

EFFECTS OF 900 MHZ RADIOFREQUENCY RADIATION ON THE RATS' LIVER

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Graphical abstract



Abstract

The goal of the study is to evaluate the liver function tests and histological alterations of the rats liver cells after exposure to mobile phone radiation. To perform the liver function test Aspartate transaminase (AST) U.V kinetic test kit produced by CYPRESS DIAGNOSTICS and spectrophotometer was used. Wistar rats (male, 5 week old, approximate body weight 30-40 g) (N = 12) were exposed to 900 MHz mobile phone radiation of Global System Mobile signal modulation (GSM), whole body average specific absorption rate SAR of 1.090W/kg. Twelve (12) male rats were used for the study divided into 3 groups of 4 rats each. Group B and Group C were exposed to 4 hours/day and 8 hours/day mobile phone radiation during calling mode for 2 months respectively while Group A served as control. There was significant reduction in total protein, globulin, while there was significantly increased in Glutamate Oxalacetate Transaminase (GOT), Alkaline Phosphatase (ALP), Albumin, total bilirubin in the rats (Group B and C) exposed to mobile phone radiation when compared to the control group but there was no histological alteration in the arrangement of cells in both control and exposed group. The results of this study suggest that, under the experimental conditions applied, repeated 900 MHz irradiation could modify liver functions.

Keywords: 900 MHz mobile phone radiation, liver, histology, liver function tests

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1.0 INTRODUCTION

Electromagnetic radiation is a term used to describe a stream of energy-bearing particles that travels outward from an electromagnetic source. The energy in these streams can vary extensively in power, and is measured by the electromagnetic spectrum. Electromagnetic radiation can be beneficial, harmless or extremely dangerous to humans, depending on the source, level of radiation, and duration of exposure [1]. Mobile phone radiations belong to microwave spectrum of electromagnetic radiation and its effects on human health is the subject of recent interest and study, as a result of the enormous increase in mobile phone usage throughout the world. According to CCA (China Consumers Association) half of cell phone's radiation is absorbed by the human body and another

quarter by the brain. Our body is exposed to cell phone radiation while making or receiving calls.

The radio waves given out by the mobile handsets are absorbed by the human body. This type of radiation has a mild heating effect on the living tissues in the body which can be linked with the rise in temperature during the mobile phone usage. However, there are indications that cell phone radiation can cause a few changes in the functioning of cells. The functions that get affected by radiation include activation of proteins, communication between the body cells, genetic functions etc [2]. Though the exact reasons behind these changes have not yet been ascertained. Although the brain is where the damage starts but it is not the only part of the body that gets adversely affected by cell phone radiations. Also, when a cell phone is worn near the

waist during its use (as may occur when a corded or a cordless headset is used), much of the outgoing radiation is being absorbed by adjacent soft tissues such as the liver, kidneys etc. which may pose health risks.

Due to numerous usage of mobile phone a lot of researches is going on to investigate the effects on the populace. Histological changes observed was by [3] in the different visceral organs including; heart, lung, liver and kidney of rats after exposure to radio frequency radiation for 4 weeks (1h/day). Another experiment investigated the possible electromagnetic fields (EMFs) effects on the kidney and testis [4]. Both groups were exposed to same frequency of radio frequency radiation for 8 h per day for 3 days and other group for 8 Hours per day for 12 days. At the end of the exposed period, significant histopathological alterations were observed in the treated animals after 3 days. The histopathological alterations in the kidney and testis were more obvious after 12 days of treatment. Report by [5] showed significantly higher serum levels of triglycerides, albumin, and total protein compared with sham-irradiated controls after exposing rats to 970-MHz electromagnetic radiation (SAR = 2.5 mW/g, 22 h daily for 70 consecutive days). According to research carried out at Institute for Medical Research and Occupational Health, Zagreb and others [6-8] observed DNA breaks in renal and liver cells, but do not affect the cell genome at the higher extent compared to the basal damage after in vivo exposure to 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m², whole body average specific absorption rate SAR of 0.6 W/kg. Oxidative injury was reported by [9-11] in liver, lung, testis and heart tissues mediated by lipid peroxidation, increased level of NO_x and suppression of antioxidant defense mechanism after exposure to 900 MHz pulsed modulated RF radiation at (SAR) level of 1.20 W/kg 20 min/day for three weeks.

The aim of this study is to assess the liver profiles and histological changes of rats exposed to 900 MHz mobile phone radiation.

