



RESEARCH ARTICLE

Effects of Exposure to Electromagnetic Waves from 3G Mobile Phones on Oxidative Stress in Fetal Rats

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ABSTRACT

This study aimed to determine the effects of electromagnetic radiation from third-generation technology mobile phones on the incidence of oxidative stress in fetal rats. Exposure to radiation of mobile phones began after the rats had acclimatized for a week and begun copulation. The study consisted of control (no radiation from a mobile phone), treatment 1 (second-generation technology, standby mode), treatment 2 (second-generation technology, active mode), treatment 3 (third-generation technology, standby mode), and treatment 4 (third-generation technology, active mode), and the exposure continued until after birth. The level of reactive oxygen species (ROS) in liver and brain of the fetus and the level of ROS in liver of the mother were measured using a solution of nitro blue tetrazolium and N,N-dimethylformamide. Liver histology preparations were made for morphometric measurements using histological morphometry. The data were analyzed using one-way ANOVA and Tukey test, and a significance level of 5%. The results showed that exposure to electromagnetic waves from mobile phones in treatment 4 negatively affected the fetus in the form of increased levels of ROS in the liver and brain, as well as altered morphology in the liver.

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INTRODUCTION

Communications technology is developing rapidly, a notable example being the evolution from second-generation technology (2G) to third-generation technology (3G). The advantages of 3G technology are the ability to communicate using fax, email, and internet. The application of 3G technology is supported by the release of cell phone as the communication device. However, the development of mobile phones is accompanied by an increasing impact of radiation. Radiation emitted by the 3G mobile phone is more than 1800 MHz whereas the 2G mobile phone type only emits waves of 900-1800 MHz (Mayuda *et al.*, 2014). Many studies have reported that a mobile phone's electromagnetic wave radiation can interfere with male reproductive development and physiology (Saleh *et al.*, 2003; Agarwal 2011; Kesari *et al.*, 2013; Houston *et al.*, 2018). In the long term, exposure to electromagnetic waves of intense radiation can result in elevated levels of reactive oxygen species (ROS). Radiation of electromagnetic waves of mobile phones can reduce antioxidant levels and increase ROS

levels in the blood, which causes oxidative stress to the blood (Bodera *et al.*, 2013).

Oxidative stress is a cause of damage to the molecular structure of tissues and cells. For example, exposure to intense radiation can damage the structure and function of the kidneys, liver, and nervous system, and can induce cancer (Agarwal *et al.*, 2008; Koca *et al.*, 2013). Kouchaki *et al.* (2016) reported that the effect of 900-950 MHz cell phone radiation continuously in long term may increase the risk of seizure attack in mice. Electromagnetic wave radiation testing of 3G mobile phones has not addressed the incidence of oxidative stress in the fetus. This study therefore aimed to examine the effects of electromagnetic radiation from 3G mobile phones on the incidence of oxidative stress in the rat fetus.

MATERIALS AND METHODS

Materials: Experimental animals used in this study were Wistar rats weighing 350-400 grams for males and females, kindly provided by the Faculty of Pharmacy, Universitas Gadjah Mada. The materials used were

alcohol, distilled water, paraffin (Sigma-Aldrich, Saint Louis, US), hematoxylin-eosin (Sigma-Aldrich) for sample staining, nitro blue tetrazolium (NBT) solution 0.2% (Sigma-Aldrich) and N,N-dimethylformamide solution (Sigma-Aldrich). As sources of electromagnetic wave radiation, we used 2G and 3G mobile phones.

Experimental design and radiation exposure

treatment: The rats were adapted in the Animal Physiology Laboratory of the Faculty of Biology, Gadjah Mada University for 7 days and grouped into 5 treatments, each treatment group in a single cage consisting of 5 females and 1 male rats. Weighing and acclimatization were conducted during the first week. In the second week, the animals began their radiation exposure treatment, with each group exposed to a different phone mode. A mobile phone was placed outside the cage, separated from the animals by mesh cage construction materials. The distance (10 cm) between the cage and the mobile phones' electromagnetic waves (875 MHz, 0.07 mW/cm^2) may generate extracellular ROS by stimulating the activity of cell membrane-associated nicotinamide adenine dinucleotide (NADH) oxidase (Consaes *et al.*, 2012). The first treatment group was not exposed to cell phone radiation. The second group was exposed to radiation from a 2G mobile phone set to standby mode (mobile in the active state, in the absence of any telephone or short message service (SMS) activity); the third treatment group was exposed to radiation from a 2G phone set to active mode (mobile in the active state with both telephone and SMS activity); the fourth treatment group was exposed to radiation from a 3G phone set to standby mode (mobile is switched on, but without browsing activity or streaming video); and the fifth treatment group was exposed to radiation from a 3G mobile phone set to active mode (mobile in the active state with both browsing and video streaming activity). Each treatment group was given the radiation exposure for 8 hours/day and the activity of each mobile phone was under human intervention.

