone hazards

Keywords: *Electromagnetic radiation (EMR); Glutathione (GSH; Mobile phone hazards; L-Cysteine; Oxidative stress*

INTRODUCTION

Mobile phone usage has been increased in the last few years, estimated of more than one billion cellular phone are in use[1]. According to global statistics, the steady increase in the use of mobile phones has increased the establishment of ground situations required. Mobile phones emit electromagnetic radiation (EMR) which is considered as one of the most dangerous types of pollution for human being. Changes in the cell components and alteration of their functions were previously investigated; resulting in the existence of many damage risks to the human vital organs[2]. It has been reported that EMR of cellular phones may affect biological systems by increasing free radicals (enhancing lipid peroxidation), and by changing the antioxidant defense systems of tissues, thus leading to oxidative stress[3].

Protein and amino acids are responsible for the synthesis of antioxidant enzymes. Glutathione and Carnosine are small peptides that act as direct scavengers of free radicals[4].

Dietary deficiency of protein shows the hazardous effect in antioxidant system of cell. Arginine and tetrahydrobiopterin deficiency directly affect nitrogen oxidative stress which implicates the superoxide production and ultimately, oxidative stress in cells/tissues[5]. Deneke has demonstrated previously that, the level of GSH in the cells, especially in hepatocytes, depends on enzymatic steps. The rate-limiting step controls GSH biosynthesis is catalyzed by ɣ-glutamyl cysteine synthetase, which is physiologically regulated by the availability of its precursor, L-CySH[6]. Restoration of GSH through the administration of L-CySH could be beneficial in diseases in which decreased tissue GSH and increased oxidative stress are involved[7]. Recent studies underline that L-CySH plays a key-role in preventing oxidative damage. This action is partly due to direct antioxidant properties via its thiol function that can scavenge free radicals, but also and certainly more

scavenge free radicals, but also and certainly more importantly as a limiting precursor of reduced GSH[8]

Moreover, L-cysteine-HCl was used in the study, where it crosses the erythrocyte membranes more efficiently as compared to N-acetyl cysteine, which must be first deacetylated before its transformation to GSH 9.

MATERIALS AND METHODS

Materials

- Elman's reagent (DTNB) (PubChem CID: 6254) was obtained from Sigma Aldrich (St. Louis, USA).
- L-Cysteine hydrochloride monohydrate (PubChem CID: 60960) was obtained from Sigma Aldrich (St. Louis, USA).
- Other chemicals (NaCl, trichloroacetic acid, orthophosphoric acid, thiobarbituric acid and n-butanol) were of analytical grade.

Experimental animals

Male Wistar Albino rats weighing 140 ± 20 g were purchased from the animal house of the Egyptian Company For Production of Vaccines, Sera and Drugs (EGY VAC), (Helwan, Cairo, Egypt). Animals were housed in cages away of near sources of EMR from the cages and maintained on stock diet and kept under fixed appropriate conditions of housing and handling till the experimental period, with free access to standard laboratory food and tap water, at a constant room temperature of 25˚C and a natural day/night cycle. The killing of the animals as well as the safely removal of killed animals were followed the lab regulations. National Institutes of Health (NIH) guidelines for the care and use of laboratory animals have been followed; besides, animal research protocol has been approved by the Animal Ethics Committee of Faculty of Pharmacy, Helwan University, and committee regulations were followed during the study.

Methodology

Microwave generator

Figure 1, microwave generator and the field densities in the chamber are measured by the microwave analyzer (Rohde & Schwarz zva67 vector network analyser 10 MHz – 67 GHz - USA) [The electronic research Centre, National Research Center (Dokki, Cairo, Egypt)]. The radiation frequency was set to be 900 MHz. mimicking mobile phone, and the electromagnetic waves were radiated through horn antenna to caged rats, for a period of 4 hours.

Experimental Design

The experimental design followed that was described recently with some modifications[10]. The rats were hosted into plastics cage with a distance of 30 cm away from the irradiation source. Rats were randomly divided into eight groups of 8 rats each: negative control group (unexposed to radiation and untreated with L-CyS-HCl) (group 1), positive control group (exposed to radiation and untreated with L-CyS-HCl) (group 2), and six prophylactic treated groups, received one milliliter I.V. injection of different concentrations of L-CyS-HCl (5, 10, 20, 30, 40 and 50 mg/ml) prepared under aseptic conditions and filtered through 0.022 µm micropore syringe filters, (group 3-8), respectively.

