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The effects of radiofrequency electromagnetic radiation emitted by mobile phones on rat parotid gland histology – an experimental study

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Abstract. – OBJECTIVE: The advancement of telecommunication technology and devices promptly transformed mobile phones into indispensable objects in our day-to-day lives, but their biological effects remain unclear. Therefore, this study aimed to investigate the potential histopathological changes induced by mobile phone radiation in the parotid gland and the nearby tissues.

MATERIALS AND METHODS: Thirty female Rattus Norvegicus rats were divided into three groups: group 1 (exposed for 30 days), group 2 (exposed for 60 days), and control group (non-exposed). Each subject was exposed to mobile phone radiation in the form of a phone call for two hours every day for their subsequent exposure time. The exposure was always directed towards the same side of the face throughout the whole exposure period. At the end of the exposure period, a comprehensive examination was conducted, including inspection of the orofacial structures, tissue sections of the parotid glands, overlying skin, oral mucosa, and cervical lymph nodes, as well as obtaining smears from the oral cavity. To highlight the presence of micronuclei within the exfoliated squamous cells of the oral epithelium, Feulgen stain was performed.

RESULTS: The results showed a significant activation of the fibroblasts in the parotid gland septa, in both exposed experimental groups, compared to the control group. We also detected significant cervical lymph node reactive changes, hyperkeratosis of the oral epithelium, and activated fibroblasts in the dermis and oral mucosa lamina propria in both experimental groups. Dermal fibrosis and lamina propria fibrosis were significantly increased in the second experimental group, compared to the control group. Moreover, vascular congestion in the parotid gland, dermal, and lamina propria fibrosis were significantly increased in the second study group compared to the first one.

CONCLUSIONS: These findings suggest that exposure to mobile phone radiation may lead to pathological changes in the parotid gland and nearby tissues of experimental rats.

Key Words:
Mobile phone, Parotid gland, Electromagnetic radiation, Histopathology.

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Introduction

The current number of mobile phone users worldwide has continued to grow exponentially in the past decades; thus, in 2022, there were around 7.26 billion mobile phone users worldwide, but it is expected to reach 7.33 billion in 2023[1]. For most mobile phone users, these devices are indispensable in their day-to-day life. Nowadays, our lives are strongly related to the use of mobile phones, which have become our first resource for gathering information, studying, researching, and getting up to date on the newest events. Moreover, mobile phones have become our primary source of entertainment, with children starting to be exposed to smartphones as an occupational activity even from the first year of life[2]. While mobile phones' potentially hazardous effects on human health have been a subject of debate in the research community, no clear conclusion has been reached. In 2011, the IARC Monograph Working Group classified radiofrequency electromagnetic radiation associated with mobile phone use, as group 2B-possibly carcinogenic to humans[3]. It is important to be able to identify the possible harmful effects that mobile phones could have. While radiation may be less harmful to adults, it is very important to be acquainted with its effects in children, mainly because of its impact on growth and differentiation processes, especially of the nervous system, skeletal muscle, bone tissue, or immune systems[4]. Christ et al[5] reported that certain areas within children's brains can experience 1.6 to 3.2 times more exposure than in adults. Additionally, children's bone marrow exposure can be up to 10 times higher than that of adults, primarily due to the decrease in electrical conductivity in human tissue as it ages.

Mobile phone radiation is defined as radiofrequency radiation on the electromagnetic spectrum (RF-EMR), and, despite being surrounded by several electromagnetic energy sources found in nature, such as the sun, the risk posed by mobile phones increases due to their proximity to the human body, particularly the head, which absorbs more than 80% of the radiation[6]. Mobile phone radiation is non-ionizing, which means that, unlike ionizing radiation, which affects atoms by removing electrons, non-ionizing radiation has no impact on atomic structures. Although regarded as safer compared to ionizing radiation, it is critical to consider the lifelong, chronic exposure we face from mobile phones[7-9].

The Specific Absorption Rate (SAR) is a standard unit that has been established to evaluate the amount of radiation to which the living body is exposed by mobile phones, and it indicates the rate of absorbed energy by the human body when exposed to radiation[7]. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines[9], SAR limitations are extremely restricted for electromagnetic fields from 100 kHz to 6 GHz being: whole-body average SAR of 0.08 W/kg, local head/torso SAR of 2 W/kg, local limb SAR of 4 W/kg.

The actual amount of energy absorbed by the human body from mobile phones has been a subject of debate since radiation is influenced by multiple factors such as proximity, frequency, exposure duration, intensity, number of emitters near the location, etc.[7,10,11]. Nowadays, it is generally admitted that a SAR of 2 W/kg in 10 g of living tissue is the maximum admissible amount for safety, and generally, it is accepted that a SAR of 4 W/kg in 10 g of living tissue represents the threshold for induction of biological effects[12-14].

Since mobile phones became widely available to the general population, researchers have been investigating the potential risks that mobile phone exposure poses to human health; the biological effects ranged from mild local warmth (increased tissue temperature or thermal effects) to possible tumor induction, but the results have been contradictory so far[15,16].

Nowadays, the literature describes two types of mobile phone RF-EMR biological effects on human tissues: thermal and non-thermal effects[17].

Thermal effects cause tissue temperature to rise by the rotation and vibration of water molecules in living bodies and can be influenced by radiation intensity and frequency, tissue thickness, water content, and the quantity of absorbed energy[7,17]. Thermal damage has been reported at an increase of 4.5 degrees Celsius (ºC) for the brain tissue[18], 3-5ºC for the induction of cataracts in the lens[19], and at least 10ºC for the skin[14]. An increase of 1 to 2ºC can lead to various physiological responses in different tissues[13,14]. Anderson and Rowley[20] reported that the cheek local temperature increases by roughly 2.6ºC after 15 minutes of using a GSM 900 phone, a device widely available before smartphones. A further increase rate of about 22-30% would have been possible if the exposure time was prolonged from 15 to 30 minutes. It is important to note that, compared to this analysis, cell phone frequencies

are substantially higher nowadays, more than double the ones used in this study.

Recent studies[8,21] highlight how mobile phone RF-EMR may induce non-thermal effects, such as cellular DNA damage, changes in the plasma membrane structure and function, and alteration of free radical metabolism[8,21-23]. Increased quantities of free radicals may have an impact on several cellular and physiological processes, including gene expression[23,24], calcium release from intracellular storage sites leading to calcium overload[8,21,22], cell differentiation[8,17,25], expression of heat shock proteins[21,26] as well as dysregulation of apoptosis signaling pathways[8,17,21,22,27].

