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Activation of liver X receptors ameliorates alterations in testicular function in rats exposed to electromagnetic radiation

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ABSTRACT

Introduction: Cell phones are essential for modern life and their usage becomes prevalent. Worries have increased concerning the harmful effects of their radio-frequency electromagnetic radiation. Liver X receptors (*LXRs*) are ligand-dependent transcription factors that have important roles in lipid metabolism and inflammation, plus several recently emerging roles. Whether it is useful to overcome the hazardous effects of cell phone radiation needs to be clarified. So, this study aimed to investigate the effect of *LXRs* activation on testicular changes in rats exposed to electromagnetic radiation.

Methods: Thirty adult male Wistar rats were subdivided into control group, experimental group: exposed to cell phone electromagnetic radiation, and treated group: as experimental group and received *LXRs* agonist T0901317. Serum testosterone (T), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH), testicular tissue malondialdehyde (MDA) and superoxide dismutase (SOD), and gene expressions of inflammatory and apoptotic markers were assessed. Seminal analysis and testicular histological examination were performed.

Results: *LXRs* activation in rats exposed to cell phone radiation improved the decreased testicular and gonadotrophic hormones, semen quality parameters, tissue SOD, and anti-apoptotic gene expression as well as the elevated tissue MDA, apoptotic and inflammatory gene expressions, and the testicular histological changes induced by exposure to electromagnetic radiation.

Conclusion: Exposure to cell phone radiation leads to harmful effects on spermatogenesis and *LXRs* activation could improve these effects via antioxidant, anti-inflammatory properties in addition to its endocrinal action.

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Apoptosis; electromagnetic radiation; liver X receptors; rat testicular function

1. Introduction

Male infertility is a rising problem around the world; the incidence of male infertility at the reproductive age has been assessed to be up to 7–8% [1]. Idiopathic male infertility is due to decreased sperm quality without organic, genetic, or endocrine etiology [2]. Several environmental factors could be considered as predisposing factors for male infertility like heat or certain chemical agents and a possible link between using cell phones and male sterility was evaluated [3]. The use of mobile phones generates an electromagnetic field (EMF) whose effects on human health have increased the interest to be investigated [4]. The fast evolution of mobile phone usage is accompanied by an equivalent surge in the density of the EMF [5]. Effect of EMF on fertilizing potential of spermatozoa and on testosterone level is controversial. Usage of cell phones by humans [2,6] or exposure to them in animal studies [1,7] leads to defective testicular function and reduction of the sperm count, motility, and viability. However, other researches proved that exposure to EMF did not induce any adverse effects on sperm quantity, quality, or morphology [8].

It was proposed that mobile phone EMF produces free radicals that finally result in oxidative stress and is associated with higher production of pro-inflammatory cytokines and reactive oxygen species (ROS) [3]. Oxidative stress results from imbalance between ROS formation and cell antioxidant defense capacity that is associated with increased germ cell apoptosis, DNA damage, and lipid peroxidation [9]. It was suggested that EMF could induce excessive inflammatory responses via macrophage activation [10]. However, effects of EMF on cytokine expression is a complex process associated with physical and biological aspects of EMF [11].

Liver X receptors (*LXRs*) are nuclear receptor superfamily and ligand-activated transcription factors that exist in two isoforms: *LXR α* and *LXR β* [12]. *LXRs* are involved in several physiological processes such as lipid metabolism and cholesterol homeostasis. In addition, they have been reported to play important roles in the modulation of inflammation [13]. *LXR α* and *LXR β* are expressed in the mouse and human testes. In the mouse, *LXR α* is mainly expressed in Leydig cells, and *LXR β* in Sertoli cells, while both are

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expressed in germ cells. *LXR* $\alpha\beta$ ^{-/-} mice become prematurely infertile with increased apoptosis of germ cells, reduced germ cell proliferation, and low testosterone levels [14]. In humans, both *LXR* α and *LXR* β are expressed in testicular biopsy specimens and are expected to have a similar role as in the mouse, so they may be related to premature infertility [15]. Moreover, *LXRs* exert anti-inflammatory effects by inhibiting production of pro-inflammatory cytokines either directly or indirectly [16].

We hypothesized that prolonged exposure to EMF will produce alteration in testicular functions and *LXRs* activation could modulate these effects. So, this study aimed to investigate the effect of *LXRs* activation on testicular changes in rats exposed to electromagnetic radiation.

