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*Research Article*

# Exogenous glutathione prevents mobile phone radiations-induced neurobehavioural deficits in mice via central antioxidant pathway

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**Keywords:**

*Mobile phone radiation, glutathione, anxiety, depression, cognition, pain perception.*

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**ABSTRACT**

**Background:** The development and rapid growth of mobile communication systems has brought about great socio-economic benefit to humans. Previous studies demonstrated potential health hazards induced by radiofrequency electromagnetic radiation (RF-EMR) emitted by such devices. We therefore hypothesize that exogenous glutathione, as an antioxidant, may potentiate these effects. This study assessed the modulatory effect of exogenous glutathione administration against chronic exposure to mobile phone radiation induced neuro-behavioural changes and oxidative stress in mice.

**Method:** Thirty-five (35) adult male mice were randomly divided into seven (7) groups of 5 mice each. They were exposed to mobile phone radiation (it2160, 2W/kg SAR, 850-1900 MHz) for four hours daily for six (6) weeks (except the control). Groups V, VI and VII were administered with glutathione (100mg/kg, orally) daily before exposure to mobile phone radiation. Neuro-behaviours (anxiety, depression, motor coordination, cognition and pain perception) were assessed using mice models.

**Results:** Chronic exposure to mobile phone radiation in this study causes increase in anxiety, depression and motor coordination in the mice. There was also a decrease in central and chemical pain perception, as well as induction of oxidative stress in the brain. However, exogenous glutathione administration modulates those neuro-behavioural deficits, decreases the brain MDA and increases brain GSH concentrations.

**Conclusion:** Glutathione administration improved neuro-behavioural deficits and brain oxidative status in mice after chronic exposure to mobile phone radiation.

## INTRODUCTION

Despite the tremendous increase in the use of mobile phones globally, knowledge of the hazardous effect of the radiation produced by such devise to human health is still scanty among its users. Most telecommunication networks use the Global System for Mobile communication (GSM). Mobile phones, or cell phones operate by the use of wireless technology involving waves of electromagnetic fields (EMF) and electromagnetic radiation (EMR) (Aly and Crum, 2016) for signal transmission. They radiate at frequency of 900-1800 MHz and belong to a wide range of microwave radiations. Even weak, low frequency magnetic fields have been described to produce complex biological effects.

Effect of mobile phones on human health can be thermal (dielectric heat), non-thermal (electric current) and genotoxic (damage to chromosome). The circulating currents induced by magnetic fields within the human body can stimulate nerves and muscles, which can lead to adverse health effects. Although international guidelines limits the power levels of wireless devices, concerns about possible detrimental effects of electromagnetic fields emitted by mobile phones on health remain unanswered. As mobile phones are mostly used close to the vicinity of the user's head, concerns about the development of cancerous brain tumours are significant (Mohril et al., 2016).

Number of researches have produce conflicting results on effects of radaition exposure to the central nervous system. The risk for developing brain cancer is 50% higher in people who talk on the phone for several hours in a day (Shivaji et al., 2020). Radiations from mobile phones has been rated grade 2B level by the International Agency for Research on Cancer (IARC), which means mobile phone is a possible human carcinogen (Shahin-Jafari et al., 2016). Because mobile phones use pulsed technology for wireless communication, there are concerns that it may pose a health risk even when exposed below the International Commission for Non-Ionizing Radiation Protection (ICNIRP) limits (Song et al., 2019).

Biological effects of exposure to mobile phone radiation include negative effects on thought, behaviour and attitude (Shivaji et al., 2020), increased DNA fragmentation and disturbed DNA repair system, disturbed nerve transmission and cell signalling, as well as increased permeability of blood brain barrier (BBB) (Schirmacher et al., 2000; Shahun-Jafari et al., 2016). Although electromagnetic radiation from cell phones possess less energy and cannot directly cause DNA damage, it can trigger strong cellular oxidative stress and release of free radicals that in a long run, can lead to DNA damage (Kesari et al., 2011; Lu et al., 2012). Despite the current global increase in awareness of possible effect of mobile phone radiation to human health, there is significant increase in mobile phone subscribers worldwide (Iqbal-Faruque et al., 2014). The specific absorption rate (SAR), measured in watt per kilogram (W/kg), is used to measure electromagnetic absorption by humans, and it shows the amount of radiation absorbed by the body per gram of body tissue (Faruque et al., 2011; Kusuma et al., 2011; Hossain et al., 2014). Safe limit is set at 1.6 – 2 W/kg. Factors that determine SAR include type of phone, network provider, position of antenna and position of the mobile phone.

To date, there is a significant number of published researches with credible scientific evidence pointing to the possibility that radio frequency electromagnetic radiation can induce harmful biological effects. Electromagnetic radiation of mobile phones has been found to have a negative effect on general health. The analysis of the aftermath of radiation from mobile phone users displayed the brain as the most susceptible to this radiation, particularly in children (Grigoriyev and Biriukov, 2013), and these effects are very likely induced via the oxidative stress pathway.

The brain's high amount of polyunsaturated fatty acids and oxygen demand makes it highly vulnerable to oxidative damage (Langbein et al., 2018). To combat oxidative stress in different cells, the glutathione (GSH) system has a major part to play (Yabuki and Fukunaga, 2013). The moderate down modulation of neuronal GSH leads to dendrite disruption in a concrete set of hippocampal neurons that cause behavioral symptoms compatible with cognitive impairment and loss of motor coordination in mice.

The hippocampal neurons require a large pool of GSH to sustain dendrite integrity and cognitive function (Fernandez et al., 2018). The detoxification of reactive oxygen species is an essential task within the brain and the involvement of the antioxidant glutathione in such processes is very important.

Glutathione is involved in the disposal of peroxides by brain cells and in the protection against reactive oxygen species. The antioxidant GSH is essential for the cellular detoxification of reactive oxygen species in brain cells. A compromised GSH system in the brain has been connected with the oxidative stress occurring in neurological diseases. Data demonstrate that besides intracellular

functions, GSH has also important extracellular functions in brain (Dringen and Hirrlinger, 2003).

