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Exposure to mobile phones and its biophysical, biochemical and hematological effects

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Abstract:

The growing number of cellular telephone production and the increasing number of users, increased the interest of studying the effect of electromagnetic fields (EMFs) emitted by cellular phones on living organisms. The potential health risks of radiofrequency electromagnetic fields emitted by electromagnetic devices are currently of considerable public interest. As a matter of fact, in recent years, histological and physiological studies have increased evaluating the effects of electromagnetic fields on human health. Among the various areas of scientific interest over the last years, the oxidative stress induced by electromagnetic radiation in biological systems is of utmost importance. This oxidative stress is a biochemical condition characterized by an imbalance in the presence of relatively high levels of toxic reactive species, mainly reactive oxygen species (ROS) and the antioxidant defense mechanisms. The enzyme systems are considered as the first line of defense of our body against ROS. The aim of this study is to investigate the influence of electromagnetic fields emitted by mobile phone on some biophysical, biochemical and hematological effects on Swiss albino mice using two different mobile phones of Global System for Mobile Communication (GSM). The study also includes the variation of both Electric Field Strength and Variation of the Power Density with Distance and the accompanied biological effects and the critical distance of safe use.

Keywords:

Cellular telephone, electromagnetic fields, power density, oxidative stress

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1. Introduction

The growing number of cellular telephone production and the increasing number of users, increased the interest of studying the effect of electromagnetic fields (EMFs) emitted by cellular phones on living organisms [1]. The potential health risks of radiofrequency electromagnetic fields (RF EMFs) emitted by electromagnetic devices are currently of considerable public interest. [2]

It should be noted that mobile phones get more attention from scientists. As the radiation level of a mobile phone during a call is higher than that of a base station [3-5] and the mobile phone is very close to user's head. However, this may not be known by the public. Further, it should be noted that the radiation of base stations expose people 24h (hour) a day. [3] This situation is more important for living in close neighborhood to a base station.

The current basic safety limits applicable to the wireless device are defined in terms of specific absorption rate (SAR), which is defined as the rate at which a person absorbs Electromagnetic energy per unit mass; where SAR averaged over X grams of tissue can be denoted by X-g (gram) SAR. The SAR in a biological body exposed to a radio frequency (RF) field depends on a number of factors, including; tissue geometry, dielectric properties and the orientation of the body relative to the source. In wireless devices at frequencies above 300 MHz (Megahertz), the absorption affects only parts of the body, which are close to the device. Hence, the most critical value is the local peak SAR limit. Localized SAR averaged over 10-g and 1g of tissue i.e. peak 10-g SAR and peak 1-g SAR not exceeding 2.0 W/kg(Watt/kg) and 1.6 W/kg respectively, are recommended by the Institute of Electrical and Electronics Engineers (IEEE), American National Standards Institute (ANSI) and Federal Communications Commission (FCC) as the upper safety limit. [6]

Many studies reported that radiation from mobile phones absorbed by the human body turns inside to heat producing biological effects. The biological effect leads to the continuation of the existence of many damage risks caused to human and their vital organs. This findings have confirmed the report of the Australian Radiation Protection in 2005v, where 70% of the waves emitted by mobile phones absorbed in the user's head which leads to an increase in the speed of nerve impulses, blood pressure and heart rate, i.e., exposure to electromagnetic waves leads to an imbalance in the circulatory system, increase in blood flow and disruption in blood pressure. [7,8]

Among the various areas of scientific interest over the last years, the oxidative stress induced by electromagnetic radiation in biological systems is of utmost importance. [9] This oxidative stress is a biochemical condition characterized by an imbalance in the presence of relatively high levels of toxic reactive species, mainly reactive oxygen species (ROS) and the antioxidant defense mechanisms. [10]

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At the level of the organism, there are antioxidant systems, of enzymatic or non-enzymatic nature, [11] which have different activities and are able to protect tissues by retarding or inhibiting the oxidation of oxidizable substrates. [12] The following enzyme systems are considered as the first line of defense of our body against ROS (reactive oxygen species) [13]:

- The superoxide dismutase (SOD): catalyzes the dismutation of superoxide anion O_2^- to hydrogen peroxide H_2O_2 .
- The catalase: responsible for the elimination of H_2O_2 by a transformation in H_2O and O_2 .
- The glutathione peroxidase: acts synergistically with SOD and accelerates the dismutation of H_2O_2 to H_2O and O_2 . [11]
- The glutathione reductase: regenerates the glutathione reduced (GSH) from GSSG (Glutathione disulfide) by NADPH(Nicotinamide adenine dinucleotide phosphate).

