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Effects of antioxidants on apoptosis caused by cell phones in stomach, colon, and liver tissues

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Abstract
Objective: This study aimed to determine the possible effects of cell phones on apoptosis in colon, stomach, and liver tissues and the protective role of vitamins C and E against these effects.
Method: Two same-model and same-mark cell phones were used, one in stand-by mode and the other in calling mode. A total of 42 male Wistar albino rats were used in the study. The rats were divided into six groups: control, antioxidant, call, stand-by, call + antioxidant, and stand-by + antioxidant. Apoptosis indexes were studied as pathological indicators.
Results: Statistically significant apoptosis was observed only in the call-mode group in stomach tissue. The beneficial effects of antioxidants were observed only in the stand-by group in liver tissue.
Conclusions: People should be careful about the biologic effects of cell phones, especially in the calling mode. Antioxidants (vitamins C and E) can help get rid of the harmful effects of radiofrequency waves.

Keywords: apoptosis, antioxidants, wistar

Introduction
Nowadays, radiofrequency (RF) waves, especially from cell phones and base stations, are one of the sources that generate electromagnetic (EM) field, which increasingly affects human beings. Various studies have described the harmful effects of these waves [1, 2]. It is proved that standard cell phones increase apoptosis in various cells and tissues of the body by generating EM radiation. However, no studies explored whether cell phones in the stand-by or calling mode caused apoptosis in liver, stomach and colon tissues. It is known that vitamins E and C have antioxidant effects and decrease apoptosis [3].
This study aimed at investigating whether EM waves from cell phones increased apoptosis in liver, stomach and colon tissues and whether vitamins E and C served as possible antioxidants to get rid of the damages caused by apoptosis.

Material and Methods
Devices used in the study were a centrifuge (Hermle Z300. Selectra), a spectrometer (Shimadzu UV-1700 model), a deep freezer (Sanyo, –80ºC), a microbalance (Mettler Toledo AB204-S), a digital pH-meter (Mettler Toledo, Seveneasy), an automatic biochemistry analyzer (Integra 800, Roche), and a hormone device (Roche E-170).
Two Nokia 8210 cell phones with specific absorption rate 1.00 W/kg (24) and Digital Communication System 1800 (Avea) were used in the study. The cell phones were continuously charged and prevented from shutting down. One cell phone was shared by the third and fifth groups and another was shared by the fourth and sixth groups. The groups subjected to cell phone radiation and those not subjected to cell phone radiation were separated from each other and kept in different rooms far from each other, at least 7.80 m apart (Fig. 1).

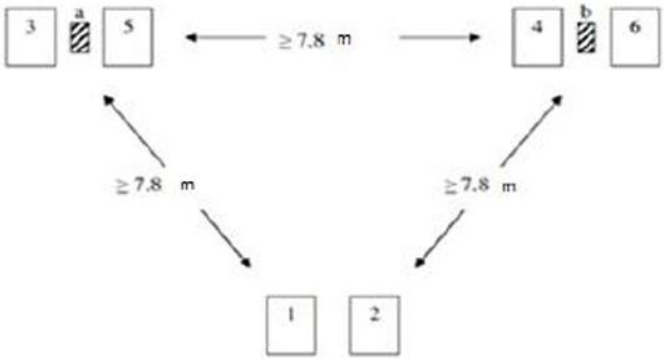

Fig 1: Schematic presentation of experiment mechanism 1: Control group, 2: Antioxidant group, 3: Call group, 4: Stand-by mode group, 5: Call and Antioxidant group 6: Stand-by+ Antioxidant group, a: cell phone on call mode, b: cell phone on Stand-by mode

A total of 42 Wistar albino rats were divided into 6 groups, each consisting of 7 rats: group 1: nothing was done to the rats in the control group; they were fed under standard laboratory conditions for 1 week. Group 2: vitamin C [20 mg/ (kg · day), intraperitoneally] and vitamin E [50 mg/(kg · day), intramuscularly] were administered as antioxidants to rats belonging to the antioxidant group. Group 3: the rats were subjected to EM radiation from the cell phone that was called for 10 min each hour while being held in the stand-by mode for 8 h. Group 4: The rats in the stand-by mode group were

subjected to EM radiation from the cell phone held in the stand-by mode. Group 5: While the rats in the call + antioxidant group were subjected to EM waves from the cell phone that was called for 10 min every hour for 8 h and held in the stand-by mode for 1 month, vitamin C [20 mg/(kg · day), intra-peritoneally] and vitamin E [50 mg/(kg · day), intramuscularly] were administered additively to these rats as antioxidants. Group 6: While the rats belonging to the stand-by mode + antioxidant group were subjected to EM waves from the cell phone in the stand-by mode continuously for 1 month, vitamin C [20 mg/(kg · day), intraperitoneally] and vitamin E [50 mg/(kg · day), intramuscularly] were administered to these rats as antioxidants.

