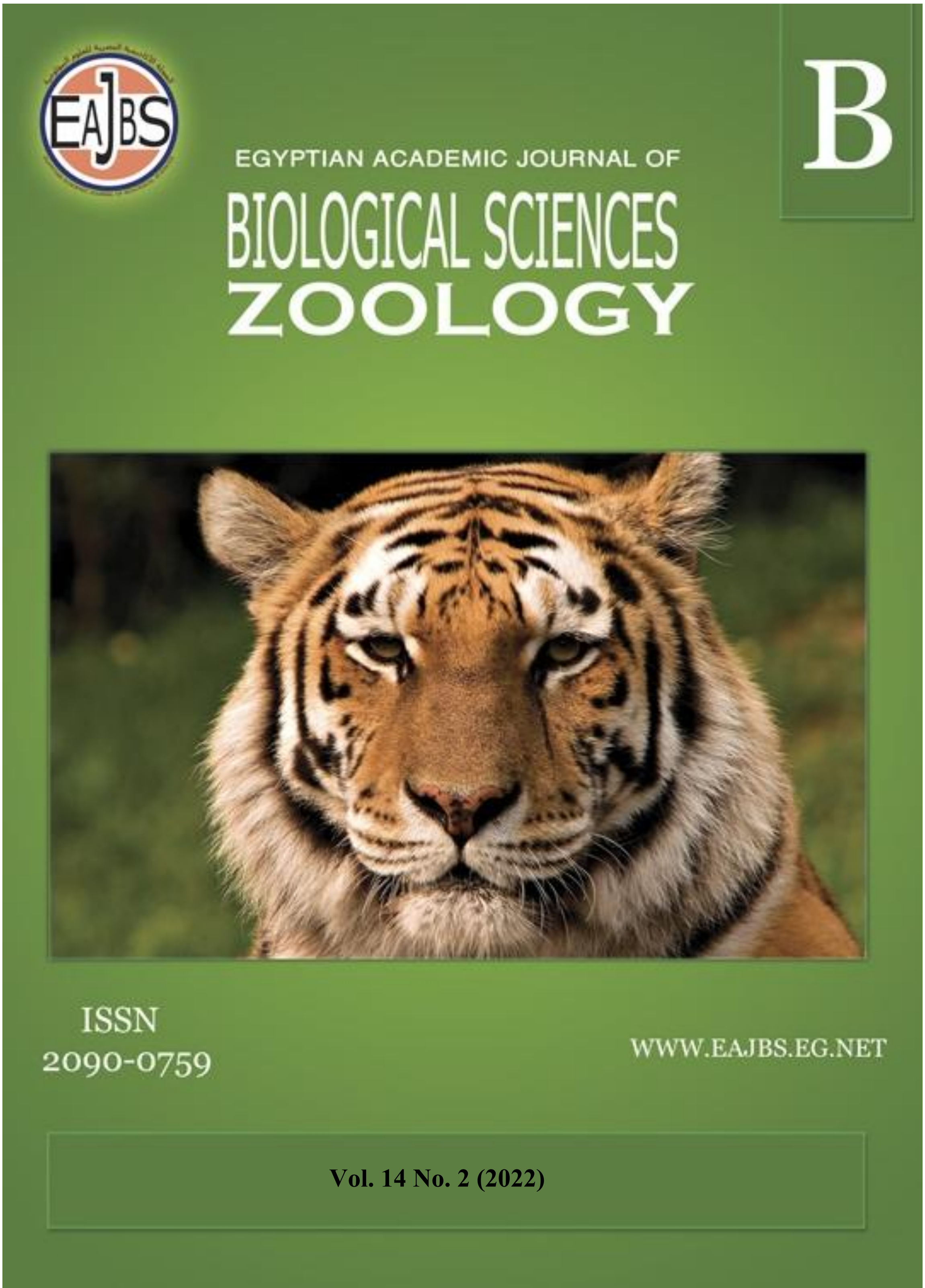

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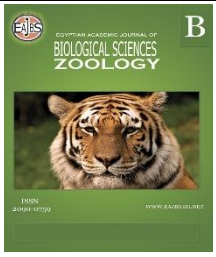

Chronic Exposure to Mobile Phone Radiation: Effects on Liver Functions and Brain of Male and Female Sprague-Dawley Rats During Vibrating and Ringing Modes

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ABSTRACT

Exposure to electromagnetic radiation (EMR) has increased exponentially in recent years. As such, the potential physiological effects of wireless emissions (GHz) on biological systems are of significant interest to researchers but remain to be fully elucidated. The current study examines the impacts of long-term EMR exposure on certain biochemical and histopathological parameters in male and female Sprague-Dawley rats, using vibrating and ringing modes. Twenty-four male rats and twenty-four female rats weighing 60 ±10 g were randomly divided into eight groups (n=6) (4 groups for male rats and 4 groups for female rats) as follows: group I served as a control group without mobile phone, groups II, III, and IV were exposed to electromagnetic radiation from mobile phone 49 minutes per day for 12 weeks. Group II was in ringing mode, group III was in vibrating mode, while group IV was in ringing and vibrating modes (both modes). The frequency of electromagnetic radiation emitted from the mobile phone was measured using an electromagnetic radiation frequency radiometer for both modes. Rats were sacrificed after 4, 6, 8, and 12 weeks. The liver, brain and blood samples were obtained to assess oxidative stress, liver functions, and histopathological damage. Results showed that EMR from mobile phone in both ringing and vibrating modes increased the oxidative stress and liver enzymes. The EMR groups had a significant decrease in catalase (CAT) activity and reduced glutathione (GSH) concentration in brain and liver tissues compared to the control group. However, the concentration of malondialdehyde (MDA) was observed to increase significantly in the treatment groups exposed to both phone modes. Additionally, levels of protein and liver enzymes (ALT & AST) significantly increased. Also, the brain and liver tissues showed degeneration. These data collectively suggests that EMR exposure from mobile phone on male and female rats causes obvious impairment in the brain and liver.

INTRODUCTION

The widespread utilization of mobile phones for prolonged periods, and the setup of communication infrastructure including base antennas and towers, have sparked worries regarding possible health implications for humans due to exposure to electromagnetic emissions. This concern is amplified by the fact that man-made electromagnetic radiation

(EMR) from various sources, including electrical lines, diagnostic and therapeutic devices, contributes significantly to the electromagnetic pollution of our environment (Moon, 2020; Paolucci *et al*., 2020 and L´opez *et al*., 2021).

Research has indicated that man-made electromagnetic fields (EMFs) pose a heightened risk to human health, as they have the capacity to disrupt cellular electrochemical homeostasis, unlike naturally occurring atmospheric electromagnetic field (EMF) (Pall, 2021). The underlying mechanism through which EMR impacts the biological systems is the implication of reactive oxygen species (ROS) (Dasdag and Akdag, 2016). Radiation can affect the distribution of free radicals in a human system, thereby disrupting essential physiological process that may produce detrimental biological effects (Sannino *et al*., 2014).

