RESEARCH ARTICLE

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Exposure of Spargue Dawley Rats to Phone Cell Electromagnetic Field Frequency and Related Physiological, Apoptotic and Molecular Profile of Para Oral Tissues: *In–vivo* Study

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Abstract

Objective: Daily exposure to cellular EMF induced a long run deleterious impact. Accordingly, the aim of the present work is to find out the deleterious drawbacks of daily exposure of human to the cellular EMF concerning the pathological, biochemical /physiological and apoptosis profile in EM and EMF/Avocado, sole Avocado and EMF exposed /recovery group of rats. **Methods:** Different age (Adult, young and Senile) groups were considered. Exposure of S, A and Y rats to EMF showed elevated ROS, MDA and reduced SOD and administration of avocado customized the changes of oxidative stress. Apoptotic activity of both tongue and PG of Y, A and senile rats exposed to EMF showed age dependent response. The apoptotic effect was arranged positively as S, Y and A, revealing higher necrotic activity, Early and late apoptosis in S, Y and A rats. PG tissue showed a noticeable resistance to EMF effect. **Conclusion:** Also, the physiological alteration was detected insignificantly among test groups where EMF/Avocado treated groups showed almost normalization of oxidative stress compared with its values in control and EMF/Avocado and EMF recovery groups. Also, pathological changes detected in exposed groups showed the same recurrency of different tissue to normal profile.

Keywords: EMF- Rats- antioxidant- cell cycle- Apoptosis- pathology

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Introduction

The usage of devices creating low- and medium-frequency electromagnetic fields (EMFs) has increased, in both the workplace and the home [1]. EMF causes harmful effects on human health. Exposure to EMF influences neural networks, body weight, tissue morphology and histology, biochemical parameters of the blood, hormones, the immune system, and the tissue repair system [2]. The alterations resulted from EMF to cells and molecules rely on the length of the exposure, penetration, and the healing capability of the tissue [3]. The EMFs generated in home environments also have some negative effects such as leukemia and tumor formation in the central neural system [4]. It has been reported that low-frequency EMF affects the cellular antioxidant defense mechanisms, that increases oxidative damage and causes DNA damage, thereby resulting in a carcinogenic effect [5-7]. As well as exposure to EMFs caused by mobile phones, it is stated to lead to lack of attention, damage in the inner ear, reduction of the speed of reflexes, blurred vision, and headaches [8]. It is shown that high-frequency EMF has a genotoxic impact on tissues [9].

Avocado is a nutritional source of vitamin E, contain eight different isoforms namely, alpha, beta, gamma, and delta-tocopherol and alpha, beta, gamma, and delta-tocotrienol [10]. Vitamin E has antioxidant properties [11], protecting cell membrane components such as poly unsaturated fatty acids (PUFA) and LDL from oxidative damage mediated by free radicals [12] induced under the effect of different stress factors. Vitamin E deficiency is associated with defected spermatogenesis, decreased testosterone production [13], and testicular degeneration [14]. Several studies reported that application of vitamin E against environmental pollutants have shown the beneficial effects of vitamin E, mainly in terms of several histological parameters, increases the epididymal sperm number [15], and the number of spermatogonia A, spermatogonia B, spermatocytes, spermatids and Sertoli cells [16], with daily sperm production [17]. Vitamin E used against TCDD-induced toxicity; has been shown to increase testicular body weight, epididymis, seminal vesicles, and ventral prostate weights. Also, vitamin E enhance elevation of GSH levels and decrease of MDA.

Aim of the study:

The purpose of this study was to investigate the possible

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effect of extremely low-frequency electromagnetic fields (ELF-EMFs), from a high-voltage source, on rat para-oral tissues (parotid gland (PG) and tongue (T)) and related oxidative stress (ROS, GSH, SOD and MDA) and the effect of antioxidants natural source (Avocado) in counteracting these effects. Also, apoptotic profile using Flow cytometry analysis and PCR.

Materials and Methods

Three groups of Spargo Dawley rat strain 10 each (Young 2weeks of age, Adult, 2 months and senile, 12 months of age) Customization of electromagnetic field production set for producing current mobile frequency at 50HZ. Avocado dried for a week and ethanol extracted. Derived extract was processed for Gas chromatography mass spectrophotometer (GCMS) to identify active compounds. Rat groups 1st set was daily exposed to EMF for 3 hrs. for 30 days. 2nd group was orally administered 0.1 ml of Avocado extract pre-exposure to EMF. The 3rd group was not exposed to EMF negative control group. Rats were scarified and target organs; T and PG were excited and processed for flow cytometry and biochemical investigations.

Avocado extract

avocado fruits extraction using ultrasonic rotary evaporator Avocado fruits were purchased from a local store then dried in the oven (Tech-Lab, stainless steel forced-air convection oven FAC-138SS) at 50 C for 14 days. High-purity analytical grade ethanol (Sigma-Aldrich, 3050 Spruce St Saint Louis, MO, 63103-2530 United States), with purity of 99.7%, was used to extract oil for 7 days. The moisture content of avocado was determined by measuring the mass of the collected samples before and after drying.