2.0 MATERIALS AND METHOD

2.1 Animal Preparation and Exposure

Twelve weaning male Albino wistar rats, weighing 30–40 g, were used for the experiment. The rats were purchased from the animal house of University of Agriculture, Abeokuta. The animals were left for five days for acclimatization in the laboratory with food and water provided ad libitum throughout the experimental period. For the care and use of laboratory animals, this study used the guidelines of the biological sciences animal house of the Covenant University. The rats were divided into three groups (A, B and C) of four rats each. The two cell phones used in the study were Nokia 1202 (China), which have a personal communications service code division multiple access (PCS CDMA) frequency band of 2G

network (900 MHz / 1800MHz digital), weight 78g and SAR 1.090W/kg (head). Group C received cell phone radiation exposure for 8 hours per day using calling mode, group B equally received cell phone radiation exposure for 4 hours per day at calling mode for eight weeks and group A acted as a control group under the same environmental conditions (Figure 1).



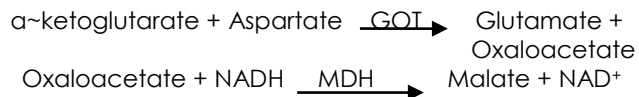
Figure 1 Exposure of Wistar rats to mobile phone radiation

2.2 Collection of Tissue

The liver tissue of rat was removed after sacrifice to determine the levels of total protein, albumin, total bilirubin, Aspartate Aminotransferase (AST), Globulin, Glutamic-Pyruvate Transaminase (GPT), Glutamate Oxalacetate Transaminase (GOT), Alkaline phosphatase (ALP). Liver tissues were collected and placed in empty glass tubes also blood serum samples were collected and all specimens were kept frozen at – 800 C.

2.3 Determination of Aspartate Transaminase (AST) [12]

Aspartate transaminase (AST) test was carried out using an Aspartate transaminase (AST) U.V kinetic test kit produced by CYPRESS DIAGNOSTICS Belgium. The kinetic determination of AST activity according to the following reaction;



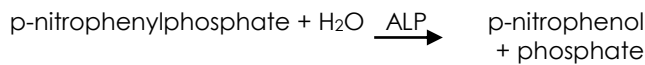
The rate of Nicotinamide Adenine Dinucleotide (NADH) consumption is determined photo metrically and is directly proportional to the AST activity in the sample. The test was carried out at a wavelength of 340nm at temperature 250C. The spectrophotometer used for the measurement was adjusted to zero with distilled water. 1ml of the working sample and 0.1ml of test sample was pipette into a corvette, mixed and allowed to stand for 1 minute. The initial absorbance was read and absorbance was read every minute for 3 minutes with stopwatch. The change in absorbance and the average absorbance difference per minute were calculated using equation 1

$$AST\left(\frac{U}{L}\right) = \frac{\Delta Abs.}{\text{min} \times 1750} \quad (1)$$

where ΔAbs is change in the absorbance

2.4 Determination of Alkaline Phosphatase (ALP/GPT)

Alkaline phosphatase catalyzes the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:



1.2 ml of the working sample and 20 μ l of test sample was pipette into a corvette, mixed and allowed to stand for 1 minute. The initial absorbance was read and absorbances were read every minute for 3 minutes with stopwatch. The differences between the absorbance's and the average absorbance differences per minute were calculated using equation 2 at wavelength of 405 nm and temperature 250C.

$$ALP\left(\frac{U}{L}\right) = \frac{\Delta Abs.}{\text{min} \times 3300} \quad (2)$$

2.5 Determination of Bilirubin

Bilirubin in the presence of sulphanic acid diazonium salt forms a red coloured azbilirubin in alkaline solutions. Of the two fractions present in serum, bilirubin-glucuronide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (bilirubin indirect). The intensity of the colour formed is proportional to the bilirubin concentration in the sample. Using a wavelength of 555nm and temperature of 25oC and corvette of 1 cm light path, it was mixed; allow to wait for 5 minutes exactly at room temperature and measure absorbance the Bilirubin was obtained using equation 3.

$$\text{Bilirubin}(mg / dl) = (\text{Abs. Sample} - \text{Abs. Sample Blank}) \times \text{factor} \quad (3)$$

2.6 Determination of Albumin

The measurement of serum albumin is based on its quantitative binding to the indicator 3',3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the

sample. It was properly mixed and incubated for 5 minutes at 25oC. The absorbance of the sample (A_{sample}) and of the standard (A_{standard}) against the reagent blank was measured and Albumin concentration was calculated using equation 4.

Albumin Concentration (g/l or g/dl)

$$= \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Concentration of standard} \quad (4)$$

A_{sample} and A_{standard} is the absorbance of sample and standard respectively.

2.7 Determination of Total Protein

Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex using wavelength 546 nm; Temperature 25oc. This test was carried out using total protein test kit produced by Randox laboratories (UK). Method according to [13, 14] was followed for the measurement using equation 5.

$$\text{Total Protein} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 6.0g / d \quad (5)$$

2.8 Histology of Liver

The method described by [15] was followed. After blood collection, the liver was carefully dissected from the abdominal region. It was fixed in normal saline for 72 hours and sliced into a thickness of 2.1 mm. The tissues were dehydrated with alcohol of graded concentrations. It was further treated with paraffin wax and cast into blocks; sections of the tissues were then cut on a microtome to 5 μ m. These were later attached to a slide and allowed to dry. The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope; photomicrographs of the sample were recorded.