For the non-enzymatic systems and contrary to the antioxidant enzymes, most of these components are not synthesized by the body and must be supplied by the diet. In this category of antioxidants the oligoelements, the reduced glutathione, [9] the ubiquinone, [14] the cytochrome c [15] and the vitamins E & C [14,16]. The ROS can constitute an important factor in tissue injury. Therefore, if they are not trapped, these species may lead to damage of lipids, proteins and DNA (Deoxyribonucleic acid). [17] The lipid peroxidation which increases due to the reduction of antioxidant defense systems, [18] generates highly reactive lipid peroxides and induces a modification of the fluidity, the permeability and the excitability of the membranes. [19] Among the products formed during the lipid peroxidation: the Malondialdehyde (MDA), which is considered as a reliable marker of the oxidative stress in tissues. [20]

The aim of this study is to investigate the influence of electromagnetic fields from mobile phones on some biophysical, biochemical and hematological properties of Swiss albino mice using two different mobile phones of Global System for Mobile Communication (GSM).

2. Materials AND Methods

2.1. Materials:

2.1.1. Mobile Phones

Two mobile phones, namely; Archos and Nokia mobile phones were used with two different specific absorption coefficients (SAC), as follows:

1. Archos Mobile Phone (Archos 50d Helium): Certified to work on the system (GSM) with the same previous frequency range (900 - 1800 MHZ). The highest SAC value is 0.36 W/kg.
2. Nokia Mobile Phone (Nokia 101): Certified to work on Global System for Mobile Communication (GSM) with frequency range of 900 - 1800 MHZ. The highest SAC value is 1.28 W/kg .

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2.2. Animals:

A total of 50 Swiss male albino mice, 6-8 weeks of age, weighing 25-30g were allowed to acclimatize for 2 weeks before experiment. The animals were housed in polyethylene cages inside a well-ventilated room. Each cage contained 5 mice. Use of experimental animals in the study protocol was carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University. Mice were randomly divided into five groups, each of 10 mice, as follows:

- **Group I:** served as a control group, i.e., without exposure to any electromagnetic waves of nearby mobile phones.
- **Group II:** exposed, in cage, to rays of Archos mobile phone for 2 weeks .
- **Group III:** exposed, in cage, to rays of Nokia mobile phone for 2 weeks.
- **Group IV:** exposed, in cage, to rays of Archos mobile phone for 4 weeks.
- **Group V:** exposed, in cage, to rays of Nokia mobile phone for 4 weeks.

Each exposure was one hour / day.

2.3. The Animal Cage:

An iron cage (diameter 10 Cm by 10 Cm height) was designed for this work. The cage was covered all around by aluminum foil and cardboard sheet to prevent dispersion of radiations from the cage. The floor of the cage was designed to place the mobile phone instrument in such a way that the mice remained at a distance less than 4 Cm from the mobile phone device.

3. Methods

3.1. Detection and Measurement of the Mobile Phone Signal

Commercial cell sensor (Cornet ED78S EMF RF Electro Magnetic Detector Meter, 2016) was used to measure the strength of electric field in (V/m) and power density in (mW/Cm^2) of electromagnetic radiation emitting from the mobile phones.

3.2. Collection of Blood and Brain Tissues

At the end of each exposure period, (2 or 4 weeks), mice were sacrificed by cervical dislocation and blood samples were collected from the animal of the studied groups from inferior vena cava by heart puncture in a non-heparinized tubes.

Part of the blood samples were collected in tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) for the determination of the biophysical properties, e.g., Red Blood Cells (RBC) relative viscosity, Aggregation Shape Parameter (ASP), Form Factor (FF), hemolysis degree, in addition to. Complete Blood Count (CBC), as a Hematological Investigation.

Serum samples were obtained by centrifugation of the blood samples at (3,000 rpm) for 30 min using refrigerated apparatus, followed by freezing at $-20\text{ }^\circ\text{C}$ for further assaying,

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e.g., the determination of serum levels of Total Antioxidant Capacity (TAC) concentration. Immediately, the brain was removed, rinsed with physiological saline solution, numbered, dated and preserved at 4 °C for determination of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) activities.

3.2.1 Biophysical Processing:

The biophysical tests were also run on the blood including relative viscosity [21] , degree of hemolysis [22] , and adhesive properties of RBCs by evaluating the aggregation shape parameter (ASP), (equation 1) by inclined slide microscopic technique [23], and form factor (FF) [22] , using equation (2)

$$ASP = 4 \pi A / P^2 \quad (1)$$

Where A and P, are the projected area and the perimeter of the projected area of the aggregate. The average value of ASP for each group counts was taken in order to characterize the shape of the aggregation of this group.

$$FF = PPA^2 / 4 \pi (PAA) \quad (2)$$

Each image projected area of aggregates (PAA) was calculated by summing the projected areas of all aggregates in the image. In addition, the projected perimeter of aggregates (PPA) was calculated for each image by summing the perimeter of all aggregates in the image.

3.2.2. Biochemical properties

The determination of the Total Antioxidant Capacity (TAC) Concentration [24,25] ((Biodiagnostic, Egypt) was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide H₂O₂. The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ was determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5,dichloro -2- hydroxy benzensulphonate to a colored product.

$$TAC = A_{blank} - A_{sample} \times 3.33mM/L \quad (3)$$

The Biochemical Investigation of the Brain Tissue was done for Superoxide Dismutase (SOD) Activity and for Lipid peroxide (Malondialdehyde) the level of SOD was also determined by a Ready-for- use colorimetric kit (Biodiagnostic, Egypt). [24] The principle of determination of superoxide dismutase (SOD) activity relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye. The level of MDA was also determined by a Ready-for- use colorimetric kit (Biodiagnostic, Egypt). [26-27] Principle of Colorimetric Determination of Lipid peroxide (Malondialdehyde) relies on reacting of Thiobarbituric acid (TBA)

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with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm.