The harmful cumulative effects of mobile phone radiation have been reported on the male[28,29] and female[30] reproductive system, heart[17,31], brain[17,32,33], salivary glands[34], kidney[17,35], eye[17,32], liver[17,32], bone marrow[17,32], etc. Neuropsychiatric disorders were also associated with mobile phone radiation exposure[32].

Biomarkers of exposure (chromosomal abnormalities, micronuclei, sister chromatid exchanges, etc.), biomarkers of susceptibility/risk (genetic variations), and biomarkers of disease are the three categories of biomarkers that are subject of research in molecular epidemiological studies[36,37]. There are no clear distinctions between these three categories; for example, chromosomal defects may be regarded as both biomarkers of exposure and indicators of cancer risk[38].

Micronuclei are sensitive biomarkers of DNA damage. Thus, the micronuclei assay has the potential to serve as an effective biomarker of exposure in order to improve the implementation of biomonitoring, diagnosis, and treatment of diseases caused by or related to genetic damage[39,40]. There are several diagnostic procedures available nowadays, including routine histological exams, exfoliative cytology, and immunohistochemistry. Oral exfoliative cytology is an effective approach for detecting micronuclei in oral epithelium, with a sensitivity of 94%, specificity of 100%, and accuracy of 95%[41]. Numerous staining methods such as May-Grunwald Giemsa (Giemsa), acridine orange, and propidium iodide have been used to evaluate micronuclei; however, the Feulgen-Fast Green stain has been recommended due to its DNA specificity[39].

Establishing a definitive cause-effect relationship is challenging, but some studies suggest that prolonged exposure to mobile phone RF-EMR may be linked to an increased incidence of cancer and other pathological conditions[34,42-44].

The purpose of our work was to explore the potential negative effects of mobile phone radiation released during phone calls on the head region that is in close contact or nearby phone location. The parotid gland is the largest and most superficially located of the three pairs of salivary glands, sitting just 4 to 10 mm under the skin[45], which may increase its vulnerability to RF-EMR. Furthermore, examination of the nearby tissues, such as the skin, the oral mucosa, and the cervical lymph nodes, might reveal a difference in adverse effects based on the power of penetrability of the mobile phone (MP) radiation. Therefore, our study aims to investigate the potential histopathological changes induced by electromagnetic radiation emitted by mobile phones on the parotid gland and adjacent structures in rats.

Materials and Methods

Animals

Rattus Norvegicus female adult rats (n=30) weighing between 230-270 grams were chosen to be used for this study due to their particularly small size which made immobilization easier without the need for sedation. Immobilization was desired in order to maintain exposure exclusively to one chosen side of each rat throughout the exposure period. The rats were obtained from the Center for Experimental Medicine and Practical Skills of "Iuliu Haţieganu" University of Medicine and Pharmacy.

The experimental animals were placed in cages in conformity with European standards[46]. The rats were acclimated under these conditions for seven days before starting the experiment.

The quarantine area was equipped with proper ventilation (10-20 air changes per hour), the temperature was optimally selected for the species (22±2°C) and the humidity was held at 50%±10. Lightning was set to satisfy animal biological needs (12 h light/dark cycles). The diet consisted of compound feed administered *ad libidum* along with free access to tap water.

The immobilization cages were made from plastic material and contained 3 slots, in accordance with the rats' dimensions; the middle slot of each cage remained empty during irradiation. The top of the cage had been perforated in two spots per slot, to allow phone insertion. The cages also had perforations on the sides, to allow ventilation.

The current study was approved by the Ethical Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania (approval number AVZ147/6.06.2022), and by the National Sanitary Veterinary and Food Safety Authority (approval number 343/19.12.2022) in conformity with ethical regulations, and fulfills the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) requirements for animal research[47]. Specific regulations and amendments used in this study were from the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology[48] (Reston, VA, USA) and all national laws regarding the protection of animals used for scientific research.

Exposure Source

Animals were exposed to electromagnetic radiation emitted by commonly used mobile phones during current times, which were fourth-generation (4G) devices. These phones used the n77/n78 telecommunication bands with frequencies ranging from 3,400 MHz to 3,800 MHz and the n1 band with frequencies of 2,100 MHz, with a declared specific absorption rate between 0.87 and 1.39 W/kg.

Experiment Protocol

A total of 30 female adult Rattus Norvegicus rats were randomly assigned into 3 groups: 2 experimental groups and one control group. Group 1 (n=13) was exposed to mobile phone radiation for 2 hours per day, 7 days per week for 30 days, and group 2 (n=13) was exposed to mobile phone radiation for 2 hours, 7 days per week for 60 days. Group 3 (n=4) represented the control group that was not exposed to mobile phone radiation and was kept in the same environmental conditions.

Each animal belonging to the study groups was assigned a number from 1 to 13, so that the even-numbered ones were exposed on their right side and the uneven ones on their left side.

For each irradiation session, two rats were placed in the left and right slots of the cage, with the middle slot remaining empty, to avoid interferences between the phones. Next, a phone call was made between two mobile phones and each of them was inserted upside-down through the top perforations, aiming the phone's antenna at the latero-cervical side of each rat that needed to be exposed (Figure 1). This way, the distance between the rats' cervical areas and the mobile phone antenna was below 5 mm, simulating hand-held mobile phones in humans. The phone call was maintained for the two hours of radiation time.

At the end of the exposure period, the rats were sacrificed with xylazine/ketamine overdose using protocols accepted by the Ethical Committee.

Pathologic Assessment

Grossing procedures included inspecting the orofacial structures, performing tissue sections of the parotid glands, overlying skin, oral mucosa, and cervical lymph nodes, and obtaining smears from the oral cavity for further investigations.

The removed parotid glands, skin, oral mucosa, and cervical lymph nodes were processed using standard procedures. Thus, the tissues were fixed in 10% buffered formol, processed, embedded in paraffin, and sectioned, resulting in 3 mm sections stained with Hematoxylin and Eosin (H&E). The H&E staining protocol included the following steps: first, the sections were deparaffinized with xylene and hydrated by immersion in different concentrations of ethanol (100%, 95%, 80%) and washed in deionized water. Secondly, the sections were stained with Hematoxylin solution composed of oxidized hematoxylin and aluminum salt, followed by bathing in tap water and distaining the excess background stain using acid ethanol. Thirdly, the sections were counterstained with Eosin solution, followed by washing, dehydrating in ethanol of different concentrations (95%, 100%), and clearing with xylene. Finally, the sections were mounted on glass slides using polystyrene and covered completely by a glass coverslip.

