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Original Research

Oxidative stress and apoptosis in electromagnetic waves exposed Zebrafish embryos and protective effects of conductive nonwoven fabric

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Abstract: The amount of technological products including television, radio transmitters, and mobile phone that have entered our daily life has increased in recent years. But these devices may cause adverse effects on human health. Electromagnetic shielding fabrics may limit and inhibit electromagnetic waves. Aim of our study was to evaluate electromagnetic wave blocking performance of nonwoven textile surfaces on zebrafish embryos that were exposed to electromagnetic waves at specific frequencies. Oxidant-antioxidant system parameters were evaluated spectrophotometrically. The expressions of *tp53* and *casp3a* were evaluated by RT-PCR. Results showed that electromagnetic shielding fabrics produced as conductive nonwoven textile surfaces improved oxidant-antioxidant status and *tp53* expression that were impaired in electromagnetic waves exposed zebrafish embryos. Also, electromagnetic shielding fabrics decreased *casp3a* expression responsible for the execution phase of apoptosis that increased in electromagnetic waves exposed zebrafish embryos.

Key words: Electromagnetic wave; Conductive fibres; Zebrafish embryo; Apoptosis; Oxidant-antioxidant parameters.

Introduction

In line with the developments in science and technology, the amount of technological products that have entered the industry and our daily life has increased. These technological products include television and radio transmitters, computer, mobile phone and base stations. These devices are suggested to cause adverse effects on human health as a result of radiation emitted (1,2). Accordingly, after noise, water, and air, electromagnetic radiation has been reported to be the fourth most critical source of public pollution (3).

Man-made electromagnetic fields have different frequencies in the form of extremely low frequency, low, medium, high and very high frequency. Electricity supplies and electrical appliances have extremely low frequency and extremely high frequency is usually associated with wireless communication (4). Although the World Health Organization did not report their adverse health effect, extremely low frequency and radiofrequency electromagnetic radiation originated from cell phones has been defined as potential carcinogens for human by the International Agency for Research on Cancer (IARC) (5). On the other hand radiofrequency electromagnetic radiation has been shown to cause DNA damage through oxidative stress (6).

Metal wire, fiber, coating, and film are among the conductive materials that are used to limit and inhibit electromagnetic waves (7). Thanks to developments in the textile industry, textile materials produced using conductive fibers and yarns can shelter the large part of the electromagnetic waves and protect the health of living organisms against the hazardous effects of electromagnetic radiation. Electromagnetic shielding is defined as the process of lowering the electromagnetic field in by obstructing the field with barriers produced using conductive or magnetic materials (3). In particular, electromagnetic shielding fabrics may be used to cover electromagnetic devices or as garment interlining to shield electromagnetic waves or they may be used under carpet to eliminate static electricity (8,9).

Zebrafish embryo has become an alternative popular model organism that is used to decrease animal experiment number and related costs within the scope of the "3Rs" principle as replacement, reduction, and refinement of animal experiments. Due to its small size, fast and external development, high fecundity, maintenance advantages and optical transparency zebrafish embryo has gained its popularity in biomedical research (10).

The aim of our study is to evaluate the electromagnetic wave blocking performance of nonwoven fabric that is produced using conductive fibers building a barrier against electromagnetic waves at specific range of frequencies.

Materials and Methods

Production of conductive nonwoven fabrics

As a raw material, silver coated staple polyamide fiber with a fineness of 1.7 dtex was used (R-Stat SAS Company/France). In order to produce nonwoven fabric from silver coated staple polyamide fibers, nonwoven production methods were preferred. The steps for the nonwoven fabric production were mainly web forming and web bonding. Carding and needle punching machines were used for web forming from silver coated staple polyamide fibers (3).

Maintenance of zebrafish

AB/AB Strain zebrafish were used in this study. Zebrafish were maintained in aquarium rack system (Zebtec, Tecniplast, Italy) under disease-free conditions at 28 ± 1 °C, 14/10 h light/dark cycle and fed with commercial flake fish food and live artemia twice a day. Reverse osmosis water that was supplemented with 0.018 mg L^{-1} Instant Ocean™ salt was used during the experiments (11). After natural spawning fertilized embryos were collected. Embryos were staged according to their development and morphological criteria as previously explained (12).