Liver, colon and stomach tissues were taken from rats under general anesthesia and fixed in 10% neutral formalin solution for 1 month. They were embedded in paraffin and cut into 4-µm slices. The slices were placed on poly-L-lysine and then kept in alcohol after deparaffinization. They were boiled in a buffer solution containing 10 mmol/L citrate in a pressure cooker for 7 min and then treated with 0.3% hydrogen peroxide for 10 min after 20-min cooling at room temperature. The sections were washed with phosphate-buffered saline (PBS) and exposed to Ultra V block for 5 min. After diluting to a ratio of 1:10 with Caspase 3 (Millipore AB3623, Chemicon, CA, USA), the sections were incubated for 60 min. A standard streptavidin biotin complex kit (Lab Vision, USA) was used after washing the sections with PBS. They were placed in 3-amino-9-ethylcarbazole chromogen for 10 min, washed with distilled water, and then closed with water-based Entellan after counterstaining with hematoxylin. Apoptotic cells were counted by evaluating 1000 cells using a Olympus BX51 microscope.

The relevant difference between the groups was evaluated with SPSS version 13.0 (SPSS, IL, USA) using the Kruskal–Wallis test. Comparison between groups was done using the Mann–Whitney *U* test.

The ethics committee has been approved.

Results

A difference in stomach tissue and number of apoptotic cells between the call and stand-by groups was observed ($P = 0.01$). No differences in colon and liver tissues was detected ($P = 0.17$, $P = 0.8$). No differences in stomach, colon, and liver tissues were detected between the control and call groups ($P = 0.09$, $P = 0.836$, and $P = 0.75$). No significant differences in stomach, colon, and liver tissues were detected between the control and stand-by groups in terms of apoptosis ($P = 0.26$, $P = 0.975$, and $P = 0.836$). No statistical difference was detected in terms of apoptosis in the stomach, colon, and liver tissues between the call and call + antioxidant groups. ($P = 0.731$, $P = 0.836$, and $P = 0.234$). No statistical difference was detected in terms of apoptosis in the stomach, colon, and liver tissues between the stand-by and stand-by + antioxidant groups ($P = 0.805$, $P = 0.101$, and $P = 0.71$). No statistical difference was detected in terms of apoptosis in stomach, liver, and colon tissues between the control + antioxidant and call + antioxidant groups ($P = 0.234$, $P = 0.138$, and $P = 0.628$). Statistical difference was detected in terms of apoptosis in liver tissues between the control + antioxidant and stand-by + antioxidant groups ($P = 0.01$). No statistical difference was detected in terms of apoptosis in stomach and colon tissues between the control + antioxidant and stand-by + antioxidant groups ($P = 0.535$, $P = 0.26$).

Discussion

The effects of cell phones emitting EM waves on people depend on their frequency. While analogue phones work at frequencies between 800 and 900 MHz, digital phones work at frequencies between 1850 and 1890 MHz [4]. A cell phone communicates with the base station with the help of RF waves, which are a form of unionized EM radiation [5]. Exposure of the human body to these waves can result in thermal and nonthermal effects [6, 7, 8]. The thermoregulation system saves the human body from the thermal effects of RF waves. Molecular concentrations that damage biologic tissues and cause headache and sleep-related disorders can exert nonthermal effects [5, 6, 7, 8, 9, 10]. Several studies have demonstrated the harmful effects of RF waves emitted from cell phones on the human body [3, 8, 11, 12, 13, 14]. This study aimed to explore the harmful effects of cell phone on liver, stomach and colon tissues by the pathological detection of apoptosis.

Apoptosis or programmed cell death is accompanied by nonpathological cell loss. Fast chromatin condensation occurs in the cell after disposal by neighborhood cells [15, 16]. Inadequate apoptosis may result in several neurodegenerative illnesses, ischemic damage, autoimmune illness, and various types of cancers [17]. This study investigated apoptosis, which is the basis of many diseases.

Defense systems that make free radicals inactive in organisms are called "antioxidants" [18]. Ascorbic acid (vitamin C) and tocopherol (vitamin E) are antioxidants that are not enzymatic. Vitamin C protects lipids against oxidation by removing the effects of radicals that initiate lipid peroxidation. Vitamin E protects fatty acids against attacks of oxygen free radicals [19, 20]. These two vitamins were used in the present study for protection against the effects of free radicals.

