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Effect of Ethanolic Extract of Avocado Pear (Persea Americana) on the Testicular Structure and Function of Different Age Groups of Albino Rats Exposed to Electromagnetic Filed (EMF) Emitted Form Cellular Phones

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ORIGINAL ARTICLE

Effect of Ethanolic Extract Of Avocado Pear (*Persea americana*) On The Testicular Structure And Function Of Different Age Groups Of Albino Rats Exposed To Electromagnetic Field Emitted From Cellular Phones

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Abstract

Background: **The possible effects of electromagnetic field (EMF) generated by mobile phones on reproductive functions have been discussed recently.**

Aim: **To study the structural and functional changes in the testes of different age groups of albino rats after exposure to cellular phone radiation and the possible protective effect of avocado extract.**

Materials and methods: **The present study was carried out on 50 healthy albino rats of different age groups. The rats were divided into five groups, with each including three subgroups, according to age.**

Results: **EMF-exposed rats showed testicular alterations, which were ameliorated using the avocado extract.**

Conclusion: **EMF emitted from cellular phones has potential adverse effects on the male reproductive functions. Avocado pear has a protective role against radiation-induced damage in testicles by its antioxidant and anti-apoptotic effects.**

Keywords: Albino rats, Avocado pear, Electromagnetic field, Ethanolic extract, Testicular

1. Introduction

Modern technology has allowed the development of many different instruments emitting electromagnetic waves (EMWs) such as mobile phones, microwaves, televisions, satellite signals, and computers. Humans are exposed to EMWs every day, more than the day before. Electromagnetic radiofrequency radiations of these devices were reported to affect negatively male fertility by some studies, whereas other studies showed no abnormalities.[1]

Exposure to extra-low frequency electromagnetic field (EMF) affects living cells and tissues. There is a worldwide increase in the number of cell phone users and the question is whether microwaves from these instruments could cause health hazards. There has been increasing interest in the biological effects and possible health outcomes of weak, high-frequency electric and magnetic fields. When the biological systems are exposed to an external magnetic field with a very large strength relative to the biomagnetic field of the cells, a disturbance in their metabolic function is expected and may lead to cell death or increase in cell division.[1]

Exposure of mice to 900−1800-MHz microwaves affected the histological structure of testis, particularly Leydig cells, and showed an apoptosis-inducing effect on the spermatogenic cells.[2]

Avocado has health benefits such as wound healing and prevention of psoriasis, wrinkles, stretch marks, scleroderma, hepatic injuries, stroke, obesity, and cancer. The health benefits of fatty avocado fruit may be due to its content of essential nutrients,

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proteins, fibers, minerals, and vitamins. Previous studies have showed that it reduces the risk of diabetes, helps in weight control, normalizes blood cholesterol levels, and is involved in liver metabolism.[3] Therefore in this study, we examined the structural and functional changes in the testes of albino rats after exposure to cellular phones radiation and the possible protective effect of avocado extract.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

The present study was carried out on 50 healthy albino rats of different age groups. The rats were divided into five groups, with each including three subgroups, according to age (neonates – adult and senile).

Group I (EMF): rats exposed to frequency of 950 MHz of EMF for 1 month.

Group II (EMF + avocado): rats treated orally with avocado extract (1 ml/kg body mass) before exposure to EMF for 1 month.[4]

Group III (avocado): rats received avocado extract only.

Group IV (control): rats left without treatment.

Group V (spontaneous recovery): rats exposed to EMF and then left for spontaneous recovery for 1 month.

2.2. Methods

2.2.1. Electromagnetic field exposures

EMF generator was adjusted for a frequency of 950 MHz, which equals that of commercially available cellular phones. The coil was placed in the center of the cage for 2 h daily. In the exposure room, other materials or devices that would interfere with the experiment environment were not allowed.

2.2.2. 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.

2.2.3. Hormonal assay

Blood samples were collected into EDTA bottles then centrifuged at 1100 rpm for 15 min. Plasma samples were assayed for testosterone using the ELISA technique.

2.2.4. Semen analysis

The caudal epididymides were blotted with filter papers and lacerated then immersed in 5-ml saline in a tube, then poured into a plate and homogenized into a suspension from which the sperm count was carried out manually under the microscope using × 400 magnification.

