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BIOCHEMICAL AND PATHOLOGICAL EFFECTS ON THE MALE RAT HEPATIC TISSUE AFTER EXPOSURE TO 900MHZ ELECTROMAGNETIC FIELD DURING ADOLESCENT PERIOD

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ABSTRACT. The aim of this study was to employ biochemical analysis and histopathological techniques to investigate the effects of the application of a 900-megahertz electromagnetic field during adolescence on the rat liver. 24 male Sprague Dawley rats aged 21 days were randomly divided into electromagnetic field, sham and control groups. No procedure was performed on the control group rats. Electromagnetic field group rats were exposed to a 900-megahertz electromagnetic field in an electromagnetic field application cage (1 hours/day, every day for 25 days). The sham group rats were placed in the same electromagnetic field cage without exposure to electromagnetic field. At the end of the experiments, the livers were removed. The livers were used for histopathological evaluation (light, electron and immunofluorescence microscopy) and biochemical analyses. Biochemical analyses revealed increased lipid peroxidation and glutathione values while catalase and superoxide dismutase values were decreased in electromagnetic field group. Electron microscopy

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evaluations identified necrotic hepatocytes with numerous cytoplasmic and mitochondrial vacuoles in electromagnetic field group sections. We also observed an enlarged endoplasmic reticulum and loss of mitochondrial matrix, in addition to the presence of vacuoles. Also, loss of inner and outer mitochondrial membrane integrity was observed. Light microscopy evaluations revealed sinusoidal capillary dilatation and degeneration in hepatocytes with cytoplasmic swelling, vacuolar degeneration and pyknotic nuclei in electromagnetic field group sections. Deterioration in the integrity of the collagen fibers was also present around the vena centralis. Additionally, electromagnetic field had no effect on body weight. Therefore, we conclude that continuous 900-megahertz electromagnetic field treatment may cause changes in oxidative stress biomarkers and the morphology of the adolescent rat liver.

1. INTRODUCTION

Electromagnetic fields (EMF) are emitted from many devices (such as TV, microwave ovens, and cell phones) used all around us. Increased cell phone usage suggests that these are the most important sources of exposure to EMF. Cell phones, initially used only for purposes of communication, now permit numerous other activities, such as watching TV, navigation, online shopping, recording, game playing, and performing calculations. This increased spectrum of their use, and the fact that they possess features that are attractive to all ages, mean that such phones affect all age groups. Research by the UK Office of Communications (OFCOM) indicates that children aged 12-15 years spend approximately 21 h a week online, and that their mobile phone usage exceeds 18 h per week. OFCOM figures for 2017 showed that 70% of adults and adolescents use mobile phones [1], which suggests that EMF exposure will therefore be very high in all age groups. This study is also significant in showing us that mobile phone usage is quite high in adolescence period. Previous studies have indicated that EMF has numerous harmful effects on biological tissues. The major sites, which is affected by the deleterious effects of EMF, are the central nervous system [2-9], testis [10] and heart [11]. Furthermore, concentration and sleep disorders, fatigue and warming ear are common outcomes of exposure to EMF [12]. Other studies have reported no effects of EMF on, for instance, locomotor memory, learning and behavior [9], hematograms and blood chemistry [13].

Other studies have investigated the effects of EMF applied at varying doses in terms of histopathological analysis. Light microscopy studies have reported inflammatory changes in hepatic tissue [14], hyperemia, and dilatation in hepatic sinusoids and small inflammatory foci in lobule centers [15-17]. Holoslovska et al. employed electron microscopy and reported varying shapes and sizes of hepatocyte

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vesicles, lipid droplets and smooth endoplasmic reticulum proliferation, and that some hepatocytes were necrotic [15].

Oxidative stress occurs in several tissues for endogenous and exogenous reasons, and gives rise to oxidative injury [18]. Biochemical changes can trigger intracellular ionic changes and lead to DNA damage [19]. Studies have shown that EMF applied at 900 MHz has been reported to cause oxidative stress by suppressing antioxidant mechanisms in liver tissue [20]. Another study reported that EMF applied at 900 MHz and 1800 MHz caused oxidative stress in liver tissue in growing rat pups [21].

Cell phone operators function at a frequency of 800-900-1800-2100-2600 MHz. A frequency of 900 MHz has most commonly been employed in studies [2, 3, 9, 22]. The adolescence in rats has been classified under three sub-periods in the previous literature, early adolescence (prepubertal animals, 21-34 days), mid-adolescence (peri-adolescence, 34-46 days), and late adolescence (early adulthood, 46-59 days) [23]. Due to the increase in mobile EMF exposure in adolescent period, the effects of this exposure on health should be continued to be investigated. Although there have been studies investigating the effects of cell phones on the liver, those involving adolescents are still to some extent insufficient. In light of all these information, the purpose of this study was to determine the histopathology and the oxidative stress effects on liver tissue due to 900 MHz EMF applied in adolescence in a rat model.