Embryo exposure

The groups were designed as the control group, electromagnetic wave exposed group (EMG group) and nonwoven textile shielded electromagnetic waves group (EMG+ NTS group). Each group consisted of 100 zebrafish embryos. For the investigation of the protective effect of conductive nonwoven fabrics against the electromagnetic waves in zebrafish embryos, a test device was designed (Figure 1). The Network Analyzer was used to generate electromagnetic waves. To direct the electromagnetic wave produced onto the zebrafish embryos, horn antenna that sends and collects the wave was used. A frequency range between 15 and 3000 MHz was used for the electromagnetic waves.

Biochemical analyses

Zebrafish embryos at 72 hpf were used for the biochemical analyses performed in this study. Replicate pools from 72 hpf zebrafish (n=5, 100 embryos per pool) were prepared and homogenized in 1mL PBS. After centrifuging the supernatant was collected and used for the determination of biochemical analyses.

Total protein determination

The method of Lowry was used for total protein determination (13). The method is based on the reaction of alkaline proteins with copper ions followed by the reduction by Folin reactive. At the end of the reaction absorbance of the product was measured at 500 nm using a spectrophotometer using albumin as standard protein. Total protein levels were used to express the results of the biochemical parameters per mg protein.

LPO determination

In order to measure malondialdehyde (MDA), which is the end product of lipid peroxidation, Yagi's method was applied (14). 1.56 x 10^5 $M^{-1}cm^{-1}$ was used as the extinction coefficient and LPO was expressed as nmol MDA/mg protein.

NO determination

NO levels were determined by the method which is based on nitrate reduction to nitrite by vanadium (III) chloride. The colored complex formed at the end was measured at 540 nm using spectrophotometer and results were given as nmol NO/mg protein (15).

SOD activity determination

SOD activity was determined by the method using riboflavin-sensitized o-dianisidine photo-oxidation. End product absorbance was measured at 460 nm using a spectrophotometer at 0 and 8th minutes of illumination. The results were given as U/mg protein (16).

Glutathione-S-transferase determination

Glutathion-s-transferase (GST) activity was evaluated by the spectrophotometric measurement of the absorbance of the conjugation product of GSH and 1-chloro-2,4-dinitro-benzenin (CDNB) at 340 nm (17).

Quantitative Real-Time PCR

Rneasy Mini Kit and Qiacube (Qiagen) were used to isolate RNA from the embryos following manufac-

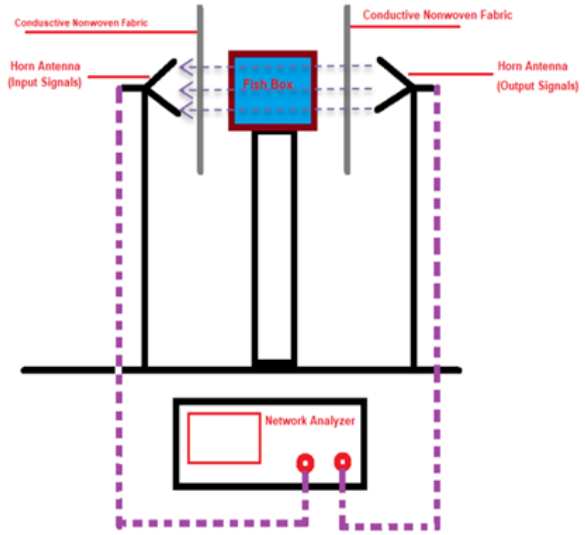

Figure 1. Schematic presentation of the device used to investigate the effects of the conductive nonwoven fabric against the electromagnetic waves in zebrafish embryos.

Table 1. Forward and reverse primers used in the study.

casp3a	forward primer	5' ATGAACGGAGACTGTGTG-3'
	reverse primer	5'TTAAGGAGTGAAGTACATCTCTTTG-3'
p53	forward primer	5' GGGCAATCAGCGAGCAAA-3'
	reverse primer	5' ACTGACCTTCCTGAGTCTCCA-3'
β actin	forward primer	5′AAGCAGGAGTACGATGAGTCTG-3'
	reverse primer	5′-GGTAAACGCTTCTGGAATGAC-3'

turer's instructions. From 1 µg of total RNA single-stranded cDNA was produced by RT^2 Profiler PCR Arrays (Qiagen). PCRs were carried out by DNA Master SYBR Green kit (Qiagen). The expressions of *tp53* and *casp3a* were measured by RT-PCR using the Qiagen Rotor Gene-Q Light Cycler instrument. As the housekeeping gene *β actin* was used. ΔΔCT method was used to calculate relative transcript levels (18). List of primers is given in Table 1.