Rats received L-CYS-HCl over dose at dose ≥30 mg showed mortality (data not shown). It was previously reported that, administration of high levels of L-CyS-HCl are responsible for many side effects [6].

The effects of microwave emitted from mobile phones on the in vivo blood biological parameters were studied and the protagonist of L-CyS-HCl as a prophylactic treatment was tested.

Effect of electromagnetic radiation on red blood cells (Hematological Studies)

Osmotic fragility test

The osmotic fragility test provides an indication of the ratio of surface area/volume of the erythrocyte (expressed as percentage haemolysis). In the osmotic fragility test, whole blood (exactly 0.02 ml of blood sample) was added to 10 ml of 0.9% of sodium chloride solutions at pH 7.4. Mixing was performed by gently inverting the test tubes for about 5 times. Test tubes were allowed to stand at room temperature (26 - 27ºC) for 30 min. Thereafter, the contents were remixed and centrifuged at 1,500 x g for 15 min. The concentration of hemoglobin in the supernatant solution was measured spectrophotometerically at λ_{540} nm (Spectrophotometer: UV/Visible spectrometer (V-630), Jasco (Tokyo, Japan). The osmotic fragility expressed as percentage hemolysis was calculated using the following formula[11]:

$$\text{Percent Hemolysis} = \frac{\text{Optical density of tested sample}}{\text{Optical density of blood in deionized water}} \text{ X } 100 \quad \text{eq (1)}$$

Blood picture

At the end of irradiation period, blood samples were collected in sterile heparinized anticlotting tubes (VOMA MED, LH China). Samples were used in the evaluation of blood profile comprised in, red blood corpuscles count (RBCs), hemoglobin (Hb) and % lymphocytes (%LY), using automated blood analyzer MSLAB07 (China, mainland)[2].