2. MATERIALS AND METHODS

2.1. Laboratory conditions and Ethics

Approval for the study was granted by the Karadeniz Technical University (KTU) Animal Experiments Local Ethical Committee (Date: 19.06.2014, Protocol Number: 2014/30). Animals were held at a mean temperature of 22 ± 2 °C and humidity of $50\% \pm 5$ by an automated system, in an automatically adjusted 12-h light and dark cycle. Standard rat palette chow (BayramoğluYem and Un Sanayi Tic. A.Ş., Erzurum, Turkey) and drinking water were used. Regular feed and water were checked daily. No visible difference was observed.

2.2. Animals and experimental design

The study began with 24 male Sprague Dawley rats aged twenty-one-day. Rats were randomly assigned into three equal groups of, control (C-G), sham (S-G) and electromagnetic field (EMF-G). No procedure was performed on C-G. Standard

Type III rat cages were used to house the rats. Animals of the same group (n = 8) were housed in a cage. Animals were able to reach feed and water for 24 hours and were constantly monitored. Animals were moved to the EMF room in their cages. For EMF application, they were taken to the EMF application cage. After the procedure, they were taken back to the living place with the same cage. S-G rats were placed in an EMF cage for 1 h a day [9, 24, 25] at the same time each day (11-12 am) throughout the experiment, but were not actually exposed to EMF. Rats from EMF-G were placed inside the EMF cage for 1 h daily throughout the study, at the same time each day (10-11 am), and were exposed to 900 MHz EMF during that time (for 25 days). EMF was applied during adolescence (21-46 days). Analyses were performed in late adolescence (early adulthood, 46-59 days). EMF was not applied in late adolescence, due to representing entry into adulthood.

2.3. Body weight analyses

Rats in all groups were regularly weighed using a digital sensitive scale (KERN & SOHN GmbH, D-72336 Balingen, Germany) every five days. The scale was calibrated before each measurement.

2.4. Electromagnetic Field application system

EMF application system was installed and applied as described in [9, 24, 25]. Intensity of electrical field was also measured using the same device (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France) with a range of 100 kHz-2.5 GHz every day during. The measurements were performed at nine different points inside the EMF-application cage, with rats inside the cage (Figure 1). Measurements were performed at 3 time points (0 min, 30 min and 60 min).



FIGURE 1.Dimensions of the Plexiglas cage used for EMF application and the positions of the nine points used to measure EMF intensity on the Plexiglas cage floor (Keleş et al., 2019).

SAR value is calculated by many methods [4, 5, 26] Special programs are applied to calculate the SAR value. In the calculation of SAR for 900MHz in these programs, 0.01 W / kg for small rats, 0.009 W / kg for medium-sized rats, and 0.006 W / kg for large rats. We have calculated SAR values with this program (RadHaz SAR Equivalence Calculator Version 1.0, \bigcirc 2000 Richard Tell Associates, Inc., Mesquite, NV, USA) [7-9, 24]

2.5. Histological Procedures

Animals used in the experiment were sacrificed by cervical dislocation. Liver tissues were removed and divided into three parts. One part was placed in 10% formaldehyde and embedded in paraffin blocks following routine preparatory procedures. The second part was placed in 100 mM phosphate buffer containing 2.5% glutaraldehyde and 1% osmium tetroxide was fixed and routine electron microscopy procedures were performed. The third part was stored at -80° C in Eppendorf tubes for biochemical analyses.

2.5.1. Light Microscopy Analysis Procedure

Liver tissues were blocked, and 5-µm thick sections were taken with a microtome (Thermo Scientific Shandon Finesse 325 microtome, United Kingdom). The sections were then stained with Masson's trichrome, periodic acid-Schiff (PAS), hematoxylin and eosin (H&E) for histopathological evaluation. Light microscope (BX53; Olympus Optical Co. Ltd, Tokyo, Japan) and a camera for microscope (DP 72, Olympus Optical Co. Ltd., Tokyo, Japan) was used for histopathological examination.

2.5.2. Transmission electron microscopy procedures

The liver tissue samples were post-fixed in 1% osmium tetroxide (Merck Darmstadt, Germany), dehydrated in an ascending degrees of alcohol series, and finally embedded in epoxy resin (Araldite CY212) with an appropriate kit (Agar Scientific, Essex, United Kingdom). For electron microscopy analysis, ultra-thin sections of 40-70 nm in thickness were cut using an ultramicrotome (LKB Nova, Sweden). Sections were taken on 200-mesh nickel grids and stained with uranyl acetate and Reynold's lead citrate. Images were prepared on a JEOL SX1010 transmission electron microscope (JEOL Ltd., Akishima, Tokyo, Japan) for photographic image capture (Kodak 4489, Eastman Kodak Company, Rochester, NY, USA) [7].

2.6. Immunofluorescence staining

In order to evaluate the nuclear organization of the chromatin, the sections were immunofluorescently stained with a DNA-binding dye DAPI (4',6-diamidino-2-

phenylindole). The sections were deparaffinized through standard procedures. Next, heat induced antigen retrieval was carried out in 10 mM Sodium Citrate buffer (pH 6.0) for 10 minutes at 95°C. Sections were permeabilized in 0.4% Triton X-100 in TBS for 20 minutes and blocked with 4% BSA-TBS-Triton X for 1 hour at room temperature. After washing the sections 30 minutes in TBS, the sections were treated with Fluoroshield mounting medium containing DAPI (Abcam), sealed and stored at 4°C in the dark. Microscopic analyses were performed using a microscope (Olympus, BX53, Japan) equipped with a digital camera (DP 80, Olympus, Japan) and Cellsens standard program (version1.17) at different magnifications.