2.2.5. Histopathological examination

The samples were prepared and stained with hematoxylin and eosin and Masson trichrome stain and examined by light microscope for any abnormalities.

2.2.6. Transmission electron microscopy

Stained sections were examined with a Jeol 1010 transmission electron microscopy at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

2.3. Statistical analysis

The data were collected and analyzed to determine the significance of differences among means. Then data were expressed as mean ± SD. Values were considered significant at *P* value less than 0.05.

3. Results

Tables 1–7.

3.1. Histopathological results

The results of sections of testis stained with hematoxylin and eosin and Masson trichrome stains are shown in Figs. 1–6.

Table 1. Comparison between studied groups regarding testosterone level.

	Group I ($N = 15$)	Group II ($N = 15$)	Group III ($N = 15$)	Group IV ($N = 15$)	Group V ($N = 15$)	*F*	*P* value
Testosterone							
Mean ± SD	1.3 ± 0.4	2.2 ± 0.7	2.0 ± 0.4	2.4 ± 0.5	1.7 ± 0.4	10.01	<0.001 HS

F, *F* value of analysis of variance test.
P value less than 0.001 is considered highly significant (HS).

Table 2. Comparison between studied groups regarding semen analysis.

	Group I ($N = 15$)	Group II ($N = 15$)	Group III ($N = 15$)	Group IV ($N = 15$)	Group V ($N = 15$)	KW	*P* value
Semen volume							
Mean ± SD	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.0	1.0 NS
Spermatic count							
Mean ± SD	10.9 ± 8.0	32.7 ± 25.1	0.5 ± 0.7	3.5 ± 5.1	5.2 ± 4.6	19.7	0.001 S
Abnormal forms of sperms							
Mean ± SD	2.7 ± 3.9	4.1 ± 3.1	0.0 ± 0.0	3.9 ± 5.7	6.7 ± 4.9	17.7	0.001 S

KW, Kruskal–Willis test.
P value more than 0.05 is considered nonsignificant (NS); *P* value less than 0.05 is considered significant (S).

Table 3. Comparison between studied groups regarding morphology.

	Group I ($N = 15$)		Group II ($N = 15$)		Group III ($N = 15$)		Group IV ($N = 15$)		Group V ($N = 15$)		χ^2	*P* value
Tunica propria												
Average	0	0%	5	33.3%	15	100%	15	100%	15	100%		
Thick	5	33.3%	10	66.7%	0	0%	0	0%	0	0%	86.7	<0.001 HS
Thick/irregular	10	66.7%	0	0%	0	0%	0	0%	0	0%		
Interstitium												
Average	10	66.7%	10	66.7%	10	66.7%	15	100%	10	66.7%		
Mildly edematous	0	0%	5	33.3%	5	33.3%	0	0%	5	33.3%	31.8	<0.001 HS
Markedly edematous	5	33.3%	0	0%	0	0%	0	0%	0	0%		

χ^2, χ^2 test.
P value less than 0.001 is considered highly significant (HS); *P* value less than 0.05 is considered significant (S).

Table 4. Comparisons of testosterone and semen analysis between studied rates in group I.

	Group I			Statistical test	*P value*
	Young ($N = 5$)	Adult ($N = 5$)	Senile ($N = 5$)		
Testosterone					
Mean ± SD	1.4 ± 0.4	0.9 ± 0.1	1.7 ± 0.3	$F = 9.7$	0.001 S
Semen volume					
Mean ± SD	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	–	–
Spermatic count					
Mean ± SD	0.0 ± 0.0	15.2 ± 0.8	17.4 ± 1.1	$F = 673.3$	<0.001 HS
Abnormal forms of sperms					
Mean ± SD	0.0 ± 0.0	0.0 ± 0.0	8.0 ± 0.7	$F = 640$	<0.001 HS

F, *F* value of analysis of variance test.
P value less than 0.001 is considered highly significant (HS); *P* value less than 0.05 is considered significant (S).

Table 5. Comparisons of testosterone and semen analysis between studied rates in group II.