The calculation of MDA in sample was calculated using the following equation:

$$\text{MDA in sample} = \frac{A(\text{sample})}{A(\text{standard})} \times \frac{10}{\text{gm of tissue used}} \quad \text{nmol / g.tissue} \quad (4)$$

3.2.3. Hematological Investigation:

Complete blood count was performed using cell analyzer (SYSMEX XP-300). [22]

4. Statistical analysis

Data were expressed as the mean \pm SD. Statistical comparisons were performed using analysis of variance (ANOVA, SAS 9.0). P-value (significance level) is smaller than 0.05 indicated statistical significance.

5. Results and Discussion

The SAR of wireless devices as the case of mobile phones depends on a number of parameters, such as distance from the radiofrequency source and power density, which is usually expressed in units of watts per kilogram (W/Kg) or milliwatts per gram (mW/g) [28].

$$\text{SAR} = \frac{(\sigma |E|^2)}{2\rho} \quad (5)$$

Where: $|E|^2$: is the maximal strength of the time harmonic electric field, σ : is the conductivity ($\Omega^{-1}\text{m}^{-1}$) of the tissue, ρ : is the mass density (Kg/m^3). This means that both of the electric field intensity and the power density affect the specific absorption rate.

The specific absorption rate (SAR) is directly proportional to intensity of the electric field, so it was of importance to describe the variation in the electric field strength with distance for the two types of mobile phones used in this work, i.e., Archos and Nokia mobile phones. Figure (1) shows that the strength of the electric field decreases gradually with distance, which is in good agreement with Seker *et al.* (2000), [28]. However, the electric field intensity of Nokia mobile phone is higher than that of Archos mobile phone with all the distances. At zero distance the electric field intensity of Nokia mobile phone started with a higher value of 11.80 ± 0.98 V/m in comparison to 4.75 ± 0.87 V/m for Archos mobile phone. The decline in electric field intensity with distance is also higher in case of Nokia with respect to Archos. The values of the electric field intensity approaches each other beginning from 8 Cm and coincide completely at distances 12 and 14 Cm, respectively, reaching minimum values of 0.74 ± 0.12 and

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0.66±0.07 V/m in case of Nokia and Archos, respectively. It must be of value to know that, according to Seker *et al.* (2000), [28], that the electric field from mobile phones were two times higher during speaking than listening.

As power density is commonly used for characterizing (RF) electromagnetic field, it is defined as power per unit area, usually expressed in terms of milliwatts per square centimeter (mW/cm²). As shown in Figure (2), the variation of power density in mW/Cm² also decreases with increasing distance. As a matter of fact, the power density of Nokia mobile phone started with a maximum value of 4.23±0.67 (mW/Cm²) at zero distance in comparison to a value of 0.27±0.03 (mW/Cm²) of Archos mobile phone. These results can be explained depending on the SAR values of Nokia and Archos mobiles. As Nokia SAR value (1.28 W/Kg) is higher than Archos SAR value (0.36 W/Kg). However, the decline in power density with distance is more rapidly in Nokia mobile phone than that the case of Archos, where minimum value is reached (approximately zero value) in the case of the two mobiles starting from 8 Cm afterwards.

It can be concluded that, the effects of electromagnetic fields on the human body depend on the electric field level, the frequency and energy. Radiofrequency fields penetrate the human body that decreases with increasing the electric field; this means that the absorption of electromagnetic energy decreases with the amount of electric field, which decreases gradually with increasing distance from the RF source, as a mobile phone, for example.

The biophysical properties of blood specifically on the RBCs of the exposed mice were affected by the exposure to electromagnetic radiation emitted from Nokia mobile and Archos mobile. The RBCs relative viscosity of RBCs in the blood samples withdrawn from the mice related to the exposed groups (II, III, IV and V) was significantly higher (at $p < 0.05$ significant level) compared to that of the control group, represented graphically in Figure (3) . On the same time the relative viscosity of the RBCs of mice exposed to Nokia mobile phone is generally significantly higher at the same confidence level for either the two exposure periods two weeks or one month. It is also observed that the RBCs relative viscosity in the exposure groups for the same type of mobile (Archos or Nokia) in long-term exposure (one month) is higher than that in short-term exposure groups (two weeks), i.e., the relative viscosity increased from 1.603±0.027 to 2.706±0.030 (Archos) and from 2.609±0.070 to 3.460±0.031 (Nokia) as a result of exposure to two weeks and one month respectively. This can be explained on the basis of time of exposure. These results are in agreement with the results reported by Lowe *et al.* (1988), [29] and Lowe. (1988). [30]