2.7. Biochemical procedures

Levels of superoxide dismutase (SOD) and catalase (CAT) enzyme activities and levels of glutathione (GSH) and lipid peroxidation (LPO) were determined by biochemical liver tissue analyses. Biochemical assay procedures were performed as described by Keleş et al. [9].

2.8. Statistical analyses

Statistical analyses were performed on SPSS 22.0 (USA) software. One-way ANOVA/ Duncan's test was used for data analysis.

3. Results

3.1. Body weight findings

The body weight of the rats was measured regularly the until last day of the experiment day. The mean body weight calculated according to the measurements taken every five days is shown in Table 1, which indicated that the body weight was increased in all groups. However, this time-dependent increase was not statistically significant between C-G and EMF-G (p = 0.732) and S-G and EMF-G (p = 1.000) (p > 0.05) (These findings is a part of Keleş et al. [24]).

TABLE 1.Body weight averages results of groups

	6 1
Groups	Mean body weight
Control (n=8)	$111 \pm 52.6 \text{ g}$
Sham (n=8)	118.5 ± 57.5 g
EMF (n=8)	118.7 ± 57.8 g

Values are expressed as mean \pm SEM.; EMF, electromagnetic field

3.2. Light microscopic findings

Histopathological analyses were performed in all three staining (H&E, Masson's trichrome and PAS). H&E-stained liver tissue sections from C-G and S-G sections exhibited a normal appearance. However, analysis of liver sections from EMF-G revealed dilatation in sinusoid extansion. In addition, we observed vacuolar degeneration and pyknotic nuclei in hepatocyte cytoplasm (Figure 2-3). No pathology was observed at Masson's trichrome staining in C-G and S-G sections. EMF-G sections showed deterioration in the integrity of collagen fibers around vena centralis but Glisson capsule was normal. Also, sections from the EMF-G animals exhibited vascular congestion (Figure 4). Sections from liver tissues stained with PAS appeared to be normal in C-G and S-G, while vacuolization in hepatocyte cytoplasm was present in EMF-G. In addition, some hepatocytes were stained with PAS, while others were not (Figure 5) (Table 2).

TABLE 2. Histopathological evaluation results of groups underlight microscope.

	GROUPS		
PATHOLOGIES	Control (n=8)	Sham(n=8)	ElectromagneticField(
FEATURES			n=8)
Pycnoticnucleus in	Yes	Yes	Yes
hepatocytes	(Oneanimal)	(Oneanimals)	(Eightanimals)
Edema in hepatocytes,	Yes	Yes	Yes
vacuolardegeneration in	(Oneanimal)	(Twoanimal)	(Seven animals)
cytoplasm			
Sinosoidal expansion	Yes	Yes	Yes
	(Twoanimal)	(Oneanimal)	(Eightanimals)
Vacuolization in	No	Yes	Yes
hepatocytecytoplasm	(Twoanimal)	(Twoanimal)	(Seven animals)
PAS (+) staining in	No	No	Yes
hepatocytes			(Sixanimals)
Vascularcongestion	Yes	Yes	Yes
-	Twoanimal	Twoanimal	Seven animal





FIGURE 2.Light microscopic micrographs of rat liver sections stained with hematoxylin and eosin from the control, sham and EMF groups (X10). Liver tissue sections control and sham a normal appearance. Analysis of sections electromagnetic field group revealed (\rightarrow) pyknotic nuclei, (*) vascular congestion and the collagen fibers around the vena centralis (\blacktriangleright) is impaired.

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FIGURE 3.Light microscopic micrographs of rat liver sections stained with hematoxylin and eosin from the Electromagnetic field groups; (A) (x10), (B and C) (x60). Analysis of sections B and C revealed (\rightarrow) pyknotic nuclei, (\blacktriangleright) edema in hepatocytes, vacuolar degeneration in cytoplasm and (*) vascular congestion.



FIGURE 4.Light microscopic micrographs of rat liver sections stained with Masson's trichrome from control (A) (X20), sham (B) (X20), and EMF (C) (X60) and EMF group (D) (X20). Liver tissue sections A and B exhibit a normal appearance the integrity of the collagen fibers around the vena centralis. However, the integrity of the collagen fibers around the vena centralis C. Liver section (D) (\rightarrow) Glisson's capsule exhibit a normal appearance and (\blacktriangleright) vascular congestion.

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FIGURE 5.Light microscopic micrographs of rat liver sections stained with PAS from the control (A), sham (B), and EMF (C, D) groups (X60). Liver tissue in sections A and B exhibits a normal appearance. On sections C and D, however, vacuolization can be seen in hepatocyte cytoplasm (\rightarrow), while some hepatocytes stained PAS (+) but no staining was observed in others.