GC/MS analysis

The bioactive compounds in the avocado seed oil were analyzed using a Gas Chromatograph interfaced with a Mass Spectrometer (GC-MS) equipment; (Schimadzu GCMS-QP2010 PLUS). The GC-MS was equipped with a split injector and an ion-trap mass spectrometer detector, and used a fused-silica capillary column with a thickness of 1.00 µm, dimension of 30 mm × 0.25 mm, and a programmed temperature range from 60 °C to 250 °C at a rate of 3.0 °C/min. The injector temperature was set at 250 °C, an ion-source temperature 230 °C and the detector temperature was 200 °C, with helium as the carrier gas flowing at a rate of 1.58 mL/ min and a sample injection volume of 1 µL. The oven temperature was programmed from 80 °C (isothermal for 1 min), with an increase of 10°C/minute, to 200 °C, then 10 °C/minute to 280 °C, ending with a 5 min isothermal at 280 °C. Mass spectra were taken at 70 eV with a scan interval of 0.5 s. Total run time was 28 min. The relative percentage of each identified compound was determined by comparing its average peak area to the total area. The identification of components was done through computerized matching of their spectra with known compounds from the NIST spectra library (2009). The fragmentation patterns of the eluted compounds were identified by comparing them with known data from the database.

Cell cycle analysis

For cell cycle and apoptotic profile, rat T and PG tissue masses were digested using digesting enzymes. The affected cells were collected for cell cycle analysis, the cells were harvested and fixed gently with 70% (v/v) ethanol in PBS, maintained at a temperature of 4°C overnight, and re-suspended in PBS containing 40 µg/ml PI (propidium iodide), 0.1 mg/ml RNase and 0.1% (v/v) Triton X-100 in a dark room. After 30 min at 37°C, the cells were analyzed using a flow cytometer (Becton-Dickinson, San Jose, CA, USA) equipped with an argon ion laser at a wavelength of 488 nm. The cell cycle and sub-G1 groups were determined and analyzed, as described previously Chakraborty et al [18].

Molecular Biology

Total RNA from 0.1 gm of target tissues (T&PG) was extracted from control, ELFEMF (250 µT) and ELFMF -Avocado treated Adult, young and senile rats for 30 days 2 h daily, using RNeasy Mini Kit (Qiagen, Germantown, MD, USA) according to manufacturer's instructions. The concentration of extracted RNA was evaluated using a Beckman dual spectrophotometer (Beckman Instruments, Ramsey, MN, USA). The expression level of apoptosis-related genes; *P53* (F: 5'-TCAGATCCTAGC GTCGAGCCC-3' & R: 5'-GGGTGTGGAATCAACCCA CAG-3'), *Bax* (F: 5'-ATGGACGGGTCCGGGGAGCA-3' & R: 5'-CCCAGTTGAAGTTGCCGTCA-3') and BCL2 (F: 5'-GTGAACTGGGGGAGGATTGT-3'& R: 5'-GGAGAAATCAAACAGAGGCC-3') and housekeeping gene; β-actin (F 5'-AGCGAGCATCCCCCAAAGTT-3'& R: 5'-GGGCACGAAGGCTCATCATT-3) were determined using real-time PCR. 10 ng of the extracted total RNA from each sample was used for cDNA synthesis using high-capacity cDNA reverse transcriptase kit Biosystems-Thermo Fischer Scientific, USA). The obtained cDNA was subsequently amplified using Sybr Green I PCR master kit (Thermo Fisher Scientific Inc., Lithuania) using Step One apparatus (Applied Biosystems), as follows: 10 min at 95°C for enzyme activation followed by 40 cycles of 15 s at 95°C, 20 s at 55°C and 30 s at 72°C for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of β-actin as housekeeping gene by the DCT method.

Determination of antioxidant activity

This immunoassay kit allows in vitro quantitative determination of reactive oxygen species, Superoxide dismutase (SOD) malondialdehyde (MDA) and reduced glutathione (GSH) levels. The microtiter plates provided in these kits have been pre-coated with an antibody specific to an antioxidant parameter. Standards or samples were added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ROS, GSH, SOD and MDA and Avidin conjugated to Horseradish Peroxidase was added to each microplates wells and incubated at 37oC

for an hour. Tetramethylbenzidine substrate solution was added to each well. Only those wells contain ROS, SOD, SOD and MDA biotin-conjugated antibodies and enzyme-conjugated Avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of a 2% sulphuric acid stop solution, and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of antioxidant items was determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

All experiments were carried out three independent times. Results were statistically analyzed using GraphPad Prism v. 6.07 (GraphPad Software, San Diego, CA, USA) with the following tests: t-test between 2 sub-groups, one-way analysis of variance (ANOVA) between more than two subgroups, regression analysis and Spear man's correlation test. All results were presented as mean ± SD. The difference was considered statistically significant at $P < 0.05$.

Results

GCMS

Chromatogram of active biological products of Avocado extract

The most prominent active products detected were phenols, 2-methyl-5-(1-methylethyl, 2-methyl-5-(1-methylethyl), 5-methyl-2-(1-methylethyl), 5-methyl-2-(1-methylethyl), Caryophyllene, BICYCLO (7.2.0) UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYL ENE-, Bicyclo(7.2.0)UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-Methylene, á-longipinene 1,4-methanoazulene, decahydro-4,8,8-trimethy l-9-methylene-Dodecane (Figure 1) (Supplementary Table 1).