2. Materials and methods

2.1. Experimental animals

The present research included 30 adult male Wistar rats aged 12–15 weeks weighing 200 ± 20 g that were obtained from animal house of faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in plastic cages (18 inches in length and 12 inches in width) five rats/cage. They were maintained under controlled room temperature ($22 \pm 2^\circ\text{C}$) and humidity (50–60%) with 12 h light and 12 h dark cycle and were fed on a standard diet with free access to water. All experimental procedures were following the guide for the care and use of laboratory animals (8th edition, National Academies Press) and have been reviewed and approved by Zagazig University institutional animal care unit committee (ZU-IACUC; Sharkia; Egypt) with approval number: ZU-IACUC /3/F/117/2019).

2.2. Experimental design

Following acclimatization for two weeks, animals were randomly allotted to: control (sham) group ($n = 10$): in which animals were exposed to cell phones in switch off mode, experimental (EM) group ($n = 10$): in which animals were exposed to electromagnetic radiation generated by cell phone and treated (EM+T0) group ($n = 10$): in which animals were exposed to electromagnetic radiation as experimental group and received *LXR* agonist T0901317. The exposure to mobile phone radiation was done in the morning between 7:00 a.m. and 10:00 a.m.

2.3. EMF exposure system

Cell phones of the same brand and model were used, their properties were as follows: the peak specific absorption rate (SAR) was 0.96 watt/kg (W/kg) and

each of them had an 890–915 MHz carrier frequency band. The electromagnetic field radiation generated by cell phones was nearly equal the average of downlink frequency of Orange network in Egypt (935–945) MHz. A call was made from one set of GSMs (Global System for Mobile Communications) mobile phones to another set of mobile phones after ensuring that each of them was powered-on and the answering mode switched on. Animals were exposed to EM radiations from a mobile phone as calling for 60 min daily for 6 weeks. The cell phones were placed inside plastic experimental cages that did not have any metallic fitting and were placed in the center of the cage to provide approximately equal distribution of radiation to the whole body of the rat. The sets of cell phones were put in special sectors in the middle of the cages to avoid any damage that could be produced by rats. Animals could move freely in the cage during the exposure period. Sham animals were exposed to cell phones in switched off mode in a similarly sized cage for the same period in a separate room like that used for the experimental groups [17].

2.4. LXR agonist administration

LXR agonist T0901317 (Sigma-Aldrich, Saint Louis, MO, USA) was dissolved in 100% dimethylsulfoxide (DMSO) at 1 g/ml and was stored at -20°C till time of administration to rats, the DMSO-containing T0901317 was diluted with phosphate-buffered saline (PBS) to a final concentration of 2% [18]. The rats in the EM+T0 group received 10 mg/kg per day *LXR* agonist T0901317 orally by gavage for six weeks just after 60 min of EMF exposure. Animals in control and EM groups received the same volume of vehicle (2% DMSO in PBS) orally by gavage at the same time for similar period as EM+T0 group.

2.5. Sample collection

At the end of experimental period, rats' final body weights were assessed then blood samples were obtained from tail veins and allowed to clot for 2 hours at room temperature before centrifugation for 20 minutes. The sera were frozen at -20°C until hormonal assay. The rats were sacrificed under anesthesia (chloral hydrate) inhalation and both testes were excised out, cleared of adhesive tissue, and weighed precisely.

Left testes were used to prepare testicular tissue homogenates by homogenization with 5 to 10 mL of cold buffer containing: 50 mmol/L potassium phosphate and 1 mmol/L EDTA with pH 7.4 followed by centrifugation for 15 minutes at 4°C . The testicular tissue homogenate was frozen at -80°C until further analytical assay.

The right testes were collected and fixed in 10% formalin solution for histopathological examination.

2.6. Hormonal assay

Serum levels of testosterone (T), Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH) were measured colorimetrically by enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, Inc. CA, USA) as manufacturers' instruction.

2.7. Semen analysis

Caudal epididymis was excised and placed in a sperm collecting bottle containing 5 mL pre-warmed Hank's Balanced Salt Solution buffer (HBSS) at 37°C. It was cut three times to release sperms. The dilution was obtained by adding 0.05 sperm fluid to 0.95 of HBSS. A hemocytometer was used to determine non-motile sperm count in diluted sperm fluid then multiplied in the dilution factor. Sperm viability was evaluated from smears on a glass microscope slide stained by eosin-nigrosin dye (1.67% eosin, 10% nigrosin, and 0.1 M sodium citrate) by light microscopy in randomly 10 selected fields microscope that evaluated for the percentage of vital (unstained) and dead (stained) spermatozoa [19]. The container of the diluted sperm fluid was submerged in hot water to kill motile sperms then left to reach room temperature and the total number of sperm was counted using hemocytometers then multiplied in the dilution factor. When gross observation showed a small sperm count in sperm fluid (low turbidity), an undiluted aliquot of the fluid was used.

The following equation was used to calculate the percentage of motile sperm: Motile sperm rate (%) = (Number of sperm – number of non-motile sperm) X100/Number of sperm.