3.3. Transmission electron microscopic findings

Under transmission electron microscopy, C-G and S-G sections exhibited typical regular nuclear and cytoplasmic features together with normal mitochondrial cristae. Typical granular endoplasmic reticulum and regular hepatocytes and sinusoids were also observed. In contrast, EMF-G sections exhibited necrotic hepatocytes with many cytoplasmic and mitochondrial vacuoles. Furthermore, we detected enlarged endoplasmic reticulum and loss of mitochondrial matrix, together with the presence of vacuoles. Also, loss of inner and outer mitochondrial membrane integrity was observed (Figure 6).



FIGURE 6.Electron micrograph showing liver ultrastucture in control (A), sham (B) and EMF (C, D) groups. (A) Electron micrograph showing regular nuclear (N) and cytoplasmic ultrastructure (arrowhead). Typical endoplasmic reticulum (arrow) and mitochondrial cristae (arrowhead) were observed. Mitochondria (m). Erythrocyte x 10.000. (B) Electron micrographs showing typical nuclear (N) and cytoplasmic ultrastructure in the sham group. Typical endoplasmic reticulum (arrow) and mitochondrial cristae (arrowhead) were observed. Mitochondria (m). Erythrocyte x 10.000. (B) Electron micrographs showing typical nuclear (N) and cytoplasmic ultrastructure in the sham group. Typical endoplasmic reticulum (arrow) and mitochondrial cristae (arrowhead) were observed. Mitochondria (m). Erythrocyte x8000. (C and D) Electron micrographs showing irregular nuclear (n) and cytoplasmic ultrastructure to necrotic hepatocytes. Hepatocytes exhibited numerous cytoplasmic (v) and mitochondrial (m) vacuoles (arrowhead). Enlarged endoplasmic reticulum (arrow) was also observed. C (X6000) and D (X 15.000).

3.4. Immunofluorescence staining findings

Since we detected morphological disruptions within the EMF-G sections, we wondered whether the nuclear organization of the chromatin was affected as well. For this reason, we performed immunofluorescence staining using DNA-intercalating dye DAPI, which identified mostly interphase cells with regularly

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FIGURE 7.Nuclear organization of the chromatin in the control, sham and EMF group liver tissues at x10, x20, x40 (DAPI). DAPI-rich regions marked by arrowheads (\blacktriangleright) indicate heterochromatin foci.

3.5. Findings on biochemical parameters

SOD, CAT, LPO, and GSH values within C-G, S-G, and EMF-G tissues was shown in Table 2. There were no statistically significant differences in hepatic tissue SOD and LPO values between C-G and S-G (p>0.05). On the contrary, liver tissue CAT values were significantly decreased in S-G compared to C-G (p < 0.05). Also, S-G liver tissue GSH values showed a statistically significant increase in comparison to C-G (p < 0.05). EMG-G biochemical analyses pointed out that GSH and LPO values were increased while SOD and CAT levels were decreased (Table 3) (p < 0.05).

	Groups				
Biochemical parameters	Control (n=8)	Sham (n=8)	EMF (n=8)		
Lipid peroxidation (nmol/mg tissue)	$8.21{\pm}0.34^a$	$9.9\pm0.85^{\rm a}$	12.64±0.47 ^b		
Catalase (µmol/min/mg tissue)	51.85 ± 1.8^{a}	$44.5\pm1.65^{\text{ b}}$	36.75±2.5 ^c		
Superoxide dismutase (mmol/min/mg tissue)	8.06 ± 0.19^{a}	7.64 ± 0.12^{a}	6.44 ±0.15 ^b		
Glutathione (nmol/mg tissue)	2.7 ± 0.05^{a}	$3.22\pm0.04~^{b}$	3.75±0.05 ^c		

TABLE	3.Biochemical	analysis	results	from	liver	tissues
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EMF-G: Electromagnetic field group, *a,b,c*: Different super script letters denote significant differences between the groups.

3.6. Findings on the Electromagnetic Field measurements

EMF measurements were taken from the inside of the EMF-application cage while the rats were inside (Figure 8). The results of our study showed that the rats were exposed to a mean EMF density of 8.8V/m (0.242 W/m2). This value is mean 1–10 V/m for variables such as location, mobile phone model, distance from base station and similar. The specific absorption rate (SAR) was calculated at 0.012 W/kg (RadHaz SAR Equivalence Calculator Version 1.0, © 2000 Richard Tell Associates, Inc., Mesquite, NV, USA).

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FIGURE 8.Geometric mean values of EMF intensity measurement for the rats inside the cages (0, 30, 60 minutes).

4. Discussion

Previous studies have shown that EMF affects many tissues such as spleen [25] liver, brain [24], cerebellum [6, 8], spinal cord [4, 7], and vertebra [27]. Our analyses revealed that 900 EMF may affect liver tissue adversely. Our showed that 900 MHz EMF expose may cause changes in oxidative stress biomarkers and the morphology of the adolescent rat liver.