Flow cytometric analysis

Flow cytometric analysis of tongue parotic gland compared with unexposed control indicated that there was an insignificant total apoptosis (P>0.05) of young Sprague Dawley albino rats compared with that of adult. In the mean times there was a significantly elevated (P<0.05) total apoptotic cells in senile rats' tongue tissues than in both young and adult groups. Similarly, adult and senile tongue tissues showed a significant early apoptosis than young tongue tissues. Finally late apoptosis of senile rat tongue tissue was significantly increased (P<0.05) than in young and adult tissues. Oppositely the parotid Gland (PG) tissue showed a significant elevated (P<0.05) total apoptotic cell % in case of young and senile rats compared with that of adult rats. Also, early, and late apoptotic % of young and senile rats was significantly elevated (P<0.05) than that in case of adult rats, while a significant (P<0.05) necrotic % was observed in both adult and senile rats than in young tissues. Concerning the EMF exposed rats, there was a significantly elevated (P<0.05) total apoptotic % of tongue and PG tissues in case of senile rats than in both young and adult rats and the total apoptotic % of tongue tissue of adult and young showed insignificant difference (P>0.05). The same pattern was noticed in early and late apoptosis but necrosis % was significantly elevated (P<0.05) in adult and senile EMF exposed group than in young group (Table 2). Concurrently the defensive mechanism of Avocado extract (Avo-E) as a source of antioxidant, showed a decreased apoptotic% of tongue and PG tissues, as there was a significant decrease (P<0.05) total apoptotic cell % in young and senile groups compared to those of EMF exposed groups, while the adult group showed insignificant changes (P>0.05). Also, parotid gland apoptotic % showed a significant decrease compared with those in EMF exposed group (P<0.05). In the same way young and senile groups showed too much decrease % than adult. Similarly Early apoptosis % showed a significant decreased value (P<0.05) than those of sole EMF exposed group, while late apoptosis was insignificantly changed in adult group. Finally, necrosis % showed a significant change compared with necrotic % of EMF exposed tongue and parotid gland tissues (Figures 2-3).

Molecular Biology

Apoptotic potential of EL-EMF showed that there was a noticeable elevated proapoptotic genes (*P53* and *Bax*) and a decreased anti-apoptotic gene expression (*Bcl2*). The gene fold change was age dependent, and application

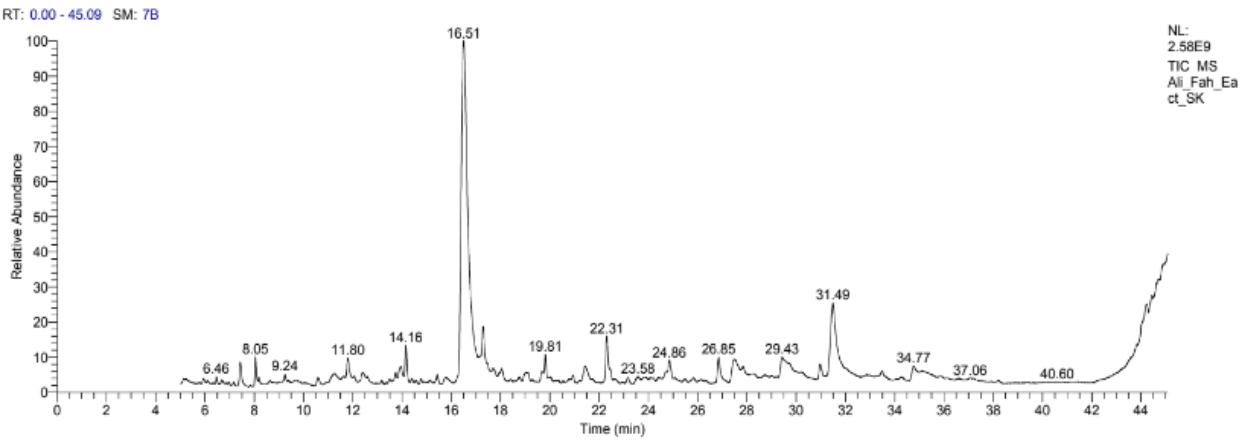

Figure 1. Chromatogram of Active Biological Products of Avocado Extract

Table 2. Evaluation of Apoptotic Profile of Tongue and Parotid Gland for Three Groups (Young, Adult and Senile) for Control and EMF Exposed Group

S	Code	Conc	Apoptosis			Necrosis
			Total	Early	Late	
1	4YC/ Tongue		8.06	0.51	1.51	6.04
2	4AC/ Tongue		4.45	0.38	0.71	3.36
3	4SC/ Tongue		13.29	0.66	2.43	10.2
	4YC/PG		6.41	0.73	0.92	4.76
	4AC/PG		2.99	0.26	0.88	1.85
	4SC/PG		6.41	0.73	0.92	4.76