Epidemiological studies have shown that noise is a nonspecific stressor, and chronic exposure to low levels of noise, such as those produced by mobile phone ringtone could induce interruption of sleep and communication, resulting in emotional response that leads to oxidative stress (Carreras *et al*., 2014 and Munzel *et al*., 2018). Ringtone treatment increased oxidative stress, as shown by lower levels of antioxidant enzymes in the brain and heart (Usman *et al*., 2020). This is consistent with the findings on environmental noise exposure, which imply noise could potentially lead to oxidative stress (Schmidt *et al*., 2013). Also, vibration is considered a physical stressor (Blaxter *et al*., 2017 and Goswami *et al*., 2020). Vibrations can hurt the circulatory system, nerves, bones and joints (Kakosy, 1989). Exposure to vibration at 250 Hz may induce oxidative stress in the peripheral nerve, which is associated with inflammation (Pacurari *et al*., 2019).

Research efforts have predominantly focused on the brain and liver tissues due to their susceptibility to EMFs-induced oxidative stress. The proximity of the brain to the EMFs source, coupled with its limited antioxidant enzyme activity and high oxygen consumption, renders it particularly vulnerable to oxidative damage (Kim *et al*., 2021). Similarly, hepatic DNA, lipids, and proteins are prime targets of reactive oxygen species (ROS) produced upon EMFs exposure (Soares-da-Silva *et al*., 2020). Several studies have highlighted the induction of oxidative stress as a result of EMFs exposure in the brain (Chauhan *et al*., 2017 and Salameh *et al*., 2022) and liver (Fahmy and Mohammed, 2021 and Berköz *et al*., 2018). Long-term oxidative stress caused lipid molecules to accumulate in liver cells, which is responsible for the peroxidation of mitochondrial enzymes (Dinčić, *et al*., 2018). It has been suggested that EMFs (2.45 GHz, during 60 min/day for 30 days) can affect the voltage-dependent channels in the brains of Wistar albino rats (Nazıroğlu *et al*., 2012).

Consequently, this investigation aimed to comprehensively evaluate the biochemical and histopathological profiles of liver and brain tissues in response to EMFs exposure. Also, to compare the effect of different modes (ringing and vibrating) waves on the oxidative stress indicators and the functions of the liver.

MATERIALS AND METHODS

1. Animals:

Twenty-four male and twenty-four female Sprague-Dawley rats, weighing 60 ±10 g were obtained from the Agricultural Chemistry Department, Biological Experimental Animal Lab, Minia University. The rats were maintained in separate cages under standard laboratory environments (temperature remains 25 ± 2 C^{O}). All rats were adapted for two weeks prior to the start of the trial. During this period, the animals were fed *ad libitum* with a standard rat chow diet (Campbell *et al*., 1961*)*. The rats used in this study were housed in a temperature and humidity-controlled habitat with a 12-hour dark/light. All operations were performed in compliance with institutional rules and the Guide for Care and Use of Laboratory Animals.

2. Experimental Design:

Forty-eight Sprague-Dawley rats (comprising 6 males and 6 females per group) were allocated into four experimental groups as shown in Fig. (1). Group I served as the control group, while Group II was exposed to mobile phone radiation in ringing mode. Group III received exposure in vibrating mode, and Group IV was subjected to a combination of both ringing and vibrating modes. Groups II through IV were exposed daily to electromagnetic field (EMF) radiation for 49 minutes over a 12-week period.

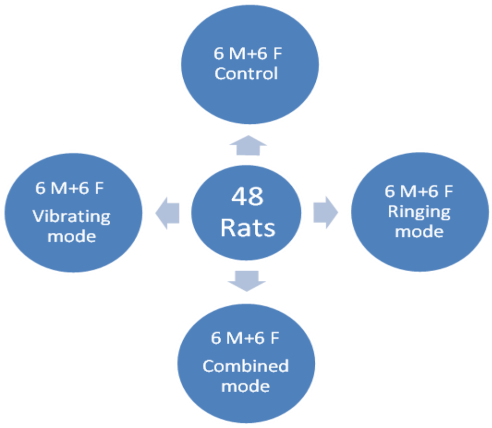

Fig. 1: Diagram showing the experimental design. (M) indicates male rats, and (F) indicates female rats.

3. Determination of Mobile Phone Irradiation:

The mobile phone used in the present work was manufactured in China (Type ZTE, Model S, Number 213). The strength of radiations emitted from this mobile was measured using a Taiwan-mode Radio Frequency EMF Strength Meter, Model 480836, EXTECH Instruments Corporation, MAO 2451, USA (Fig. 2).

The radiations were measured at 13 cm spacing from the mobile in both the ringing and vibrating modes (Siddiqi *et al*., 2017). The used EMF strength meter records strength values of radiation at one frequency. This is why it was not possible to measure the strength of radiations emitted from the mobile during simultaneous operation of both the ringing and vibrating modes because they differ in their frequency.

Fig.2: (A) Mobile phone employed in the experiment; **(B)** Radio frequency EMF strength meter.

4- Measurement of Body Weights:

The body weight in grams of each rat in every experimental group was initially estimated (at 0 week) and after 4, 6, and 12 weeks.

5. Collection of Samples:

Animals were sacrificed following induction of anesthesia with ether. Blood samples were obtained independently from each rat's jugular vein in non-heparinized tubes and permitted to clot at room temperature. The blood was centrifuged at 4000 rpm for 30 minutes. Serum samples were collected and preserved at − 80 °C for biochemical examination. After blood sampling, two parts of each brain and liver tissues were taken. The first portion was fixed in 10% formalin for histopathological investigation, while the second portion was promptly frozen at −80 °C to assess oxidative stress and antioxidant parameters.

6. Biochemical Assays:

Brain and liver tissues were put in iced normal saline and homogenized in cold phosphate buffer, pH 7.4. The homogenates were then centrifuged for 10 minutes at 3000 rpm at 4°C. The supernatant was collected for the evaluation of oxidative and antioxidant parameters.

6.1. Liver Function Tests:

6.1.1. Determination of Aminotransferases:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined enzymatically using reagent kits (Bio Merieux chemical company, France) according to Reitman and Frankel (1957). The concentration of ALT and AST were calculated as (U/ml).

6.1.2. Determination of Serum Total Protein and Albumin Contents:

Serum total protein was determined using the biuret reagent, following the procedure described by Gornall *et al.*, (1949), while serum albumin concentration was determined according to the procedure outlined by (Doumas *et al.,* 1971).

6.2. Measurement of lipid peroxidation (Malondialdehyde):

Malondialdehyde (MDA) was measured enzymatically at an absorbance of 534 nm in the homogenates of liver and brain by the method introduced by (Bartles *et al.,* 1972 and Larsen, 1972). The concentration of MDA was calculated as (nmol/ g.tissue).

6.3. Measurement of Glutathione Reduced (GSH):

Glutathione reduced was determined enzymatically in the homogenates of liver and brain by the method introduced by (Beutler *et al.,* 1963). Its absorbance can be measured at 405 nm. Glutathione reduced (GSH) concentration was expressed as mg/g tissue.

6.4. Measurement of Catalase (CAT):

The activity of CAT was evaluated in brain and liver tissues according to (Beuchamp and Fridovich, 1971) and was expressed as (U/g).

7. Histopathological Examination:

Rat brain and liver autopsy samples were collected from different groups and fixed in formalin for 24 hours. Washing was done in tap water, followed by dehydration with serial dilutions of alcohol (methyl, ethyl, and 100% ethyl). Specimens were cleared in xylene and embedded in paraffin at 56° in a hot air oven for 24 hours. Tissue blocks made of paraffin bees wax were sectioned at thickness of 4 microns using a slide microtome. The prepared tissue sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin. Microscopic examination was then performed using a light electric microscope (Banchroft *et al*., 1996).