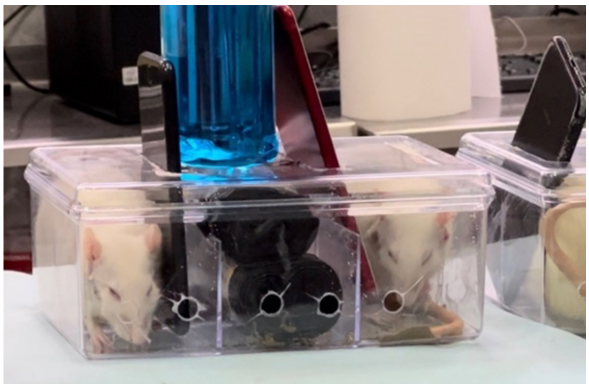


Figure 1. Distribution of subjects in the immobilization cages during irradiation sessions.

To highlight the presence of micronuclei within the exfoliated squamous cells of the oral epithelium, Feulgen stain was performed. First, the smears were rinsed in distilled water for 10 minutes, washed for 1 minute in M-HCl at ambient temperature, and immersed in preheated M-HCl for 8 minutes at 60°C. The next part included immersion of smears in M-HCl for 1 minute, Schiff Reagent McManus for 45 minutes to an hour, and washing them in bisulfite solution for 2 minutes (3 times). Next, the sections were rinsed in tap water for 5 minutes, introduced in distilled water, counterstained in light green SF yellowish stain 1%, for 1 minute, followed by dehydration in ethanol of different concentrations (95%, 100%) and clarification in xylene. Finally, the smears were covered by a glass coverslip.

The sections were examined using Leica DM750 microscope (Leica Microsystems; Wetzlar, Germany) and captured with Real-time Digital Pathology System Aperio LV1 (Leica Biosystems; Richmond, VA, USA).

For the parotid gland, the collected variables were represented by: cytonuclear atypia of the acinar cells, alterations of excretory ducts, fibroblast activation, inflammatory reactions and fibrosis in the septa, hemosiderin deposits, and vascular congestion. In the cervical lymph node, we evaluated follicular hyperplasia, paracortical hyperplasia, sinus histiocytosis, and sinus expansion, while the parameters regarding the skin and the oral mucosa were represented by cytonuclear atypia of epithelial cells, the occurrence of micronuclei, hyperkeratosis, fibroblast activation, inflammatory reactions, edema, fibrosis, and vascular congestion. When achievable, the changes were scored semi-quantitatively using a four-tiered system: 0 for absent changes; 1 for mild changes; 2 for moderate changes, and 3 for severe changes.

Statistical Analysis

Data regarding the subjects, along with the pathological parameters were introduced in a Microsoft Excel spreadsheet (Microsoft 365). The associations between the variables were determined using the Fisher Exact Test, in IBM SPSS V. 25 (IBM Corp., Armonk, NY, USA). Statistical significance was considered when the *p*-value was 0.05 or below.

Results

Macroscopy

No differences were observed regarding weight, physical activity, food, and water intake in the study groups *vs.* the control group in this experiment. During dissection, the exposed cervical area of the subjects belonging to the study groups showed grossly visible changes in the lymph nodes' diameter, consistent with latero-cervical adenopathy. Slight congestion was also spotted. The parotid glands showed no macroscopic changes.

Microscopy

The histopathological changes observed in the parotid gland, the overlying skin, the oral mucosa, and latero-cervical lymph nodes, along with their associated *p*-values are depicted in Table I.

Parotid Gland

Microscopic assessment of the parotid glands in the control group (Figure 2) revealed septal congestion (3 out of 4 cases) and hemosiderin deposits (2 out of four cases).

In the first study group (animals exposed to radiations for 30 days), we observed mild (one case, 7.69%) and moderate (one case, 7.69%) cytonuclear atypia of the acinar cells (Figure 3A-B) and dilated excretory ducts with intraluminal inflammatory cells (Figure 3C) in 5 animals. Within the stromal compartment, this group also presented congestion (2 cases, 15.38%); hemosiderin deposits (2 cases, 15.38%) (Figure 3B); mild (one case, 7.69%), moderate (3 cases, 23.07%) and severe (one case, 7.69%) lymphoplasmacytic inflammatory infiltrate associated with a few neutrophils, as well as reactive fibroblast proliferation (all cases, 100%, $p<0.001$) and fibrosis (one case, 7.69%) in the connective tissue septa.

The second study group (60 days exposed animals) presented moderate (2 cases) and severe (1 case) cytonuclear atypia of the acinar cells (Figure 4A-B) in 3 cases and no duct dilatation. We also detected congestion in 10 animals (76.92%) (Figure 4A-C); hemosiderin deposits (4 cases, 30.77%) (Figure 4A); mild (one case, 7.69%) (Figure 4A), moderate (2 cases, 15.38%) and severe (3 cases, 23.07%) lymphoplasmacytic inflammatory infiltrate associated with rare neutrophils; reactive fibroblast proliferation (all cases, 100%, $p<0.001$) (Figure 4C) and fibrosis (5 cases, 38.46%) in the connective tissue septa. Vascular congestion was significantly more abundant in the second exposed group compared to the first study group ($p=0.004$).

Table I. Histopathological effects observed in the study groups.

Region	Morphologic changes	Frequency withing group 1, n (%)	*p*-value: group 1 *vs.* control	Frequency withing group 2, n (%)	*p*-value: group 2 *vs.* control	*p*-value: group 1 *vs.* group 2
Parotid gland	Cyto-nuclear atypia in the acinar cells	2 (15.38%)	1	3 (23.07%)	0.54	1
	Dilated excretory ducts with intraluminal neutrophiles	5 (38.46%)	0.26	0	nc	nc
	Activation and proliferation of the septal fibroblasts	13 (100%)	**0.00042**	13 (100%)	**0.00042**	nr
	Septal inflammatory infiltrate	5 (38.46%)	0.26	6 (46.15%)	0.23	1
	Septal fibrosis	1 (7.69%)	1	5 (38.46%)	0.26	0.16
	Hemosiderin deposits	2 (15.38%)	0.55	4 (30.77%)	1	0.64
	Vascular congestion	2 (15.38%)	0.28	10 (76.92%)	0.17	**0.004**
Epidermis	Cyto-nuclear atypia	4 (30.77%)	0.51	5 (38.46%)	0.26	1
Oral epithelium	Cyto-nuclear atypia	1 (7.69%)	1	3 (23.07%)	0.54	0.59
	Micronuclei	0	nc	2 (15.38%)	1	0.48
	Hyperkeratosis	13 (100%)	**0.00042**	13 (100%)	**0.00042**	nr
Connective tissue of the dermis and oral mucosa lamina propria	Activation and proliferation of fibroblasts	13 (100%)	**0.00042**	13 (100%)	**0.00042**	nr
	Inflammatory infiltrate	7 (53.84%)	0.1	8 (61.53%)	0.08	1
	Edema	8 (61.53%)	0.35	0	0.08	**0.0016**
	Fibrosis	0	nc	10 (76.92)	**0.014**	**0.0001**
	Vascular congestion	7 (53.84%)	1	8 (61.53%)	0.64	1
Cervical lymph nodes	Follicular and paracortical hyperplasia, sinus histiocytosis and expansion of the sinuses	13 (100%)	**0.00042**	13 (100%)	**0.00042**	nr