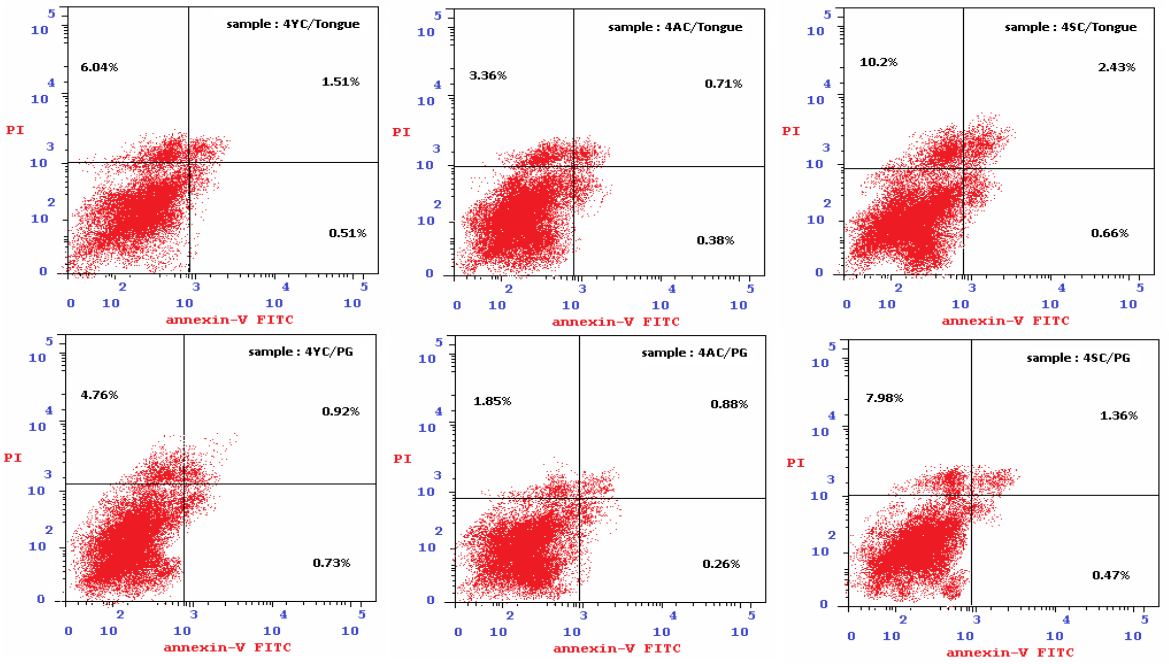

Figure 2. Evaluation of Apoptotic Profile of Tongue and Parotid Gland for Three Groups (Young, Adult and Senile) for Control and EMF Exposed Group

of avocado extract showed a significant normalization of expression profiles (P<0.05) Figures (4, 5 and 6).

Antioxidant activity

Data recorded revealed that there was an insignificant elevated ROS value post animal's exposure to EMF but insignificantly(P>0.052). Also, the administration of Avocado decreased ROS in EMF exposed group. There was an insignificant difference in RS value of control and ROS post administration of Avocado group (P>0.05). Concurrently, SOD of control and Avocado administrated group showed insignificant difference (P>0.05). Meanwhile, SOD level was decreased post exposure to EMF (P>0.05). Administration of Avocado showed insignificant elevated SOD value (P>0.05) (Table 3). In the same way exposure to EMF induced decreased GSH compared with control group (P>0.05), while administration of Avocado induced insignificant elevated GSH post administration of Avocado, while group administered Avocado showed insignificant difference compared with control group. Similarly, there was a significantly elevated MDA (P<0.05) post rats' exposure

Table 3. Generation of ROS and SOD Levels Post Exposure to EMF, EMF-Avocado Combination and Avocado Alone versus Control Cells

Antioxidant	ROS A Pg/ml	ROS Y pg/ml	ROS S pg/ml	SOD A µm/gm	SOD Y µm/gm	SOD S µm/gm
Cont.	452±0 36.6	459±37.5	448±52.6	98.5±0.756	100± 6.88	98.63±4.38
EMF	495±47.7	466 ±15.33	522 ± 57.6	78.32±0.625	72.69 ± 3.57	69± 2.78
EMF-Avocado	477±66.22	482 ± 29.88	502 ± 44.6	88.56±0.425	82.69± 4.22	79± 4.29
Avocado	438± 29.8	423 ±38.9	436 ± 38.9	96.75±0.425	94.8 ± 3.69	86±4.68
Recovery	462±33.9	472 ±40.2	466 ±43.7	101.24±.4.25	96±2.95	97± 6.5