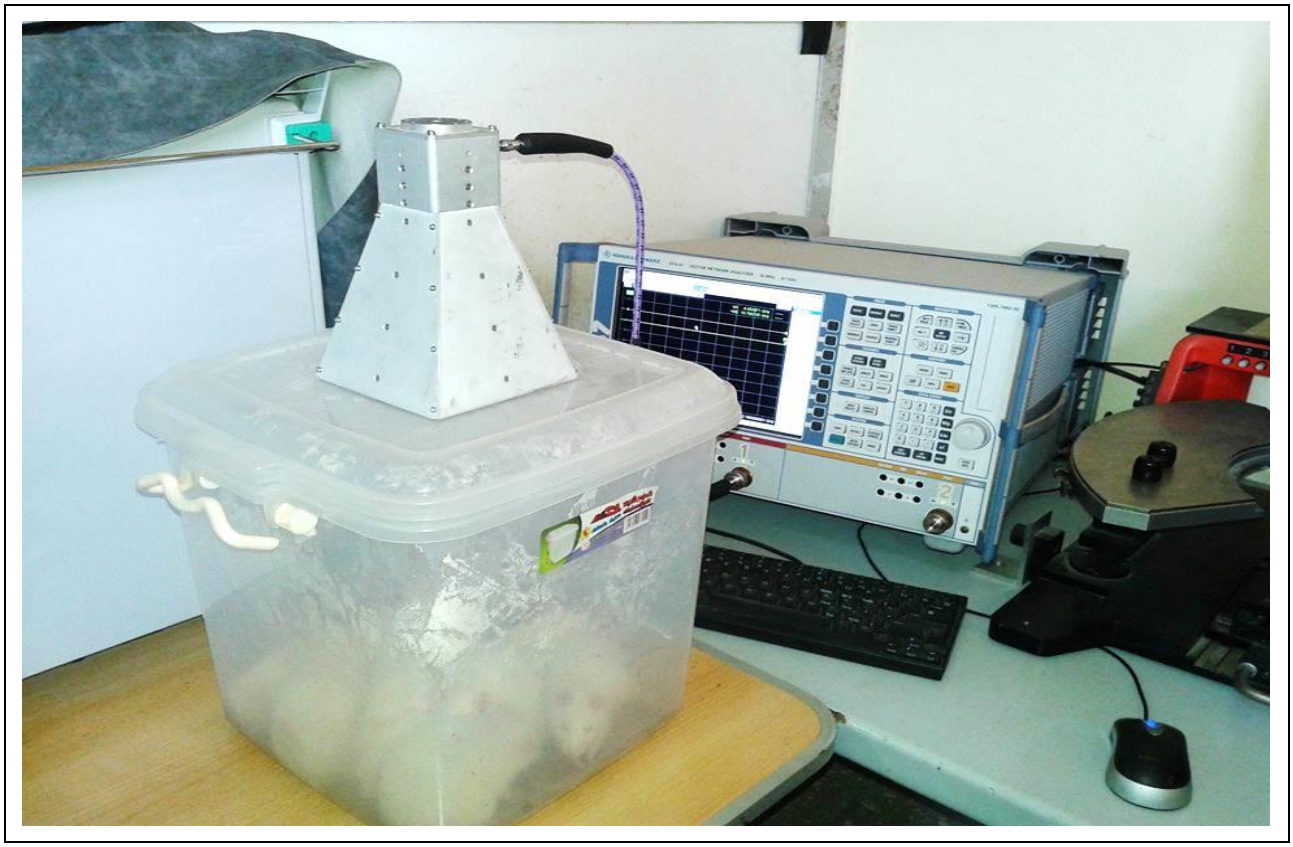

Figure 1: Microwave generator with microwave analyzer (ROHDE & SCHWARZ ZVA67 vector network analyser 10 MHz – 67 GHz - USA). The electromagnetic waves signals generate a radiation frequency at 900 MHz. mimicking mobile phone, and the electromagnetic waves were radiated through horn antenna to caged rats.

Blood biochemical analysis

Whole blood samples were collected into heparinized tubes for estimation of reduced GSH levels and blood serum malondialdehyde (MDA) levels using previously reported methods with some modifications [12].

Determination of reduced GSH

Collected blood samples were lysed in four its volume of ice-cold water. Then, allow standing for five minutes and centrifuge at 3000 rpm for 15 minutes. Aliquots of 0.1ml were added to 0.5ml of 10% trichloroacetic acid and 0.5ml distilled water. The mixture was allowed to stand for five minutes and centrifuging at 3000 rpm for 15 minutes. 0.2ml freshly prepared Ellman's reagent (0.4% w/v) and 1ml phosphate buffer pH 8 (0.1M) were added to each 0.5 ml aliquots. The mixtures were mixed well for 5-10 minutes and were measured spectrophotometrically at λ_{405nm}.

Malondialdehyde level determination

Malondialdehyde (MDA) is a degradative product of peroxidation of polyunsaturated fatty acids (PUFAs) in cell membrane, ending in the generation of oxidative stress indicators, as hydro-peroxides. Reactive oxygen species (ROS) occur naturally, but their accumulation is a marker for oxidative stress. Blood samples were collected in non-heparinized tubes and allowed to clot for thirty minutes, then centrifuged at 4000 rpm for 15 minutes at 4˚C. 0.2 ml aliquots were added to 3 ml of 1% orthophosphoric acid, 1ml of thiobarbituric acid and 0.5 ml distilled water, the mixtures were mixed well and put on boiling water bath for 45 minutes. Four ml of n-butanol was added to the mixtures after cooling and centrifuged at 4000 rpm for 4 minutes, the organic pink layer was separated for measuring the formed MDA coloured complex at λ_{532nm}.

Statistical data analysis

Results were expressed as mean standard deviation (± SD) of quintuplicates (n=8), and were evaluated using the Statistical Package for Social Sciences (SPSS) (IBM SPSS, v 20.0 software, Inc, Chicago IL, USA). Probability at significance level of (P<0.05) was considered as statistically significant.