3.0 RESULTS AND DISCUSSION

3.1 Albumin Level

Figure 2 displays the albumin level showing a significant reduction in the Group B those exposed for 4 hrs, while there is significant increase in the Albumin level of Group C, which had radiation for 8 hrs when compared to the control, suggesting that cell phone radiation is capable of modifying the albumin level of liver. This is very important protein that helps keeps fluid level in the body stable and carries many substances in the body. Reduction in albumin level may be as a result of lack of protein (deficiency) or malnutrition. This can lead to chronic liver disease.

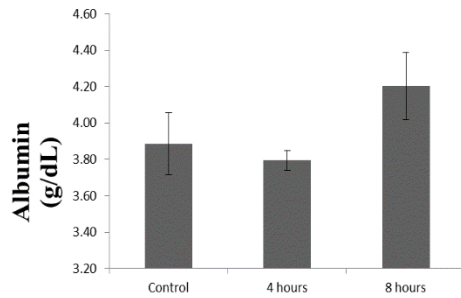


Figure 2 Variation in the Albumin level

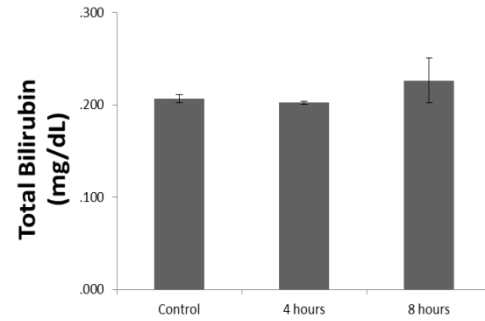


Figure 4 Variation in the total Bilirubin level

3.2 Total Protein Level

Figure 3 presents the total protein level in the control and exposed animals. There was a significant decrease in the total protein level in Groups B and C that were exposed to radiation. This indicates a reduction in the production of protein by the liver in the group exposed to mobile phone radiation. This shows how well the liver is good in production of proteins needed to fight infections and perform other function. Lower than normal level may indicate liver damage or disease [16].

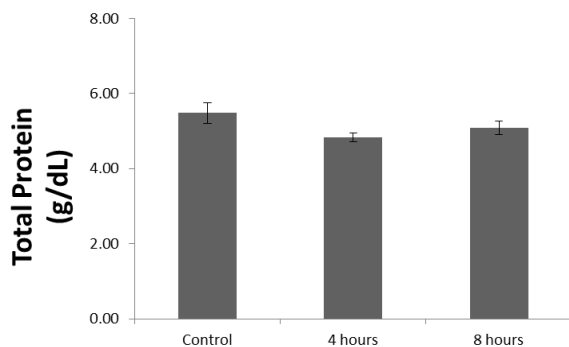


Figure 3 Variation in the total protein level

3.4 Alkaline Phosphatase (ALP) Level

Figure 5 displays the ALP level in the liver of the control and exposed animals. There is no significant increase in Alkaline Phosphate in the Group B which shows no negative effect on the liver, while in the Group C there is an increase in the ALP enzyme. This suggests that it depends on the exposure period. It is an enzyme found mainly in the bile ducts of the liver, increase in ALP and another liver enzyme Gamma GT (GTT) can cause obstructive or cholestasis liver disease, where bile is not properly transported from the liver because of the obstructive (blockage) of the bile duct it can lead to liver disease.

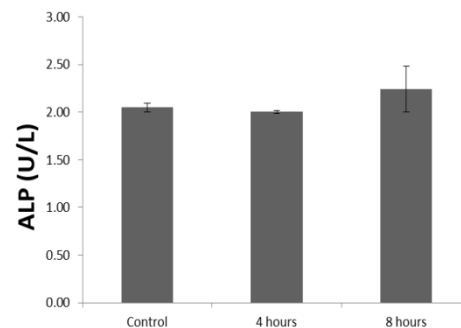


Figure 5 Variation in the ALP level

3.3 Total Bilirubin Level

Figure 4 displays the total bilirubin level in the control and exposed animals. There was a slight change in the total bilirubin level in the Group A (control) and Group B (4 hours), meanwhile in Group C, there was a significant increase in the total bilirubin level compared to the control which suggests that the effect depends on time of exposure, the longer the exposure the more the effect incurred. This is formed from hemoglobin and the main pigment in bile (a yellow/green substance made by the liver). An increase of bilirubin causes jaundice, a yellowing of the eyes and skin in liver disease.