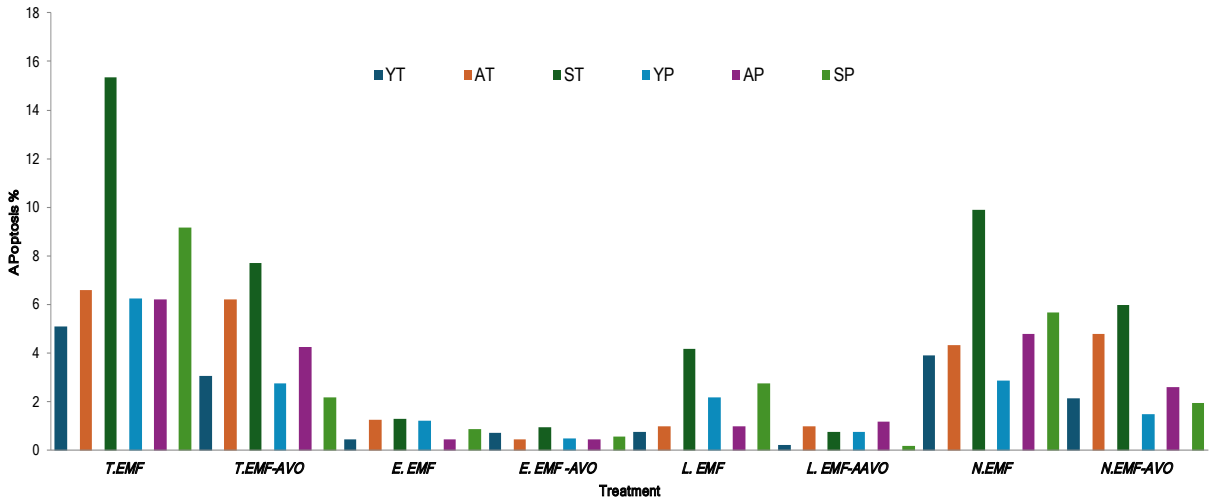

Figure 3. Evaluation of Apoptotic Profile of Tongue and Parotid Gland for Three Groups (Young, Adult and Senile) Post Exposure to EMF, EMF-Avocado Combination and Avocado Alone versus Control Cells.

to EMF($P<0.05$), followed by an insignificant decrease of MDA post administration of Avocado ($P>0.05$) while MDA level of control and Avocado administered group was insignificant (Table 4).

Discussion

Extremely low frequency electromagnetic fields (EL-EMF) have been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC) and this has raised some concerns about its health effects on employees extensively exposed to these fields at thermal power plants. Also, it was reported that EL-EMF produced from mobile phones is a source of increased magnetic pollution. The possible adverse health effects of the EL-EMFs are thermal effects, as biological systems are influenced by EMF due to the induced thermal related damages [19], although non-thermal effects have

Table 4. Generation of GSH and MDA Levels Post Exposure to EMF, EMF-Avocado Combination and Avocado Alone versus Control Cells.

Antioxidant	GSH A µm/gm	GSH Y µm/gm	GSH S µm/gm	MDA nm/gm	MDA nm/gm	MDA nm/gm
Cont	3.95±0.425	3.62±0.21	3.72 ±0.15	1.52±0.211	1.38 ±0.19	1.26± 0.23
EMF	3.35±0.325	3.99±0.28	4.2±0.56	2.65±0.458	2.79±0.58	2.62± 0.41
EMF Avocado	3.64±0.425	3.35±0.38	3.7±0.56	2.47±0.425	2.36 ±0.34	1.99 ±0.32
Avocado	3.82±0.425	3.79 ±0.41	3.69±0.33	1.62±0.425	1.58 ±0.24	1.61±0.22
Recovery	3.9±0.425	3.86±0.31	3.92±0.51	1.495±0.425	1.52 ±0.19	1.58 ±0.32

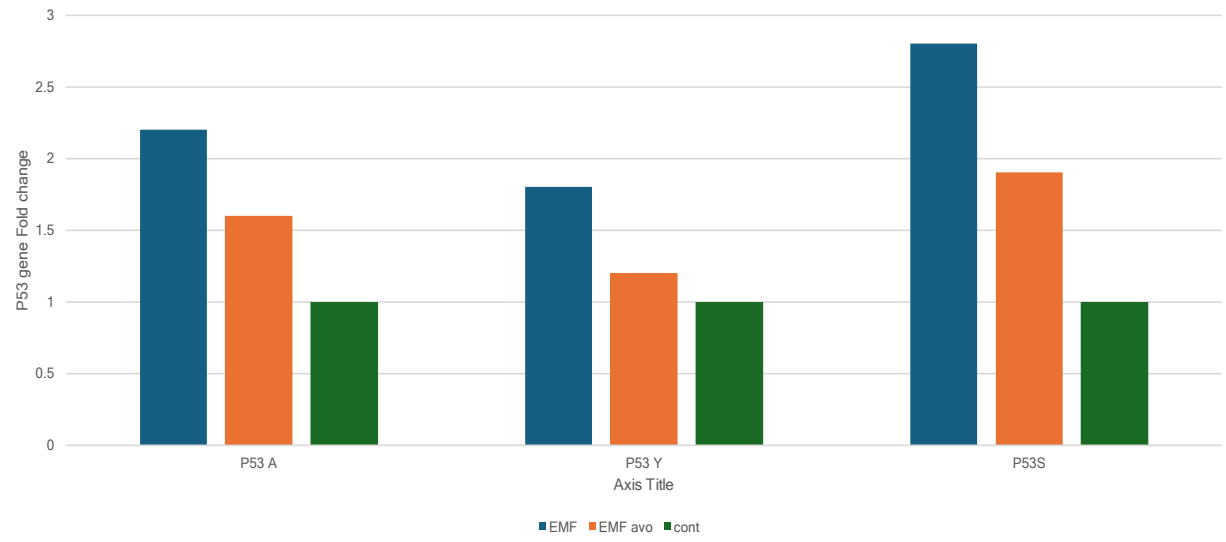

Figure 4. Evaluation of Pro-Apoptotic Gene *P53* for Three Groups (Young, Adult and Senile) Post Exposure to EMF, EMF-Avocado Combination versus Control Cells.