The RBCs aggregation shape parameter (ASP) and Form factor (FF) of the RBCs of blood samples withdrawn from all the exposed groups were significantly higher (at $p < 0.05$ significant level) than that in the control group, as illustrated graphically in Figs (4-

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5). These two parameters followed the same behavior of the relative viscosity of the RBCs either with the two types of the mobile phones used or with respect to the exposure periods. In our opinion, the increase in either the aggregation shape parameter or the form factor is due mainly to the decrease in the surface charge of the RBCs membrane causing more aggregation due to the decreasing repulsive forces responsible for making neighboring RBCs quite apart. [31]

The other biophysical parameter that suffered changes as a result of exposure to the EMR from Nokia and Archos mobile phones is the hemolysis degree of the RBCs. The same behavior was also observed where the hemolysis degree was significantly higher (at $p < 0.05$ significant level) than the control level. This parameter was also higher with the Nokia than Archos mobile phone, with increasing level with increasing exposure time in both types of mobile phones used as shown in Figure (6).

The afore-said discussion reveals that, in general, the exposure to mobile phones causes an increase in biophysical parameters of the RBCs of the blood of the exposed mice, namely; relative viscosity, aggregation shape parameter, form factor, and the hemolysis degree, with respect to the control. The increase was significant at ($p < 0.05$ significant level) with the increasing level, in each parameter, is proportional to the exposure period, with also higher levels in case of Nokia than that of Archos mobile phone. .

It was also of interest to investigate the exposure to mobile phones on some biochemical parameters of the brain tissue of mice. The brain can absorb the electromagnetic waves emitted from mobile phones more than other internal organs because the mobile phones are typically used near the head, consequently to the brain.

As shown in figure (7-9), Exposure to RF radiation from Archos and Nokia mobile phones resulted in a significant increase at ($p < 0.05$) in the level of MDA in the brain tissue with respect to the control level, with higher level in case of the Nokia than that for Archos mobile phone. The level of increase also increases with increasing exposure period. These results are judged by significant reduced levels at ($p < 0.05$) of both the total antioxidant capacity activity and the superoxide dismutase activity with more decreasing levels both with the exposure period and Nokia and Archos mobile phones, respectively. These results indicate that the mice groups exposed to RF radiation from either the two types of mobile phones used in this work were under oxidative stress.

The oxidative stress is a biochemical condition characterized by an imbalance in the presence of relatively high levels of toxic reactive species, mainly reactive oxygen species (ROS) and the antioxidant defense mechanisms. [32] At the level of the organism, there are antioxidant systems, of enzymatic or non-enzymatic nature, which have different activities and which are able to protect tissues by retarding or inhibiting the oxidation of oxidizable substrates. [33] The mechanisms by which the electromagnetic fields cause the cellular responses in different tissues such as brain is

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still unknown, but several hypotheses have been proposed, including the extension of the life of the reactive oxygen species that are causing the macromolecular damages. [34]

Therefore, from the aforesaid discussion, the results of our study indicated that the electromagnetic waves emitted from mobile phones are associated with increased production of the free radicals and high levels of the lipid peroxidation in the brain. The significant increase of the MDA ($p < 0.05$) and the significantly decreased activities of the total antioxidant activity side by side with decreased level of superoxide dismutase capacity in the brain of mice exposed for 1 hour/day for either two or four weeks compared to controls could be due to high rate of oxidative metabolism activity and a higher concentration of polyunsaturated fatty acids of the membrane of the neuronal cells easily oxidizable. [12] The change in SOD and TAC activity may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure.

Our results are in agreement with other studies, which suggest that the exposure of rats to electromagnetic waves at a frequency of 900 MHz in the brain, causes depression of their antioxidant systems due to an increase of the lipid peroxidation and formation of the free radicals with an increase of the rate of MDA and decrease of the catalase activity. [35,36]

As shown in Table (1), The present work studied also the effect of exposure to RF radiation from Archos and Nokia mobile phones on 12 blood factors of the exposed mice. The results indicated that in the RBCs indices, no steady variation in the RBCs counts occurred, with significant reduction in the counts occurred only in the group of mice exposed to Nokia mobile phone for one month exposure. Hemoglobin concentration showed more reliable results where it was reduced significantly with both the mobile exposures to nearly the same levels. The lower levels of hemoglobin indicate that the animals, as a whole, suffered anemia which leads to other diseases.

The hematocrit reduced significantly only in the group of mice exposed to Nokia radiation for one month compared to the control group. The mean corpuscular volume of the RBCs suffered significant reduction only in the groups of mice exposed to Nokia mobile phone for either two or four weeks. On the other hand, the mean corpuscular hemoglobin of the RBCs was reduced in all the exposed group of mice with Archos or Nokia mobile phones, with the much lower level with the mice group exposed to Archos for one month. Also the mean corpuscular hemoglobin concentration was reduced significantly in all the exposed groups with much lower levels in the groups of mice exposed to Archos mobile phone for two or four weeks. The reduced levels of both MCH and MCHC are due mainly to the reduced level of the hemoglobin which is reflected on reduced level of both mean corpuscular hemoglobin and mean corpuscular

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hemoglobin concentration of the exposed animals, leading to the existence of anemia. In fact the present results are in agreement with the data published by Tohidi *et al.* (2016), [37] who reported significant changes in both MCH and MCHC in mice exposed to cellphone radiation.