Group 1 (n=13): exposed to mobile phone radiations for 30 days; Group 2 (n=13): exposed for 60 days, in relation to the control group (n=4). The value in bold shows $p<0.05$. A *p*-value lower than 0.05 leads to the rejection of the null hypothesis of independence and suggests that there is evidence to support a relationship or association between the variables being analyzed. nc = not calculated, variable absent in all subjects of the study group; nr = not statistically relevant.

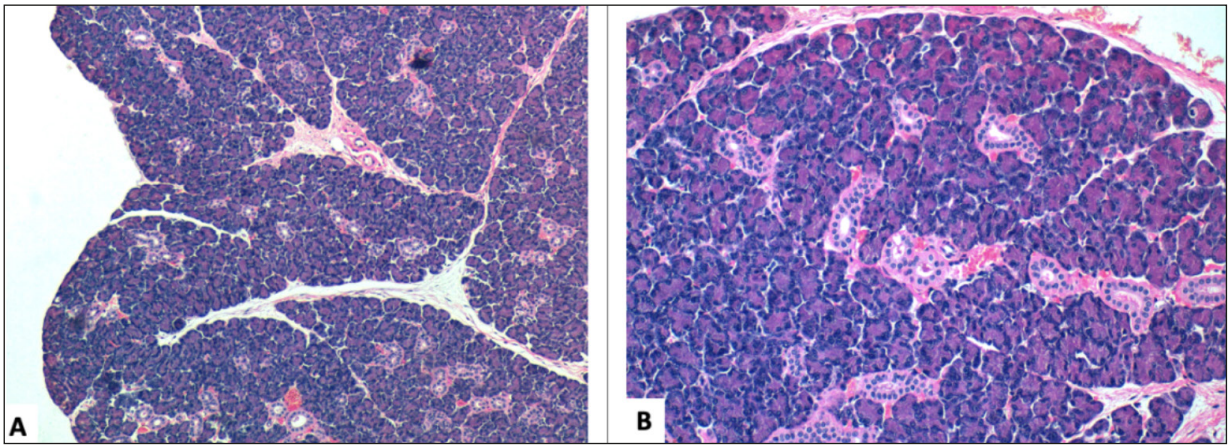


Figure 2. Microscopic aspect of the parotid gland in the control group: the normal architecture of the parotid acinar cells and excretory ducts; septal congestion; (**A**) H&E, 10×; (**B**) H&E, 20×.

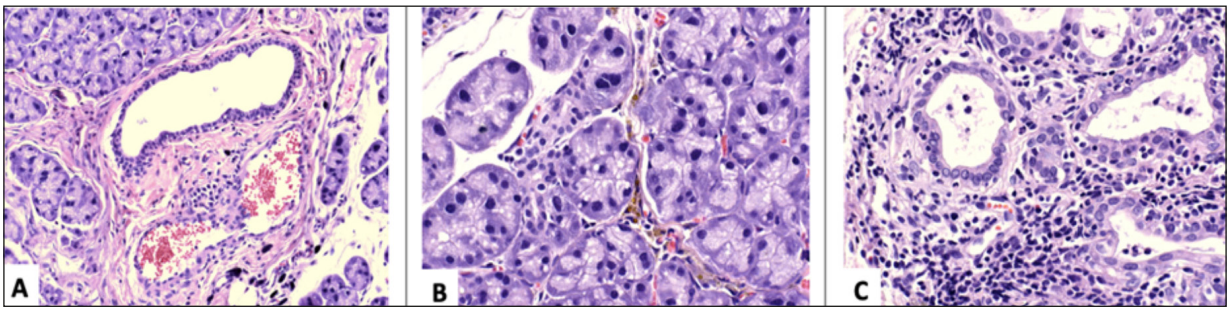


Figure 3. Histopathological sections from the parotid glands, obtained from the first study group, exposed for 30 days; (**A**) mild and moderate cytonuclear atypia of the acinar cells, vascular congestion, and reactive fibroblast proliferation, H&E, 20×; (**B**) mild and moderate cytonuclear atypia of the acinar cells, hemosiderin deposits, H&E, 40×; (**C**) dilated excretory ducts with intraluminal inflammatory cells; interstitial moderate lymphoplasmacytic inflammatory infiltrate, H&E, 40×.

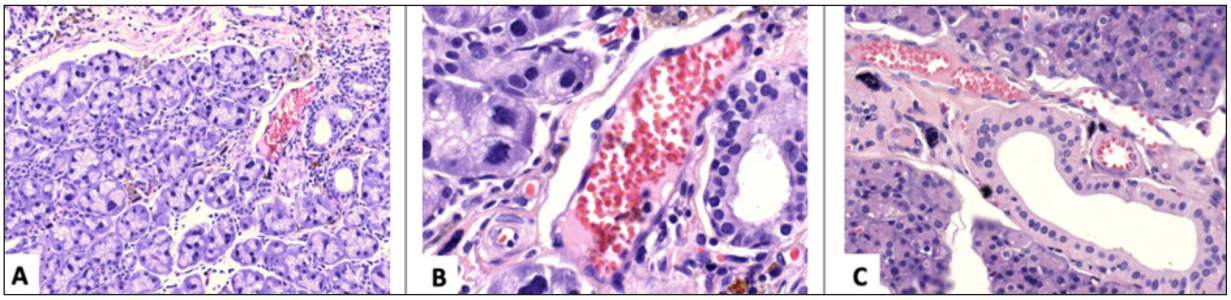


Figure 4. Histopathological sections of the parotid glands, obtained from the second study group, exposed for 60 days; (**A**) moderate and severe cytonuclear atypia of the acinar cells, septal fibrosis, vascular congestion, hemosiderin deposits, mild lymphoplasmacytic inflammatory infiltrate, H&E, 20×; (**B**) moderate and severe cytonuclear atypia of the acinar cells, vascular congestion, H&E, 40×; (**C**) reactive fibroblast proliferation; vascular congestion, H&E, 40×. **A-B**, These images have been captured at a higher magnification level.