8. Statistical Analysis:

Analysis of variance (ANOVA) was carried out using SAS (Statistical Analysis System) SAS Institute Inc. (SAS, 2018). The differences between the respective means were determined using Duncan (1957) and considered significant when $p < 0.05$, for mean ± standard deviation.

RESULTS

Effect of EMFs on Body Weight:

Table (1) indicated a remarkable increase in body weight observed in both male and female rats as the number of exposures increased irrespective of the mode of exposure;

ringing, vibrating or combined mode, over the test period (12 weeks). However, for the same number of exposures e.g., 42, the obtained data revealed a decline in the body weight of male and female rats for the three modes of exposure against the control group. Also, at the end of the experiment, after 84 times of exposure, the body weight values of the male rats significantly decreased in the three investigated exposure modes versus the unexposed one. The decrease was statistically significant for the ringing and vibrating modes, and highly significant for the combined mode. This represented a decrease of body weight gain due to exposure to EMFs in comparison with the control group of 3.8%, 16.9% and 23.23% for the respective three modes.

According to Table (1), the body weight of female rats after 84 times of exposure decreased significantly in the three modes of exposure against the unexposed group (significant for ringing mode and highly significant for vibrating and combined modes). This represented a decrease of body weight gain as a result of EMFs exposure when compared with the control group of 7.6%, 17.5% and 32.6% for the ringing, vibrating and combined modes. Our results indicated that male and female rats respond differently to EMFs and the female rats were more sensitive than males.

Table 1: Temporal variation of body weight (g) in male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

Number of exposures	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
0 times (0 week)	Male Female	67.83±0.98 69.33±0.81	70±0.89 69.5±0.54	68±2.82 68.5±0.83	69.33±1.03 69±1.41
28 times (4 weeks)	Male Female	145.83±1.47 149.33±1.96	136.83^{*}±2.92 139.5^{*}±1.87	126.83^{*}±1.94 131.83^{*}±1.94	120.5^{**}±1.87 122.16^{*}±3.06
42 times (6 weeks)	Male Female	219.5±2.42 277.66±2.06	208.16^{*}±1.47 181.5^{*}±49.97	193.16^{*}±2.04 197^{**}±2.82	157.66^{**}±2.94 171.66^{**}±2.06
84 times (12 weeks)	Male Female	325.66±1.21 330.83±1.47	313.33^{*}±1.21 305.83^{*}±1.72	270.66^{*}±0.81 273^{**}±2.36	208^{**}±1.41 223^{**}±2.68

: significant **: highly significant *: very highly significant*

Biochemical Assays:

Effect of EMFs on Serum Alanine Aminotransferase (ALT):

Table (2) illustrated the serum alanine aminotransferase activity (ALT) over the test period which extended up to 12 weeks. However, ALT activity elevated significantly with the increase in the number of exposures to electromagnetic fields (EMFs) irrespective of the mode of exposure, ringing, vibrating or combined ringing and vibrating mode. For the same number of exposures, 28 times, the ALT activity of male rats recorded an increase for the three investigated exposure modes against the control group (significant for ringing mode, highly significant for vibrating mode and very highly significant for combined mode). This represented an increase of ALT activity due to exposure to EMFs above that of the control group of 39.65%, 88.55% and 123.19% for the respective three modes. The effect of EMFs on the increase in ALT activity is ranked in ascending order to be at first for ringing mode followed by vibrating mode and then the combined mode of exposure. This ascending order of EMFs on ALT activity remained the same irrespective of the number of exposure times which increased up to 84 over the test period of 12 successive weeks as illustrated in Table (2).

Results reported in Table (2) revealed the ALT activity in female rats over the 12-

week test period. As observed for male rats, the ALT activity increased significantly with the increase of the number of exposure times to EMFs irrespective of the exposure mode. For 28 times of exposure, the increases of ALT activity were respectively 33.23%, 79.98% and 115.50% over that of the control group for the ringing, vibrating and combined modes. Also, for 56 times of exposure to EMFs, the above-mentioned increase of ALT levels recorded 66.35%, 227.65% and 157.15% above that of the control group. For 84 times of exposure to EMFs over the 12 weeks test period, the levels of ALT were 81.70, 127.47 and 128.15 U/L for ringing, vibrating and combined modes of exposure vs 21.13 U/L for the control group.

Table 2. Temporal variation of serum ALT activity (U/L) in male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at $P<0.05$.

Number of exposures	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
28 times (4 weeks)	Male Female	24.97±0.37 25.67 ± 0.87	34.87*±0.60 34.20* ± 1.22	47.08**±1.83 46.20* ± 1.46	55.73***±3.02 55.32** ± 2.66
42 times (6 weeks)	Male Female	24.92±0.44 25.53± 0.69	45.73*±1.93 42.47* ± 0.56	85.77**±2.02 83.65** ± 0.67	68.72***±0.56 65.65*** ± 2.99
56 times (8 weeks)	Male Female	25.18±0.33 25.57±0.57	61.52**±0.88 58.74* ± 1.86	107.93***±1.44 108.63**± 0.52	90.77***±1.04 89.17*** ± 1.89
84 times (12 weeks)	Male Female	25.85±0.51 21.13 ± 4.12	82.14**±1.33 81.70** ± 1.14	125.27***±4.69 127.47** ± 1.24	136.12***±8.72 128.15*** ±0.51

: significant **: highly significant *: very highly significant*

Effect of EMFs on Serum Aspartate Aminotransaminase (AST):

As regards to the effect of EMFs on AST activity in male rats, Table (3) illustrated the AST activity over the 12-week test period. The same as that for ALT, the AST levels increased significantly with the increase of the number of exposures to EMFs, irrespective of the exposure mode. For 28 times of exposure to EMFs over a 4-week test period, the AST activity increased significantly for the ringing mode and highly significant for vibrating and combined modes of exposure in comparison with the control group. This represented corresponding increase in AST level of 30.10%. 95.23% and 127.19% for the three modes of exposure over that of the control group. For 42 times of exposure to EMFs, the increase in AST activity due to EMF exposure over that of the control group were respectively 62.36%, 115% and 191.3% for the ringing, vibrating and combined modes. For 56 times of exposure to EMFs, the ringing, vibrating and combined modes resulted in an increase in AST level by 86.30%, 166.07% and 207.10%, respectively compared to the value of the control group. For 84 times of exposure to EMFs, the above-mentioned increase of AST levels recorded 101.76%, 222.85% and 286.52% above that of the control group.

The effect of EMFs on increasing the AST activity was ranked in ascending order the same as the ALT activity where the ringing mode came first followed by the vibrating mode and then the combined mode of exposure. Table (3) showed the effect of EMFs on serum aspartate transaminase activity in female rats. These data indicated that the same trend was observed as in ALT activity of female rats for the ringing, vibrating and combined modes of exposure. Also, we can say that the influence of EMFs on the increase in AST activity ranked in ascending order to be at first ringing mode followed by vibrating and then the combined mode of exposure. It was noted that no sex-dependent difference was confirmed for either ALT or AST activity.