2.8. Oxidative stress assay in testicular homogenate

Oxidative stress induction was indicated by the amount of lipid peroxidation (LPO) by analysis of malondialdehyde (MDA). It was assessed in testicular homogenates spectrophotometrically by using commercially available ELISA kits (Bio diagnostic, Cairo, Egypt).

Antioxidant assessment was indicated by superoxide dismutase (SOD) activity in testicular tissue. It was measured in testicular homogenates spectrophotometrically by using commercially available ELISA kits (Bio diagnostic, Cairo, Egypt).

2.9. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from testicular homogenate specimens using the RiboZol™ RNA extraction reagent (Amresco, Solon, Cleveland, Ohio, USA) as the manufacturer's instructions. RNA was then reverse transcribed using complementary DNAs (cDNAs) by the SensiFAST™ cDNA synthesis kit (Bioline, London, UK). Real-time PCR was performed using 10 µl of SYBER Green PCR Mix (SensiFAST™ SYBER Lo-ROX Kit, Bioline London, UK). The SYBER green data were analyzed with a relative quantification to β-actin as an internal control. The sets of primers used were listed in Table 1. The relative gene expression ratio is calculated from the real-time PCR using the 2-ΔΔCt method [20].

2.10. Histopathological examination

Testes were fixed in 10% buffered formalin solution overnight, dehydrated, and embedded in paraffin. The paraffin-embedded samples were sectioned at 3 µm thickness with a microtome and the slices were subjected to routine hematoxylin and eosin (H&E) staining. The slides were evaluated under a light microscope by a single pathologist who was blinded to the experimental details. Histopathological assessment of spermatogenesis was evaluated in 30 randomly selected seminiferous tubules from each group using Johnsen's mean testicular biopsy score (MTBS) [1]. MTBS was calculated by dividing sum of all scores by the total number of seminiferous tubules examined (Table 2).

2.11. Statistical analysis

The results are presented as descriptive statistics (mean ± standard deviation). Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 25 (SPSS, Inc., IBM Company, Chicago, IL, USA). The normal

Table 1. Primer sequences.

Gene	Forward	Reverse
NF-κB	5' -GAAAGTAAGGATGTGGGAGGAG-3'	5' - GTGGATGATGGCTAAGTGTAGG-3'
IL6	5' - TCCTACCCCACTTCCAATGCTC-3'	5' - TTGGATGGTCTTGGTCTTAGCC-3'
TNF-α	5' -AAATGGGCTCCCTCATCAGTTC-3'	5' -TCTGCTTGGTGGTTTGTCTACGAC-3'
Caspase 3	5' - CAGAGCTGGACTGCGGTATTGA -3'	5' - AGCATGGCGCAAAGTACTG -3'
Bcl-2	5' -CGCCCGCTGTGCACCGAGA-3'	5' -CACAACTCTCCCGAGTTCACC-3'
β-actin	5' -CCATCAACGACCCCTTCATT-3'	5' -GACCAGCTTCCCATTCTCAG-3'

Bcl-2, B-cell lymphoma protein 2; IL6, interleukin 6; NF-κB, nuclear factor-kappa B; TNFα, Tumor necrosis factor α.

Table 2. Histological criteria and Johnsen's score for spermatogenesis assessment.

Score	Characters
Score-10	Complete spermatogenesis with many spermatozoa present.
Score-9	Slightly impaired spermatogenesis with many late spermatids, disorganized epithelium.
Score-8	Less than five spermatozoa per tubule, few late spermatids.
Score-7	No spermatozoa, no late spermatids, many early spermatids.
Score-6	No spermatozoa, no late spermatids, few early spermatids.
Score-5	No spermatozoa or spermatids, many spermatocytes.
Score-4	No spermatozoa or spermatids, few spermatocytes.
Score-3	Spermatogonia only.
Score-2	No germinal cells, Sertoli cells only.
Score-1	No seminiferous epithelium.

Table 3. Effect of LXR activation in EMF-exposed rats on body, right and left testicular weights.

	Control	EM	EM+T0	p-value
Body weight (initial) (g)	198.2 ± 11.1	201.7 ± 8.9	198.8 ± 12.9	0.756
Body weight (final) (g)	263.7 ± 4.9	264.8 ± 4.8	265.7 ± 3.6	0.657
Weight gain (g)	65.5 ± 3.6	63.9 ± 4.7	67 ± 3.7	0.255
Weight of right testis (g)	1.69 ± 0.06	1.72 ± 0.09	1.71 ± 0.08	0.667
Weight of left testis (g)	1.68 ± 0.08	1.71 ± 0.09	1.72 ± 0.09	0.467

n = 10 in each group. Data are represented as mean±SD. P-value is significance difference of ANOVA (F test). Significance is considered when (P < 0.05).

distribution of data from each group was confirmed using the Kolmogorov-Smirnov normality test. Since the test indicated that variables followed normal distribution, comparisons among the experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by least significance differences (LSD) test to evaluate statistical difference between two groups. P-value <0.05 was considered significant.