This study was therefore designed to investigate the effects of exposure to mobile phone radiation in different modes on neuro-behavioural outcomes of laboratory mice and possible modulation of the mobile phone radiation effects by exogenous glutathione administration.

## MATERIALS AND METHODS

*Animals and experimental design*

A total of thirty-five (35) apparently healthy adult male albino mice (20-30g weight) were obtained from a private Animal House in Zaria, Northern Nigeria. They were housed in polypropylene cages in the animal house of the department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. The animals were left to acclimatize for one week before commencement of the experiment and were fed with standard commercial vital feeds and water *ad libitum*. All animal experiments were carried out in accordance International Guidelines and ethical approval was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2021/005).

The animals were randomly selected and divided into seven groups of five (n=5) mice per group:
Group I: Normal Control
Group II: Ringtone mode
Group III: Vibration mode
Group IV: Silent mode
Group V: Glutathione + ringtone mode
Group VI: Glutathione + vibration mode
Group VII: Glutathione + silent mode.

*Exposure to mobile phone radiation*

Using Itel mobile phone (it2160) with specific absorption rate (SAR) of 2W/kg and 2G Network Bands, of frequency 850-1900 MHz, the animals in groups II to VII were all exposed to 4 hours mobile phone radiation of an average of 300 missed calls per day for six (6) weeks. Animals in groups V, VI and VII were administered glutathione (250 mg/kg) thirty minutes prior to mobile phone radiation daily.

*Experimental protocol*

**Elevated plus maze for anxiety:** The elevated plus maze (EPM) used in this study was made up of four arms (two open and two enclosed). The maze was kept elevated 55 cm above the floor. The experiment was performed according to the method described by Aduema *et al*. (2018). Mice were placed in the central square of the EPM facing the open arm. Number of entries and time spent in the arms were recorded. The maze was cleaned with 70% ethyl alcohol and permitted to dry before the next animal was allowed to explore.

**Hole board test for anxiety:** The circular hole board with 16 evenly spaced holes (3 cm diameter and 2.2 cm depth), was used for the study. The mice were placed in the center of the hole board and allowed to freely explore the apparatus for 5 min. The number of times an animal dipped its head into the holes was automatically counted and recorded by the instrument (Doukkali et al., 2015).

**Open field test:** The open field apparatus consists of floor space with the dimension of 40 cm x 40 cm and 30 cm in height. The floor space is divided into 16 squares equally. Each mouse was placed at the center of the apparatus and allowed to explore for 5 minutes. The following parameters were noted: 1) Centre crossing 2) Line crossing and 3) Rearing (Bagewadi *et al.,* 2015).

**Tail suspension Test for depression:** Mice were suspended from a metal rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape 17 cm on the model of the test which is a bar placed on wood with dimension (55 height x 60 width x 12 cm depth). The duration of the test was 6 minutes and immobility time was recorded. Immobility is defined as the absence of any limb or body movements, except those caused by respiration (Yusha'u et al., 2021).

**Cognition test by EPM:** Long term visuo-spatial memory was tested using elevated plus maze. It consists of a central square (5 × 5cm), made of two open arms (25 x 5 cm) and two enclosed arms of the same size with 15cm high wooden walls. The entire plus maze was elevated 55cm above the floor. On the acquisition (training) day, each mouse was placed at the end of one open arm, facing outward. The time taken for the mouse to move from the open to the enclosed arms was recorded within 90 seconds. The mice were allowed to explore the apparatus for 20 seconds. For every trial, the maze was wiped with 70% ethanol, and allowed to dry to remove any olfactory cue before another mouse was placed in the maze. Twenty-four (24) hours later, the retention test was performed and the time taken to enter the enclosed arm was also observed and recorded (Ishaku et al., 2018).

**Fore-paw grip test for motor coordination:** The Fore-paw grip test is used to assess motor co-ordination and motor function. The animal was suspended with its fore on the fore-paw grip apparatus and timed till it lost its grip.

**Beam walk assay for motor coordination:** The beam walk apparatus used in the study was made of wood, and consist of 75cm strips of flat (25mm wide) and round (20mm diameter) platforms were placed from start to goal box. A support was used to raise the beam 40cm from the floor together with the Goal box (Ishaku et al., 2018).

**Test for central pain using hot plate:** The apparatus is a circular hot plate maintained at $45^0$C ($\pm 2^0$C). Mice were individually placed on the hot plate apparatus. The stopwatch was started immediately after introducing the mouse to the apparatus and was stopped when the required behaviour was observed. The cutoff point for the test per mouse was thirty seconds, to avoid tissue damage. The time taken for the mice to jump off, raise limbs (foot-shakes) or lick a paw, was recorded (Wadioni *et al.*, 2017).

**Acetic acid induced writhing test for pain:** Pain was assessed by injection of 1% acetic acid solution (0.1 mL/kg, *i.p.*) to the mice. The number of writhes (each of which is characterized by a wave of contraction of abdominal musculature followed by extension of the hind limbs) were counted 5 min after acetic acid injection for a period of 10 min (Umar et al., 2013).

*Preparation of brain homogenate*

At the end of the experiments, all the animals were anaesthesized by IP injection of ketamine (75mg/kg) and diazepham (5mg/kg). The skulls were dissected and brains carefully extracted. The right hemisphere of each mouse was weighed and homogenized in phosphate buffered with the addition of 0.3ml of 0.3mM adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM adrenaline and 0.2ml of distilled water. The absorbance was measured at 480 nm at 30 seconds as initial and 180 seconds as final absorbance.

*Statistical analysis*

Results are presented as mean ± standard error of mean (SEM). All the data were analysed by one way ANOVA, followed by Tukey's post-hoc test for multiple comparison. SPSS version 23 was used for the analysis. Graphs were plotted using microsoft excel.

## RESULTS

### Anxiety and depression

Effect of mobile phone radiation and glutathione administration on arm entries in elevated plus maze (EPM) model of anxiety showed a statistically significant decrease in open arm entries in all the groups ($p < 0.05$) compared to the control (5.00±1.14), indicating increase saline (pH = 7.4), then the mixture was centrifuged at 1000 x *g* to obtain the supernatant which was used for biochemical analysis of malondyaldehyde (MDA), superoxide dismutase (SOD) and and reduced glutathione (GSH).