Table 3. Temporal variation of serum AST activity (U/L) in male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

Number of exposures	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
28 times (4 weeks)	Male	33.32± 2.44	$43.35^{*}\pm 0.49$	$65.05^{**}\pm 1.06$	$75.70^{**}\pm 1.92$
	Female	31.03± 1.65	$44.68^{*}\pm 1.05$	$64.20^{**}\pm 0.76$	$73.90^{**}\pm 1.57$
42 times (6 weeks)	Male	32.20± 2.03	$52.28^{*}\pm 3.74$	$69.23^{**}\pm 4.36$	$93.8^{***}\pm 2.51$
	Female	28.63± 2.027	$45.90^{*}\pm 1.42$	$66.83^{**}\pm 1.06$	$79.53^{**}\pm 1.67$
56 times (8 weeks)	Male	33.80± 2.49	$62.97^{*}\pm 3.71$	$89.93^{**}\pm 5.27$	$103.8^{***}\pm 3.64$
	Female	28.03±2.82	$46.80^{*}\pm 1.31$	$94.37^{**}\pm 0.87$	$104.28^{***}\pm 3.02$
84 times (12 weeks)	Male	32.43± 1.87	$65.43^{*}\pm 1.68$	$104.70^{***}\pm 3.76$	$125.35^{***}\pm 10.1$[illegible]
	Female	32.68± 1.67	$63.53^{*}\pm 0.47$	$103.33^{***}\pm 2.86$	$125.25^{***}\pm 9.22$

: significant   **: highly significant   *: very highly significant*

Effect of EMFs on Total Protein and Albumin:

The effects of EMFs on total protein and albumin levels of male and female rats were determined, and the obtained results were given in Tables (4 & 5). The data indicated that both total protein and albumin levels increased significantly with the increase of the number of exposures to EMFs irrespective of the mode of exposure; ringing; vibrating or combined mode ringing and vibrating (P<0.05 for total protein and P<0.05 and P<0.01 for albumin).

At the end of the test period (84 times of exposure), the total protein content in the male and female rats increased for the three investigated exposure modes against the control groups (P<0.05). This represented an increase in total protein content due to EMFs exposure above that of the control group of 22.69%, 15.88% and 25.77% in the male rats and 13.56%, 21.53% and 17.22% in the female rats for the respective three modes.

According to Table (5), at the end of the experiment the albumin level increased by 41.32% & 57.57% for the ringing mode, 50.00% & 55.09% for vibrating mode and 55.71% and 68.73% for the combined mode.

Table 4. Temporal variation of serum total protein (g/dl) in male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

Number of exposures	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
28 times (4 weeks)	Male	6.70± 0.25	6.23 ± 0.76	$6.8^{*}\pm 0.71$	$7.23^{*}\pm 0.68$
	Female	5.47±0.62	$6.52^{*}\pm 0.31$	$6.71^{*}\pm 1.40$	$6.55^{*}\pm 0.50$
42 times (6 weeks)	Male	5.91 ± 0.49	$6.48^{*}\pm 0.15$	$6.84^{*}\pm 0.11$	$6.96^{*}\pm 0.11$
	Female	6.44 ±0.49	6.66 ± 0.26	$7.07^{*}\pm 0.60$	$6.78^{*}\pm 0.33$
56 times (8 weeks)	Male	6.34 ± 0.22	$6.71^{*}\pm 0.14$	$7.13^{*}\pm 0.38$	$7.39^{*}\pm 0.09$
	Female	6.43 ± 0.24	6.87± 0.28	$6.99^{*}\pm 0.13$	$7.14^{*}\pm 0.07$
84 times (12 weeks)	Male	6.17 ± 0.36	$7.57^{*}\pm 0.82$	$7.15^{*}\pm 0.26$	$7.76^{*}\pm 0.16$
	Female	6.27 ± 0.22	$7.12^{*}\pm 0.57$	$7.62^{*}\pm 0.15$	$7.35^{*}\pm 0.36$

: significant   **: highly significant   *: very highly significant*

Table 5: Temporal variation of serum albumin (g/dl) in male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

Number of exposures	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
28 times (4 weeks)	Male Female	4.14 ± 0.26 3.97 ± 0.24	5.25^{*} ± 0.47 5.32^{*}± 0.24	5.25^{*} ± 0.30 5.46^{*} ± 0.32	6.06^{**} ± 0.21 5.91^{**} ± 0.11
42 times (6 weeks)	Male Female	4.38 ± 0.12 4.07 ± 0.14	5.38^{*} ± 0.29 5.84^{*} ± 0.15	5.96^{*} ± 0.28 6.27^{*} ± 0.09	5.9^{**} ± 0.061 5.96^{**} ± 0.09
56 times (8 weeks)	Male Female	4.59 ± 0.41 4.39± 0.48	5.83^{*} ± 0.11 6.14^{*} ± 0.09	6.37^{*}± 0.10 5.86^{*} ± 0.60	6.44^{**} ± 0.433 6.24^{**} ± 0.07
84 times (12 weeks)	Male Female	4.38 ± 0.19 4.03 ± 0.12	6.19^{*} ± 0.17 6.35^{*} ± 0.20	6.57^{*} ± 0.11 6.25^{*} ± 0.13	6.82^{**} ± 0.24 6.8^{**} ± 0.21

: significant **: highly significant *: very highly significant*

Effect of EMFs on the Oxidative / Antioxidative Systems:

Glutathione (GSH) is the first line of defense system against free radicals and other oxidative species. Malondialdehyde (MDA), a stable metabolite of the free radical mediated lipid peroxidation cascade was commonly employed as an indicator of lipid peroxidation.

Table (6) presented the effect of EMFs on brain homogenate lipid peroxidation marker (MDA). It is quite clear that the rats treated with combined mode of EMFs exposure demonstrated a highly significant rise in MDA content in male and female when compared with the control group.

With respect to liver homogenate, the corresponding values of MDA were 3.96±0.010 in male and 4.01±0.091 in female against the control groups 0.70±0.02 and 0.75±0.051, respectively (Table 7).

On the other hand, the obtained data indicated that the combined mode of EMFs exposure resulted in a highly significant decline in GSH content in the brain homogenate in male and female as compared with the control rats.

Concerning the liver homogenate, the corresponding values of GSH were 0.91±0.01 in male and 0.96±0.02 in female versus the control groups 0.70±0.02 and 0.75±0.051, respectively, Table (7).

Assay for catalase activity (Table 6) showed a significant decrease of catalase in the brain of male and female rats over the test period which extends up to 12 weeks. The obtained data revealed that the decreased values of CAT activity in the brain of male rats were due to EMFs exposure in relation to the control group were 7.38 %, 52.92% and 73.28% for the ringing, vibrating and combined modes, respectively. Also, the tabulated data in Table (7), showed a remarkable decrease in the CAT activity in the liver of male and female rats for the three investigated exposure modes compared to the control group. This represents percentage decrease in CAT activity due to EMFs compared with the control group as 25.39%, 63.20% and 76.90 % for the respectively three modes of exposure in female rats.

Table 6. Temporal variation of MDA (nmol/g. tissue) and GSH (mg/mg wet tissue) and CAT (U/g) in brain homogenate of male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
MDA	**Male** **Female**	0.69±0.015 0.74±0.027	1.18*±0.025 1.48*±0.026	2.98*±0.027 3.03*±0.020	4.51**±0.015 4.47**±0.036
GSH	**Male** **Female**	4.19±0.031 4.23±0.051	2.75*±0.030 2.90*±0.075	1.46*±0.015 1.96*±0.047	0.90**±0.021 0.93**±0.012
Catalase	**Male** **Female**	3.25±0.017 3.18±0.017	3.01* ± 0.150 2.99*±0.015	1.53**± 0.032 1.38**±0.032	0.87***±0.020 0.98 ***±0.020

: significant **: highly significant *: very highly significant*

Table 7: Temporal variation of MDA (nmol/g. tissue) and GSH (mg/mg wet tissue) and CAT (U/g) in liver homogenates of male and female rats recorded for the control group and the groups with different modes of exposure to EMFs. Each value represents the mean of 6 replicates ± SD. Significance at P<0.05.