6. Conclusions

The potential health risks of radiofrequency electromagnetic fields emitted by cellular phones on living organisms do exist. The measurements on the signals emitted from the Archos and Nokia mobile phones revealed that both the electric and the power density were higher in case of Nokia mobile and both decreases with increasing distance. All the biophysical parameters of the RBCs, e.g, the relative viscosity, Aggregation Shape Parameter (ASP), Form Factor (FF), and the Hemolysis Degree, were increased with increasing exposure time, with more effect on using Nokia mobile phone. The Total Antioxidant Capacity (TAC) Concentration, and the Superoxide Dismutase (SOD) Activity were decreased, and the Malondialdehyde (MDA) was increased with increasing exposure period indicating that the exposed animals were under oxidative stress, with the more effects with the Nokia mobile phone. The hematological parameters suffered some changes in the Hb, MCV, and MCHC, indicating that some hematological diseases accompany the exposure to mobile phone radiation. The results of this work lead us to recommend the following:

To use the mobile phone safely, it is preferable to make the mobile phone at about 6 Cm apart from the head, which reduces the power density, consequently, the SAR to minimum value. It is of importance to perform periodic CBC at least every six months to give a clear picture of the blood counts, especially Hb and WBCs. The biochemical and biophysical parameters studied in this work can also give indication of the possible hazards in case of using the mobile phone for long periods per day or very close to the head. It is highly recommended to choose a mobile phone with low specific absorption coefficient (SAC). Avoid using your phone in areas where the signal strength is poor. When your phone is searching for the network, its antennas produce radiations at a maximum limit.

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Table (1). RBCs counts and indices

Parameters Groups	RBC X 10 ⁶ (unts)	Hemoglobin Hb (g/dl)	Haematocrit HT (%)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Group I	7.272 ±1.419	12.520 ±2.032	36.400 ±6.229	50.240 ±1.828	17.300 ±0.731	34.440 ±0.856
Group II	7.674 ±0.775	11.920 ±2.121	36.200 ±5.718	46.820 ±3.785	15.460 ±1.454 ^a	32.940 ±1.367
Group III	6.646 ±0.859	10.740 ±1.260	32.000 ±4.359	48.100 ±1.134	16.200 ±0.987 ^c	33.680 ±1.514
Group IV	7.054 ±0.369	10.820 ±0.634	36.800 ±2.683	52.320 ±3.662 ^b	15.340 ±0.709 ^{a,c}	29.420 ±2.080 a,b
Group V	7.827 ± 1.285	10.933 ± 2.219	36.333 ± 6.807	63.167 ± 28.959	13.900 ± 0.557 ^a	29.867 ±0 .808 a,c

pa <0.05 all Groups compared to Group I

pb <0.05 (Group III and Group IV) compared with Group II

pc <0.05 n (Group III and Group IV) comp

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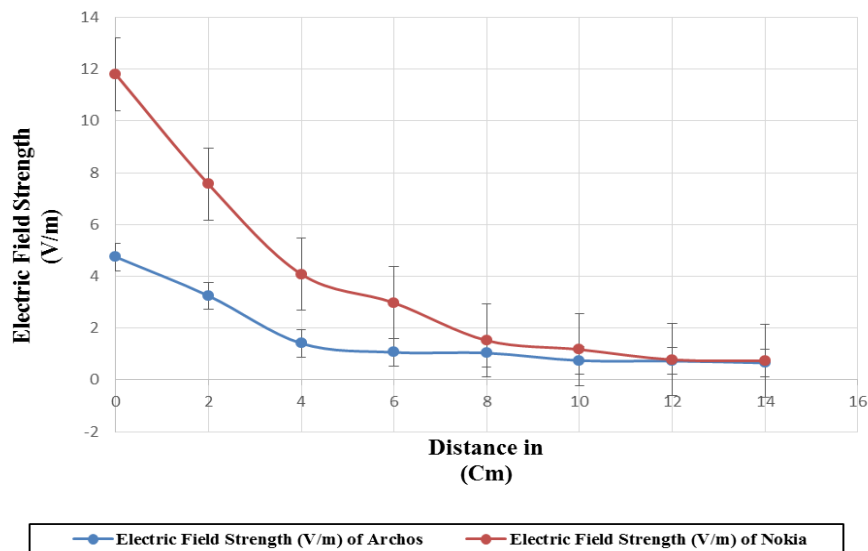


Figure (1). Variation of the Electric Field Strength (V/m) with Distance away from Archos and Nokia mobile phones