*Determination of oxidative stress*

Tissue MDA was determined according to the method described by Atawodi (2011). Exactly 2 $cm^3$ of 15% trichloroacetic acid (TCA) was measured into a test tube, 2 $cm^3$ of thiobabitutric acid (TBA) and 100 µl of serum were added. The mixture was incubated at 80ºC for 30 minutes on a water bath and allowed to cool for 30 minutes, followed by centrifugation at 1000 x *g* 10 minutes. The clear supernatant was collected and the absorbance was measured at 535 nm using a spectrophotometer.

Brain GSH concentration was determined according to the method of Ellman (1959), as described by Rukkumani *et al.* (2004). 10% TCA was added to the homogenate and centrifuged at 1500 rpm for 5 minutes. Thereafter, 1ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm.

The activity of SOD was determined in the brain tissue according to the method described by Fridovich, (1989). 0.05M carbonate buffer (pH 10.2) and Adrenaline (0.3mM) solution were prepared freshly and 0.2ml of sample was added to 2.5ml of 0.05M carbonate buffer. The reaction was started

in anxiety by mobile phone radiation exposure. Similarly, mean number of closed arm entries was significantly higher in the control group (8.00±2.93) compared to all the other groups ($p < 0.05$), except the vibration only (VIB) group (5.80±0.58), which, though is also lower than the control group but the difference was not statistically significant ($p = 0.23$). The group exposed to vibration only (VIB) had higher mean closed arm entries compared to the group exposed to ring tone and glutathione (RT+GSH; 1.80±0.37), and the difference was statistically significant ($p = 0.03$) (figure 1).

In the hole board test, mean number of head dips in the SIL group (31.60±3.31) was significantly lower compared to all the other groups ($p < 0.05$). On the other hand, mean number of head dips in the SIL+GSH group (58.20±1.98) was significantly higher than all the other groups ($p < 0.05$) except the RT+GSH, which, though also lower (50.80±3.39), the difference was not statistically significant ($p = 0.18$) (figure 2). Result for tail suspension

test showed significant decrease ($p < 0.05$) in immobility time by glutathione after exposure to mobile phone radiation in silent mode (figure 3). In the open field test, mean number of line crossing was significantly higher in the VIB+GSH and SIL+GSH groups (96.00±9.22 and 82.60±5.45 respectively) compared to the other groups, and the differences were statistically significant ($p < 0.05$). The control group had significantly higher mean number of centre crossing (10.00±0.71) compared to all the other groups ($p < 0.05$) (table 1).

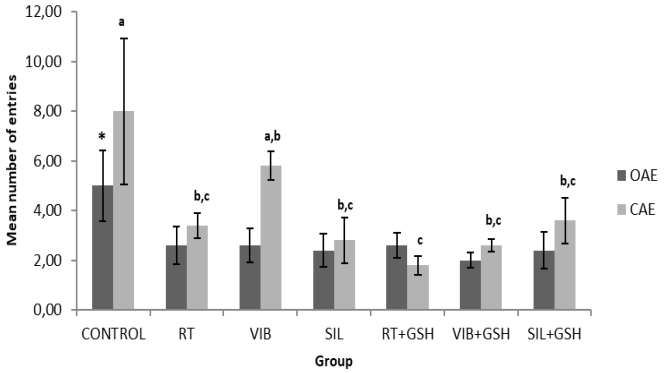


**Figure 1:** Effect of mobile phone radiation and glutathione administration on arm entries in elevated plus maze model of anxiety in mice. Mean±SEM.
*Statistically significant compared to other groups (OAE; ANOVA; $p < 0.05$).
Bars that have different letters [a,b,c] are statistically significantly different (CAE; ANOVA; $p < 0.05$).
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione, OAE; open arm entries, CAE; closed arm entries.

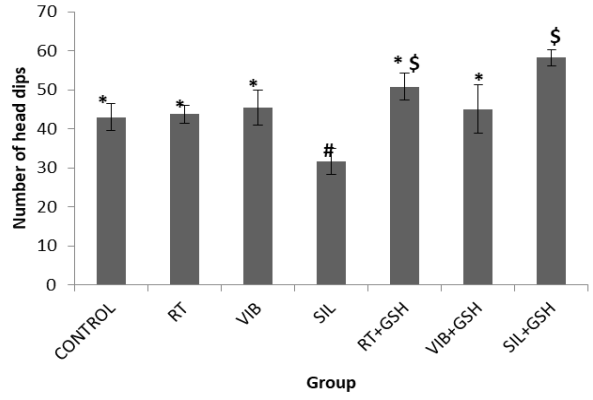


**Figure 2:** Effect of mobile phone radiation and glutathione administration on number of head dips in hole board test for anxiety in mice. Mean±SEM.
Bars with different signs [*,#,$] are statistically significantly different (ANOVA; $p < 0.05$).
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione

**Table 1:** Effect of mobile phone radiation and glutathione administration on open field test in mice. Mean±SEM.

| GROUP | LINE CROSSING (mean±SEM) | CENTRE CROSSING (mean±SEM) |
|---|---|---|
| CONTROL | 45.80±6.14[a] | 10.00±0.71[a] |
| RT | 53.40±3.01[a] | 3.00±0.45[b] |
| VIB | 48.60±6.06[a] | 2.80±0.37[b] |
| SIL | 35.80±5.74[a] | 3.80±0.86[b] |
| RT+GSH | 38.40±9.56[a] | 4.60±0.68[b] |
| VIB+GSH | 96.00±9.22[b] | 2.80±0.49[b] |
| SIL+GSH | 82.60±5.45[b] | 4.00±0.71[b] |

Values not having the same superscript letter [a,b,c] are statistically significantly different with respective parameters in the same column
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione

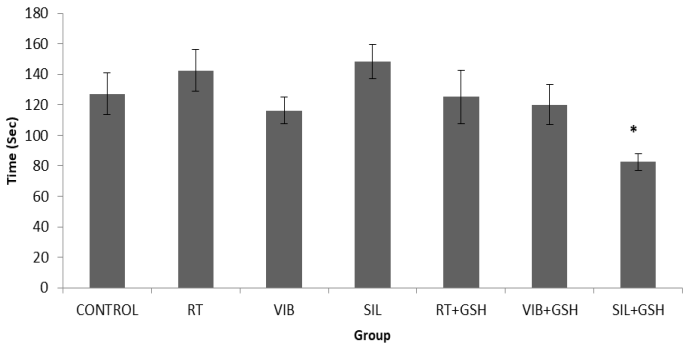


**Figure 3:** Effect of mobile phone radiation and glutathione administration on immobility time in tail suspension test model of depression in mice. Mean±SEM.
*Statistically significant compared to other groups (ANOVA; $p < 0.05$).
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione

## Cognition

EPM test for memory showed statistically significant increase in mean acquisition time in the RT (18.80±3.60; $p = 0.025$), RT+GSH (19.57±3.26; $p = 0.016$) and VIB+GSH (21.40±3.68; $p = 0.005$) groups compared to the control (9.93±0.81). Mean retention time was significantly higher in the RT+GSH group compared to the VIB+GSH group (16.97±3.76 and 10.04±0.72 respectively; $p = 0.03$) (figure 4).

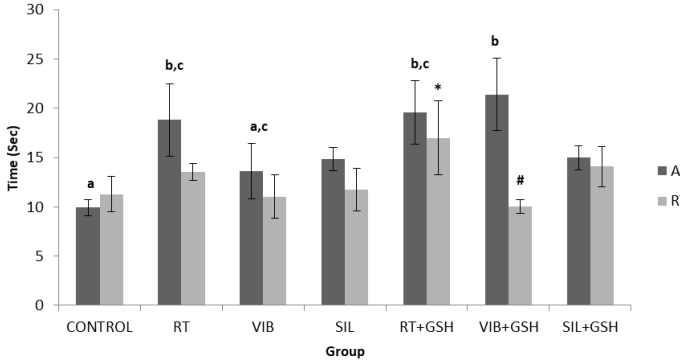


**Figure 4:** Effect of mobile phone radiation and glutathione administration on acquisition and retention time in elevated plus maze model of memory in mice. Mean±SEM.
Bars with different letters [a,b,c] are statistically significant (AT; ANOVA; $p < 0.05$)
*# Mean difference is statistically significant (RT; ANOVA; $p < 0.05$).
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione, AT; acquisition time, RT; retention time

## Pain perception

Exposure to mobile phone radiation in silent mode caused statistically significant increase in hot plate latency (5.34±0.50) compared to control (2.33±0.28; $p = 0.001$), which was not decreased by glutathione administration (4.71±0.97; $p = 0.006$) (figure 5). In acetic acid induced writhing reflex test for pain, mean number of contractions were significantly increased by glutathione after exposure to mobile phone radiation in ring tone mode (39.20±3.28; $p = 0.015$) compared to ring tone only (28.60±3.40) (figure 6).

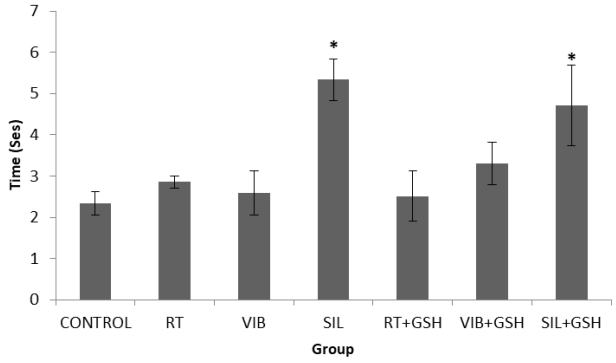


**Figure 5:** Effect of mobile phone radiation and glutathione administration on hot plate latency in mice model of thermal pain perception. Mean±SEM.
*statistically significant compared to control group (ANOVA; $p < 0.05$)
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione

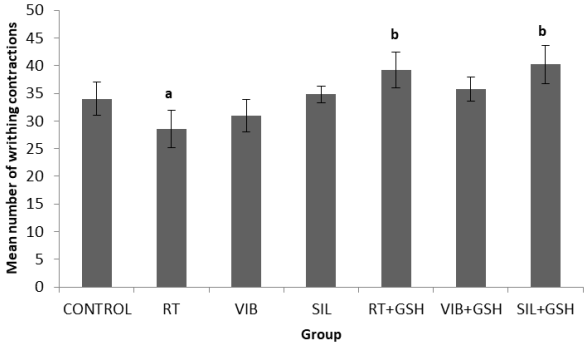


**Figure 6:** Effect of mobile phone radiation and glutathione administration on number of contractions in acetic acid induced writhing reflex in mice model of chemical pain perception. Mean±SEM.
Bars with different letters (a,b) are statistically significant (ANOVA; $p < 0.05$)
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione

## Oxidative stress

There was statistically significant increase in serum MDA concentration by exposure to mobile phone radiation at ring tone mode (18.09±0.85; $p = 0.00$) and vibration mode (14.28±1.05; $p = 0.00$) compared to the control (7.31±1.21). However, glutathione administration significantly decreased the brain MDA concentration due to exposure to ring tone mode (9.46±0.55) and vibration mode (5.61±0.94) compared to their respective exposures without glutathione ($p < 0.05$) (figure 7).