	Gender	Groups			
		I Control	II Ringing Mode	III Vibrating Mode	IV Combined Mode
MDA	**Male** **Female**	0.70±0.020 0.75±0.051	1.09*±0.021 1.21*±0.021	2.24*±0.030 2.62*±0.032	3.96**±0.010 4.01**±0.091
GSH	**Male** **Female**	4.16±0.015 4.17±0.021	2.27*±0.027 2.32*±0.040	1.92*±0.020 1.97*±0.038	0.91**±0.01 0.96**±0.021
Catalase	**Male** **Female**	3.91±0.025 3.94±0.025	2.97*±0.010 2.94*±0.031	1.50**±0.021 1.45**±0.032	0.92***±0.02 0.91*** ±0.025

: significant **: highly significant *: very highly significant*

Determination of Mobile Phone Irradiation:

It is quite clear that the strength of EMFs radiation shown in Fig (3) for the vibrating mode was higher than those for the ringing mode in Fig (4). This confirms the experimental results reported in this study.

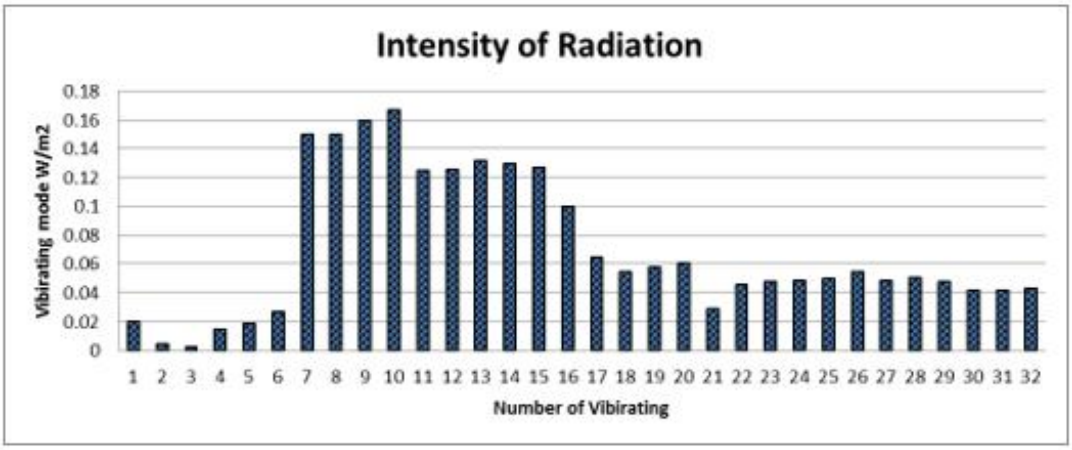

Fig. 3: Histogram showing temporal decrease of the EMF strength during mobile vibrating mode.

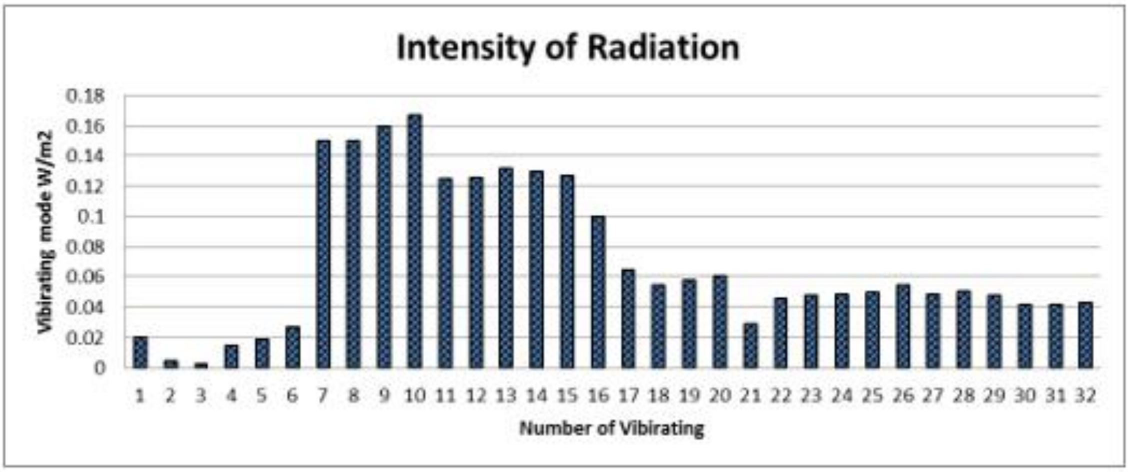

Fig. 4: Histogram showing temporal decrease of the EMF strength during mobile ringing mode.

Histopathological Examination:

Histopathological findings showed a strong correlation with the biochemical results. Microscopic examination of liver tissue from the control group revealed a normal histological architecture of the hepatic lobule (Fig. 5A). While rat liver tissues exposed to ringing mode showed leucocytes in hepatic sinusoids, distorted architecture and dilated sinusoids (Fig. 5B). Liver of rat exposed to vibrating mode showed sinusoidal leucocytosis (Fig 5C).

Sections of control brain (Fig 6A) showed no histopathological changes. The brain of rat exposed to ringing mode showed necrosis of neurons (Fig 6B) whereas, the brain of rat exposed to vibrating mode showing focal gliosis and neuronophagia of pyknotic neurons (Fig 6C).

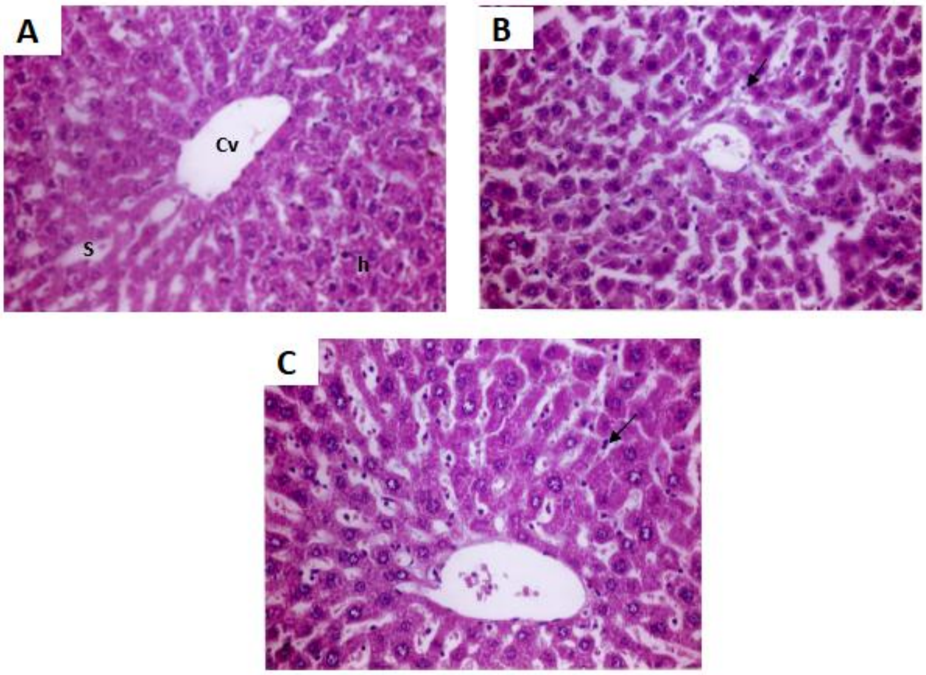

Fig. 5: A. The photomicrograph of liver section from the control group showing normal structure of the liver with normal hepatocytes (h), central vein (Cv), and hepatic sinusoids (s). **B**. The photomicrograph of liver section from rat exposed to ringing mode showing leucocytes in hepatic sinusoids (arrow). **C**. The photomicrograph of liver section from rat exposed to vibrating mode showing sinusoidal leucocytosis (arrow) (H & E x 400).