3. Results

3.1. Effect of cell phone radiation and LXR activation on body and testicular weights

Exposure to mobile radiation as well as LXR activation induced insignificant change in final body weight, weight gain, and testicular weights (p > 0.05). (Table 3)

3.2. Effect of cell phone radiation and LXR activation on hormonal assay

Exposure to radiation emitted from cell phones led to significant decrease in serum levels of FSH (p = 0.007), LH (p = 0.005) and testosterone (p = 0.003) in comparison to control rats. LXR activation induced

significant increase in serum levels of FSH, LH and testosterone (p < 0.001) in comparison to EM group (Table 4).

3.3. Effect of cell phone radiation and LXR activation on sperm quality

Rats exposed to cell phone radiation exhibited significant decrease in sperm count, motility, and viability (p < 0.001) in comparison to control rats. LXR activation induced significant increase in sperm quality parameters (p < 0.001) in comparison to EM group (Table 4&Figure 1).

3.4. Effect of cell phone radiation and LXR activation on oxidative stress markers

Testicular tissue MDA level was significantly higher (p < 0.001) while SOD activity was significantly lower (p < 0.001) in rats exposed to cell phone radiation when compared with the control group. LXR activation led to significant decrease in MDA level (p < 0.001) and significant increase in SOD activity

Table 4. Effect of LXR activation in EMF exposed rats on hormonal assay, sperm quality, and testicular oxidative stress markers.

	Control	EM	EM+T0	p - value
FSH (ng/mL)	3.95 ± 0.56	3.1 ± 0.61 ^a	4.36 ± 0.76 ^b	0.001
LH (mIU/mL)	5.14 ± 0.64	4.2 ± 0.48 ^a	5.49 ± 0.88 ^b	0.001
Testosterone (ng/mL)	9.3 ± 1.57	7.23 ± 0.98 ^a	9.66 ± 1.44 ^b	0.001
Sperm count (×10 ⁶ /mL)	75.2 ± 5.35	52.9 ± 7.65 ^a	71.6 ± 6.41 ^b	<0.001
Sperm motility (%)	79.5 ± 6.79	63.1 ± 4.68 ^a	74.9 ± 4.67 ^b	<0.001
Viability (%)	85.9 ± 3.34	73.2 ± 5.51 ^a	84.1 ± 3.51 ^b	<0.001
MDA (nmol/g wet tissue)	9.9 ± 2.33	28.2 ± 4.8 ^a	11 ± 3.37 ^b	<0.001
SOD (U/g wet tissue)	29.3 ± 5.93	19 ± 5.44 ^a	28.4 ± 4.65 ^b	<0.001

n = 10 in each group. Data are represented as mean±SD. FSH, follicular stimulating hormone; LH, luteinizing hormone; MDA, malondialdehyde; SOD, superoxide dismutase. P-value is significance difference of ANOVA (F test). Significance is considered when (P < 0.05): (a) significant when compared with control group, (b) significant when compared with EM group.

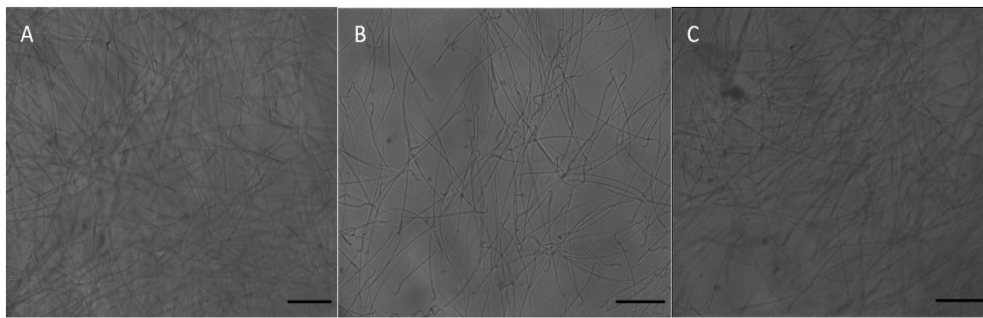


Figure 1. Optical micrograph showing sperm in the studied groups, A, control group; B, EM group; C, EM+T0 group $\times 400$, scale bar = 50 μm .

($p < 0.001$) in EM+T0 group when compared with EM group (Table 4).