Body weight is serious indicator of an animal's health status. Previous research has revealed that EMF (50Hz) exposure had no effect on body weight or liver weight [28]. Similarly, Margonato et al. [29] observed no change in body weight of rats exposed to 50 Hz and 5 T EMF for 32 weeks. In line with these previous findings, our data show that 900 MHz exposure of rats to EMF caused no effected in body weight.

Kim et al. [30] conducted a study in which they applied an intermediate frequency of 20 kHz at 6.25 microTrms for 20 days in a carousel radiator. Their results showed that EMF had no effect on urine (ketone bodies, protein, bilirubin, specific gravity, pH, urobilinogen and serum glucose) and blood parameters (hemoglobin, leucocyte count, mean corpuscular mean corpuscular hemoglobin concentration, hemoglobin, hematocrit, thrombocyte count and mean corpuscular volume, glucose, blood urea nitrogen, total protein, creatinine, total cholesterol, alkaline phosphatase, total bilirubin, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase). They also concluded that EMF had no histopathological effect for organs such as lung, spleen, heart, kidney, testis, ovary, liver and brain.

Previous studies have reported that EMF causes deleterious effects in the liver tissue [14, 15, 17, 20]. Histopathological examinations have reported that exposure to 900 MHz can lead to inflammatory changes in the liver and damage to the Langerhans islets in particular [14]. In another study, Holovska et al. [15] applied 2.45 GHz EMF and reported moderate hyperemia, dilatation of hepatic sinusoids and small inflammatory foci in hepatic lobules, together with changes in hepatocyte structure. Additional studies reported localized inflammatory cell infiltration and consequent apoptosis in the liver even at EMF frequency as low as EMF 50 MHz [16] and regional hepatocyte fragmentation and moderate portal inflammation with 1 mT EMF [17]. In our study, examination of EMF-G tissues under the light microscope exhibited vasculer congestion and sinosoidal expansion. In addition, we identified vacuolar degeneration and pyknotic nuclei in the hepatocyte cytoplasm. Furthermore, some hepatocytes were stained with PAS, while others were not. Impaired integrity of collagen fibers around the vena centraliswas also observed. However, we did not identify any major alteration to the nuclear organization of the chromatin.

Electron microscopy examinations of the liver tissue exposed to 2.45 GHz EMF revealed vesicles of different shapes and sizes, lipid droplets and smooth endoplasmic reticulum proliferation, and occasional necrotic hepatocytes [15]. Topal et al. [31] reported dilatation in endoplasmic reticulum, necrotic hepatocytes and vacuolization in mitochondria in the postnatal period of rats exposed to 900 EMF in the prenatal period. A different study on adolescent rat liver showed that EMF group exhibited occasional loss of the typical radial arrangement of hepatocytes, sinusoid expansion, edema, vascular congestion and cytoplasmic vacuolization [32]. In our work, C-G and S-G liver sections, however, exhibited several cytoplasmic and mitochondrial vacuoles and necrotic hepatocytes. Furthermore, we observed endoplasmic reticulum dilatation and mitochondrial matrix loss. The integrity of the internal and external mitochondrial membranes

was also compromised. Although all these results were similar to previous studies on rats [15, 16, 30, 32] no such study was conducted in adolescence.

MDA is one of the products of lipid peroxidation and is widely used as a mark of oxidative stress occurring in tissues [33]. When oxidative metabolism significantly increases, blood supply of the tissues decrease, production of free radicals accelerates and antioxidant defense systems fail [34]. EMF cause oxidative stress both at tissue and cellular levels [31]. The EMF-G male rat biochemical analysis results showed that GSH and LPO values increased while SOD and CAT values decreased. High LPO values are normally indicative of oxidative stress in hepatic tissue. Studies have reported that exposure to EMF causes oxidative stress in the liver [19, 19, 20, 21] while 50 Hz MFs had no significant effect on the majority of blood biochemical parameters [35]. The same researchers reported that EMF had an effect on creatinine and cholesterol blood levels. However, this change was minimal and was not clinically significant [35]. Another finding of our study on the S-G biochemical analyses showed that GSH values increased while CAT levels decreased, which suggests that The S-G group may have oxidative stress due to stress. Similar to our results, Gözen et al. [36] reported that oxidative stress occurs in S-G groups in the liver.

The antioxidant enzymes SOD, CAT and GSH are components of the cellular defense system [37]. In our study, SOD and CAT values decreased in EMF-G, while GSH levels were high. Similar findings were reported for SOD [38]. and CAT [39] levels. Experimental animal studies were determined different effects of EMF on GSH levels, depending on the length of the treatment. In particular, glucose-6-phosphate dehydrogenase (G6PD) has been reported to be induced as a result of GSH synthesis adaptation following long-term exposure [40]. Studies using chronic methods have observed an increase in cardiac GSH levels, while acute studies have reported both increased and decreased GSH levels [41]. Yilmaz et al. reported an increase in GSH levels in liver tissue [42].

Research has revealed that rates of cell phone use among young people and even children are increasing all the time [1]. This is a source of concern, since cell phones have been reported to have deleterious effects on biological tissues in numerous previous studies [10-12]. Although they are generally portrayed as harmless, the reality is rather different. The results of the present study, revealed morphological changes, and increased oxidative stress in liver tissue exposed to 900 MHz EMF during adolescence.