During week 1 the weighing and acclimatization were conducted. In week 2, the radiation exposure process began when males were placed together with females, and pregnancy was confirmed using the vaginal smear method to determine day zero of pregnancy. Radiation exposure continued until after birth. Surgery was conducted on the maternal and fetal rats.

Measurement of ROS: We also measured the levels of ROS in the liver and brain of the fetus, and the liver of the mother, using a solution of NBT in N,N-dimethylformamide. First, the liver and brain of fetus were taken from every parent who gave birth. This was followed by removing the liver of the mother after she had given birth. Maternal liver, fetal liver, and fetal brain were ground with 10 drops of distilled water. Next, 100 μl of ground tissue suspension was placed in a microtiter plate and 100 μl 0.2% NBT was added. The mixture was incubated at room temperature for 30 min. A 50 μl aliquot of the liquid was removed and mixed with 1 ml of N,N-dimethylformamide. After centrifugation at 10,000 rpm for 2 min, the supernatant was used to measure the absorbance at 620 nm (OD_{620}) using dimethylformamide as a blank (Ispir and Dorucu, 2005).

Morphometry measurement: Further histological preparations were conducted to measure the central venous diameter, sinusoid, and liver cells using histological morphometry. The histological samples were then photographed using 3.7 AmScop with 10x magnification. The diameters of histological preparations were then measured using raster image 3 (Bhadoria *et al.*, 2015).

Statistical analysis: Experimental data were expressed as means and standard errors. The data were analyzed using one-way ANOVA and Tukey-test at a significance level of 5% in SPSS 13.0 software (SPSS, 2006).

RESULTS

There were differences in NBT activity in fetal liver and brain, as well as in maternal liver, between treatments, as presented in Tables 1. According to the results obtained from the NBT spectrophotometer test in Tables 1, oxidative radical production in fetal liver and brain were highest in treatment 5 (3G active; 1.54 ± 0.004 and 0.158 ± 0.010 , respectively), which was also the case for maternal liver (1.74 ± 0.057), while the controls that were not exposed to mobile phone electromagnetic wave radiation had the lowest NBT activity in fetal liver and brain (0.016 ± 0.003 and 0.057 ± 0.007); for maternal liver the value was 0.05 ± 0.001 . Therefore, it can be concluded that exposure to electromagnetic waves from active mode 3G mobile phones has an impact on oxidative stress in the liver and brain of the fetus as well as in the liver of the mother. This is consistent with the results of liver tissue morphometry, measured using a histomorphological method as shown in Table 2.

Table 1: Effects of mobile phone radiation in fetal liver, fetal brain, and maternal liver (OD_{620})

Treatment	Mean \pm SE		
	Fetal liver	Fetal brain	Maternal liver
Control	0.016 ± 0.003^a	0.057 ± 0.007^a	0.05 ± 0.001^a
2G Standby	0.065 ± 0.02^c	0.064 ± 0.007^a	0.13 ± 0.026^b
2G Active	0.11 ± 0.02^b	0.071 ± 0.007^a	0.22 ± 0.019^c
3G Standby	0.15 ± 0.01^b	0.114 ± 0.015^b	0.42 ± 0.05^d
3G Active	1.54 ± 0.004^d	0.158 ± 0.010^c	1.74 ± 0.057^e

Note: Each value represents the mean \pm SE of five replicates per treatment. (2G) Second Generation Handheld Phone, (3G) Mobile Phones Third Generation. Different letters in columns indicate significance ($P < 0.05$).

Fetal liver histomorphological analysis (Table 2) revealed a difference between the control group, where the length of the central venous diameter was $17.92 \pm 0.98 \mu\text{m}$ and the 3G active-mode treatment group, where the length was $30.83 \pm 0.91 \mu\text{m}$. In addition, the diameter of the hepatocytes was higher in the control group ($17.03 \pm 0.78 \mu\text{m}$) than in the 3G active-mode treatment group ($7.03 \pm 0.12 \mu\text{m}$). Fetal liver sections also revealed that there is damage at the cellular level due to electromagnetic wave radiation exposure from 3G mobile phones in active mode (Fig. 1). The histomorphological analysis of maternal liver and sections showed the same trend with the fetal liver results (Table 3 and Fig. 2).