3.5. Effect of cell phone radiation and LXR activation on mRNA gene expressions

- (A) Assessment of inflammation: relative gene expressions of NF- κ B, IL-6, and TNF- α were increased in rats exposed to cell phone radiation ($p < 0.001$) in comparison to control group. LXR activation led to significant decrease in NF- κ B, IL-6, and TNF- α expressions ($p < 0.001$) in EM+T0 group when compared with EM group (Figure 2).
- (B) Assessment of mitochondria-dependent apoptosis: relative gene expression of Caspase 3 was significantly higher ($p < 0.001$) while relative gene expression of Bcl-2 was significantly lower ($p < 0.001$) in rats exposed to cell phone radiation when compared with the control group. LXR activation led to significant decrease in Caspase 3 expression ($p < 0.001$) and significant increase in Bcl-2 expression ($p < 0.001$) in EM+T0 group when compared with EM group (Figure 2).

3.6. Effect of cell phone radiation and LXR activation on testicular histological assessment

Rats in control group showed normal structure of the testes, rats exposed to mobile radiation showed rupture of the basement membrane, dropped off spermatogenesis cells, intertubular fibrosis, and Leydig cells hyperplasia. Rats exposed to mobile and LXR activation showed seminiferous tubules lined by nearly normal layers of spermatogenesis cells with mature sperms, the basement membrane of nearly normal thickness, no Leydig cells hyperplasia, and slight focal drop of spermatogenesis cells. Rats exposed to cell phone radiation exhibited significant decrease in Johnsen's score ($p < 0.001$) in comparison to control rats. LXR

activation induced significant increase in Johnsen's score ($p < 0.001$) in comparison to EM group (Figure 3).

4. Discussion

Here in the present study, we hypothesized that exposure to cell phone radiation has hazardous effects on male fertility and LXR activation could be protective against these effects. We classified these protective effects into four categories including testicular endocrine function, anti-inflammatory and antioxidant status, anti-apoptosis effect besides sperm quality parameters assessment.

Our histological assessment showed that rats exposed to mobile radiation exhibited significant decrease in spermatogenesis with increased disorganization and separation of germ cells in the seminiferous tubules that resulted in spermatogenesis arrest. These changes are similar to that reported by other studies [19,21,22] while Tumkaya et al. reported no histological abnormalities in testicles of rats exposed to mobile phones [23]. However, LXR activation diminished the EMF-induced damages. These findings could be explained by the cross-links between testicular endocrine status and spermatogenesis as serum levels of testosterone could reveal the normal and/or disturbed spermatogenesis [24]. Moreover, spermatogenesis is hormonally regulated by the hypothalamic-pituitary-gonadal (HPG) axis, with pulsatile secretion of gonadotropins (LH and FSH) leading to androgen synthesis by Leydig cells. In view of these facts, we assessed the testicular endocrine status by evaluating the serum levels of FSH, LH and testosterone [25].

Similar to previous reports about chronic exposure of male Wistar rats to cell phone electromagnetic radiation [26], the exposure to cell phone EMF resulted in diminished serum level of gonadotropic hormonal profile (FSH, LH and testosterone). Moreover, several studies reported the dropped testosterone level in electromagnetism-exposed animals [8,27–29].