	Group II			Statistical test	*P* value
	Young ($N = 5$)	Adult ($N = 5$)	Senile ($N = 5$)		
Testosterone					
Mean ± SD	2.3 ± 0.4	2.9 ± 0.2	1.5 ± 0.7	$F = 9.6$	0.003 S
Semen volume					
Mean ± SD	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	–	–
Spermatic count					
Mean ± SD	0.0 ± 0.0	56.0 ± 6.5	42.0 ± 5.7	$F = 169.8$	<0.001 HS
Abnormal forms of sperms					
Mean ± SD	0.0 ± 0.0	6.6 ± 1.1	5.8 ± 0.8	$F = 97.3$	<0.001 HS

F, *F* value of analysis of variance test.
P value less than 0.001 is considered highly significant (HS); *P* value less than 0.05 is considered significant (S).

Table 6. Comparisons of testosterone and semen analysis between studied rates in group III.

	Group III			Statistical test	*P* value
	Young ($N = 5$)	Adult ($N = 5$)	Senile ($N = 5$)		
Testosterone					
Mean ± SD	1.9 ± 0.1	2.5 ± 0.4	1.7 ± 0.3	$F = 12.6$	0.003 S
Semen volume					
Mean ± SD	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	–	–
Spermatic count					
Mean ± SD	0.0 ± 0.0	1.4 ± 0.5	0.0 ± 0.0	$F = 32.7$	<0.001 HS
Abnormal forms of sperms					
Mean ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	–	–

F, *F* value of analysis of variance test.
P value less than 0.001 is considered highly significant (HS); *P* value less than 0.05 is considered significant (S).

Table 7. Comparisons of testosterone and semen analysis between studied rates in group V.

	Group V			Statistical test	*P* value
	Young ($N = 5$)	Adult ($N = 5$)	Senile ($N = 5$)		
Testosterone					
Mean ± SD	1.7 ± 0.5	1.5 ± 0.4	1.8 ± 0.2	$F = 0.96$	0.410 NS
Semen volume					
Mean ± SD	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	–	–
Spermatic count					
Mean ± SD	10.8 ± 1.3	0.0 ± 0.0	4.8 ± 0.8	$F = 183$	<0.001 HS
Abnormal forms of sperms					
Mean ± SD	10.0 ± 0.7	0.0 ± 0.0	10.0 ± 0.7	$F = 500$	<0.001 HS

F, *F* value of analysis of variance test.
P value less than 0.001 is considered highly significant (HS); *P* value more than 0.05 is considered nonsignificant (NS).

3.1.1. Group I

Fig. 1.

3.1.2. Group II

Fig. 2.

3.1.3. Group III

Fig. 3.

3.1.4. Group IV

Fig. 4.