Results

Results of lipid peroxidation analysis

Lipid peroxidation increased significantly in the EMG group (0,16±0,01 nmol MDA/mg pr) when compared with the control group (0,13± 0,005 nmol MDA/mg pr).On the other hand lipid peroxidation decreased significantly in the EMG+NTS group (0,13±0,01 nmol MDA/mg pr) when compared with the EMG group (Figure 2).

Results of nitric oxide analysis

Ntiric oxide levels increased significantly in the EMG group (2,73±0,18 nmol /mg pr) when compared with the control group (1,97± 0,18 nmol /mg pr). In the EMG+NTS group, a significant decrease was observed in the nitric oxide levels (2,17 ± 0,15 nmol /mg pr) when compared with the EMG group (Figure 3).

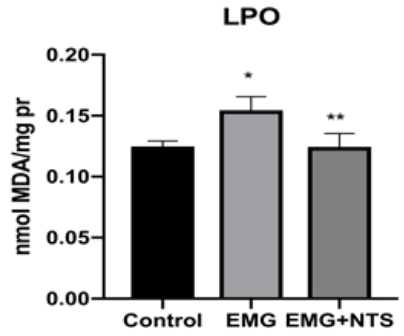

Figure 2. Lipid peroxidation (LPO) levels of the Control, EMG and EMG+NTS groups. Replicate pools of 72 hpf zebrafish (n=5, 100 embryos per pool) were used. Values are expressed as mean±standart deviation. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group. LPO: Lipid peroxidation.

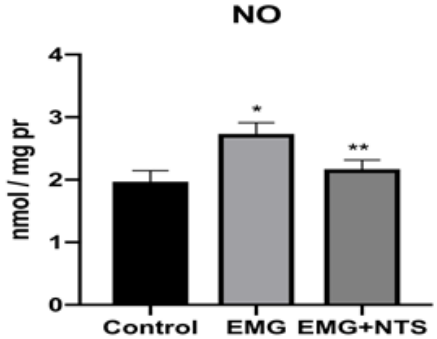

Figure 3. Nitric oxide (NO) levels of the Control, EMG and EMG+NTS groups. 72 hpf zebrafish embryos were analyzed as replicate pools (n=5, 100 embryos per pool). Values are expressed as mean±standart deviation. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group. NO:Nitric oxide.

Results of superoxide dismutase analysis

Superoxide dismutase activity decreased significantly in the EMG group (0,37 ± 0,04 U/mg pr) when compared with the control group (0,69 ± 0,06 U / mg pr). In the EMG+ NTS group, significantly decreased superoxide dismutase activity (0,48±0,04 U/mg pr) was observed when compared with the control group but increased significantly when compared with the EMG group (Figure 4).

Results of Glutathione-S-Transferase analysis

Glutathione-S-Transferase activity increased significantly in the EMG group (0,06±0,004 U/mg pr) when compared with the control group (0,03± 0,002 U/mg pr). In the EMG+ NTS group, When compared with the control group significantly increased glutathione-S-Transferase activity was observed (0,05±0,002 U/mg pr) but the activity decreased significantly when compared with the EMG group (Figure 5).

Results of *tp53* expressions

tp53 expression decreased significantly in the EMG group when compared with the control group. In the EMG+ NTS group, a significant increase was observed in *tp53* expression when compared with the EMG group and a significant decrease was observed when compared

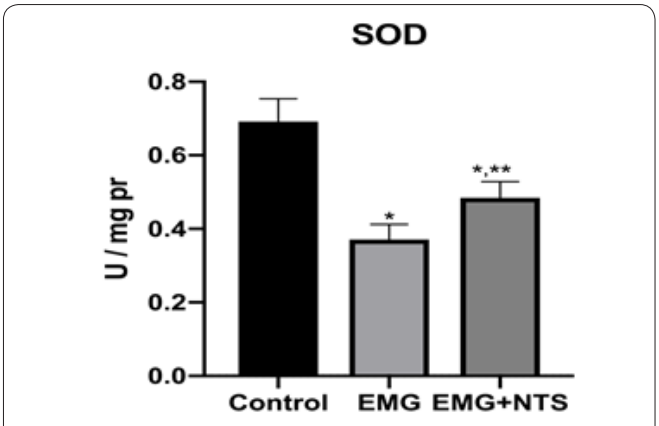

Figure 4. Superoxide dismutase (SOD) activities of Control, EMG and EMG+NTS groups. 72 hpf zebrafish embryos were analyzed as replicate pools (n=5, 100 embryos per pool). Values are expressed as mean±standart deviation. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group. SOD: Superoxide dismutase.