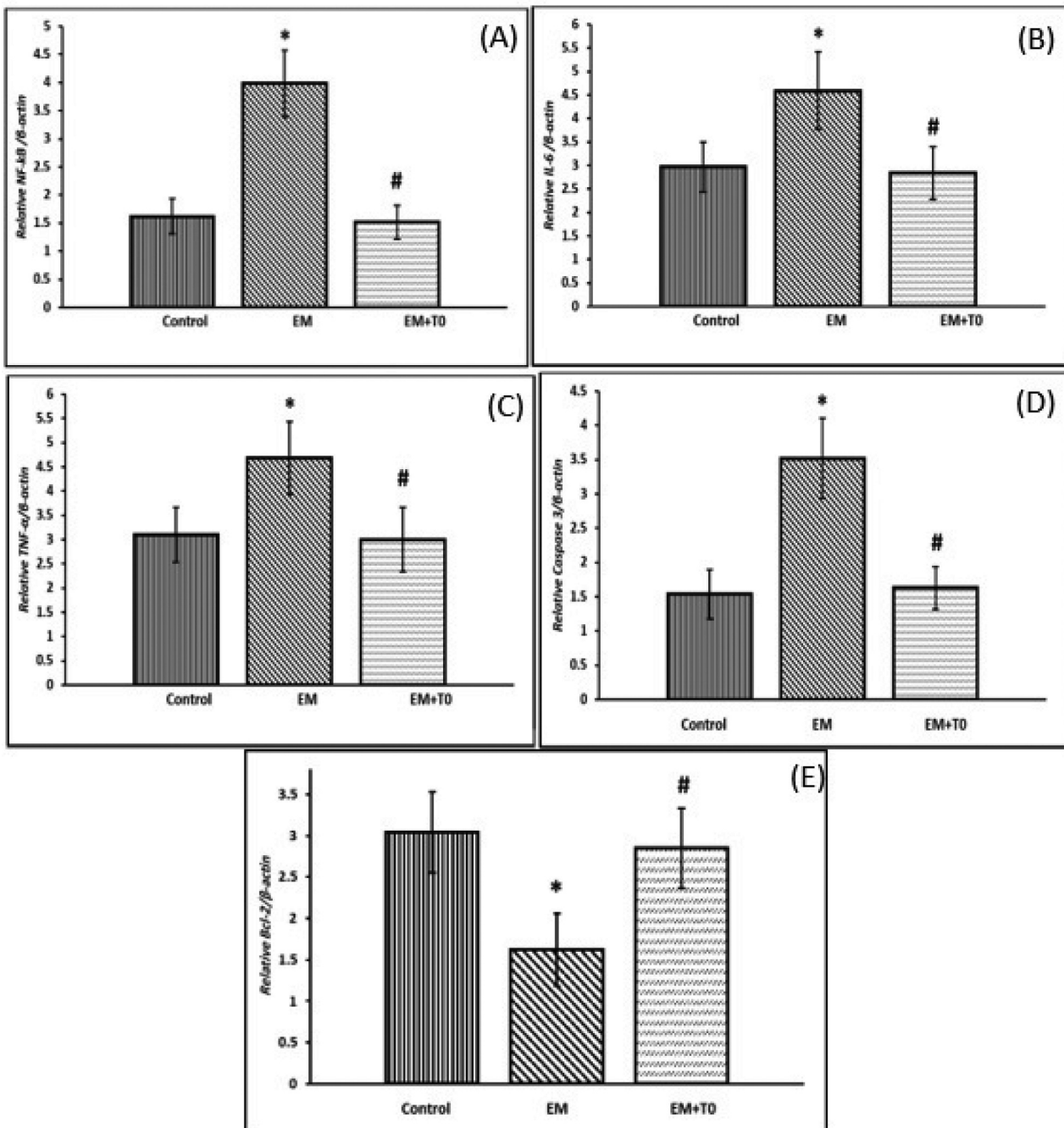


Figure 2. Effect of cell phone radiation and LXR activation on gene expressions of NF- κ B (A), IL-6 (B), TNF- α (C), Caspase 3 (D) and Bcl-2 (E). Data are represented as mean \pm standard deviation. Significance ($P < 0.05$): (*) significant when compared with control group, (#) significant when compared with EM group.

However, the effect of exposure to cell phone EMF on gonadotropic hormonal level is controversial. It was reported that 30 days' exposure to mobile phone radiation for 6 h/day leads to insignificant decrease in serum testosterone levels [19]. Another study on rats showed that EMF significantly lowered serum testosterone with no noticeable changes in FSH, LH levels [22]. Moreover, it was found that mice [30] and rats [31] exposed to EMF exhibited an increase in testosterone levels.

Meanwhile, LXR agonist up-regulated the lowered serum FSH, LH and testosterone levels in rats exposed to EMF reflecting the ameliorative effect of LXR

activation on endocrine status, which in turn notably improved the histological changes. In legalization with our findings, Volle et al. have reported that LXR agonist T0901317 induces a 13.3-fold increase in intratesticular testosterone in wild-type mice and low FSH and testosterone levels were observed in LXR $\alpha\beta$ - mice [14].

In the current study, remarkable elevation of testicular MDA (oxidative stress marker) and significant reduction in testicular SOD activity (endogenous free radical scavenger) were revealed in electromagnetism-exposed rats suggesting that the defective