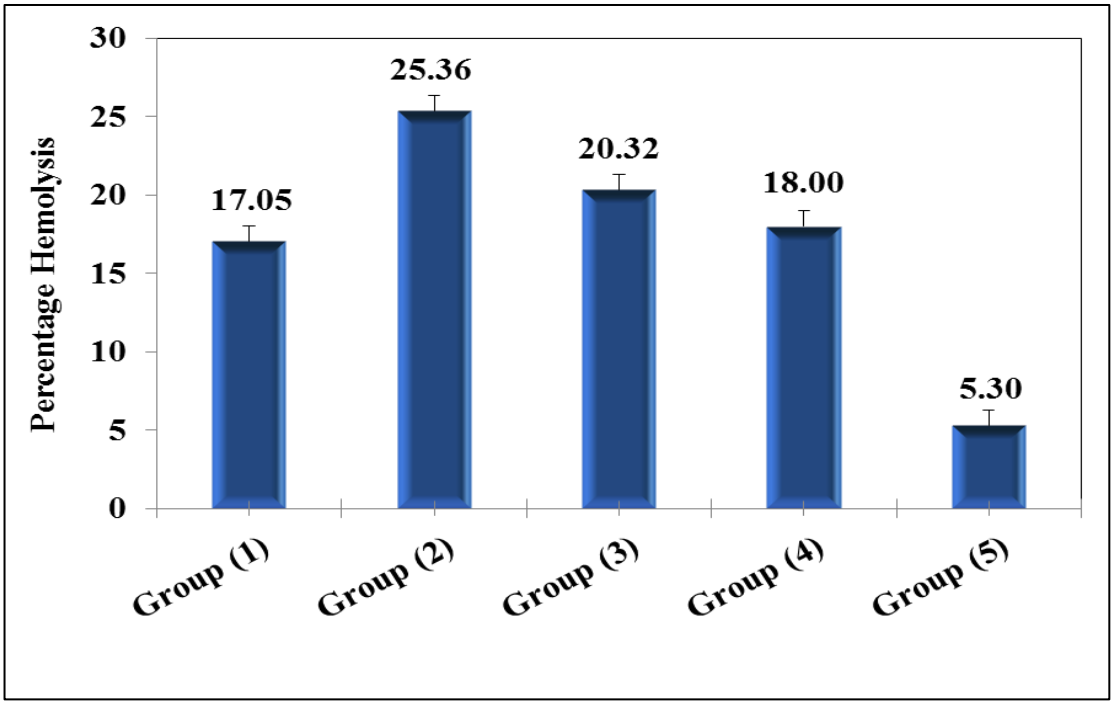

Figure 2: Effect of L-CyS-HCl concentration on erythrocyte osmotic fragility (EOF) in rats, indicated as blood hemolysis, after I.V. administration of a single different concentrations of L-CyS-HCl and exposed to 900 MHz RF/MW continuous fields for four hours. (Mean ± S.D., n=8)

RESULTS

The study showed mortality for rats that received L-CyS-HCl doses more than 20 mg/ml. that doses ≥ 30 mg/ml can be considered as over doses, data not shown.

Effect of electromagnetic radiation on red blood cells (Hematological Studies)

Osmotic fragility test results

Figure 2 shows the protective effect of L-CyS-HCL on erythrocytes, from oxidative fragility damage due to ROS produced by EMR in rats. The results revealed reduction in percent hemolysis, in the following order: group 5 > 4 > 3, with significant difference in percent hemolysis values (P<0.05) compared with negative control groups (group 1), by applying One Way ANOVA followed by Post Hock (Dunnett) test for multiple comparison. Figure 2 shows sharp descending in the percentage hemolysis in groups 3, 4 and 5 (exposed to radiation and treated with 1 ml of L-CyS-HCl (5, 10, 20 mg/ml), respectively, compared to group 2 (positive control group). The data revealed ascending in percent protection against hemolysis (75%, 80%, 82% and 95%) for groups (2, 3, 4 and 5), respectively, compared to (group 1) that exhibited 83% protection against hemolysis.

Blood picture

Table 1, shows that, values of RBCs and Hb count were decreased by exposure to EMR while LY percentage was increased. Using One Way ANOVA followed by Post Hoc (Dunnett) test for multiple comparison; positive control (group 2) in addition to rats received 5 mg and 10 mg of L-CyS-HCL and exposed to EMR (groups 3 and 4, respectively) showed significant difference in the RBCs, Hb and LY values compared with their alternatives of negative control (group1). Fruitfully, rats received 20mg L- CyS-HCL (I.V.) and exposed to EMR (group 5), showed non-significant difference compared with negative control (P>0.05) unlike those rats of positive control (group 2).