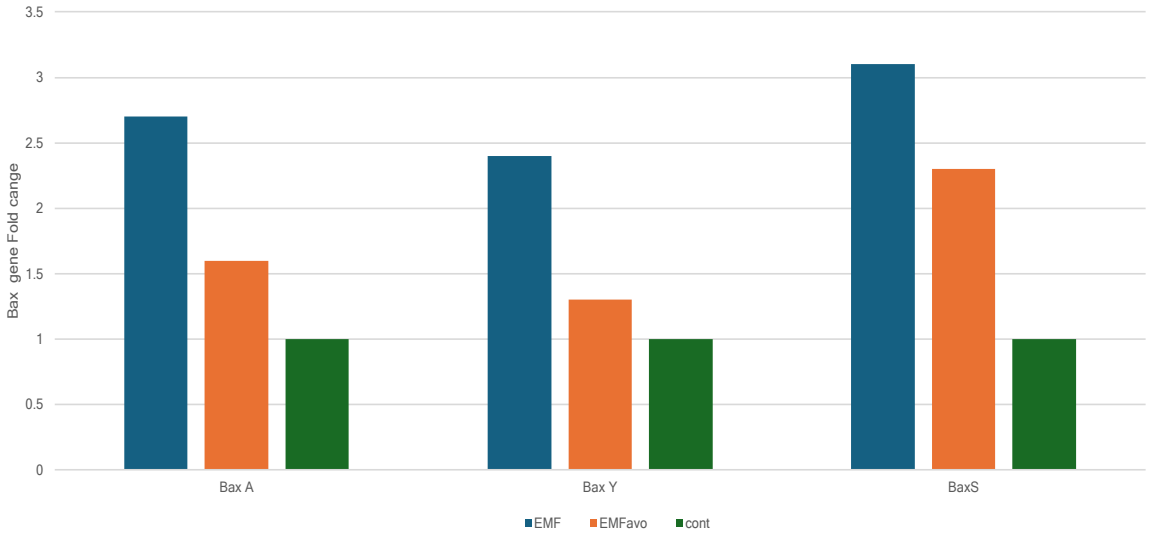

Figure 5. Evaluation of Pro-Apoptotic Gene Bax for Three Groups (Young, Adult and Senile) Post Exposure to EMF, EMF-Avocado Combination versus Control Cells.

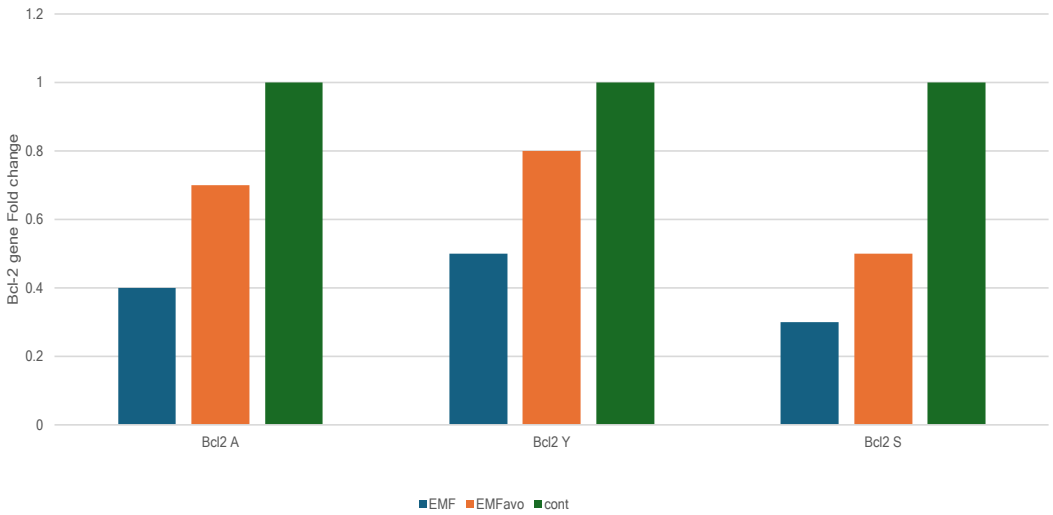

Figure 6. Evaluation of Anti-Apoptotic Gene *Bcl2* for Three Groups (Young, Adult and Senile) Post Exposure to EMF, EMF-Avocado Combination versus Control Cells.

also been studied [20]. The cellular target of EMF is still controversial. Several studies have reported that the effect of EMF produced energy is much lower than those can produce thermal changes in different organs [21]. Thermal effects can produce physiological alterations both in Vivo and in vitro [22] inducing histopathological changes but that still not confirmed [23]. Despite the reports of insensitivity to the thermal effect of EMF on tissue biology, they have not been considered in the existing EMF safety standard; rather it is principally based on the thermal effect of EMF [24].