5. Conclusion

Our aim in this study was to investigate the histopathological and biochemical effects of 900 MHz EMF applied in adolescence on the rat liver. Our analyses revealed that EMF caused pathological changes, and oxidative stress in liver tissue. These findings are important in showing the effects of cell phone use in adolescence on the liver. We hope that the present study will be useful in the planning of larger studies in the future.

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References

- [1] OFCOM., Children and Parents: Media Use and Attitudes Report. Adults' Media Use and Attitudes Report. https://www.ofcom.org.uk/research-and-data/media-literacyresearch/childrens/children-parents-2017.https://www.ofcom.org.uk/research-anddata/media-literacy-research/adults/adults-media-use-and-attitudes (accessed: 22.11.2018).
- [2] Baş, O., Sönmez, F.O., Aslan, A., İkinci, A., Hancı, H., Yıldırım, M., Kaya, H., Akça, M., Odacı, E., Pyramidal cell loss in the cornu ammonis of 32-day-old female rats following exposure to a 900 Megahertz electromagnetic field during prenatal days 13-21, Neuroquantology, 4 (2013), 591-599.https://doi.org/10.14704/nq.2013.11.4.701.
- [3] İkinci, A., Odacı, E., Yıldırım, M., Kaya, H., Akça, M., Hancı, H., Aslan, A., Sönmez, F.O., Baş, O., The effects of prenatal exposure to a 900 Megahertz electromagnetic field on hippocampus morphology and learning behavior in rat pups, Neuroquantology, 4 (2013), 582-590.https://doi.org/10.14704/nq.2013.11.4.699.
- [4] Odacı, E., İkinci, A., Yıldırım, M., Kaya, H., Akça, M., Hancı, H., Sönmez, O.F., Aslan, A., Okuyan, M., Baş, O., The effects of 900 Megahertz electromagnetic field applied in the prenatal period on spinal cord morphology and motor behavior in female rat pups, Neuroquantology, 4 (2013), 573-581.https://doi.org/10.14704/nq.2013.11.4.698.
- [5] Şahin, A., Aslan, A., Baş, O., İkinci, A., Özyılmaz, C., Fikret, S.O., Çolakoğlu, S., Odacı, E., Deleterious impacts of a 900MHz electromagnetic field on hippocampal pyramidal neurons of 8-week-old Sprague Dawley male rats, Brain Research, 1624 (2015), 232-8. https://doi.org/10.1016/j.brainres.2015.07.042.
- [6] Odacı, E., Hancı, H., İkinci, A., Sönmez, O.F., Aslan, A., Şahin, A., Kaya, H., Çolakoglu, S., Baş, O., Maternal exposure to a continuous 900 MHz electromagnetic field provokes neuronal loss and pathological changes in cerebellum of 32-day-old

female rat offspring, Journal of Chemical Neuroanatomy, 75 (2016), 105-10. https://doi.org/10.1016/j.jchemneu.2015.09.002.