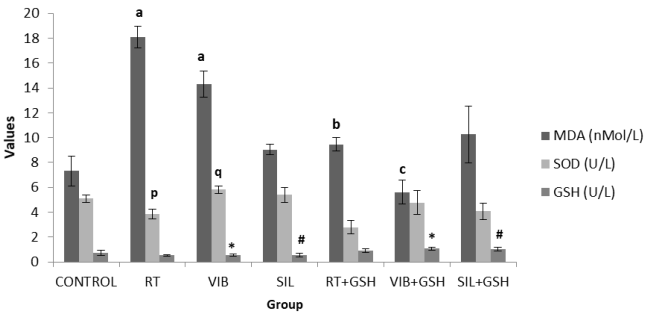


**Figure 7:** Effect of mobile phone radiation and glutathione administration on serum MDA, SOD and GSH in mice. Mean±SEM. a: statistically significant compared to control group (MDA; ANOVA; $p < 0.05$). b: statistically significant compared to RT group (MDA; ANOVA; $p < 0.05$). c: statistically significant compared to VIB group (MDA; ANOVA; $p < 0.05$). p,q: bars with different letters are statistically significant (SOD; ANOVA; $p < 0.05$). Bars with the same symbol (*,#) are statistically significant (GSH; ANOVA; $p < 0.05$).
RT; ring tone, VIB; vibration, SIL; silent, GSH; glutathione, MDA; Malondealdehyde, SOD; Superoxide dismutase

## DISCUSSION

In this study, we investigated how exposure to mobile phone radiation in different modes (ring tone, silent, vibration) affect neurobehavioural outcomes using animal models. We also examine the possible modulation of the effects by exogenous glutathione supplementation, as well as possible involvement of the central antioxidant

mechanism. The outcome of our study shows that chronic exposure to mobile phone radiation causes increase in anxiety and depression, a decrease in cognition index and central and chemical pain perception. We also observe disturbances in the brain oxidative status by the chronic mobile phone radiation. These negative effects were found to be modulated by exogenous glutathione administration.

Chronic exposure to mobile phone radiation on silent mode in the present study caused increase in anxiety-like behaviour in the mice, an effect that was prevented by glutathione administration. A study by Gupta et al. (2019) shows that sub-chronic exposure of rats to EMR at 2450 MHz frequency cause an increase in anxiety-like symptoms. Our result is also in agreement with the findings of Jadidi et al. (2014) that showed increase in anxiety in male and female mice due to exposure to radiaiton at 900 MHz, but disagree with the study of Esmaili et al. (2017), that showed no significant effect of mobile phone radiaiton at 900, 1800 and 2100 MHz on anxiety-like behaviour in rats. In two other previous studies by Cassel et al. (2004) and Cosquer et al. (2005), exposure to electromagnetic fields at 2.45 GHz does not significantly alter performance of rats in radial maze and elevated plus maze respectively. A relationship between smart phone use and anxiety in humans has also been demonstrated (Elhai et al., 2016).

Anxiety like behaviour in rodents can be assessed by measuring number of head dips in a hole board test, which is inversely proportional to anxiety (Casarrubea et al., 2009). Other studies showed that mobile phone radiation at 900 MHz can cause oxidative damage and reduced SOD activity in the brain tissue of rats (Ilhan et al., 2004), and liver (Okem et al., 2005). Conversely, exposure to mobile phone radiation at 900, 1800 and 2100 MHz produced no effect on serum SOD level in rats (Esmaili et al., 2017). The anti-oxidant system have capacity to repair oxidative damage that may be caused by low intensity mobile phone radiation exposure (Johansson et al., 2008). Exposure to mobile phone radiation at 900MHz was ineffective in causing alpha-Int 1 gene sequence variation (Shahin-Jafari, 2016).

Although it is a well known fact that brain dysfunction can increase anxiety in humans, studies that examine effects of exposure to mobile phone radiation on anxiety-like behavoiurs in rodents are still inconclusive. Subacute exposure to static magnetic field does not significantly alter SOD in the liver and kidney of pregnant rats, but cause increase in GSH (Charter et al., 2006).

Effects of exposure to electromagnetic fields on sensory perception and intellectual capacity have also been explored. There was an observed increased pain threshold (decrease sensory perception), as well as decrease in cognition index in the present study. In a study to investigate effect of electromagnetic radiation on memory in rodents, it was reported that exposure to radiation at 950 MHz from base station antenna did not affect long term potentiation (LTP) in rats (Jadidi et al., 2007). Also, acute whole body exposure to radiation at 950 MHz produced no effects on acquisition and consolidation of spatial memory in rats (Jadidi et al., 2009). Glutathione is an important cellular antioxidant and changes in its levels are linked to structural alterations in brain areas associated with cognition.

The outcomes observed in our study could be due to alterations in cellular oxidative status in the brain of the animals. Exposure of rabbits to 1800 MHz of radiation from GSM has been shown to cause increase in lipid peroxidation in the brain (Jagetia, 2022), and the effects were not due to increase in temperature, but likely due to oxidative stress and upregulation of inflammatory cytokines (Yuan et al., 2020). Significant increase in lipid peroxidation as well as reduction in anti-oxidant enzymes was observed in humans exposed to electromagnetic radiation at 900 MHz (Zothansiama et al., 2017), as well as increase in reactive oxygen species and decrease in antioxidant enzymes in serum (Kumar et al., 2010) and brain (Gürler et al., 2014) of rats. Rats exposed to radio frequency electromagnetic field were reported to have increase in breakage of DNA strands and oxidative damage in the brain (Paulraj and Bahari, 2006; Deshmukh et al., 2013). In another study, exposure of rats to EMF increase lipid peroxidation (Sahin et al., 2016) and upregulate inflammatory cytokines in the brain (Megha et al., 2015). Exposure to GSM radiation at 2100 MHz causes oxidtive damage and lipid peroxidation in the frontal lobe of rat's brain, reduced GSH concentration and increased ROS (Alkis et al., 2019; Sharma et al., 2020; Singh et al., 2020).

Oxidative stress ensues when there is imbalance in the cellular generation of reactive oxygen species and the antioxidant defense system resulting in accumulation of free radicals/ROS causing cellular damage (Denu & Hematti, 2016; Facchin et al., 2018). Oxidative stress as a result of exposure to radiofrequency-electromagnetic wave from mobile phone usage have been reported by various investigators (Jeong et al., 2018; Mailankot et al., 2009; Shivashankara et al., 2015). For instance increase in lipid peroxidation, and decrease in GSH have been in

testicular cells of rat due to exposure to RF-EMR from mobile phones (Mailankot et al., 2009). Similarly, increased lipid peroxidation was found in the saliva of mobile phone users without changes in GSH level (Shivashankara et al., 2015).

Here also, increase lipid peroxidation (MDA level) and superoxide dismutase level as well as decrease in GSH were observed in rats exposed to mobile phone radiations. Interestingly, adminstration of antioxidant glutathione reduced the impact of mobile phone radiation on oxidative stress. Decrease in all the measured indices of oxidative stress were prevented by glutathione adminstration. This expected as glutathione is essential in the preservation of cellular redox balance, detoxification and ultimately cell protection (Irvine, 1996).