Use of avocado extract as antioxidant against DNA damage was incompliance with Hosseinabadi et al. [25] despite their use of vitamin E & C as antioxidant for monitoring the thermal effect on the employee of thermal power plant. DNA damage was measured in peripheral blood lymphocytes using comet assay and apoptosis using flow cytometry. Based on the results, tail intensity and tail length in the vitamin E group, and all comet assay indices in the vitamin E + C and vitamin C groups (except DNA damage index) significantly decreased after the intervention, while the comet assay indices did not change significantly in the control group. None of the flow cytometry indices including early apoptosis, late apoptosis and necrosis changed after intervention in either group. The use of antioxidant vitamins such as E and C, can increase the activity of the non-enzymatic antioxidant defense system, and can manage DNA damage caused by exposure to extremely low frequency magnetic fields, but has no effect on apoptosis. It seems that consumption of vitamin E affected all investigated comet assay indices and can be probably considered as the best intervention.

In the same context, and regrading the physiological alteration it was reported by Magda et al. [26] that avocado

antioxidant content potential can protect against inducible damages and can restore the testicular enzymatic activities of SOD and GPx to almost control their levels, in addition, decreasing the MDA and caspase-3 activity and increased germ cells numbers can be managed by supplementation of antioxidant of Avocado source. Recent studies on human semen have suggested an increased production of ROS in human semen due to cell phone radiation [27]. Also, Claudia et al. [28] reported that avocado oil is a source of several bioactive compounds with antioxidant effects, decreases oxidative stress by improving the function of the mitochondrial electron transport chain (ETC) and decreasing ROS levels in the mitochondria of hepatic and renal diseased rats. inducing attenuating mitochondrial dysfunction, oxidative stress and inflammation. Also, according to our results, El Zahaby et al. [29] reported that exposure of rate at different ages (adult, young and senile) to EMF equivalent to phone frequency showed an elevated Oxidative stress parameter. Also, induced histopathological changes including testicular, tunica albuginea with mild subcapsular and interstitial edema and average-sized tubules with average germinal lining. Thick tunica propria, spermatogonia, primary spermatocytes, many spermatozoa, and average interstitium with average Leydig cells. Average tunica albuginea with markedly congested subcapsular blood vessels, average-sized tubules with average germinal lining, and average interstitium, thick collagen in tunica albuginea and in blood vessel wall, and average collagen in tubular wall. At the same time, testis showed average collagen in tunica albuginea, all these changes showed back changes to almost normal state in avocado administered group compared to the rest of groups followed by normalization of spermatogenesis and testosterone level, also reported the effect was age dependent. The apoptotic effect detected using flow cytometry and apoptotic gen profile under the effect of EMF was attributed to the increased oxidative stress. The effect of EMF on cell biology / apoptosis was investigated on the physiological level in presence of avocado extract acting as antioxidant reducing the elevated oxidative stress diminishing the apoptosis process and cell damage. The decreased in apoptotic process items are in agreement with the results of Falone et al. [30] despite our study was In Vivo performed , while they used in vitro application of EL-EMF recording increased viability of SH-SY5Y cells and suggested that the shift towards a more reduced intracellular environment deduced by measuring glutathione S-transferase, glutathione peroxidase and glutamyl cysteine synthetase, affecting the signal transduction pathway, thus increasing the expression of anti-apoptotic and repair-related proteins Bcl-2 and p53 in addition to these findings, the decrease in the number of apoptotic cells and the increase in viability can be attributed to an increase in heat-shock protein hsp70 levels induced by ELF-EMF. The increase in hsp70 levels under the effect of ELF-EMF is in accordance with the results of Goodman et al. [31] and Tokalov and Gutzeit. [32] Guo et al. [33] showed that hsp70 significantly increased activities of glutathione peroxidase and glutathione reductase. They suggested that this might provide a new insight into the cytoprotection induced by hsp70. In addition, the anti-apoptotic role of hsp70 is via interfering with the apoptotic cascade [34]. Regarding the apoptotic genes induced initiated by ELF-EMF in Tongue and PG and were normalized using Avocado extract our result was in agreement with Lara-Márquez et al. [35] despite they used in vitro model; Caco-2 (colon cancer) in their study recording Chemotherapy used in CRC patients has severe side effects, and an alternative derived from avocado lipid extract (LEAS) induced anti-apoptotic activity through the activation of caspases 8 and 9. Also, induced loss of membrane mitochondrial potential(MMPs), inhibited fatty acid oxidation and increased the superoxide production and mitochondrial ROS. Furthermore, LEAS stimulated secretion of cytokines IL-6 (~500%), IL-8 (~400%) and IL-10 (~150%); whereas IL-1β secretion was inhibited (~50%). The results suggest that LEAS induces apoptosis on Caco-2 cells, indicating that avocado is a source of functional food products that can reduce the risk for development of cancer.