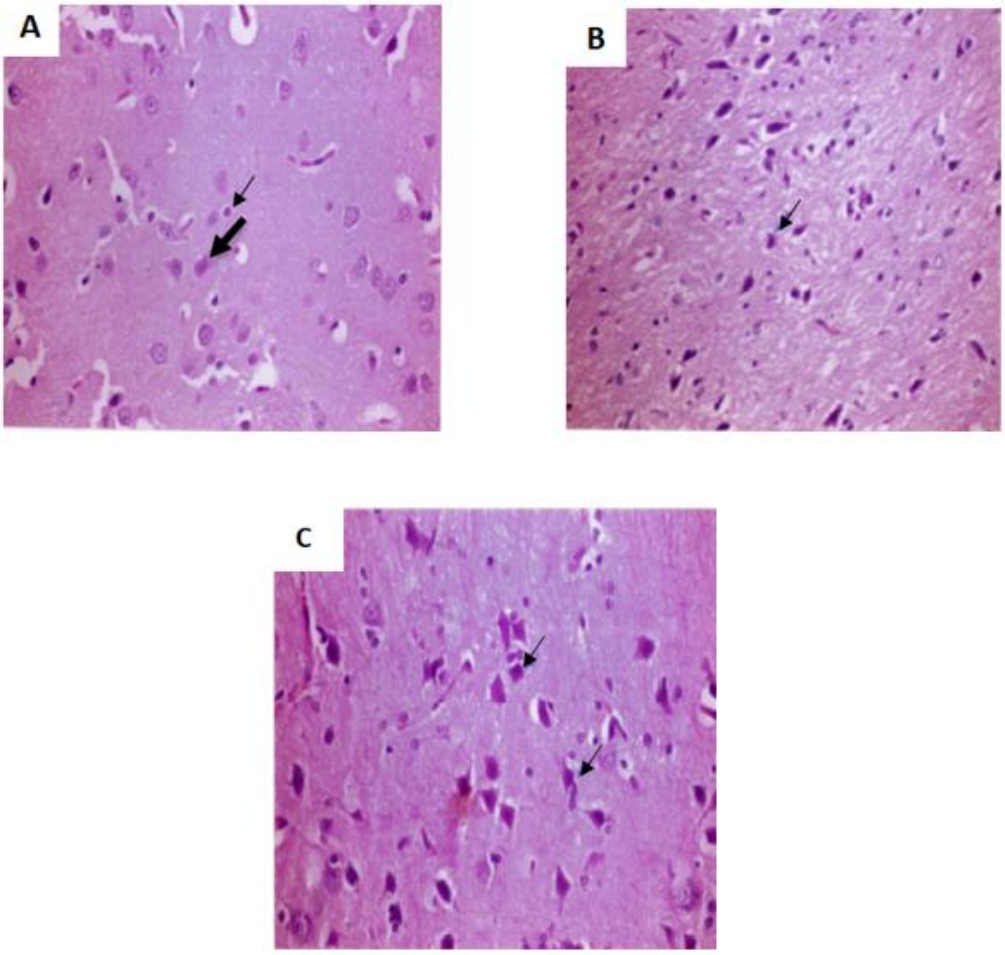

Fig. 6: A. The photomicrograph of brain section from the control group showing normal glial cell (thin arrow) and neuronal cell (thick arrow). **B**. The photomicrograph of brain section from rat exposed to ringing mode showing necrosis of neurons (arrow). **C**. The photomicrograph of brain section from rat exposed to vibrating mode showing focal gliosis and neuronophagia of pyknotic neurons (arrows) (H & E x 400).

DISCUSSION

Given the widespread utilization of mobile phones and their frequent proximity to the body, they are considered a primary source of EMFs exposure among the population. The human body can function as an efficient antenna, allowing electromagnetic radiation to be absorbed and distributed throughout various tissues. As a result, radiation emitted from mobile phones can penetrate living tissues and potentially affect biological systems at the cellular level (Sarookhani *et al*., 2011). The absorption of EMFs energy leads to a rise in tissue temperature, which is known as the thermal effect (Dilli, 2021). However, the majority of man-made wireless communication - EMFs exposures at environmentally relevant levels are non-thermal as they do not induce any tissue heating (Israel *et al*., 2013 and Wust *et al*., 2021). One of the key proposed mechanisms of EMFs action is the generation of reactive oxygen species (ROS), which has been documented in several studies (Houston *et al*., 2018; Li *et al*., 2018 and Yuan *et al*., 2020). EMFs- induced ROS initiates a cascade of processes that deplete GSH, catalase, SOD, GST, GPx, and subsequently increase lipid peroxidation (Jagetia, 2022 and Salameh *et al*., 2022).

Our findings indicated that exposure to EMFs for 12 weeks in ringing and vibrating modes led to suppressed antioxidant enzyme function and an elevation in lipid peroxidation, thereby intensifying oxidative stress in the liver and brain tissues of male and female rats. Furthermore, analysis revealed that serum levels of protein and transaminases (AST and ALT) spiked in response to EMFs exposure indicating liver injury.

In our experiment, we also noticed a decline in body weight after exposure to electromagnetic fields (EMFs). This reduction in weight gain, relative to the unexposed control group, might be linked to stress in the rats, which could lead to decreased appetite and reduced food intake as a result of EMFs exposure from mobile phones. Some researchers have suggested that electromagnetic radiation (EMR) acts as a mild stressor in organisms (Quesnel-Galvan *et al.,* 2021), and it is well-documented that chronic stress can lead to weight loss in animals (Willner, 2017 and Vieira *et al.,* 2018).

Our results align with earlier research that has demonstrated a link between EMFs exposure and reduced body weight in rats. For instance, Kumlin *et al.* (2007) and Adaramoye *et al*. (2012) reported that whole-body exposure to EMFs significantly reduced weight gain in exposed animals. Supporting this, Shahryar *et al*. (2009) found that EMFs exposure can enhance fat breakdown and glycolysis, leading to increased metabolic activity and body temperature. Ilhan (2004) also showed that exposure to 900 MHz microwave frequencies (used in mobile phone) resulted in weight loss in rats. More recently, Bektas *et al*. (2022) observed alterations in energy metabolism and appetite in rats exposed to 3.5 GHz radiofrequency radiation. Based on our findings, EMFs exposure may induce oxidative stress and suppress the body's antioxidant defence mechanisms, ultimately contributing to reduced body weight in exposed animals. Also, it was evident that control rats exhibited a steady gain in body weight over time, consistent with normal growth patterns. Chronic exposure to a 900 MHz RF-EMF for 280 days has been shown to induce weight variations in adult rodents, as reported by Sommer *et al.* (2004). Similarly, Usikalu *et al*. (2010) found a steady increase in body weight among both control and exposed rats subjected to 900 MHz radiofrequency (RF) radiation from a digital mobile phone (4 and 8 hours per day) for two months. However, they noted a slight decline in weight gain in the exposed group during weeks 5 to 8. Gerardi *et al.* (2008) also reported an overall increase in body weight in rats following long-term EMR exposure, a finding supported by Hasan *et al*. (2021), who suggested that extended exposure may lead to metabolic adaptations.