Blood biochemical analysis

GSH level determination

Using One Way ANOVA followed by Post Hoc (Dunnett) test, **Table 2**, illustrates that, glutathione concentration was decreased significantly in positive control group (group 2) (P<0.001) compared to negative group (group 1). The results could be attributed to the depletion of glutathione acting as a direct scavenger of ROS. On the other hand, glutathione levels started to replenish in groups of rats treated with L-CyS-HCl (5 & 10 mg) (group 3 and 4) , respectively. Rats receiving I.V. 20mg/ml of L-CyS-HCl and exposed to EMR (groups 5)

Table 1: Values of blood picture before and after exposure to mobile phone electromagnetic radiation

Group	Hb (g/dL)	RBCS (10^{12}/L)	(%) LY
GP1: Negative Control	15.4 ± 0.87	9.13 ± 1.03	73.8 ± 2.13
GP2: Positive Control	8.7 ± 0.51[d]	5.12 ± 0.26[d]	83.5 ± 3.02[C]
GP3: 5 mg	9.2 ± 0.39[d]	5.71 ± 0.39[C]	77.5 ± 2.51[a]
GP4: 10 mg	10.6 ± 0.42[d]	6.07 ± 0.42[C]	75.4 ± 3.2[a]
GP5: 20 mg	13.2 ± 0.61[b]	9.22 ± 1.03[a]	70.8 ± 2.78[a]

Hb = Hemoglobin
RCCs = Red blood cells count
% YL = % Lymphocytes
Statistical Analysis by applying One Way ANOVA followed by Post Hoc (Dunnett) comparative test with Group 1
a=non-significant differences (p>0.05)
b=significant differences (p<0.05)
c=significant differences (p<0.01)
d=significant differences (p<0.001)

showed non-significant difference in GSH blood levels comparing to the negative control group (P>0.05).

MDA level determination

The MDA level was increased significantly for positive control rats (group 2) as well as group 3 compared with negative control rats (group 1) (P<0.001) and (P<0.05) as shown in **Table 2**, by applying One Way ANOVA followed by Post Hoc (Dunnett) test. On the other hand, groups 4, and 5 treated with L-CyS-HCl conc. of 10mg & 20mg, respectively, showed a lower MDA levels in plasma that was non-significant differ from those of negative control (group 1) (P>0.05).

DISCUSSION

Rats received L-cysteine hydrochloride over dose ≥ 30 mg/ml showed mortality. Where, it was previously reported that, administration of high levels of L-CyS-HCl are responsible for many side effects, which may be related to the perturbation of extracellular fluid redox status or metabolic acidosis. Wherever, large increases in free thiols in the circulation are associated with toxic effects. These effects may be the result of thiyl radical-mediated reactions but could also be due to destabilizing effects of increases in thiol/disulfide ratios in the plasma, which normally is in a more oxidized state than intracellular compartments. Changes in the thiol redox gradient across cells could also adversely affect any transport or cell signaling processes, which are dependent on formation and rupture of disulfide linkages in membrane proteins [6].

Effect of electromagnetic radiation on red blood cells (Hematological Studies)

Osmotic fragility test results

The significant difference in percentage hemolysis (indicative of osmotic fragility) observed between different groups of rats with lower values in both negative control & prophylactic treated rats, compared with positive control group, could be recognized to the enormous ROS produced in the positive control animals as a result of oxidative stress, which is responsible for tissue damage and have injurious effects on erythrocyte cytomembrane.

Previously it was found that, the membrane of erythrocyte is rich in polyunsaturated fatty acids which is liable to lipid peroxidation and this cause the loss of membrane fluidity and cellular lysis[13]. Since oxidative stress occurs when the antioxidant defense systems in the body are stunned by FRs[14], L-CyS-HCl administration to experimental rats apparently, reduced the intensity of oxidant stress by enhancing the antioxidant defense mechanisms and minimized the destruction of erythrocyte[11].