## CONCLUSION

The present study assessed the modulatory effect of exogenous glutathione administration against chronic exposure to mobile phone radiation induced neuro-behavioural changes and oxidative stress in mice. Our result showed neuro-behavioural deficits and brain oxidative stress after the mice were exposed to mobile phone radiation for four hours daily for a period of six weeks. We also showed that administration of the antioxidant glutathione play significant role in protecting the brain from these neuro-behavioural deficits, an effect that is achieved via the antioxidant pathway. Further studies are required to ascertain the possible role of glutathione in DNA damage due to chronic mobile phone radiation.

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## REFERENCES

Aduema, W., Osim, E.E., & Nwankwo, A.A. (2018). Using the Elevated plus Maze Task in Assessing Anxiety and Fear in Swiss White Mice. *Journal of Complementary Medicine and Alternative Healthcare*, *6*(1), 555678. DOI: 10.19080/JCMAH.2018.06.555678

Alkis, M.E., Bilgin, H.M., Akpolat, V., Dasdag, S., Yegin, K., Yavas, C.M., & Akdag, M.Z. (2019). Effect of 900-, 1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain. *Electromagnetic Biology and Medicine*, *38*(1), 32–47. https://doi.org/10.1080/15368378.2019.1567526.

Aly, A., & Crum, R. (2016). Research review for possible relation between mobile phone radiation and brain tumor. *International Journal of Information Technology Convergence and Services, 6*,(4), 1–16. https://doi.org/ 10.5121/ijitcs.2016.6401.

Atawodi, S.E. (2011). Evaluation of the hypoglycemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-ofa" polyherbal tea (A-Polyherbal) in alloxan-induced diabetic rats. *Drug Invention Today*, *3*(11), 270–276.

Bagewadi, H. G., Ak, A. K., & Shivaramegowda, R. M. (2015). An experimental study to evaluate the effect of memantine in animal models of anxiety in Swiss albino mice. *Journal of Clinical and Diagnostic Research: JCDR*, *9*(8), FF01-5. DOI: 10.7860/JCDR/2015/13233.6287

Casarrubea, M., Sorbera, F., & Crescimanno, G. (2009). Structure of rat behavior in hole-board: I) multivariate analysis of response to anxiety. *Physiology & behavior*, *96*(1), 174-179. https://doi.org/10.1016/j.physbeh.2008.09.025

Cassel, J. C., Cosquer, B., Galani, R., & Kuster, N. (2004). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter radial-maze performance in rats. *Behavioural brain research*, *155*(1), 37-43. https://doi.org/10.1016/j.bbr.2004.03.031

Chater, S., Abdelmelek, H., Douki, T., Garrel, C., Favier, A., Sakly, M., & Rhouma, K. B. (2006). Exposure to static magnetic field of pregnant rats induces hepatic GSH elevation but not oxidative DNA damage in liver and kidney. *Archives of Medical Research*, *37*(8), 941-946. https://doi.org/10.1016/j.arcmed.2006.05.010

Cosquer, B., Galani, R., Kuster, N., & Cassel, J. C. (2005). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter anxiety responses in rats: a plus-maze study including test validation. *Behavioural brain research*, *156*(1), 65-74. https://doi.org/10.1016/j.bbr.2004.05.007

Denu, R. A., & Hematti, P. (2016). Effects of Oxidative Stress on Mesenchymal Stem Cell Biology. *Oxidative Medicine and Cellular Longevity*, 2989076. https://doi.org/10.1155/2016/2989076

Deshmukh, P. S., Megha, K., Banerjee, B. D., Ahmed, R. S., Chandna, S., Abegaonkar, M. P., & Tripathi, A. K. (2013). Detection of low level microwave radiation induced deoxyribonucleic acid damage vis-a-vis genotoxicity in brain of Fischer rats. *Toxicology International*, *20*(1), 19–24. https://doi.org/10.4103/0971- 6580.111549.

Doukkali, Z., Taghzouti, K., Bouidida, E. L., Nadjmouddine, M., Cherrah, Y., & Alaoui, K. (2015). Evaluation of anxiolytic activity of methanolic extract of Urtica urens in a mice model. *Behavioral and Brain*

*Functions*, *11*(1), 1-5. DOI 10.1186/s12993-015-0063-y

Dringen, R., & Hirrlinger, J. (2003). Glutathione pathways in the Brain. *Biological Chemistry*, *384*(4), 505-516. doi: 10.1515

Elhai, J. D., Levine, J. C., Dvorak, R. D., & Hall, B. J. (2016). Fear of missing out, need for touch, anxiety and depression are related to problematic smartphone use. *Computers in Human Behavior*, *63*, 509-516. https://doi.org/10.1016/j.chb.2016.05.079

Esmaili, H.M., Masoumi, H., Jadidi, M., Miladi-Gorji, H., & Nazari, H. (2017). The effects of acute mobile phone radiation on the anxiety level of male rats. *Middle East Journal of Rehabilitation and Health*, *4*(2), e43478. http://jrehabilhealth.neoscriber.org/en/articles/1...