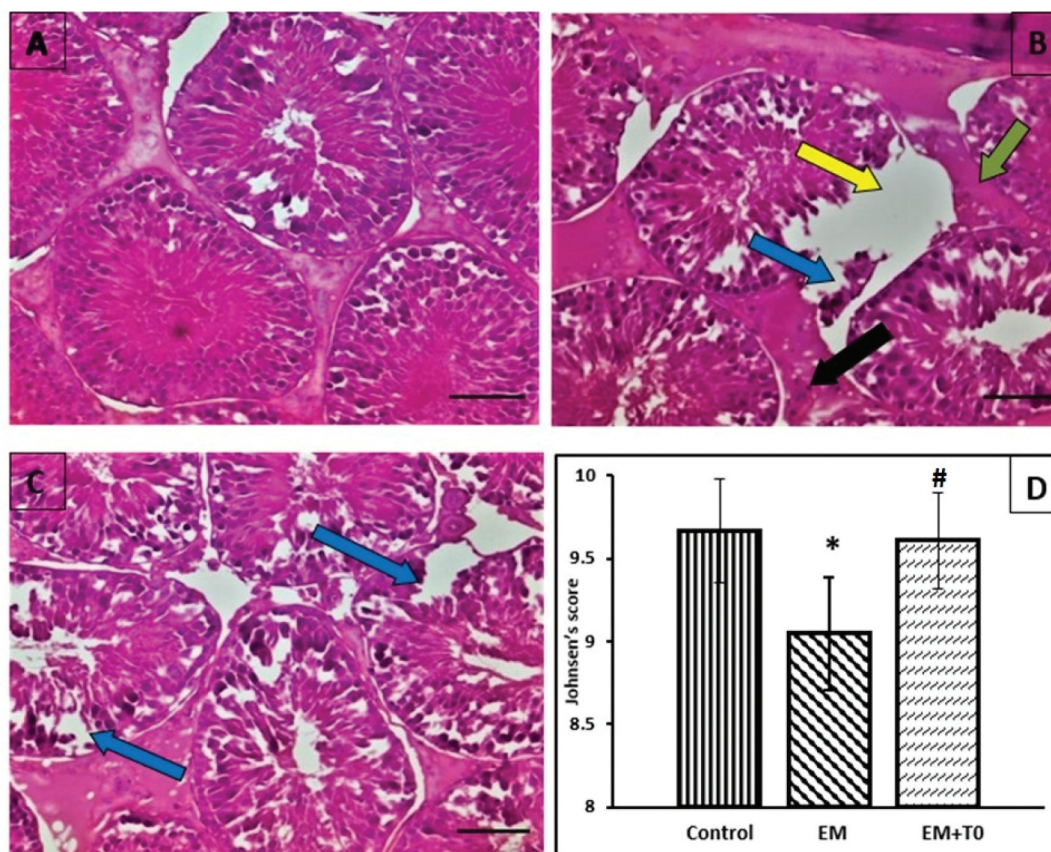


Figure 3. Photomicrograph of H & E stain x400 magnification of rat right testis in (A) control group showing normal structure of the testes, (B) mobile radiation exposed group showing: rupture of the basement membrane (yellow arrow), dropped off spermatogenic cells (blue arrow), inter tubular fibrosis (green arrow) and leydig cells hyperplasia (black arrow), (C) mobile radiation exposed and LXR_s activated group showing seminiferous tubules lined by nearly normal layers of spermatogenic cells with mature sperms, the basement membrane of nearly normal thickness, no leydig cells hyperplasia and slight focal drop of spermatogenic cells (blue arrow), (D) bar chart showing comparison of Johnsen's mean testicular biopsy score: data are represented as mean \pm standard deviation. Significance ($P < 0.05$): (*) significant when compared with control group, (#) significant when compared with EM group.

spermatogenesis function is associated with augmented oxidative stress and deterioration in antioxidant activity, that leads to a surge in oxidative stress. However, the animals in T0901317-treated group signified decreased MDA content and improved SOD activity.

Many researchers have reported elevated levels of ROS as well as a reduction in the antioxidant capacity after EMF exposure at different frequencies and power levels [3,20,26,32]. Similarly, in a human study; Agarwal et al. revealed an increase in ROS levels together with a reduction in ROS-TAC score after exposure to EMF from cellular phones while total antioxidant capacity (TAC) of semen revealed no significant changes from the unexposed group [33]. However, other studies failed to find any significant increase in ROS levels in sperm post cell phone radiation exposure in rats [34] or human [35].

LXR_s activation is typically known to have valuable effects in several chronic diseases via regulating cholesterol homeostasis and anti-inflammatory action. In this study, we established that LXR_s have an antioxidant potential in testicular tissue of rats exposed to

cell phone radiation. LXR_s antioxidant action was proved in other tissues as peripheral nerve [36], myocardial ischemia reperfusion injury [37], and diabetic nephropathy [38] in studies performed on LXR_s knockout mice. The antioxidant effect of LXR_s agonists was also evaluated in liver fibrosis [39] and hypertensive rats [40].

On the basis of that EMF could modulate gene expression and cytokine production and oxidative stress is also associated with high levels of pro-inflammatory cytokines that are related to unfavorable semen parameters [11], relative gene expressions of NF- κ B, IL-6, and TNF- α were assessed. Higher NF- κ B, IL-6, and TNF- α expression in testicular homogenates of rats exposed to mobile phone EMF were noticed in the current study while administration of LXR_s agonist showed anti-inflammatory action and improved these changes.

