EFFECT OF LYCOPERSICON ESCULENTUM EXTRACT ON APOPTOSIS IN THE RAT CEREBELLUM, FOLLOWING PRENATAL AND POSTNATAL EXPOSURE TO AN ELECTROMAGNETIC FIELD
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Abstract
At present, the developing human brain is regularly exposed to mobile telephones, pre- and postnatally. Several studies have demonstrated the acute effects of EMF exposure during pre- or postnatal periods; however, the chronic effects of EMF exposure are less understood. Thus, the aim of the present study was to determine the chronic effects of EMF on the pre- and postnatal rat cerebellum. The control group was maintained in the same conditions as the experimental groups, without the exposure to EMF. In the EMF group, the rats were exposed to EMF during pre- and postnatal periods (until postnatal day 80). In the EMF2 group, the rats were exposed to EMF pre- and postnatally; in addition, however, they were provided with a daily oral supplementation of Lycopersicon esculentum extract (~2 g/kg). The number of caspase-3-labeled Purkinje neurons and granule cells present in the rats in the control and experimental groups were then counted. The neurodegenerative changes were studied using cresyl violet staining, and these changes were evaluated. The results indicated that apoptosis and neurodegeneration in rats exposed to EMF during pre- and postnatal periods may be reduced by Lycopersicon esculentum extract therapy.

Introduction and Purpose
Numerous studies have demonstrated that electromagnetic field (EMF) exposure does not significantly increase the apoptosis rate in cells of different cell types and cell lines (1–3). However, there have been several studies that have revealed evidence to the contrary (4–6). Several studies have investigated the acute effects of EMF exposure during the pre- and postnatal periods (7–9). By contrast, little is understood regarding the chronic effects of EMF exposure. The developing human brain is now regularly exposed to mobile telephones pre- and postnatally (7). Thus, the aim of the present study was to determine the chronic effects of EMF exposure on pre- and postnatal rats.

Several studies indicated that tomato and lycopene may act as neuroprotective agents, due to their anti-oxidant, anti-inflammatory, and free radical scavenging effects (10). In the current study, we investigated the protective effects of Lycopersicon esculentum extract on the development of apoptosis and neurodegeneration in the EMF–exposed rat cerebellum, during pre- and postnatal development.

Methods
The study comprised three groups of Albino Wistar rats, one control and two experimental (EMF1 and EMF2) groups. In the control group, the rats were kept in the same conditions as the experimental groups, but without exposure to EMF. In the EMF1 group, the rats were exposed to EMF during pre- and postnatal periods (until postnatal day 80). In the EMF2 group, the rats received the same EMF exposure as the EMF1 group; however, they were also provided with daily oral supplements of Lycopersicon esculentum extract (~2 g/kg) during the pre- and postnatal periods. All experimental protocols received full approval from the Animal Ethical Committee of Dumlupinar University, Turkey, No. 02.11.2009/9.

EMF exposure. A commercially available cellular telephone with Global System for Mobile communications (GSM)-900 digital technology was used for EMF exposure. The rats were placed on the inside walls of the cages, and the rats were exposed to the effects of the cell phones throughout the pre- and postnatal periods, until they were 80 days old. During the study, the exposure procedure comprised the phones being in standby mode for the entire duration, with the exception of 30 min per day when they were in talking mode.

Counting of caspase-3–labeled cells. Paraform sections were immunostained with Peroxidase Rabbit Kit (Diagnostic Biosystems, KF-50A) for the anti-caspase-3 rabbit polyclonal antibody (1:50 dilution; Diagnostic Biosystems). We randomly selected six 200×200 μm2 fields from the three coronal sections through the Purkinje and granule cell layers of the cerebellum for each rat, and the caspase-3–labeled cells were counted. Cresyl violet staining. The paraffin sections were stained for RNA/DNA with cresyl violet to reveal the dark neurons.

Statistical analysis. Statistical analysis was performed by analysis of variance (ANOVA) with post hoc Tukey test.

Results
Cresyl violet staining was used for evaluation of the presence of dark neurons. The staining of the cerebellums of the rats in the control group revealed normal neuronal morphology in the Purkinje neurons. This was indicated by the pale blue appearance of the Purkinje neurons (Fig. 1A). In the EMF1 group, the Purkinje neurons demonstrated dark neuron degenerative changes. This was indicated by the intensely stained (dark), shrunken and irregular neuronal cytoplasms of the group dark Purkinje neurons (Fig. 1B). In the EMF2 group, dark Purkinje neurons were dispersed among intact neurons in the cerebellum. There was a reduced number of dark Purkinje neurons in the EMF2 group, in comparison with the EMF1 group (Fig. 1C). Caspase-3–labeling revealed an absence of cell staining in the control group (Fig. 2A), and positive cell staining in the EMF1 (Fig. 2B) and EMF2 (Fig. 2C) groups. The numbers of caspase-3–labeled neurons were observed to be 0.04±0.02, 2.05±0.09 and 0.69±0.06 for the control, EMF1 and EMF2 groups, respectively (mean ± SEM; Fig. 4). The caspase-3–labeled Purkinje neurons and granule cells were more prevalent in the EMF1 and EMF2 groups, compared with the control group (P<0.001; Figs. 3 and 4). There was a significant reduction in the number of caspase-3–labeled Purkinje neurons and granule cells in the EMF2 group, as compared with the EMF1 group (P<0.001; Figs. 3 and 4).

Conclusions
McNamee et al reported that there was no increase in apoptosis in the cerebellums of immature mice subjected to acute EMF exposure (3), whilst Joubert et al observed that EMF exposure did not significantly increase the apoptosis rates in rat primary neuronal cultures (2). However, Bas et al demonstrated that postnatal exposure to 900-MHz EMF reduced the number of pyramidal cells in the cornu ammonis (CA) of the female rat hippocampus (6). In the present study, the EMF1 group demonstrated a significant increase in the number of caspase-3–labeled Purkinje neurons and granule cells present in the cerebellum. This increase in the number of dark neurons indicated a greater apoptotic effect on the cerebellar cells than short-duration exposure, even at the same frequency.

Lycopersicon esculentum and lycopene consumption are correlated with certain diseases that are associated with cerebral neurodegeneration, such as Alzheimer’s disease. Lycopene protects against neurotoxicity by inhibiting the ROS-initiated mitochondrial apoptotic pathway. Caspase-3 is considered to be a key protease responsible for a number of the biological and morphological changes of apoptosis and cell death. The present study revealed that Lycopersicon esculentum extract reduced apoptosis in the Purkinje neurons and granule cells in the EMF2 group.

In conclusion, this study demonstrated that Lycopersicon esculentum exerted a protective effect against EMF-induced apoptosis and neurodegeneration in rat Purkinje neurons and granule cells, during pre- and postnatal periods. Further investigation is required to evaluate the neuroprotective efficacy of Lycopersicon esculentum in vivo.

References