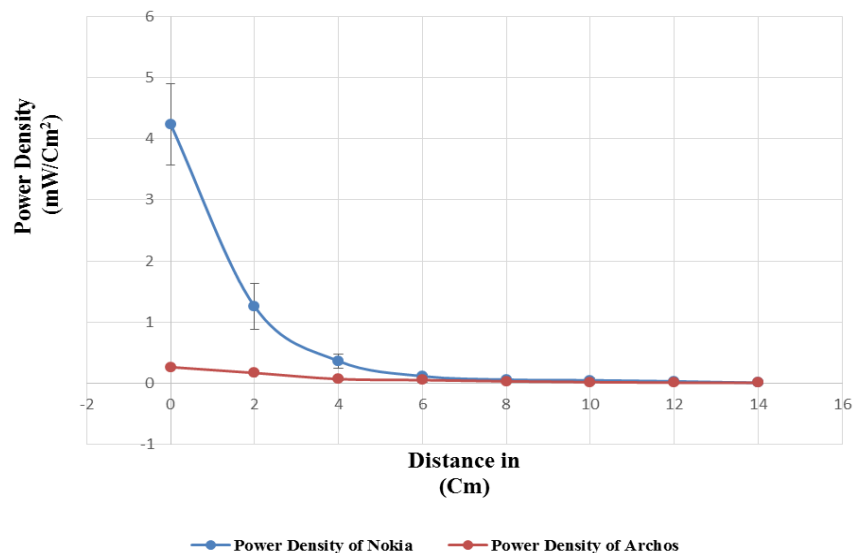


Figure (2). Variation of the Power Density with Distance away from Archos and Nokia mobile phones

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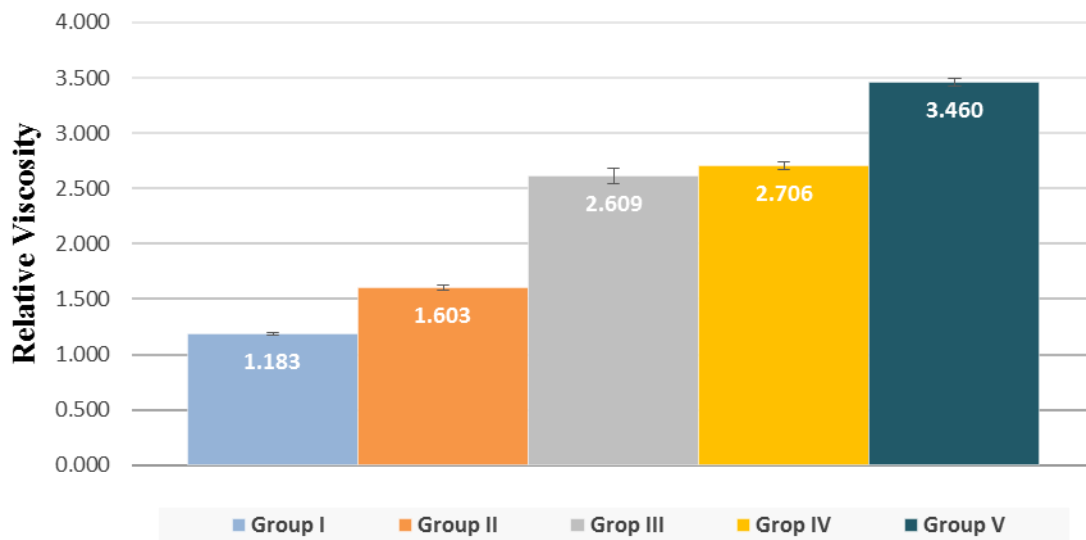


Figure (3). Relative viscosity after exposure to Archos and Nokia mobile phones

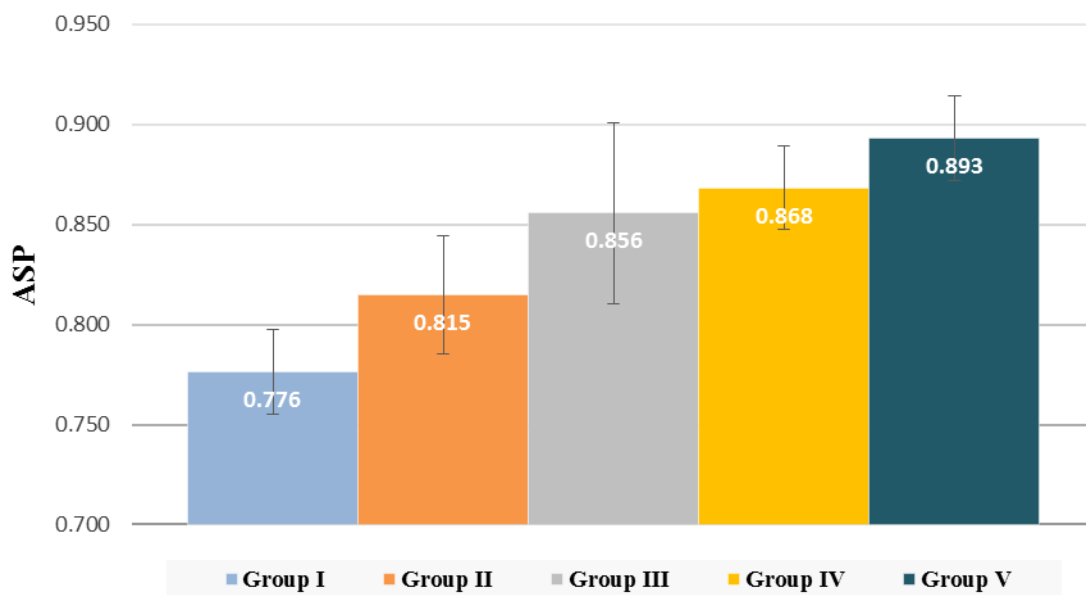


Figure (4). Aggregation Shape Parameter after exposure to Archos and Nokia mobile phones.