Facchin, F., Bianconi, E., Romano, M., Impellizzeri, A., Alviano, F., Maioli, M., Canaider, S., & Ventura, C. (2018). Comparison of Oxidative Stress Effects on Senescence Patterning of Human Adult and Perinatal Tissue-Derived Stem Cells in Short and Long-term Cultures. *International Journal of Medical Sciences*, *15*(13), 1486–1501. https://doi.org/10.7150/ijms.27181

Faruque, M. R. I., Islam, M. T., & Misran, N. (2011). Electromagnetic (EM) absorption reduction in a muscle cube with metamaterial attachment. *Medical Engineering and Physics*, *33*(5),646-652. https://doi.org/10.1016/j.medengphy.2010.12.004

Fernández, C., de Salles, A. A., Sears, M. E., Morris, R. D., & Davis, D. L. (2018). Absorption of wireless radiation in the child versus adult brain and eye from cell phone conversation or virtual reality. *Environmental research*, *167*, 694-699. https://doi.org/10.1016/j.envres.2018.05.013

Fridovich, I. (1989). Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal of Biological Chemistry*, *264*(14), 7761-7764. https://doi.org/10.1016/S0021-9258(18)83102-7

Grigoriyev, I.G., & Biriukov, A.P. (2013). Mobile communication and public health: On the risk assessment of anthropogenic electromagnetic pollution on the environment. *Medical Radiology Radiation Safari*, *58*(6); 44-61

Gupta, S. K., Patel, S. K., Tomar, M. S., Singh, S. K., Mesharam, M. K., & Krishnamurthy, S. (2019). Long-term exposure of 2450 MHz electromagnetic radiation induces stress and anxiety like behavior in rats. *Neurochemistry international*, *128*, 1-13. https://doi.org/10.1016/j.neuint.2019.04.001

Gürler, H. Ş., Bilgici, B., Akar, A. K., Tomak, L., & Bedir, A. (2014). Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. *International journal of radiation biology*, *90*(10), 892-896. https://doi.org/10.3109/09553002.2014.922717.

Hossain, M. I., Mohammad, R. I. F., Islam, M. T., & Hanafi, N. H. M. (2014). Application of auxiliary antenna elements for SAR reduction in the human head. In *Advanced Materials Research* (Vol. 974, pp. 288-292). Trans Tech Publications Ltd.

Ilhan, A., Gurel, A., Armutcu, F., Kamisli, S., Iraz, M., Akyol, O., & Ozen, S. (2004). Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. *Clinica chimica acta*, *340*(1-2), 153-162. doi:10.1016/j.cccn.2003.10.012.

Iqbal-Faruque, M. R., Aisyah-Husni, N., Ikbal-Hossain, M., Tariqul-Islam, M., & Misran, N. (2014). Effects of mobile phone radiation onto human head with variation of holding cheek and tilt positions. *Journal of applied research and technology*, *12*(5), 871-876. https://doi.org/10.1016/S1665-6423(14)70593-0

Irvine, D.S. (1996). Glutathione as a treatment for male infertility. *Reproduction*, *1*, 6-12.

Ishaku, A. A., Mabrouk, M., Mohammed, A., Tanko, Y., & Umar, A. H. (2018). Modulatory role of vitamins A and E on memory and motor functions of cyanide induced neurotoxicity in adult swiss mice. *Nigerian Journal of Physiological Sciences*, *33*(2), 183-188.

Jadidi, M., Firouzabadi, S., Rashidipour, A., Bolouri, B., & Fath, E. Y. (2007). Low-power density of 950 MHz radiation does not affect long-term potentiation in rat dentate gyrus. *Iran. J. Radiat. Res., 2007; 5 (3): 119-124*

Jadidi, M., Firouzabadi, S., Rashidipour, A., Bolouri, B., Fath, E. Y., & Sajadi, A. A. (2009). Does whole body exposure to GSM-950 MHz electromagnetic fields affect acquisition and consolidation of spatial information in rats? *Iran Journal of Radiation Research*, *7*(1), 57-62

Jadidi, M., Miladi-Gorji, H., Mahdinezhad, M., & Torkmandi, H. (2014). Effects of mobile phone jammer on the anxiety level of male and female mice. *Physiology and Pharmacology*, 354-363. http://eprints.semums.ac.ir/id/eprint/303

Jagetia, G. C. (2022). Genotoxic effects of electromagnetic field radiations from mobile phones. *Environmental Research*, *212*, 113321. https://doi.org/10.1016/j.envres.2022.113321

Jeong, Y. J., Son, Y., Han, N.-K., Choi, H.-D., Pack, J.-K., Kim, N., Lee, Y.-S., & Lee, H.-J. (2018). Impact of Long-Term RF-EMF on Oxidative Stress and Neuroinflammation in Aging Brains of C57BL/6 Mice. *International Journal of Molecular Sciences*, *19*(7), E2103. https://doi.org/10.3390/ijms19072103

Johansson, A., Forsgren, S., Stenberg, B., Wilen, J., Kalezic, N., & Sandström, M. (2008). No effect of mobile phone-like RF exposure on patients with atopic dermatitis. *Bioelectromagnetics*, *29*(5), 353-362. https://doi.org/10.1002/bem.20402

Kesari, K. K., Kumar, S., & Behari, J. (2011). 900-MHz microwave radiation promotes oxidation in rat brain. *Electromagnetic biology and medicine*, *30*(4), 219-234. https://doi.org/10.3109/15368378.2011.587930

Kumar, S., Kesari, K.K., & Behari, J. (2010). Evaluation of genotoxic effects in male Wistar rats following microwave exposure. *Indian Journal of Experimental Biology*, *48*, 586–592. https://pubmed.ncbi.nlm.nih.gov/20882761/.

Kusuma, A.H., Sheta, A,-F., Elshafiey, I., Siddiqui, Z., Alkanhal, M., Aldosari, S., Alshebeili, S.A., & Mahmoud, S. (2011). "A new low SAR antenna structure for wireless handset applications." *Progress in Electromagnetics Research*, *112*, 23-40. http://dx.doi.org/10.2528/PIER10101802

Langbein, K., Hesse, J., Gussew, A., Milleit, B., Lavoie, S., Amminger, G. P., Gaser, C., Wagner, G., Reichenbach, J.R., Hipler, U,-C., Winter, D., & Smesny, S. (2018). Disturbed glutathione antioxidative defense is associated with structural brain changes in neuroleptic-naive first-episode psychosis patients. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *136*, 103-110. http://dx.doi.org/10.1016/j.plefa.2017.10.005

Lu, Y. S., Huang, B. T., & Huang, Y. X. (2012). Reactive oxygen species formation and apoptosis in human peripheral blood mononuclear cell induced by 900 MHz mobile phone radiation. *Oxidative medicine and cellular longevity*, 740280. https://doi.org/10.1155/2012/740280

Mailankot, M., Kunnath, A. P., Jayalekshmi, H., Koduru, B., & Valsalan, R. (2009). Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. *Clinics (Sao Paulo, Brazil)*, *64*(6), 561–565. https://doi.org/10.1590/s1807-59322009000600011

Megha, K., Deshmukh, P. S., Banerjee, B. D., Tripathi, A. K., Ahmed, R., & Abegaonkar, M. P. (2015). Low intensity microwave radiation induced oxidative stress, inflammatory response and DNA damage in rat brain. *Neurotoxicology*, *51*,158-165. https://doi.org/10.1016/j.neuro.2015.10.009.