- [7] İkinci, A., Mercantepe, T., Unal, D., Erol, H.S., Şahin, A., Aslan, A., Baş, O., Erdem, H., Sönmez, O.F., Kaya, H., Odacı, E., Morphological and antioxidant impairments in the spinal cord of male offspring rats following exposure to a continuous 900-MHz electromagnetic field during early and mid-adolescence, Journal of Chemical Neuroanatomy, 75 (2016), 99–104. https://doi.org/10.1016/j.jchemneu.2015.11.006.
- [8] Aslan, A., İkinci, A., Baş, O., Sönmez, O.F., Kaya, H., Odacı, E., Long-term exposure to a continuous 900 MHz electromagnetic field disrupts cerebellar morphology in young adult male rats, Biotechnic & Histochemistry, 16 (2017), 1-7. https://doi.org/10.1080/10520295.2017.1310295.
- [9] Keleş, A.İ., Yıldırım, M., Gedikli, Ö., Çolakoğlu, S., Kaya, H., Baş, O., Sönmez, O.F., Odacı, E., The effects of a continuous 1-h a day 900-MHz electromagnetic field applied throughout early and mid-adolescence on hippocampus morphology and learning behavior in late adolescent male rats, Journal of Chemical Neuroanatomy, 94 (2018), 46-53. https://doi.org/10.1016/j.jchemneu.2018.08.006.
- [10] Hanci, H., Kerimoğlu, G., Mercantepe, T., Odaci, E., Changes in testicular morphology and oxidative stress biomarkers in 60-day-old Sprague Dawley rats following exposure to continuous 900-MHz electromagnetic field for 1 h a day throughout adolescence, Reproductive Toxicology, 81 (2018), 71-78.https://doi.org/10.1016/j.reprotox.2018.07.002.
- [11] Misek, J., Belyaev, I., Jakusova, V., Tonhajzerova, I., Barabas, J., Jakus, J., Heart rate variability affected by radiofrequency electromagnetic field in adolescent students, Bioelectromagnetics, 39 (2018), 277-288.https://doi.org/10.1002/bem.22115.
- [12] Durusoy, R., Hassoy, H., Özkurt, A., Karababa, A.O., Mobile phone use, school electromagnetic field levels and related symptoms: a cross-sectional survey among 2150 high school students in Izmir, Environmental Health, 16 (2017), 51. https://doi.org/10.1186/s12940-017-0257-x.
- [13] Lai, J., Zhang, Y., Zhang, J., Liu, X., Ruan, G., Chaugai, S., Tang, J., Wang, H., Chen, C., Wang, D.W., Effects of 100-μT extremely low frequency electromagnetic fields exposure on hematograms and blood chemistry in rats, Journal of Radiation Research, 57 (2016), 16-24.https://doi.org/10.1093/jrr/rrv059
- [14] Mortazavi, S.M., Owji, S.M., Shojaei-Fard, M.B., Ghader-Panah, M., Mortazavi, S.A., Tavakoli-Golpayegani, A., Haghani, M., Taeb, S., Shokrpour, N., Koohi, O., GSM 900 MHz microwave radiation-induced alterations of insulin level and histopathological changes of liver and pancreas in rat, Journal of Biomedical Physics & Engineering, 6 (2016), 235-24.
- [15] Holovska, K., Almasiova, V., Cigankova, V., Benova, K., Racekova, E., Martoncikova, M., Structural and ultrastructural study of rat inver influenced by electromagnetic radiation, Journal of Toxicology and Environmental Health, 78 (2015), 353-356. https://doi.org/10.1080/15287394.2014.979272.
- [16]Cevik, A., Aydin, M., Timurkaan, N., Apaydin, A.M., Yuksel, M., Pathological and immunohistochemical effects of electromagnetic fields on rat liver, Indian Journal of Animal Research, 51 (2017), 1134-1137. https://doi.org/ 10.18805/ijar.B-765
- [17]Semnani, V., Hasanzadeh, H., Darbandi-Azar, A., Seyyedi, S., Emadi, A., Rezaei-

Taviran, M., Does continuous extremely low-frequency electromagnetic fields exposure harms liver tissue? A Microscopic Assay, Middle East Journal of Rehabilitation and Health, 5 (2018), e63105.https://doi.org/10.5812/mejrh.63105

- [18]Uysal, N., Şişman, A.R., Gönenç, S., Acikgoz, O., Kayatekin, B.M., Yalaz, G., Effects of repeated maternal separation on oxidative stress in adolescent male and female rat brains, Journal of Neurological Sciences [Turkish], 25 (2008), 155-162.
- [19]Panagopoulos, D.J., Analyzing the health impacts of modern telecommunications microwaves, Advances in Medicine and Biology,17 (2011),1-55.
- [20] Esmekaya, M.A., Ozer, C., Seyhan, N., 900 MHz pulse-modulated radiofrequency radiation induces oxidative stress on heart, lung, testis and liver tissues, General physiology and biophysics, 30 (2011), 84-9.https://doi.org/10.4149/gpb20110184.
- [21] Cetin, H., Nazıroğlu, M., Celik, O., Yüksel, M., Pastacı, N., Ozkaya, M.O., Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring, Journal of Maternal-Fetal and Neonatal Medicine, 27 (2014), 1915-21.https://doi.org/10.3109/14767058.2014.898056.
- [22] Kivrak, E.G., Altunkaynak, B.Z., Alkan, I., Yurt, K.K., Kocaman, A., Onger, M.E., Effects of 900-MHz radiation on the hippocampus and cerebellum of adult rats and attenuation of such effects by folic acid and Boswellia sacra, Journal of microscopy and ultrastructure, 5 (2017), 216-224. https://doi.org/10.1016/j.jmau.2017.09.003.
- [23] Tirelli, E., Laviola, G., Adriani, W., Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents, Neuroscience and Biobehavioral Reviews, 27 (2003), 163-178. https://doi.org/10.1016/s0149-7634(03)00018-6.
- [24] Keleş, A.İ., Nyengaard, J.R., Odacı, E. (a), Changes in pyramidal and granular neuron numbers in the rat hippocampus 7 days after exposure to a continuous 900-MHz electromagnetic field during early and mid-adolescence, Journal of Chemical Neuroanatomy, 26 (2019), 101: 101681. https://doi.org/10.1016/j.jchemneu.2019.101681.
- [25] Keleş, A.İ., Sapmaz, T., Erol, H.S., Süt, B.B., Keleş, G., Odacı, E., Polat, S., Halıcı, M.B. (b), The effect of 900-megahertz electromagnetic field exposure in the first and middle adolescent period on the spleen in male rats: A biochemical and histopathological study, Duzce Medical Journal, 21 (2019), 192-196.https://doi.org/10.18678/dtfd.608616.
- [26] Usman AD, Wan Ahmad WF, Ab Kadir MZA, Mokhtar M., Wireless phones electromagnetic field radiation exposure assessment, American Journal of Engineering and Applied Sciences, 2 (2009), 771-774. https://doi.org/10.3844/ajeassp.2009.771.774.
- [27] Keleş Aİ (2020). Morphological changes in the vertebrae and central canal of rat pups born after exposure to the electromagnetic field of pregnant rats. Acta Histochemica, 122; 151652. https://doi.org/doi.org/10.1016/j.acthis.2020.151652.
- [28] Cakir, D.U., Yokus, B., Akdag, M.Z., Sert, C., Mete, N., Alterations of hematological variations in rats exposed to extremely low frequency magnetic fields (50 Hz),

Archives of Medical Research, 40 (2009), 352-6.https://doi.org/10.1016/j.arcmed.2009.07.001.