Our results indicate that male and female rats respond differently to EMFs exposure, with female rats appearing more sensitive than males. This heightened sensitivity may be linked to a greater vulnerability to chronic mild stress. Female rats were found to be more vulnerable to chronic mild stress as previously reported by Mahdavi *et al.* (2014). Similarly, research by Yenilmez (2022) demonstrated that body weight in birds was influenced by sex following EMF exposure. Another study has also emphasized that the biological responses of animals to electromagnetic radiation (EMR) are affected by sex (Sirva and Seyham, 2011), likely due to significant hormonal differences between males and females.

Enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are well-established biomarkers of hepatocellular function (Traesel *et al*., 2016). These cytosolic enzymes are normally confined within hepatocytes, and their elevation in serum indicates increased membrane permeability, which is frequently related to cellular injury or death. A previous study has reported that exposure to electromagnetic fields (EMFs) can lead to liver cell damage, likely mediated by enhanced oxidative stress within liver tissues (Fahmy and Mohammed, 2021). Similarly, in the current investigation, a significant increase in AST and ALT activity was observed in both male and female rats, indicating hepatocellular damage induced by EMFs exposure. These findings are consistent with those reported by Pooladi *et al*. (2018), Tawfik *et al*. (2018) and Hassan and Islam (2020). Supporting evidence also comes from Ozman and Kavrik (2020), who documented elevated liver enzyme levels in rats exposed to 2.45 GHz Wi-Fi radiation for one hour every day over a period of 30 days.

The mechanism underlying these changes involves increased liver cell membrane permeability, allowing intracellular enzymes to leak into the sinusoids and subsequently into systemic circulation (Tawfik *et al*., 2018). These alterations suggest that mobile radiation can disrupt the liver's antioxidative balance, leading to enzyme elevation as a marker of liver damage. Our results showed that more damage is increased as a result of increasing the number of exposure times. So, the use of mobile phone must be done under caution. Interestingly, no significant sex-dependent differences were observed in ALT or AST activities, suggesting that the hepatocellular response to EMFs may not differ substantially between male and female rats under the conditions tested.

Concerning the impacts of EMFs on serum protein levels, the current investigation showed that exposure to EMFs led to the rise in both total serum protein and albumin concentrations. These findings are consistent with those of Moussa (2009), who reported similar increases in male albino rats after 4 and 8 weeks of microwave radiation exposure, as well as with results from Hashem and El-Sharkawy (2009) who recorded elevated serum total protein levels in mice following exposure to low-frequency electromagnetic fields (4 h/day for 30 days). Additionally, Dong *et al*. (2022) observed elevated protein levels in healthy dogs following 10 weeks of EMFs exposure.

This EMFs-induced hyperproteinaemia may be referred to alterations in protein metabolism triggered by physiological stress. Electromagnetic fields have been reported to interfere with protein phosphorylation, alter the structure of the plasma membrane, and disrupt the initiation of intracellular signalling pathways responsible for protein synthesis (Hashem and El-Sharkawy, 2009). These molecular changes may collectively contribute to the observed increase in serum protein concentrations.

Contrary to our findings, Boguslaw and co-workers have detected a significant reduction in total protein levels. For instance, steel workers exposed to EMFs (50 Hz frequency, average of 6.8 hours/day for 5 days) exhibited lower levels of total protein (Boguslaw *et al*., 1999).

Previous studies have reported that exposure to electromagnetic fields (EMFs) enhances oxidative stress in liver and brain tissues (Fahmy and Mohammed, 2021 and Salameh *et al*., 2022). In the present study, a significant elevation in lipid peroxidation levels was detected in the liver and brain homogenates of both male and female rats subjected to EMFs. This increase displayed a time-dependent increase linked to exposure duration, suggesting that prolonged EMFs exposure—such as that from mobile phones—could trigger tissue damage through oxidative stress and lipid peroxidation. These findings are in agreement with other study by Shedid *et al*. (2019).

Reactive oxygen species (ROS) have been shown to initiate peroxidation of unsaturated fatty acids present in cell membranes. Malondialdehyde (MDA), a stable byproduct of this lipid peroxidation mechanism is widely recognized as an indicator to assess oxidative stress levels in biological tissues. In our study, the observed increase in MDA levels was joined by a significant decrease in glutathione (GSH), an essential intracellular antioxidant. This depletion of GSH likely reflects its consumption in detoxifying excessive ROS generated during EMFs exposure. GSH plays a critical role as a protective cellular mechanism against oxidative injury. Reduced GSH levels render the animals more sensitive to oxidative stress, especially damage triggered by ROS-driven lipid peroxidation. Previous study has linked physiological stress with reduced GSH concentrations in brain tissue (Quesnel-Galvan *et al*., 2021). Our findings are supported by Saikhedkar *et al*. (2014), who reported increased MDA levels in the liver and brain, and a significant reduction in brain GSH in male rats exposed to mobile phone radiation. Interestingly, the same research described only a slight increase in liver GSH levels compared to controls, suggesting tissue-specific responses. Consistent with our observations, Salameh *et al*. (2022) reported elevated hepatic MDA levels in animals exposed to EMFs, which may be attributed to a reduction in total hepatic antioxidants. These changes may result from EMFs effects on gene expression, protein synthesis, and enzyme activity, leading to increased ROS production and lipid peroxidation. Similarly, Alkis *et al*. (2021) found that exposure of Sprague Dawley rats to 1800 MHz GSM EMFs for 2 hours per day over 7 months significantly increased lipid peroxidation and total oxidants, while reducing total antioxidant capacity in the liver.

The mechanisms underlying EMFs-induced oxidative stress are complex. EMFs are believed to alter electric currents in biological tissues, disrupting chemical structures and impairing cellular functions. For instance, electric fields can exert oscillating forces on free ions across membranes, disturbing ion channels and causing membrane damage. These effects can disrupt biochemical signalling and ultimately impair liver and brain cell function (Schuermann and Mevissen, 2021). Further supporting this, Berkoz *et al.* (2018) demonstrated that chronic EMFs exposure leads to oxidative stress and damage in liver, brain, and renal tissues. Likewise, Moussa (2009) showed a dose-dependent increase in MDA and a concurrent decrease in GSH concentrations following exposure to microwave radiation (3–5 GHz), reinforcing the notion that prolonged EMFs exposure exacerbates oxidative damage.

Conversely, catalase (CAT) activity was significantly decreased in both liver and brain tissues of male and female rats exposed to EMFs emitted from mobile phones. Catalase, a crucial endogenous antioxidant enzyme, plays a vital role in cellular defense by catalyzing the decomposition of hydrogen peroxide into water and oxygen. The reduction in CAT activity observed in our study suggests an impaired antioxidant defense system in response to prolonged EMFs exposure and indicating oxidative stress-induced enzyme depletion. These findings are consistent with those of Saikhedkar *et al.* (2014), who also reported reduced catalase activity in the liver and brain tissues of male rats exposed to mobile phone radiation. Meral *et al.* (2007) also provided evidence supporting a decrease in CAT activity in the blood and in tissue homogenates of the heart and brain following exposure to GSM signals at 890–915 MHz for 12 hours per day over 30 days. The observed decline in CAT activity may indicate an overconsumption of the enzyme in response to elevated H_2O_2 levels, reflecting a weakened antioxidant defense system under EMFs exposure.