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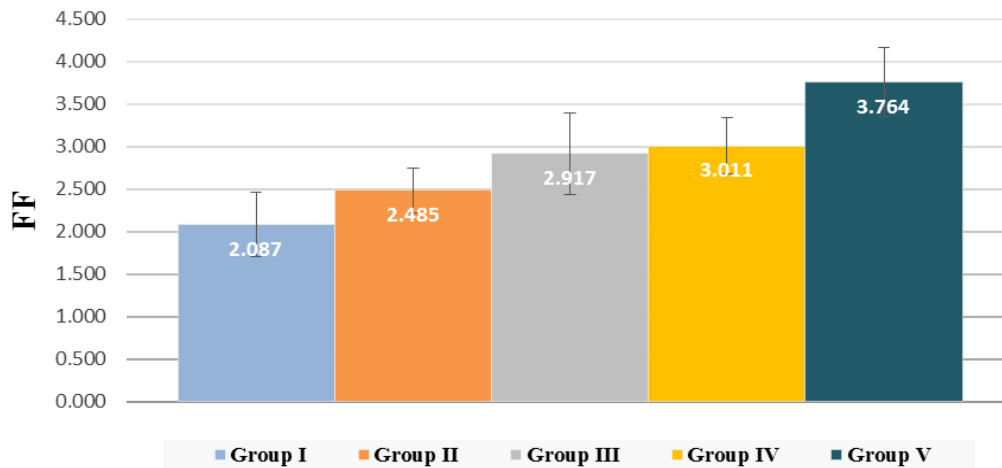


Figure (5). RBCs Form Factor after exposure to Archos and Nokia mobile phones.

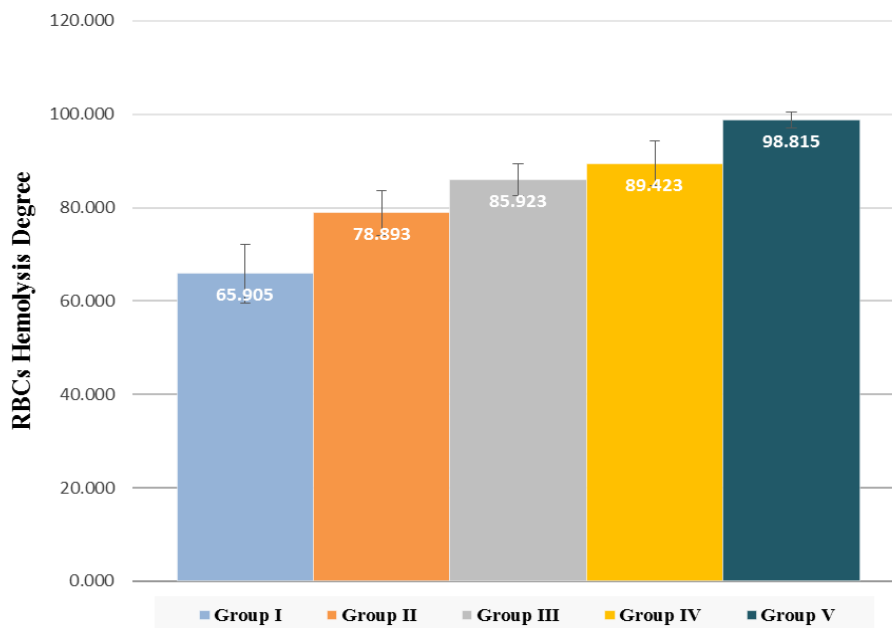


Figure (6). RBCs Hemolysis Degree after exposure to Archos and Nokia mobile phones.

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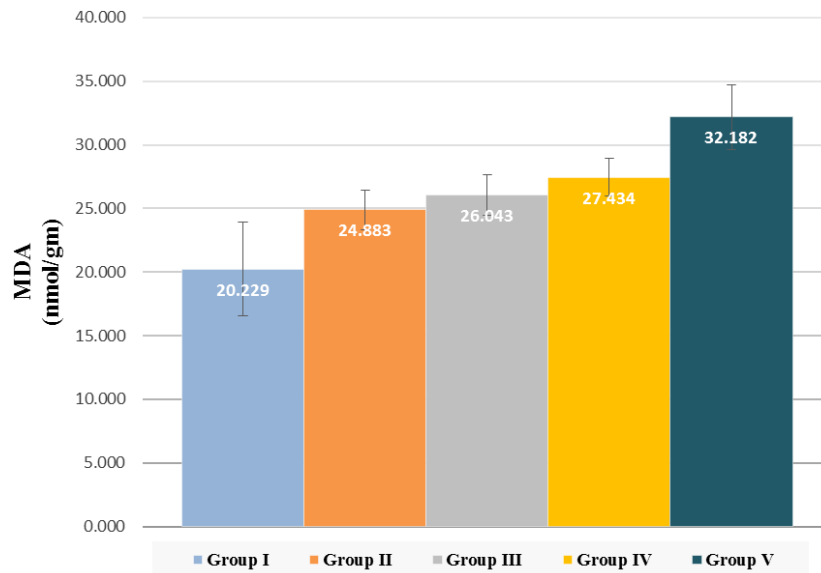