Skin

Regarding the histopathological alterations of the lateral-cervical skin overlying the parotid gland, we detected edema in 2 cases and dermal congestion in 3 cases belonging to the control group (Figure 5A). Four animals (30.77%) from the first exposure group (30 days) displayed mild cytonuclear atypia (slightly increased hyperchromatic nuclei, with nuclear irregularities), mainly in the basal layer of the epidermis (Figure 5B). Within the dermis, the majority of the animals from study group 1 presented congestion, edema, mild lymphoplasmacytic inflammatory infiltrate, and reactive fibroblast proliferation (Figure 5C). In the 60 days exposed group, we identified moderate cytonuclear atypia (moderate nuclear pleomorphism, with loss of polarity, moderately increased nuclear size, hyperchromatic or vesicular nuclei, nuclear irregularities, and scattered mitotic figures) in the basal and spinous layers of the epidermis in 4 animals (Figure 5D-E) and mild cytonuclear atypia in only one case (7.69%). Congestion, moderate lymphoplasmacytic inflammatory infiltrate, and reactive fibroblast proliferation with fibrosis in the dermis were observed in 8 (61.53%); 8 (61.53%); 13 (100%) and 10 (76.92%) cases, respectively (Figure 5F). None of the rats from group 1 showed fibrosis, as opposed to animals from group 2 ($p<0.001$).

Oral Mucosa

There were no changes in the oral epithelium of the subjects belonging to the control group (Figure 6). However, half of them showed congestion and edema in the lamina propria.

We noticed hyperkeratosis in the oral surface epithelium (Figure 7) of all radiation-exposed rats ($p<0.001$). In addition, the 60-day exposed group showed parakeratosis and acanthosis of the oral surface epithelium (Figure 7C-D). Within the 30-day exposed rats, only one (7.69%) presented mild cytonuclear atypia in the basal and parabasal layers of the surface epithelium

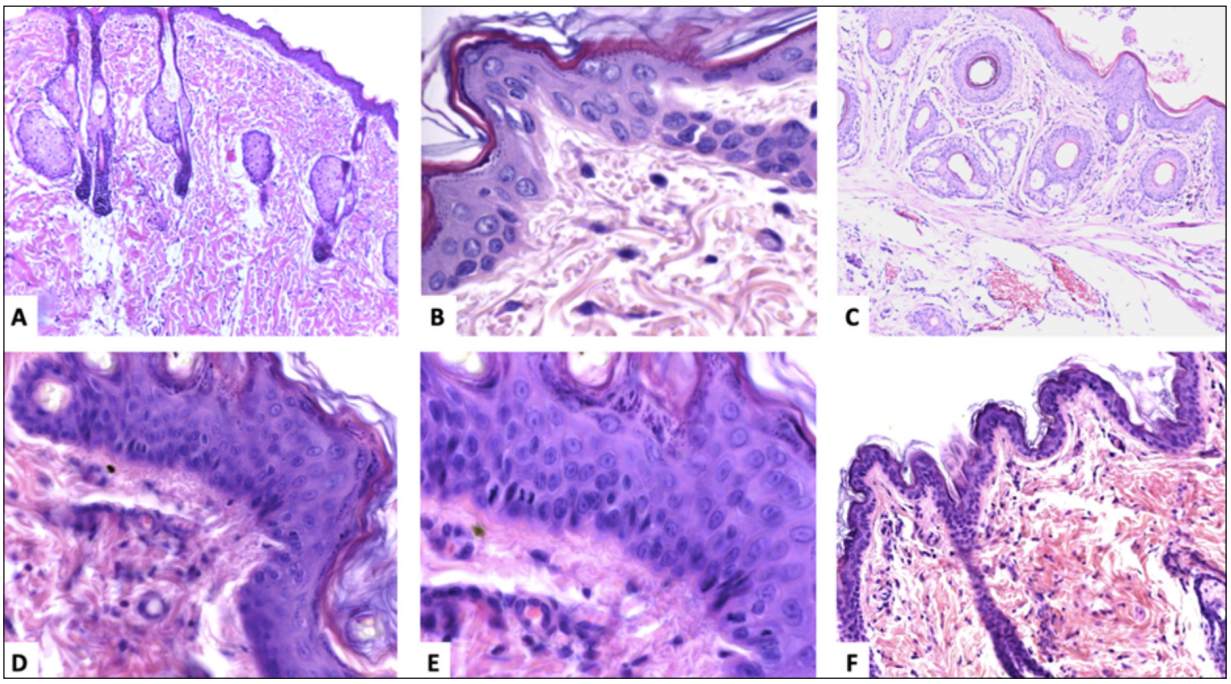


Figure 5. Histopathological sections of the skin; (**A**) histology from a tissue sample belonging to the control group, H&E, 10×; (**B**) mild cytonuclear atypia of the basal layer of the epidermis in the first exposure group (30 days), H&E, 20×; (**C**) dermal edema, congestion, mild lymphoplasmacytic inflammatory infiltrate, and reactive fibroblast proliferation in the first exposure group (30 days), H&E, 10×; (**D**) moderate cytonuclear atypia and mitotic figures within the epidermis in the second exposure group (60 days), H&E, 40×; (**E**) moderate cytonuclear atypia and mitotic figures in the basal and spinous layers of the epidermis in the second exposure group (60 days), H&E, 60×; (**F**), mild lymphoplasmacytic inflammatory infiltrate and reactive fibroblast proliferation, with fibrosis in the dermis, in the second exposure group (60 days) H&E, 10×. **D-E**, These images have been captured at a higher magnification level.