These findings collectively support the conclusion that chronic EMFs exposure can compromise antioxidant defence mechanisms, particularly by reducing catalase activity, and thereby contribute to oxidative damage in the brain and liver of male and female rats. The increase of oxidative species was noticed all over the experimental period and in a time- dependent manner.

Additionally, our results indicated a clear association between the mode of EMFs exposure and oxidative stress, likely mediated by disruption of the redox balance and leading to physiological disturbances. Our results further suggest that the degree of damage rises with the duration of exposure, supporting the hypothesis that EMFs-induced changes may initiate a cascade of biochemical disruptions. The observed reduction in antioxidant enzyme levels together with the concurrent elevation of ROS in the brain and liver point to oxidative damage as a primary mechanism of EMFs toxicity. Usman *et al.* (2020) proposed that radiation, vibration, and sound generated by GSM mobile phones can collectively contribute to this oxidative stress. Supporting this, Narayanan *et al*. (2009) found that mechanical stimulation from sound and vibration can excite cell receptors and impair the microtubule network between cells. Di Carlo *et al.* (2001) also reported that simultaneous exposure to vibration and electromagnetic fields (EMFs) has been shown to induce stress protein expression, thereby contributing to oxidative stress that may change biological functions, particularly in the brain. Interestingly, vibration had a greater influence when paired with sound (e.g., ringtone exposure), suggesting that mobile phone use in both vibration and sound modes may amplify oxidative stress. This synergistic effect has been observed by other researchers as well. Shahin *et al*. (2017) demonstrated that long-term exposure to 1800 MHz mobile phone radiation led to varying degrees of biological impact depending on the operative mode dialling, receiving, or standby. These differences are likely related to variations in pulse patterns, signal fluctuations, and connectivity durations, all of which may influence the intensity of physiological responses.

Histopathological examination of liver tissues revealed findings that strongly correlate with the biochemical results obtained in the current study. In the control group, liver sections displayed a normal histological architecture. In contrast, liver tissues from rats exposed to EMFs exhibited notable pathological alterations, including lymphocytic infiltration, sinusoidal leucocytosis, dilated sinusoids, and architectural distortion—features indicative of hepatic injury. These observations align with previous report documenting the adverse effects of EMFs exposure on liver tissue (Adebayo *et al*., 2019). The increase in ALT activity observed in EMFs-exposed groups reflects hepatocellular damage, supporting the cytotoxic effects of radiation on liver cells, often associated with apoptosis and necrosis (Hashem and El-Sharkawy, 2009).

Prolonged EMFs exposure, particularly from mobile phones, may lead to progressive liver damage. Studies have shown that this damage intensifies over time (Forgacs *et al*., 2006 and Buckus *et al*., 2017). For instance, Meo *et al*. (2010) reported mild inflammatory infiltrates, consisting of lymphocytes and histiocytes in the portal tracts of Wistar albino rats subjected to long-term mobile phone radiation. Similarly, El-Bedwi *et al*. (2011) observed hepatocellular degeneration, necrosis, and inflammatory response characterized by cell infiltration in the portal areas after three months of radiation exposure. Further evidence from Adebayo *et al*. (2019) revealed pronounced histopathological alterations including dilated sinusoids, congested central veins, distorted hepatic architecture, and hepatocyte structural changes in both male and female albino rats exposed to 1800 MHz EMR.

These findings reinforce the conclusion that chronic EMFs exposure can induce significant hepatic damage, both at the biochemical and histological levels, with effects becoming more severe over time and with increased exposure duration.

Our histopathological examination of the brain further confirmed the oxidative injury triggered by electromagnetic radiation (EMR). Brain sections from control rats exhibited normal histological architecture. In contrast, sections from rats subjected to mobile phone radiation in ringing and vibrating modes showed signs of neurodegeneration. This was evident by the presence of neurons with darkly stained, pyknotic nuclei—hallmarks of cellular degeneration. These findings suggest that EMR exposure may play a role in neuronal injury, likely mediated by oxidative stress mechanisms.

This interpretation is consistent with prior studies linking excessive generating of reactive oxygen species (ROS) during EMR exposure to neuronal apoptosis. Hanafy *et al*. (2019) demonstrated that low-frequency magnetic field exposure (2 h/day for 45 days) significantly increased ROS levels and induced apoptosis in neural cells in mice. Similarly, Sahin *et al*. (2016) observed pyknotic neurons in the brains of rats exposed to 900 MHz EMR. Othman *et al*. (2017) also confirmed that radiofrequency radiation promotes neurodegeneration by increasing ROS, disrupting protein synthesis, and impairing neuronal metabolism. Further, Terzi *et al*. (2016) and Kandeel *et al*. (2017) found a reduction in neuron numbers in rats exposed to EMF levels comparable to those emitted by mobile phones. Awad and Hassan (2008) observed a significant positive correlation between EMR exposure duration and the number of dark neurons. These neurons, often interspersed among healthy cells, appeared shrunken and intensely stained indicative of irreversible neuronal damage. Structural brain damage may also result from increased blood–brain barrier (BBB) permeability. Salford *et al*. (2003) and Eberhardt *et al.* (2008) demonstrated that EMR exposure facilitates leakage of large plasma proteins like albumin into the brain parenchyma, leading to tissue injury. An increase in albumin levels has been recorded in our study. Additionally, Warille *et al*. (2017) and Sistani *et al*. (2019) discussed EMF-induced disruption of calcium homeostasis, implicating altered voltage-gated calcium channels as a potential contributor to neuronal dysfunction. Collectively, these findings reinforce the hypothesis that chronic EMR exposure can lead to significant neurohistological changes, likely through oxidative stress, calcium dysregulation, and compromised blood–brain barrier integrity.

To verify the emission of EMFs from different operational modes of the mobile phone used. This investigation assessed the intensity of the emitted radiofrequency waves (RFW). Our results revealed that the mobile phone in vibrating mode emitted a significantly higher intensity of RFW compared to the ringing mode. It should be emphasized that the mobile phone used in this study was a low-quality, Chinese-manufactured device, likely produced without strict adherence to international safety standards. This may explain why the measured radiation levels considerably exceeded the internationally accepted safe exposure limit of 0.0047 W/m^2. Such elevated emission levels pose a potential health risk, not only to users but also as demonstrated in this study to rats exposed under experimental conditions. The observed physiological and biochemical disturbances in exposed animals further support this conclusion. Additionally, elevated Specific Absorption Rate (SAR) values have been associated with increased free radical formation, contributing to oxidative stress and tissue damage (Ammari *et al*., 2008). These findings emphasize the potential dangers of using poorly regulated mobile devices, particularly when operated in high-emission modes such as vibration.

Conclusion

This study suggests that electromagnetic radiation (EMR) can significantly impact enzyme activity and disrupt various biochemical processes. The detected changes in oxidative stress indicators, antioxidant enzyme levels, and histopathological changes in liver and brain tissues highlight the potential for EMR to induce physiological disturbances, particularly with prolonged exposure. Overall, the outcomes highlight the significance of EMF-emitting devices and the need for continued investigation into their long-term biological effects.

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