Figure (7). Malondialdehyde (MDA) in the brain tissue after exposure to Archos and Nokia mobile phones

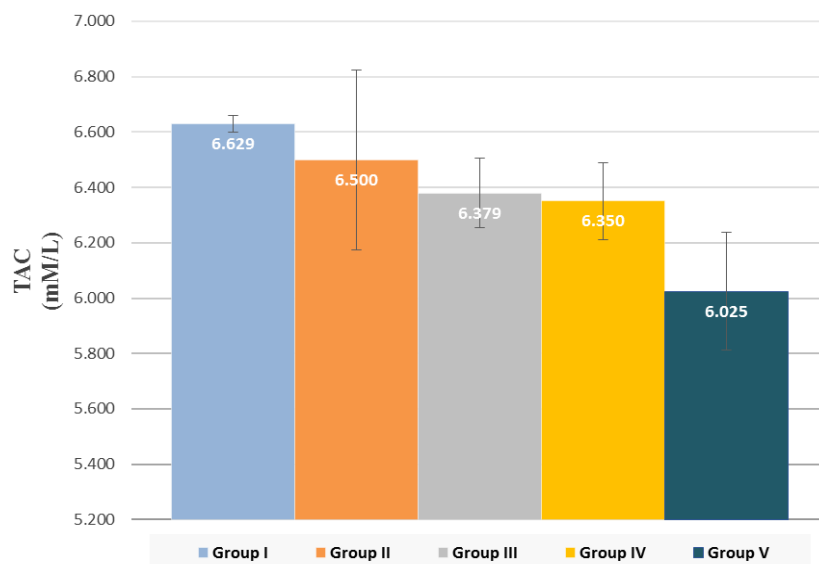


Figure (8). Total Antioxidant Capacity in the brain tissue after exposure to Archos and Nokia mobile phones

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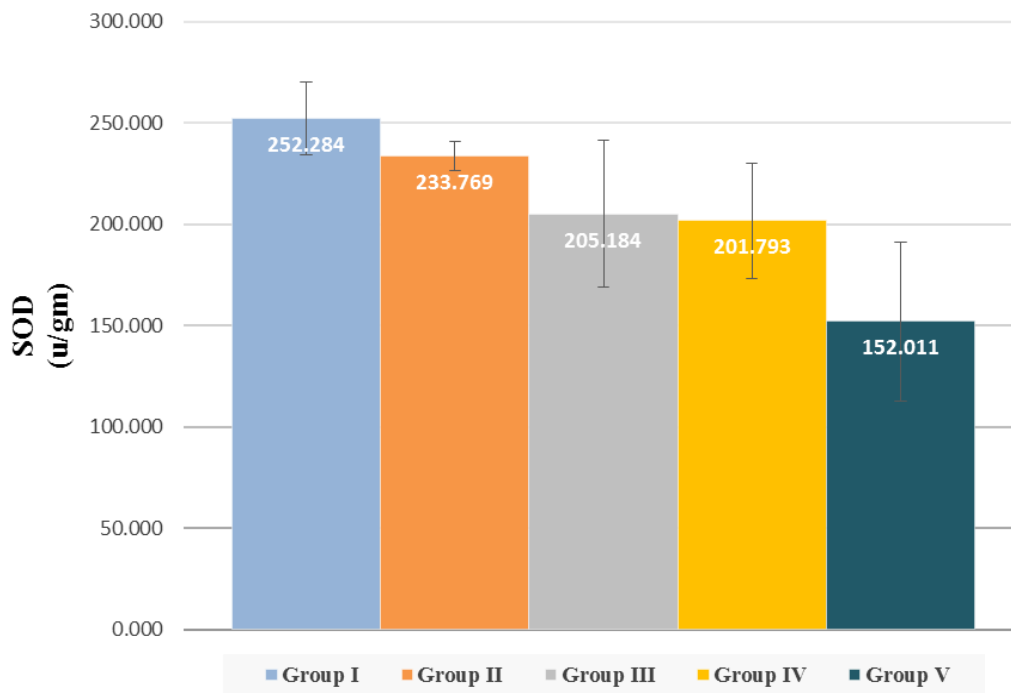


Figure (9). Superoxide Dismutase Activity in the brain tissue after exposure to Archos and Nokia mobile phone.