- [29] Margonato, V., Nicolini, P., Conti, R., Biologic effects of prolonged exposure to ELF electromagnetic fields in rats. II. 50 Hz magnetic fields, Bioelectromagnetics, 16 (1995), 343-355.https://doi.org/10.1002/bem.2250160602.
- [30] Kim, S.H., Lee, H.J., Choi, S.Y., Gimm, Y.M., Pack, J.K., Choi, H.D., Lee, Y.S., Toxicity bioassay in Sprague-Dawley rats exposed to 20 kHz triangular magnetic field for 90 days, Bioelectromagnetics, 27 (2006), 105-11.https://doi.org/10.1002/bem.20182.
- [31] Topal, Z., Hanci, H., Mercantepe, T., Erol, H.S., Keleş, O.N., The effects of prenatal long-duration exposure to 900-MHz electromagnetic field on the 21-day-old newborn male rat liver, Turkish Journal of Medical Sciences, 45 (2015), 291-7.https://doi.org/10.3906/sag-1404-168.
- [32] Okatan, D.Ö., Kulaber, A., Kerimoglu, G., Odacı, E., Altered morphology and biochemistry of the female rat liver following 900 megahertzelectromagnetic field exposure during mid to late adolescence, Biotechnic& Histochemistry, 24 (2019), 1-9.https://doi.org/10.1080/10520295.2019.1580767.
- [33] Ohkawa H, Ohishi H, Yagi K., Assay for lipid peroxide in animal tissues by thiobarbutiric acid reaction. Anal. Biochem. 95 (1979), 351-358.https://doi.org/10.1016/0003-2697(79)90738-3.
- [34] Cetinkunar, S., Tokgoz, S., Bilgin, B.C., Erdem, H., Aktimur, R., Can, S., Erol, H.S., Isgoren, A., Sozen, S., Polat, Y., The effect of silymarin on hepatic regeneration after partial hepatectomy: is silymarin effective in hepatic regeneration? International Journal of Clinical & Experimental Medicine, 8 (2015), 2578-2585.
- [35] Luo, X., Ma, L., Gao, P., Zhang, Y., Effects of subchronic extremely low-frequency electromagnetic field exposure on biochemical parameters in rats, Toxicology and Industrial Health, 33 (2017), 365-372.https://doi.org/10.1177/0748233716645479.
- [36] Gözen, H., Demirel, C., Akan, M., Tarakçıoğlu, M., Effects of pulsed electromagnetic fields on lipid peroxidation and antioxidant levels in blood and liver of diabetic rats, European Journal of Therapeutics, 23 (2017), 152-158.https://doi.org/10.5152/EurJTher.2017.139.
- [37] Urso, M.L., Clarkson, P.M., Oxidative stress, exercise, and antioxidant supplementation, Toxicology, 189 (2003), 41-54. https://doi.org/10.1016/S0300-483X(03)00151-3.
- [38] Martínez-Sámano, J., Torres-Durán, P.V., Juárez-Oropeza, M.A., Elías-Viñas, D., Verdugo-Díaz, L., Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: a preliminary report, International Journal of Radiation Biology, 86 (2010), 1088-94.https://doi.org/10.3109/09553002.2010.501841.
- [39] Koyu, A., Ozguner, F., Yilmaz, H., Uz, E., Cesur, G., Ozcelik, N., The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress in rat liverexposed to the 900 MHz electromagnetic field, Toxicology and Industrial Health, 25 (2009), 429-34.https://doi.org/10.1177/0748233709106821.
- [40] Salvemini, F., Franzé, A., Iervolino, A., Filosa, S., Salzano, S., Ursini, M.V., Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-

phosphate dehydrogenase expression, The Journal of Biological Chemistry, 274 (1999), 2750-7.https://doi.org/10.1074/jbc.274.5.2750.

- [41] Iliskovic, N., Li, T., Khaper, N., Palace, V., Singal, P.K., Modulation of adriamycininduced changes in serum free fatty acids, albumin and cardiac oxidative stress, Molecular and Cellular Biochemistry, 188 (1998), 161–166.
- [42] Yilmaz, A., Tumkaya, L., Akyildiz, K., Kalkan, Y., Bodur, A.F., Sargin, F., Efe, H., Uydu, H.A., Yazici, Z.A. Lasting hepatotoxic effects of prenatal mobile phone exposure, The Journal of Maternal-Fetal Medicine, 30 (2017), 1355-1359.https://doi.org/10.1080/14767058.2016.1214124.