(Figure 7A-B). Most animals from group 1 displayed congestion, edema, mild lymphoplasmacytic inflammatory infiltrate, and reactive fibroblast proliferation in the lamina propria. In the 60-days exposed rats, we highlighted moderate cytonuclear atypia in one case (7.69%) and severe cytonuclear atypia (severe nuclear pleomorphism with loss of polarity, nuclear molding and overlapping, large nuclei, hyperchromatic or vesicular nuclei with prominent nucleoli, nuclear irregularities, and frequent mitosis) in 2 cases (15.38%), affecting between two thirds to the entire thickness of the epithelium (Figure 7C-D). In this group, the majority of the animals presented congestion, moderate inflammation, and reactive fibroblast proliferation with fibrosis in the chorion.

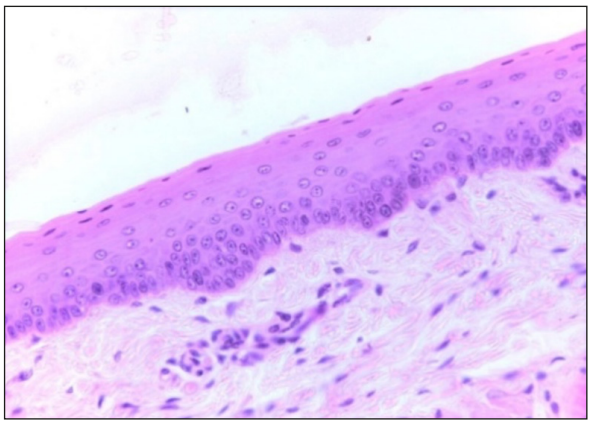


Figure 6. Histological aspect of the oral epithelium and lamina propria in the control group, H&E, 20×.

Moreover, in both rats with severe cytonuclear atypia (15.38% cases), we found micronuclei within the exfoliated squamous cells of the oral surface epithelium. The micronuclei appeared as small, purple spheroidal structures in the cytoplasm adjacent to the nuclei (Figure 8).

Lymph Nodes

As opposed to the control group, where we did not encounter notable pathological changes (Figure 9A), all radiation-exposed rats presented latero-cervical lymph node alterations (p<0.001), including

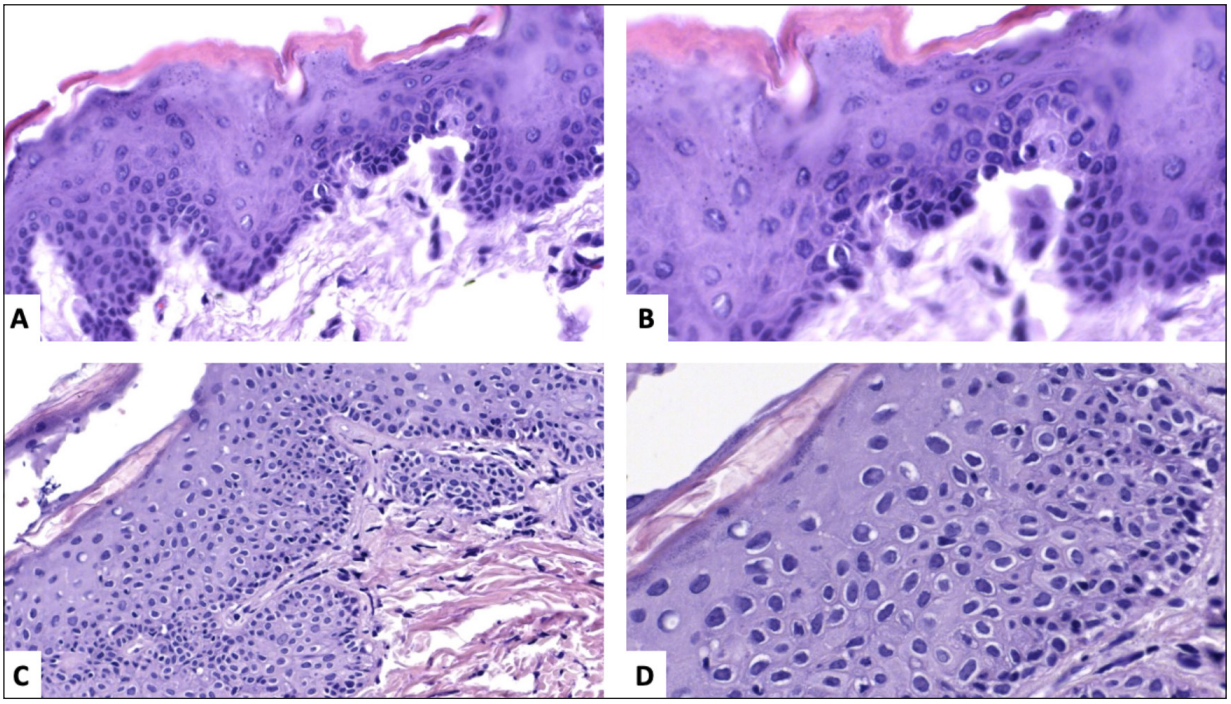


Figure 7. Histopathological sections from the oral mucosa. First study group (30 days): mild cytonuclear atypia in the basal and parabasal layers of the epithelium; hyperkeratosis (**A**), H&E, 40×; (**B**), H&E, 60×. Second study group (60 days): moderate and severe cytonuclear atypia in the basal and parabasal layers of the epithelium; parakeratosis and acanthosis (**C**), H&E, 40×; (**D**), H&E, 60×. **A-B**, and (**C-D**) have been captured at a higher magnification level.

diffuse paracortical hyperplasia, reactive follicular hyperplasia, and prominent sinuses, lined by hyperplastic sinus histiocytes (Figure 9B-C).

Discussion

Mobile phone use has surged in recent decades, with projections indicating further growth in the near future[49]. Up to now, the possible pathological effects are explained by the thermal and non-thermal processes that emerge during phone conversations. The heat leads to increased capillary blood flow, while other incriminating mechanisms include molecular alterations, oxidative stress, activation of the parasympathetic axis, and decline of the sympathetic system[50,51].

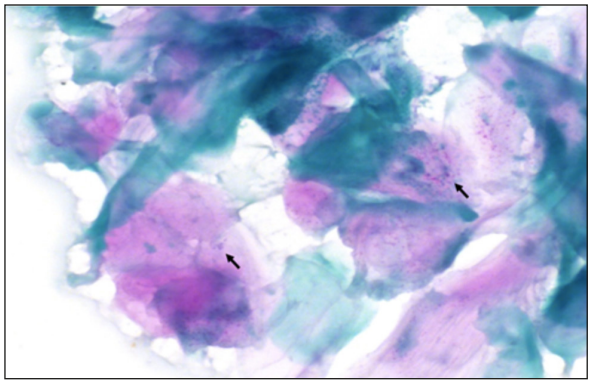


Figure 8. Smear from the oral mucosa; arrows: micronuclei within the cytoplasm of oral exfoliated epithelial cells. Feulgen stain; 60×.