The effect of EMF radiation on inflammatory cytokines was investigated in vivo and in vitro and the conclusions are controversial [11]. It was reported that exposure to EMF radiation results in downstream effects such as increased production of more free

radicals and cytokines [41]. Moreover, Kim et al. showed in their study on mice that exposure to EMFs can increase the production and expression of pro-inflammatory cytokines (IL-1b, IL-6, and TNF- α) [10]. In human study, it was reported that long-term exposure to EMFs probably affects immune responses, by stimulating the production of pro-inflammatory cytokines [42].

On the contrary, lower plasma NF- κ B and interleukin IL-6 were observed in occupationally exposed workers to EMFs for twenty years [43]. Moreover, a study performed on male workers with high occupational EMF exposure reported that chronic exposure to EMF could affect reproductive functions by decreasing male plasma testosterone but no significant associations of EMF exposure with inflammatory pathway biomarkers were observed [44].

*LXR*s exert anti-inflammatory effects through inhibiting nuclear factor- κ B -dependent induction of pro-inflammatory genes [16]. Studies on male reproductive system from *LXR* $\alpha\beta$ -/- mice revealed another possible role for *LXR*s in testicular macrophages as *LXR*s may reduce NF- κ B signaling and reduce levels of the pro-inflammatory cytokines IL-6 and TNF- α [45].

In inflammatory conditions, the mitochondria are exposed to free radicals, leading to mitochondria-dependent apoptosis. There was a relationship between free electrons released from mitochondria and the excessive production of ROS as 1–3% of all electrons in the electron transport chain of mitochondria generate superoxide radicals [46]. As a compensatory mechanism, some proteins such as Bcl-2 are systematized on mitochondrial membrane as antiapoptotic factor to maintain its integrity [47]. Moreover, the caspase 3 activates the cytoplasmic endonucleases leading to damage to the nuclear materials and proteins as well as DNA fragmentation [48].

Minding these facts, here in the current study we investigated relative gene expression of caspase 3 and Bcl-2 in rat testicular tissue and our observations revealed a significant reduction in Bcl-2 with significant increase in caspase 3 expressions after exposure to electromagnetic radiation and these changes were improved by *LXR*s agonist administration. The apoptotic effect of cell phone radiation was previously reported [3,49]. However, Falzone et al. found no evidence of any outcome of EMF exposure on apoptosis activity such as caspase activation, DNA fragmentation, or ROS generation in human spermatozoa [50].

The anti-apoptotic effect of *LXR*s could be attributed to its antioxidant and anti-inflammatory action, however, it was reported that *LXR*s may regulate the balance between the proliferation and apoptosis of germ cells. In *LXR*s knockout mice, lack of *LXR*- β reduced proliferation and lack of *LXR*- α amplified

apoptosis and these changes cannot be compensated leading to severe impairment of spermatogenesis [14].

Lastly, the sperm quality that is affected by all the previous changes has been evaluated by assessing sperm count, motility, and viability. The sperm quality evaluation showed that sperm count, motility, and viability were decreased in rats exposed to cell phone radiation while *LXR*s agonist administration improved sperm quality parameters.

Reliable with our findings, it was reported that cell phone radiation decreased sperm parameters in humans [3,5,33] and rats [20,21]. In contrast to our results, exposure to EMF did not induce any negative effects on sperm quantity, quality, and morphology [8,51,52]. *LXR*s improving action on sperm quality could be attributed to its antioxidant, anti-inflammatory properties besides its endocrinal action.

5. Conclusion

In conclusion, the usage of cell phones that becomes essential for modern life is associated with obligatory exposure to EMF leads to harmful effects on spermatogenesis and *LXR*s activation could improve these effects via antioxidant, anti-inflammatory properties as well as its endocrinal action. Further studies to elucidate the effect of natural modulators of liver X receptors on cell phone radiation-induced alterations in male reproductive quality are recommended.

Compliance with ethical standards, ethical approval

All experimental procedures were following the guide for the care and use of laboratory animals (8th edition, National Academies Press) and have been reviewed and approved by Zagazig University institutional animal care unit committee (ZU-IACUC; Sharkia; Egypt) with approval number: ZU-IACUC/3/F/117/2019).

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributor

Conception and design: R.S.G., N.M.M. acquisition, analysis, and interpretation of data: N.M.M., A.E.S, R.S.G. Drafting the work or revising: R.S.G., A.E.S, N.M.M. Final approval of the manuscript: N.M.M, R.S.G., A.E.M.

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Conception and design: R.S.G., N.M.M. acquisition, analysis, and interpretation of data: N.M.M., A.E.S, R.S.G. Drafting the work or revising: R.S.G., A.E.S, N.M.M. Final approval of the manuscript: N.M.M, R.S.G., A.E.M.

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