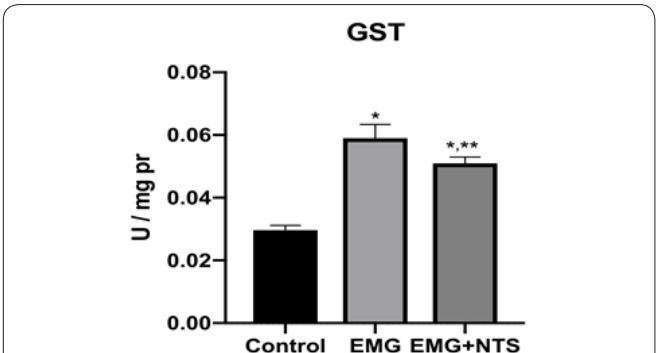

Figure 5. Glutathione-S-Transferase (GST) activities of Control, EMG and EMG+NTS groups. 72 hpf zebrafish embryos were analyzed as replicate pools (n=5, 100 embryos per pool). Values are expressed as mean±standart deviation. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group. GST: Glutathione S-transferase.

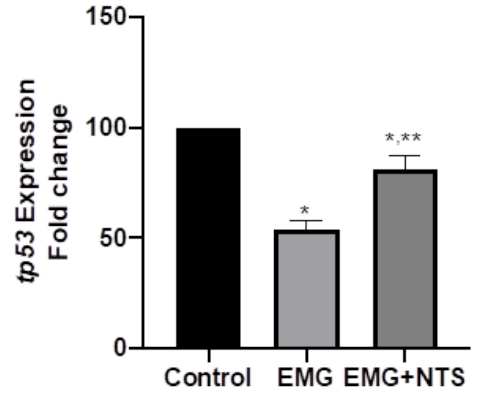

Figure 6. *tp53* transcript quantified by RT-PCR. *β-actin* was used as the house keeping gene and results are expressed as change from their respective controls. Average values were calculated from three experiments. Data presented are mean ± SD. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group.

with the control group (Figure 6).

Results of *casp3a* expressions

casp3a expression decreased significantly in the EMG group when compared with the control group. In the EMG+ NTS group, a significant decrease was observed in *casp3a* expression when compared with the EMG group and a significant increase was observed when compared with the control group (Figure 7).

Discussion

This study showed that electromagnetic shielding fabrics produced as conductive nonwoven fabric improved oxidant-antioxidant status and *tp53* expression that were impaired in electromagnetic waves exposed zebrafish embryos. Also, electromagnetic shielding fabrics decreased *casp3a* expression which increased in electromagnetic waves exposed zebrafish embryos.

Despite the use of higher animals in toxicological research for many years, zebrafish has gained popularity as a reliable vertebrate model to determine, developmental and general toxicity and for initial drug screening in the embryo (19). The effects of electromagnetic fields (EMF) exposure have been also evaluated in zebrafish and zebrafish embryos (20,21). Together with these results, the results of our study confirm the use of zebrafish in research related to the potential health effects of EMF exposure.

EMF exposure is very common in the world and every day more than 3 billion people have been reported to be exposed to EMF across the world (22). On the other hand, EMF emitted by different natural and artificial sources may lead to crucial alterations and harmful effects in biological systems. Reactive oxygen species (ROS) regulate many cellular functions for cellular homeostasis. Defined as peroxidation of membrane phospholipids, lipid peroxidation leads to alterations in the conductivity of membrane and loss of membrane integrity (23). EMF has been reported to impair antioxidant defense mechanisms and cause overproduction of ROS leading to oxidative stress (24).

Our study has shown increased lipid peroxidation and nitric oxide levels in electromagnetic waves exposed zebrafish embryos. Our results are compatible with the results of Özgüner et al. who reported increased renal nitric oxide and malondialdehyde levels in EMF exposure model (25). In our study, electromagnetic waves exposure led to decreased SOD activity. SOD catalyzes the production of molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) from superoxide (O^{2-}) radical. EMF exposure has been shown to decrease SOD activity in rat brain (26, 27). Decreased antioxidant enzymes and increased ROS have also been reported in rat kidneys that were exposed to 900-MHz EMF for 30 min/day for 1 month (25). Glutathione S-transferases (GST) catalyze the conjugation of reduced glutathione for detoxification and in our study increased GST activity was observed in electromagnetic waves exposed zebrafish embryos.