DISCUSSION

Currently, mobile phone network has evolved into the third generation (3G), which has many features such as

internet access that help human needs. However, the concomitant increase in mobile phone radiation may cause negative side effects. It was reported that the radiation emitted by a 3G mobile phone is more than 1800 MHz, whereas the 2G mobile phone type only emits waves of 900-1800 MHz (Mayuda *et al.*, 2014). Some studies have shown that an increase in the production of ROS in rats is caused by electromagnetic radiation exposure from mobile phones (Ertlav *et al.*, 2018; Jeong *et al.*, 2018). The cell phone radiation range from 900 to 1800 MHz may increase the cell death program, the oxidative stress of mitochondrial and the entry of Ca²⁺ through TRPV1 action (Ertlav *et al.*, 2018). Exposure to electromagnetic waves of mobile phones will stimulate an increase in nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), which is located on the plasma membrane; increased NADPH oxidase will produce superoxide by transferring electrons from NADPH into cells through the plasma membrane and also binds to oxygen molecules to generate superoxide. The increase of superoxide production may spontaneously configurate hydrogen peroxide, which will react with reactive oxygen to produce ROS (Desai *et al.*, 2009). Li *et al.* (2015) reported that rats exposed to electromagnetic waves for 10 weeks showed increased levels of malondialdehyde (MDA). Furthermore, electromagnetic waves from mobile phones will affect the activation of glutathione peroxide (GSH-Px) and superoxide dismutase (SOD) in hepar and lien sera, which will increase oxidative stress. In addition, radiation from mobile phones also activates heat shock proteins; this results in inhibition of apoptosis, which causes damage to hepatocytes in mouse models (Aberumand *et al.*, 2016; Ding *et al.*, 2018; Li *et al.*, 2018). In this research, we measured ROS production using the NBT assay. According to Ispir and Dorocu (2005), NBT activity may be used to measure radical oxidative production. This result was also supported by a previous study by Muñoz *et al.* (2000), which stated that the NBT spectrophotometric assay may be used to demonstrate the production of anion superoxide (O₂⁻). The measurement of O₂⁻ is considered to be an accurate

method for estimating the cell's capability to generate a respiratory burst.

Exposure of rats to electromagnetic waves for 30 min every day for a period of 3 months resulted in morphological changes in liver and pancreas (Meo *et al.*, 2010). This was supported by Bhadoria *et al.* (2015), who found that damage to the structure of the liver can be observed by measuring the length of the diameter of the central vein, central venous short diameter, the length of diameter of hepatocyte cells, hepatocyte cell short diameter, length sinusoid diameter and short diameter sinusoid.

The earliest known and most studied mechanism of cell or tissue damage due to free radical attack is lipid peroxidation. Lipid peroxidation is most prevalent in cell membranes, and especially in unsaturated fatty acids, which are essential components of the plasma membrane (Gaschler and Stockwell, 2017). This peroxidation affects the fluidity of the membrane, as well as membrane structure and function. Free radicals can damage cells by damaging the plasma membrane. Damage to the plasma membrane can occur by means of free radicals that are covalently bonded to enzymes or receptors located in the plasma membrane, thus changing the activity of these membrane components. The increase of ROS levels in the body can cause damage to the structure and function of organs such as the kidney, retina, liver, and reproductive system, and can also impair embryo development (Agarwal *et al.*, 2005). Cell phone radiation for 90 minutes each day for 30 days in BALB/c mice may increase the seizure attack (Kouchaki, *et al.*, 2016).

The US Federal Communications Commission, as reported in (Idayati, 2011), have tested the radiation levels emitted by some mobile phones. The strength of mobile phone radiation is still defined as being within a safe limit, although research in this area continues to be developed to look at the safety of mobile phone mast radiation.

Conclusions: The results of this study concluded that electromagnetic wave radiation from active-mode 3G mobile phones can result in oxidative stress and damage at the cellular level in the rat maternal and fetus.