3.5 Globulin Level

The result of globulin level is shown in Figure 6 with the Group A (control) shows that there was a significant decrease in the Globulin level in both Groups exposed to mobile phone radiation. It was inferred from this test that the longer the period of exposure to radiation the lower the globulin level which may leads to liver disease.

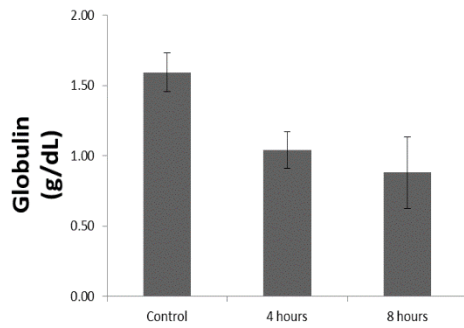


Figure 6 Variation in the Globulin level

3.6 GOT Level

Figure 7 presents the GOT level in the control and exposed animals. There was a significant increase in the GOT level in both groups exposed to mobile phone radiation compared to the control group. This shows that the longer the period of exposure to mobile phone the higher the GOT level of the animal.

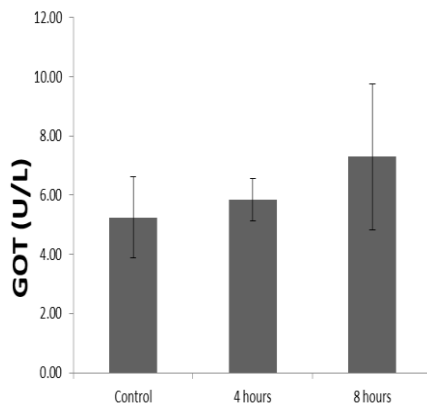


Figure 7 Variation in the GOT level

3.7 Histology Test

Histology is the study of microscopic structures and cells and tissues of plants and animals. It is often carried out by examining a thin slice (called a section) of tissue under a light microscope or an electron microscope, in order to distinguish different biological structures more easily and accurately. oedema, cloudiness and glomeruli congestion was not observed in the liver of the exposed animals. Figure 8 is the results obtained from the histology tests which showed that there were no remarkable significant changes in the arrangement of liver cells in the control and animals exposed to mobile phone radiation under our experimental condition.

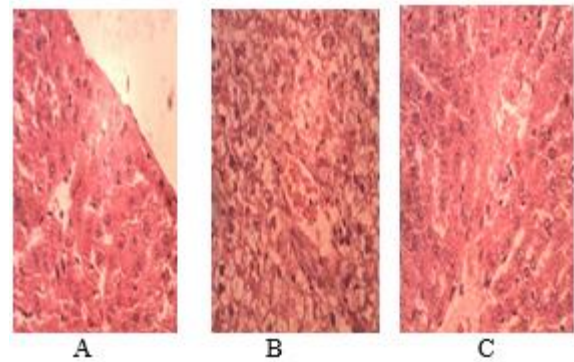


Figure 8 Photomicrographs (100X) of liver of group A, B and C

The liver function test generally refers to a group of blood tests that measure and check the levels of certain enzymes and proteins in the body, higher or lower than normal levels, can indicate problems. Different diseases of the liver will cause different types of damage and will affect liver function tests accordingly. It is possible to suggest which disease may be present from a liver function test but these tests are not the conclusive way of diagnosing liver disease. They are helpful; they are useful for monitoring someone with a liver disease. Usually the liver tests give an indication of how much the liver is inflamed and possibly either damaged or changed in its normal functions. In this study mobile phone radiation produces significant effects on the exposed animals when compared to control animals as observed from the liver function tests carried out, this is probably due to heating effects generated by the mobile phone during the exposure. The heating effect is unavoidable since all phones produce appreciable heat depending on the materials used in making them and how long the phone is in use. In this particular study the exposure was done in the same way humans receive phone calls.

The result obtained in this study is in consonance with that obtained by [6] where DNA breaks in renal and liver cells was observed after exposure in vivo to 915 MHz with Global System Mobile signal modulation (GSM) and that obtained by [9] where oxidative injury in liver, lung, testis and heart tissues mediated by lipid peroxidation, increased level of NOx and suppression of antioxidant defense mechanism were reported after exposure to 900 MHz pulsed modulated RF radiation at (SAR) level of 1.20 W/kg 20 min/day for three weeks.

4.0 CONCLUSION

It can be concluded that mobile phone radiation could modify the liver function as it produced significant reduction in total protein, globulin, GPT ratio while significantly increased GOT, ALP, Albumin, total bilirubin in the rats (Group B and C) exposed to mobile phone radiation when compared to the control group

but there was no alteration in the arrangement of cells in both control and exposed group. Wisdom calls for prudent avoidance of lengthen period of time used on mobile phones since the longer the period spent the pronounced the effects.

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