Mohril, S., Sankhla, M. S., Chaturvedi, B., & Kumar, R. (2016). Hazardous Effect of Mobile Phone Radiation on Human Health–A Review. *International Journal of Electronics & Communication*, *4*(10), 9-14.

Oktem, F., Ozguner, F., Mollaoglu, H., Koyu, A., & Uz, E. (2005). Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. *Archives of Medical Research*, *36*(4), 350-355. doi:10.1016/j.arcmed.2005.03.021.

Paulraj, R., & Behari, J. (2006). Single strand DNA breaks in rat brain cells exposed to microwave radiation. *Mutation Research/Fundamental and Molecular Mechanism of Mutagenesis, 596*, 76–80. https://doi.org/10.1016/j.mrfmmm.2005.12.006.

Rukkumani, R., Aruna, K., Varma, P.S., Rajasekaran, K.N., & Menon, V.P. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacy and Pharmaceutical Science*, *7*(2), 274-283.

Sahin, D., Ozgur, E., Guler, G., Tomruk, A., Unlu, I., Sepici-Dinçel, A., & Seyhan, N. (2016). The 2100 MHz radiofrequency radiation of a 3G-mobile phone and the DNA oxidative damage in brain. *Journal of chemical neuroanatomy*, *75*, 94-98. https://doi.org/10.1016/j.jchemneu.2016.01.002.

Schirmacher, A., Winters, S., Fischer, S., Goeke, J., Galla, H.J., Kullnick, U., Ringelstein, E.B., & Stögbauer, F. (2000). "Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood–brain barrier in vitro." *Bioelectromagnetics: Journal of the Bioelectromagnetics Society, The Society for Physical Regulation in Biology and Medicine, The European Bioelectromagnetics Association*, *21*(5), 338-345. https://doi.org/10.1002/1521-186X(200007)21:5%3C338::AID-BEM2%3E3.0.CO;2-Q

Shahin-Jafari, A., Bayat, M., Shahhosseiny, M. H., Tajik, P., & Roudbar-Mohammadi, S. (2016). Effect of long-term exposure to mobile phone radiation on alpha-Int1 gene sequence of Candida albicans. *Saudi Journal of Biological Sciences*, *23*(3), 426-433.

Sharma, A., Shrivastava, S., & Shukla, S. (2020). Exposure of radiofrequency electromagnetic radiation on biochemical and pathological alterations. *Neurology India*, *68*(5), 1092–1100. https://doi.org/10.4103/0028-3886.294554.

Shivaji, D.R., Malpani, S.G., & Dharashive, V. (2020). A Review: Effects of Mobile Phone on Human Health. *International Journal of Scientific Development and Research*, *5*(10), 167-170. https://www.ijsdr.org/papers/IJSDR2010023.pdf

Shivashankara, A. R., Joy, J., Sunitha, V., Rai, M. P., Rao, S., Nambranathayil, S., & Baliga, M. S. (2015). Effect of cell phone use on salivary total protein, enzymes and oxidative stress markers in young adults: A pilot study. *Journal of Clinical and Diagnostic Research: JCDR*, *9*(2), BC19-22. https://doi.org/10.7860/JCDR/2015/10872.5580

Singh, K.V., Prakash, C., Nirala, P.J., Nanda, R.K., Rajamani, P. (2020). Acute radiofrequency electromagnetic radiation exposures cause neuronal DNA damage and impair neurogenesis in the young adolescent rat brain. bioRxiv. https://doi.org/10.1101/2020.11.07.370627.

Song, P., Liu, C., Zhao, T., Guo, H., & Chen, J. (2019). Research on pulse response characteristics of wireless ultraviolet communication in mobile scene. *Optics Express*, *27*(8), 10670–10683. https://doi.org/10.1364/OE.27.010670.

Umar, A. H., Mabrouk, M., Danjuma, N. M., & Yaro, A. (2013). Studies on the analgesic and anti-inflammatory properties of hydro-alcohol extract of Caralluma dalzielii NE Br (Asclepiadaceae) in rats and mice. *British Journal of Pharmacology and Toxicology*, *4*(5), 169-175.

Wadioni, A., Iniodu, C. F., & Opara, J. K. (2017). Pain Sensation in CD1 Mice Following Longterm Consumption of Beans Diet in the Tail Flick and Hot PlateTest. *International Journal of Clinical and Experimental Physiology*, *4*, 21-24. 10.4103/ijcep.ijcep_8_17

Yabuki, Y., & Fukunaga, K. (2013). Oral administration of glutathione improves memory deficits following transient brain ischemia by reducing brain oxidative stress. *Neuroscience*, *250*, 394-407. https://doi.org/10.1016/j.neuroscience.2013.07.017

Yuan, L.Q., Wang, C., Lu, D.-F., Zhao, X.-D., Tan, L.-H., & Chen, X. (2020). Induction of apoptosis and ferroptosis by a tumor suppressing magnetic field through ROS-mediated DNA damage. *Aging (Albany NY)*, *12*(4), 3662–3681. https://doi.org/10.18632/aging.102836.

Yusha'u, Y., Muhammad, U. A., Mustapha, S., Umar, A. H., Imam, M. I., Umar, B., Alhassan, A.W., Saleh, M.I.A., & Ya'u, J. (2021). Alpha-lipoic acid attenuates depressive symptoms in mice exposed to chronic unpredictable mild stress. *Journal of African Association of Physiological Sciences*, *9*(1), 58-68.

Zothansiama, Zosangzuali, M., Lalramdinpuii, M., & Jagetia, G. C. (2017). Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations. *Electromagnetic Biology and Medicine*, *36*(3),295-305. https://doi.org/10.1080/15368378.2017.1350584.