Table 2: Morphometry of rat fetal liver sites (µm) following exposure to electromagnetic waves from 2G and 3G mobile phones

Treatment	Parameter (mean±SE)					
	Central venous diameter length	Central venous diameter short	Diameter length sinusoid	Diameter short sinusoid	Diameter length cells hepatocytes	Diameter short cells hepatocytes
Control	17.92±0.98 ^a	11.82±0.97 ^a	17.42±0.40 ^a	2.19±0.33 ^a	17.03±0.78 ^a	11.54±0.32 ^a
2G Standby	21.59±1.22 ^b	19.47±0.27 ^b	44.38±1.07 ^c	2.26±0.38 ^a	11.09±0.90 ^b	9.92±0.71 ^b
2G Active	25.48±0.45 ^b	19.94±0.44 ^b	44.98±1.39 ^c	0.43±4.53 ^b	8.42±0.24 ^c	7.35±0.35 ^c
3G Standby	27.87±0.68 ^c	21.83±0.59 ^c	55.89±1.42 ^d	5.07±0.41 ^c	7.97±0.64 ^d	6.32±0.35 ^d
3G Active	30.83±0.91 ^d	22.92±0.90 ^d	59.21±1.28 ^b	7.04±0.44 ^d	7.03±0.12 ^d	6±0.64 ^d

Note: Each value represents the mean±SE of five replicates per treatment. (2G) Second generation handheld phone, (3G) third generation mobile phone. Different letters in columns indicate significance (P<0.05).

Table 3: Morphometry of rat maternal liver sites (µm) following exposure to electromagnetic waves from 2G and 3G mobile phones

Treatment	Parameter (mean±SE)					
	Central venous diameter length	Central venous diameter short	Diameter length sinusoid	Diameter short sinusoid	Diameter length cells hepatocytes	Diameter short cells hepatocytes
Control	19.06±0.05 ^e	17.02±0.01 ^d	53.18±0.40 ^e	3.09±0.03 ^e	20.98±0.08 ^d	17.01±0.02 ^e
2G Standby	22.59±0.22 ^d	20.05±0.07 ^c	62.12±0.01 ^d	5.81±0.01 ^d	20.09±0.90 ^d	16.96±0.04 ^d
2G Active	26.98±0.05 ^c	29.94±0.14 ^c	74.08±0.61 ^c	7.05±0.04 ^c	18.03±0.06 ^c	15.04±0.05 ^c
3G Standby	31.07±0.08 ^b	34.03±0.99 ^b	95.23±0.02 ^b	9.07±0.15 ^b	16.01±0.02 ^b	14.01±0.08 ^b
3G Active	38.83±0.01 ^a	39.72±0.21 ^a	122.01±0.08 ^a	12.04±0.31 ^a	15.14±0.14 ^a	12.02±0.01 ^a

Note: Each value represents the mean±SE of five replicates per treatment. (2G) Second Generation Handheld Phone, (3G) Mobile Phones Third Generation. Different letters in columns indicate significance (P<0.05).