Since the electromagnetic radiation emitted by mobile phones acts locally, most of the anticipated modifications arise in the head and neck region. Studies[52] report that a high amount of cell phone radiation is absorbed by the head and neck area, as well as by the hand.

In our study, we focused on the histopathological changes of the parotid gland, an organ located superficially in close vicinity to the placement of mobile phones in humans, as well as on changes in the orofacial region and latero-cervical region. To comply with this principle, we used special cages for the immobilization of the experimental rats, and the mobile phones were located unilaterally at the cervical area during exposure.

So far, data regarding the effects of mobile phone radiation on the parotid gland are conflictual. While some papers stress the importance

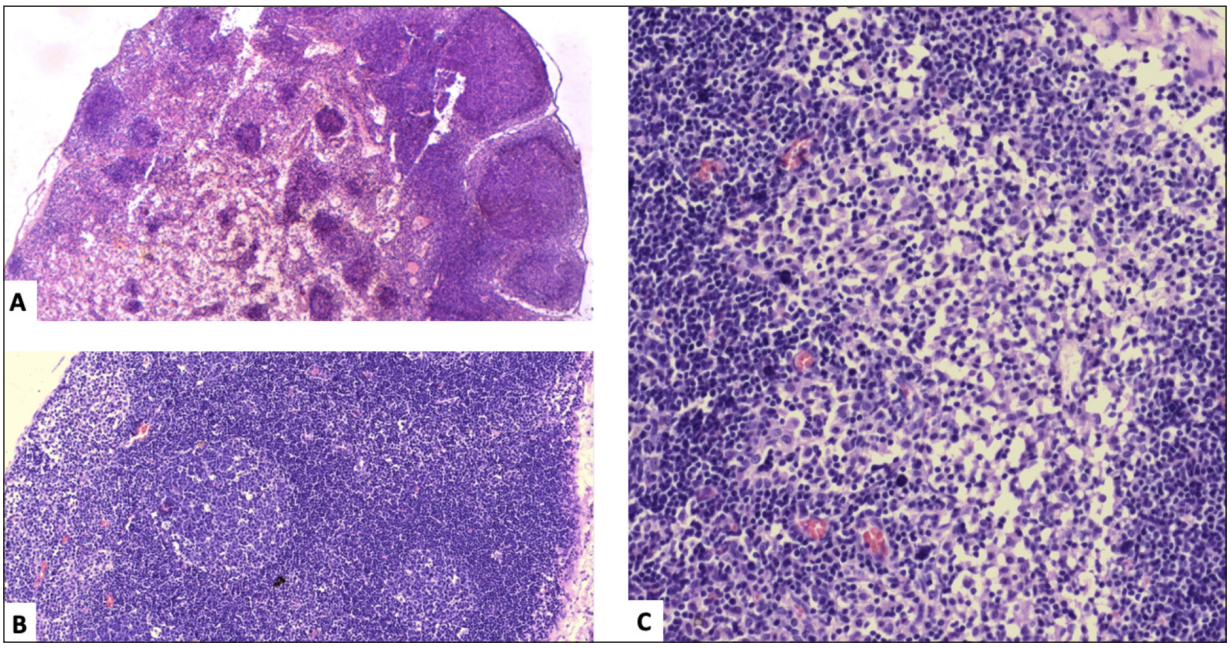


Figure 9. Photomicrographs of latero-cervical lymph nodes; (**A**) normal architecture of the lymph node parenchyma in the control group, H&E, 10×; (**B**) reactive follicular hyperplasia and diffuse paracortical hyperplasia in the first study group (30 days), H&E, 10×; (**C**) prominent lymph node sinuses, lined by hyperplastic sinus histiocytes in the first study group (30 days) H&E, 20×.

of the negative mechanisms in both human and animal subjects, other studies[53] found no correlations between mobile phone use and structural or functional transformations. Meanwhile, studies concerning morphological changes *per se* are scarce in the current academic writing.

We demonstrated that phone radiation induces morphological alterations in both the parotid gland and the surrounding orofacial structures of experimental rats. Our experiment reveals important activation and proliferation of the fibroblasts in the connective tissue component of all the examined tissues, increased vascular congestion in the 60-day exposed group, and cytonuclear atypia in a few epithelial cells belonging to the studied organs. Furthermore, if we focus on the interstitial findings (edema in the first study group, exposed for 30 days, and fibrosis in the second group, exposed for a longer time), there seems to be a continuity regarding the pathologic processes. Moreover, long-term exposure seems to accelerate the pathophysiologic effects, as suggested by the occurrence of micronuclei in the smears of the oral epithelium in the second exposure group.

Comparable to our research, Aydogan et al[54] discovered numerous morphological changes in the parotid glands of short and long-term radiation-exposed rats. However, their findings report statistical significance regarding only one parameter (variation in cell size) when correlating long-term radiation-exposed rats with the control group. Moreover, the radiofrequency radiation was induced by a generator and not by actual mobile phones[54]. Another study[55] that evaluated the effect of mobile phone-induced electromagnetic field on the parotid gland in rat models recognized several harmful processes, some of them similar to ours, partly explained by the authors because of the high levels of reactive oxygen species. Structural alterations depicted with the help of light microscopy in the parotid glands of subjects from their exposed group included irregular acinar contours, cytoplasmic vacuoles and hyperchromatic nuclei of the acinar cells, dilated ducts, and prominent septa, with congested blood vessels and dense collagen fibers. The latter were highlighted by Masson's trichrome histochemical stain and were absent in the control group. Interestingly, periodic acid-Schiff (PAS) staining showed increased mucous secretion in the acinar cells. Electron microscopy further illustrated shrunken acini and nuclear atypia, consolidating the previously mentioned changes[55]. An Egyptian study conducted by Fathy et al[56] on experimental rats showed degeneration of acini and duct dila-

tion in sections of the parotid gland following prenatal and postnatal radiofrequency radiation.