Table 2: Blood GSH levels and blood serum MDA levels in rats of different groups

Group number	GSH conc. (mg/mL)	MDA conc. (mMol/L)
Group 1 (Negative control)	0.255 ± 0.033	6.12 ± 0.60
Group 2 (Positive control)	0.103 ± 0.017[d]	8.75 ± 0.49[d]
Group 3	0.142 ± 0.026[C]	7.52 ± 0.55[b]
Group 4	0.143 ± 0.032[C]	6.88 ± 0.28[a]
Group 5	0.316 ± 0.037[a]	6.48 ± 0.34[a]

GSH conc.: Glutathione Concentration
MDA conc.: Malondialdehyde Concentration
Statistical Analysis by applying One Way ANOVA followed by Post Hoc (Dunnett) comparative test with Group 1
***a**=non-significant differences (p>0.05)*
***b**=significant differences (p<0.05)*
***c**=significant differences (p<0.01)*
***d**=significant differences (p<0.001)*

The obtained results revealed that CyS-HCl as an effective antioxidant in various biological systems protected erythrocytes from oxidative damage due to EMR, also revealed that 20mg/ml dose possess the optimized effect that could be administered safely in order to prevent erythrocytes fragility due to ROS produced by EMR in rats.

Blood picture

The present study indicated that, the whole blood picture values excluding % LY values of positive control exposed rats have been shown to decrease compared to those of negative control and treated groups as presented in **Table 1**.

From a biological point of view, blood can be considered as a tissue comprising various types of cells (RBCs, WBCs, and platelets) and a liquid of intercellular material (plasma)[10]. The hypothesis illustrated that most physiological functions in living organisms are electrochemical in nature. Disturbance of intrinsic electrical or chemical process within the cell structure has the potential to disrupt cell function leading to malfunction of organ systems; Previously, it was believed that thermal alteration of cells and tissue heating may be the chief injurious mechanism[15].

Regular exposure to electromagnetic field can increase plasma volume with a consequent decrease in the concentration of Hb and RBCS in blood as stated previously[16]. 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, it alters the cell membrane potential and distribution of ions[17].

Previous studies showed that constant exposure to EMR increased the Lymphocytes pecentage (LY(%)). Which was associated with lymphatic leukemia, or inflammation of the lymph gland. As well, Lymphocytes rising by numerical prolixferation, thus.cellular mutations ocurred[2], the presented results confirm these outcomes

Blood biochemical analysis

GSH level determination

The present results showed that rats received 20 mg of L-CyS-HCl appear to possess significant higher GSH concentrations (P<0.05) compared to positive control (group 2), resulting in lower oxidative damage, **Table 2**.

Radiofrequencies emitted from cellular phones may disturb biological systems by increasing FRs, as being very reactive unstable molecular species they initiate chain reactions to yield new radicals [18]. Glutathione are small peptide molecules, directly act to scavenge the reactive metabolites[4]. GSH-Px converts H2O2 to water and oxygen. GSH-Px uses H_2O_2 to oxidize reduced glutathione (GSH), downregulated GSH concentration consequently, results in the insufficient detoxification and accumulation of H_2O_2 in the system.

As the endogenous GSH has a protecting role in scavenging radicals and in molecular repair, the depletion of GSH concentration may be owing to the higher consumption of GSH for scavenging the FRs production. However, such scavenging can result in a superoxide-dependent chain production of H2O2 and oxidized glutathione, which would stress the cells by oxidation[19].

MDA level determination

The obtained results for the effect of L-CyS-HCl on MDA level were in agreement with that recorded previously[19]. Kerman and Senol.,(2012) recently illustrated that protected rats by melatonin (antioxidant) exposed to EMR continuous field showed a significant decrease in MDA levels in blood samples (an aldehydic secondary products, accepted as an indicator for oxidative stress), thus protection acting indirectly against oxidative stress by reducing the lipid peroxidation product[20]. The peroxidation of lipids is predominantly more harmful because the formation of lipid peroxidation products leads to a facile propagation of FRs reactions, the fatty acid carbon chain extemporaneously was cleaved during lipid peroxidation process and produce highly toxic pentane, ethane, α,β unsaturated fatty acid aldehydes[21].

CONCLUSION

EMR radiated rats receiving 20 mg parenteral L-CyS-HCl showed high protection against hemolysis with normal blood picture, GSH and Malondaialdehyde levels, compared to radiated untreated rats. Hence, 20 mg parenteral L-CyS-HCl can be used as a protective strategy against mobile phone hazards.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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