Various studies in the literature have shown that RF waves from cell phones cause apoptosis in several tissues [3, 11]. The effects of cell phones in stand-by and calling modes on liver, stomach, and colon tissues were researched separately, and it was demonstrated that cell phones caused apoptosis in either mode. Statistically significant apoptosis was observed only in the calling mode in stomach tissue. Apoptosis caused in the calling mode can be explained, but it is hard to explain why it occurred only in stomach tissues. On the other hand, statistical difference was detected in terms of apoptosis in liver tissues between the control + antioxidant and stand-by + antioxidant groups. This condition that we can not explain precisely may be due to phone exposure is more in stand-by mode. Dasdag *et al*. [14] explored the effects of exposure to 900-MHz waves produced by a GSM operator on spermatogenesis in rat seminiferous tubules. It was evaluated by staining activated caspase 3 in testicular tissues immunocytochemically. It was speculated that exposure to RF waves produced by cell phones did not cause apoptosis during spermatogenesis in rats. Esmerkaya *et al*. [21] explored the possible histopathological effects of 900-MHz RF waves on the thyroid gland using light microscopy, electron microscopy, and immunocytochemical

methods. Hypothyroidism was detected, and inhibition of hormone synthesis was shown in the group exposed to 900-MHz RF waves. Moreover, an increase in activation of caspases 3 and 9 was observed in cells. The present study investigated the effects of cell phones on apoptosis by evaluating the caspase 3 activity.

Oral *et al*. [3] explored whether 900-MHz waves increased apoptosis and oxidative stress in endometrial tissues of rats and also studied the effect of vitamins C and E on these two parameters. They found that the waves increased apoptosis and oxidative stress in rat endometrial tissues, while vitamins C and E decreased these effects. The present study showed that exposure to RF waves increased oxidative stress in liver, stomach, and colon tissues but statistically significant beneficial effects of antioxidants (vitamins C and E) were observed only in liver tissue.

Large-scale studies are needed to validate the present findings. Moreover, it is recommended that people should be careful about the biologic effects of cell phones, especially in the calling mode, communication devices not using RF waves should be preferred, and cell phones should be kept away from the body. Antioxidants can reduce the harmful effects of RF waves in liver tissue.

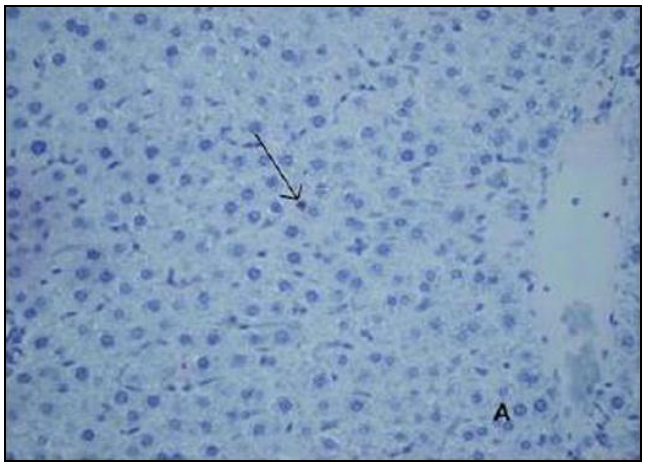

Fig 2: Rare apoptotic cell in liver tisssue

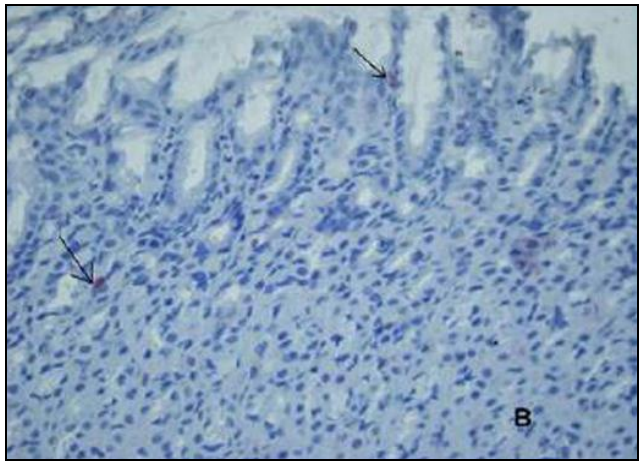

Fig 3: Rare apoptotic cells in stomach mucosa

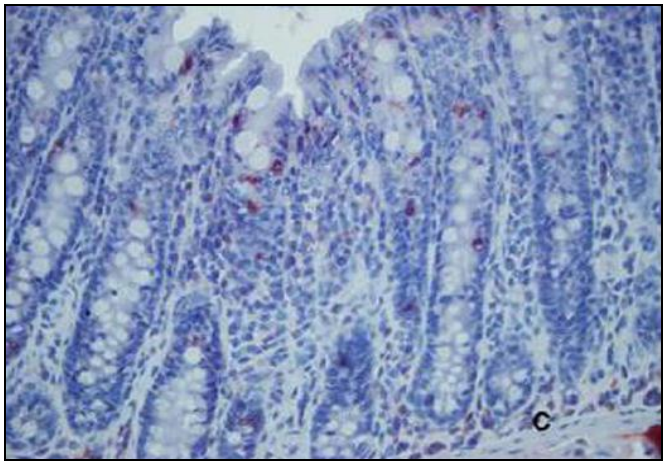

Fig 4: Frequent apoptotic cell in colon mucosa

Conflict of Interest

All the authors declare no conflict of interest regarding the publication of this study.

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