Overall, it can be hypothesized that an increase in hsp70 induced by ELF-EMF would cause a reduction in the number of apoptotic cells by triggering a shift towards a more reduced environment thus causing expression of anti-apoptotic proteins, and by directly influencing the apoptotic cascade. On the other hand, ELF-EMF increased the number of apoptotic cells in oxidative stress induced cell populations. This can be attributed in part to the slight increase in ROS levels in H2O2-induced cells exposed to ELF-EMF. However, it is evident that further studies are required to explore the mechanism that caused the increase in the number of apoptotic cells. It was noticed that the effect on oxidative stress parameters was not major, that was in accordance with Luukkonen, et al. [36] reporting that oxidative stress (OS) was prominent on application of EL-EMF > 1mT. Also, long term application of <1mT can induce OS. Despite this, all detected deteriorative effects are moderate and majority of changes were below 50%. Simko and Mattsson. [37]. Consequently, the produced amounts of ROS by ELF- EMF are not high enough to induce major DNA damage. Although this the moderate elevation of ROS due to exposure to long term exposure to EL-EMF cannot trigger cell death, it may induce cellular resistance against oxidative damage through upregulation of antioxidant pathways and induction of cellular stress response and small change in ROS levels stated above can promote different cell signaling pathways especially by means of superoxide ions [38, 39] .

Concerning the protective effect of avocado 2.5 and 5% and the related impacts on organs pathology, it was reported by Al hussanin et al. [40] that Avocado peels have effectively improved liver function and protect against liver tissues damage induced by carbon tetrachlorid. In contrast catalase and glutathione transferase were significantly decreased. Histopathological examination revealed degeneration of hepatocytes of rat livers treated with carbon tetrachloride

Also, Kesari et al. [41] reported that analysis of antioxidant enzymes; glutathione peroxidase and SOD showed a noticed depleted values accompanied with elevated concentration of catalase and MDA. Similarly,

histone kinase (HK) showed a significant decrease in the ELF-EMF exposed test groups. Also, reported that exposure to EMF induced a significant decreased micronuclei change in sperm cell cycle in exposed groups, that may be too due to the elevated level of free radicals. In the mean times the findings of Kesari et al. [41] on antioxidants, MDA, HK, micronuclei, and the sperm cell cycle are clear indications of an infertility pattern that is initiated due to an overproduction of reactive oxygen species (ROS). Similarly, it was reported that radiofrequency electromagnetic waves produced from cell phones may affect the fertilizing potential of spermatozoa. Oppositely there is evidence from several high-quality studies suggesting a lack of EMF effects on testicular function in experimental animals provided that the exposures do not induce hyperthermia. While the responses to RF fields are identical to those induced by heating using conventional means. Very few recent studies have addressed this issue, supporting the conclusion that male fertility and testicular function are not affected in the absence of hyperthermia [42]. In contrast, several reports indicated that EM waves have a broad range of deleterious effects on male reproductive system and spermatogenesis inducing a significant change in the sperm cell cycle [43] . ROS are continuously neutralized by antioxidants present in body tissues [44] . Whenever the production of ROS exceeds the scavenging capacity of antioxidants, oxidative stress (OS) will result [45]. In 1992, researchers found that electromagnetic fields increase the free radical activity in cells [46]. Within the last decade, in Vivo animal studies have shown that oxidative stress (OS) develops in response to cell phone radiation [47] . As EMR may disturb ROS metabolism by increasing the production of ROS or by decreasing the activity of antioxidant enzymes. Also, Kesari et al. [41] found that the effect of EMF on spermatogenesis may be based on the nature of vulnerable testicular tissue that is dependent on oxygen to maintain spermatogenesis and oxidative stress induced in testicular injury due to toxic effects of reactive oxygen metabolites. Also, they found a decreased GSH and elevated Catalase and MDA. Also, Eser et al. [48] reported the protective effects of avocado/soybean unsaponifiable (ASU) on the prefrontal cortex (PFC) after global brain ischemia/reperfusion (I/R) injury in rats. And the level of MDA and TNF-α levels as well as the number of apoptotic neurons were observed to have decreased significantly in groups administered avocado/soyabean while SOD activities have been found to decrease significantly compared to ischemia/reperfusion induced. In contrast catalase and glutathione transferase were significantly decreased. Regarding the apoptotic profile, our reported data agreed with Jinnfer et al. [49] recording that radiation of parotid salivary glands induced a marked deleterious effects resulting in a diminished quality of patient life accompanied with marked increases in phosphorylated p53 (serine18) and apoptosis, which was suppressed in transgenic mice expressing a constitutively active mutant of Akt1 (myr-Akt1). Also, they reported that the pro-apoptotic p53 target genes PUMA and *Bax* expression in parotid salivary glands of mice at early time points following dose dependent therapeutic radiation and the hypothesis that p53 mediates an apoptotic pathology in normal salivary glands in vivo.

In conclusion, ELF-EMF exposure for 3 h / day for one month can induce histopathological and physiological and molecular alterations relative to age. Avocado bioactive content can modulate the drawbacks of phone cells induced physiological and apoptosis, upset of oxidative stress and application of avocado could normalize the drawback of ELF-EMF exposure alterations for nearly normalized values due to its antioxidant potential.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

Ethical Declaration

This study will be carried out after ethical approval of the ethical committee, faculty of medicine, South Valley University.

Conflict of interest

All authors declare that there is no conflict of interest.

Availability of data

Data is available upon request.

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