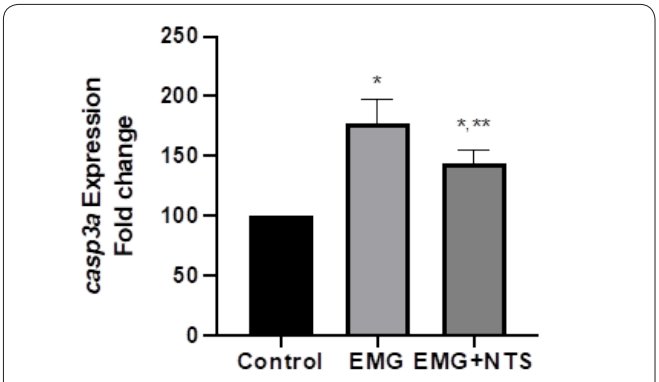

Figure 7. *casp3a* transcript quantified by RT-PCR. *β-actin* was used as the housekeeping gene and results are expressed as change from their respective controls. Average values were calculated from three experiments. Data presented are mean ± SD. *p<0.05 significantly different from the control group, ** p<0.05 significantly different from the EMG group.

In our study, decreased *tp53* expression and increased *casp3a* expression were observed in electromagnetic waves exposed zebrafish embryos. *tp53* is a tumor suppressor gene that encodes a sequence specific transcription factor, having antiproliferative and proapoptotic effects. The expression of *tp53* may b*e* stimulated by a variety of stress signals (28). TP53 induces apoptosis and in nearly 50% of human cancers TP53 has been reported to be mutated. Decreased *tp53* expression and increased *casp3a* expression in our study may suggest that electromagnetic waves exposure induced a p53-independent apoptosis. Increased lipid peroxidation and NO levels indicate excessive ROS formation that activate Bax leading to the release of cyctocrom c from the mitochondria. Caspase-3 is an endoprotease known as the executioner caspase in apoptosis that regulates the network between inflammation and apoptosis and eventually leads to DNA fragmentation or degradation of cytoskeletal proteins (29). Exposure to the EMF of 1950-MHz has not been reported to promote tumor formation however, continuous exposure has been reported to damage astrocyte mitochondria and lead to apoptosis via caspase-3-dependent pathway with the contribution of Bax and Bcl-2 (30).

Han et al. investigated the effects of 50 Hz-EMF exposure on apoptosis on breast cancer cells. They found no difference in mRNA expression of proapoptotic gene Bax and anti-apoptotic gene Bcl-2, and caspase-3 protein levels by western blot and suggested that 50 Hz-

EMF exposure had no effect on cell apoptosis (31). On the other hand, it has been shown in previous studies that EMF exposure may lead to transcriptional changes in some apoptosis-related genes including Bax and Bcl-2 that are proapoptotic and antiapoptotic respectively (30,31).

Textile materials produced using conductive fibers shelter an important part of the electromagnetic waves and therefore may protect the health of living organisms against the deleterious effects of electromagnetic radiation. Accordingly, our study was first to show that electromagnetic shielding fabrics may prevent oxidative stress and apoptosis and may improve *tp53* expression that was impaired by electromagnetic waves exposure in zebrafish embryos.

The electromagnetic shielding nonwoven fabrics used in this study may be used as a garment interlining, cover on devices or material under carpet to protect from static electricity (3). Based on the results of our preliminary study we suggest the availability of these fabrics for their protective effects on oxidative stress and apoptosis. However, we believe that investigating the effects of different frequencies of electromagnetic waves will provide more detailed results on the subject.

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Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's contribution
Ünsal Veli Üstündağ performed the exposure, biochemical and RT-PCR analyses, Mustafa Sabri Özen, produced conductive nonwoven fabrics, İsmail Ünal performed the exposure, biochemical and RT-PCR analyses; Perihan Seda Ateş performed the biochemical and RT-PCR analyses, A.Ata Alturfan contributed to discussion on biochemical results and edited the paper, Mehmet Akalın contributed to the discussion on conductive nonwoven fabrics, Erhan Sancak produced conductive nonwoven fabrics and installed the EMG exposure design, Ebru Emekli-Alturfan directed the study.

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