A recent review[57] that focused on evaluating the impact of the radiation emitted by mobile phones on the salivary glands highlighted their negative effect. Most of the studies included in the systematic review[57] (n=11) showed changes in the parotid gland salivary flow rate and its composition. The authors stress the importance of these changes since salivary gland disorders induce complications, mainly concerning the oral cavity[53]. In another review conducted by Mishra et al[58], the authors extended the search of the effects of electromagnetic radiation emitted by mobile phones on orofacial structures, although the main focus of the work remained the parotid gland. Most of the studies (79%) included in that paper[58] were conducted on human subjects, while the rest were animal studies: 16% were performed on experimental rats and only one (4%) on rabbits. They found adverse effects in the salivary glands, oral mucosa, and facial nerves. Out of the 24 studies included, 15 (62.5%) showed alterations or adverse effects, such as gross morphological changes in the parotid gland, nuclear abnormalities in the head and neck tissues, differences in salivary enzymes and interleukin-10 (IL-10) levels, facial nerve dysfunctions, reduced or increased salivary flow or in contrary, increased blood flow rate, vasodilatation, and focal bleeding[58].

From another perspective, some studies[33,43,58,59] investigate carcinogenesis, the end of the spectrum, and the most feared possible consequence of prolonged use of mobile phones. Numerous quests to solve this uncertainty remain, however, unanswered since, up to date, the exact relationship between mobile phone use and tumor induction and progression is not elucidated. Another study conducted by Al-assaf et al[60] evaluated the effects of electromagnetic fields emitted by mobile phones by assessing the Ki-67 index in sections of the parotid glands of rabbits, finding a significant difference regarding cell proliferation between the experimental, exposed group, and the control group. There is, however, an extensive journey to cross between simply correlating increased cell proliferation with tumorigenesis. In a case-control study, Sadetzki et al[34] suggests that there is a positive association between cellular phone use and the risk of parotid gland tumors. A Korean study[61] evaluated human cases of pleomorphic adenoma, the most frequent benign tumor of the major salivary glands, and correlated heavy phone users with tumor volume. Additionally, they found a strong correlation between the side of mobile phone use and the side where the tumor arose (left *vs.* right), suggesting that radiation may interfere with tumor behavior. In our study, we demonstrated the presence of micronuclei, common indicators of genetic instability[62,63] in smears belonging to two animals exposed for 60 days to mobile phone radiations, although this finding did not bear statistical significance. Similar to our study, other papers[64-66] did not find significant associations between the frequency of micronuclei in cells belonging to the oral mucosa in human mobile phone users. When assessing micronuclei, an important variable that needs to be included in the analysis is the age of the subjects, as highlighted by Bonassi et al[67]. In their study, which included over 5,000 human samples of exfoliated oral cells, it was demonstrated that the age of the subjects was highly associated with the observed frequency of the micronuclei[67].

Regarding the functionality of the parotid gland, several observational cross-sectional studies using human subjects demonstrated that the use of hand-held mobile phones increased the salivary flow rate and led to alterations of the salivary pH, amylase, total protein concentration, lipase or C-reactive protein levels, suggesting that chronic exposure to electromagnetic radiation leads to pathologic changes in the parotid gland[68,69]. In contrast, other studies[70] found a diminution of the salivary secretion. Ranjitha et al[71] performed ultrasonographic evaluations in order to assess the influence of handheld mobiles in the parotid, reporting significant differences in both gland volume and systolic velocity of blood flow in the dominant side of human subjects.

Our approach bears several limitations, mainly the ones derived from the design of this study, which included small sample sizes in both the study groups and the control group. However, the experimental design permitted a uniform adherence to the study protocol. We consider that the amount of time of exposure to radiation and the thorough pathologic assessment of tissue slides and smears ensure significance to the presented findings. Compared to similar papers found in the literature that examined solely the parotid gland, we also included adjacent structures, such as cervical lymph nodes, regional skin, and oral mucosa. Although some of the studied parameters did not show statistical significance, we consider that the pathologic confirmation of the structural alterations can represent a starting point for future studies. Nevertheless, we believe

that proper assessment and integration of certain variables in subsequent analyses, such as type of devices used, time, duration, area, and frequency of exposure, along with uniform protocols for both human and animal study models would lead to more accurate conclusions.

Despite conflicting findings, scientific data generally highlights the negative effects of mobile phone use and recommends cautious use. Interestingly, while most research focuses on the parotid gland, studies also emphasize molecular, biological, morphological, and functional changes in various other systems.

Cognitive impairment, increased anxiety, as well as reduction of grey matter and cellular injury in histopathologically evaluated brain tissue in test animals are some examples of unfavorable outcomes of radiation exposure in the nervous system[72-75]. Additionally, studies found subsequential changes in several organs and systems outside the head and neck area, including the digestive system[76], urogenital system[77], skeletal system[78], and even in the hematological system[79].

Conclusions

Our study revealed several histopathological alterations induced by mobile phone radiation on tissues that are in the immediate proximity of the mobile phone's location during conversation mode, such as the parotid gland, as well as surrounding orofacial structures and latero-cervical region. The presence of micronuclei suggests possible DNA damage and raises the need for further studies in order to determine the real long-run effects of mobile phone radiation on human health. Therefore, a question mark will continue to stand above the exact comprehension of mobile phone use and the long-term consequences this brings. In the meantime, the implementation of measures and preventive actions for using mobile phones should be of great interest, not only for adults but especially for the younger generations.

Authors' Contributions

Conceptualization: L.I.M., C.S.M., M.A.N., and F.I.; methodology: L.I.M., M.A.N., C.S.M., LM.G., G.D., and M.M.O.; formal analysis: A.C., L.P.M., and C.M.; investigation: C.S.M., R.T.S., L.P.M., G.D., and C.M.M.; data curation: A.C., L.P.M., R.T.S., and C.S.M.; writing-original draft preparation: L.I.M., C.S.M., R.T.S., L.P.M., C.M., F.I., and G.A.; writing-review and editing: C.S.M., A.I., C.M.M., L.P.M., visualization: C.S.M., M.A.N, C.M.M., and L.I.M.; supervision: C.S.M., M.A.N., L.I.M. and I.R.B.; project administration: L.I.M., and C.S.M.; funding acquisition: L.I.M. and I.R.B. All authors have read and agreed to the published version of the manuscript.

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Ethics Approval

The animal study protocol was approved by the Institutional Review Board of "Iuliu Haţiega-nu", University of Medicine and Pharmacy (approval number AVZ147/06.06.2022, dated June 6, 2022) and by the Romanian National Sanitary Veterinary and Food Safety Authority (approval number 343/19.12.2022, dated December 19, 2022).

Informed Consent

Not applicable.

Data Availability

Data is available on request from the corresponding author.

AI Disclosure

The authors declare that no form of generative artificial intelligence tool was used in writing or drafting material for this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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