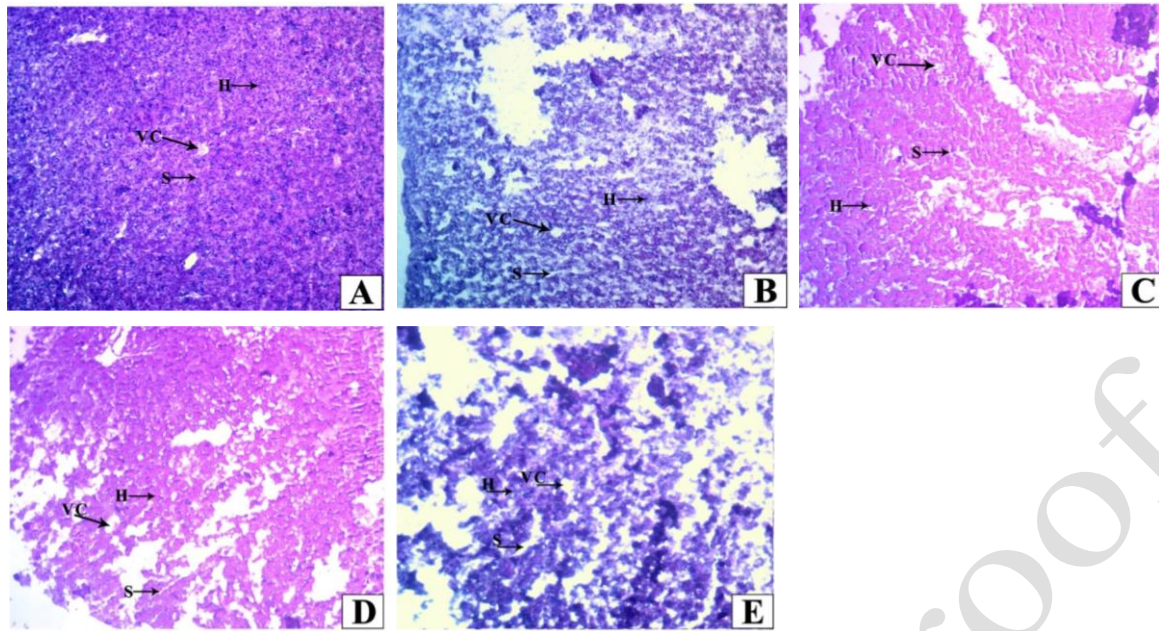


Fig. 1: Fetal liver histology visualized by hematoxylin-eosin staining. (A) Control without radiation. (B) Treatment with radiation from a 2G mobile phone in standby mode. (C) Treatment with radiation from a 2G mobile phone in active mode. (D) Treatment with radiation from a 3G mobile phone in standby mode. (E) Treatment with radiation from a 3G mobile phone in active mode. (VC) vein central, (H) hepatocytes, (S) sinusoid. Scale bars, 100 μ m.

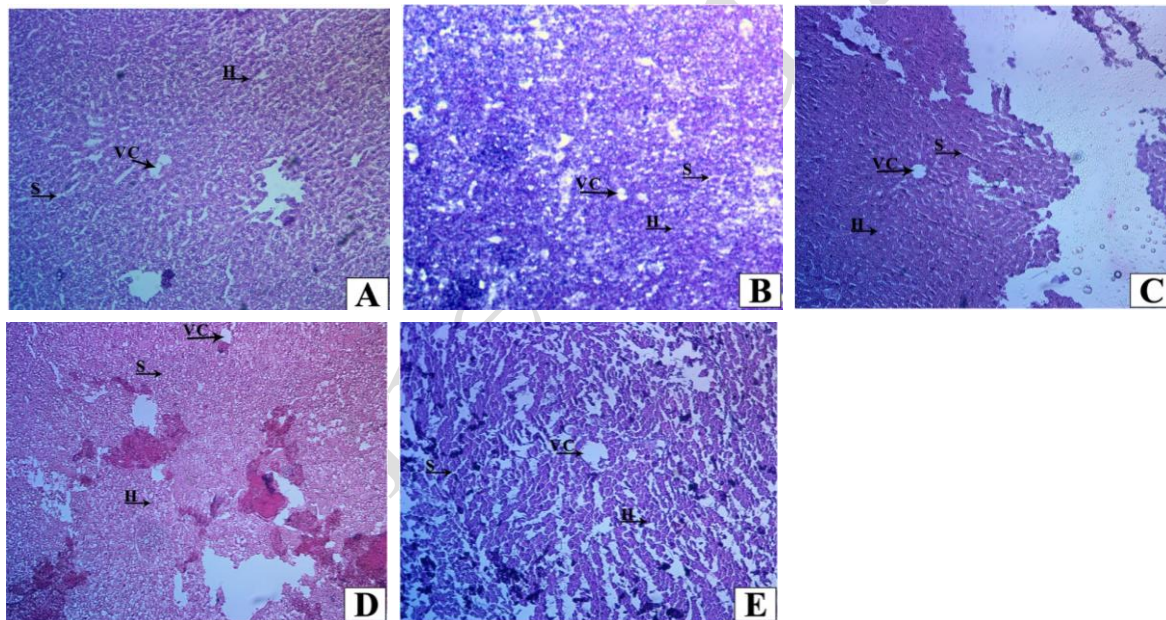


Fig. 2: Maternal liver histology visualized by hematoxylin-eosin staining. (A) Control without radiation. (B) Treatment with radiation from a 2G mobile phone in standby mode. (C) Treatment with radiation from a 2G mobile phone in active mode. (D) Treatment with radiation from a 3G mobile phone in standby mode. (E) Treatment with radiation from a 3G mobile phone in active mode. (VC) vein central, (H) hepatocytes, (S) sinusoid. Scale bars, 100 μ m.

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Authors contribution: IFS and HTS design the experiment, IFS and GP performed the experiment, IFS analyzed the data, while IFS and HTS wrote the manuscript. All author approved the final version of the manuscript.

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