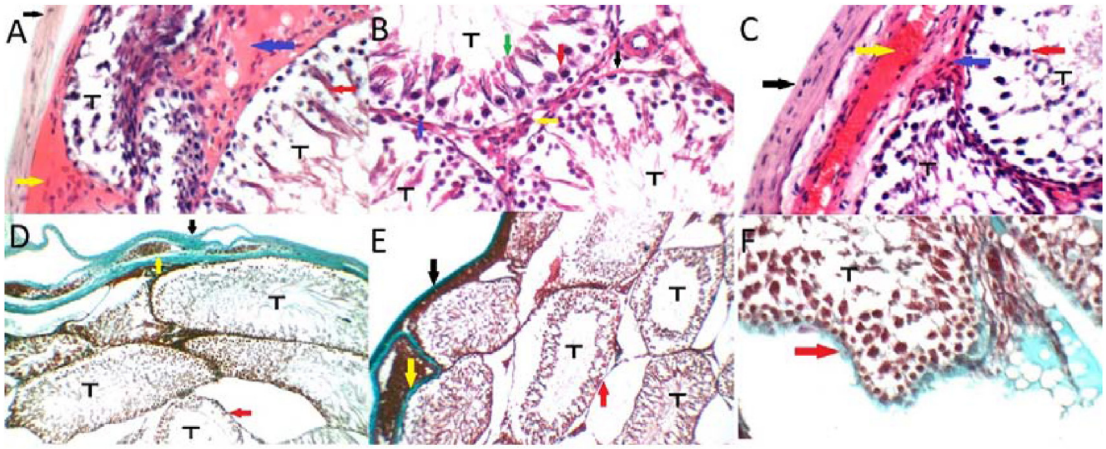

Fig. 1. Sections of testis of group I showing (a) average tunica albuginea (black arrow) with mild subcapsular (yellow arrow) and interstitial edema (blue arrow) and average-sized tubules (T) with average germinal lining (red arrow). (b) Thick tunica propria (black arrow), spermatogonia (blue arrow), primary spermatocytes (red arrow), many spermatozoa (green arrow), and average interstitium with average Leydig cells (yellow arrow). (c) Average tunica albuginea (black arrow) with markedly congested subcapsular blood vessels (yellow arrow), average-sized tubules (T) with average germinal lining (red arrow), and average interstitium (blue arrow). (d) Thick collagen in tunica albuginea (black arrow) and in blood vessel wall (yellow arrow), and average collagen in tubular wall (red arrow). (e) Testis showing average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (f) Thick collagen in tubular wall (red arrow) (a, b, and c, hematoxylin and eosin stain, × 400 and d, e, Masson trichrome stain, × 200, and f, × 400). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

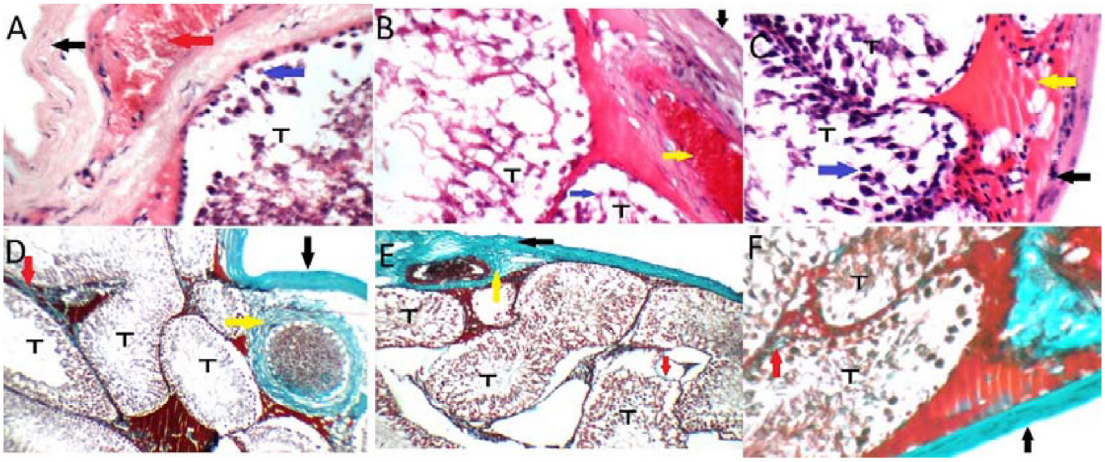

Fig. 2. Sections of testis of group II showing (a) thick tunica albuginea (black arrow) with markedly congested subcapsular blood vessels (red arrow), and average-sized tubules (T) with scattered apoptotic germinal lining (blue arrow). (b) Average tunica albuginea (black arrow) with markedly congested subcapsular blood vessels (yellow arrow) and average-sized tubules (T) with scattered apoptotic germinal lining (blue arrow). (c) Average tunica albuginea (black arrow) with mild subcapsular edema (yellow arrow) and average-sized tubules (T) with average germinal lining (blue arrow). (d) Thick collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow) and in tubular wall (red arrow). (e) Thick collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (f) Average collagen in tunica albuginea (black arrow) and in tubular wall (red arrow). (a, b, and c, hematoxylin and eosin stain, × 400 and d, e, Masson trichrome stain, × 200 and f, × 400).

3.1.5. Group V

Fig. 5.

3.2. Transmission electron microscopy results

Fig. 6.

4. Discussion

Our results showed that EMF exposure and avocado extract treatment increased the level of testosterone. In this study, long-term exposure to low-frequency EMF increased testosterone levels. In agreement with our findings, Nisbet et al.[5] reported that exposure to EMF of 1800 and 900 MHz for 2 h continuously per day for 90 days caused an increase in testosterone level, and Forgács et al.[6] reported that exposure to 1800-MHz GSM-like microwave caused an increase in testosterone level.

However, exposure to circularly polarized, 50 Hz magnetic fields continuously for 6 weeks in rats Kato et al.,[7] exposure to static magnetic fields 50 Hz

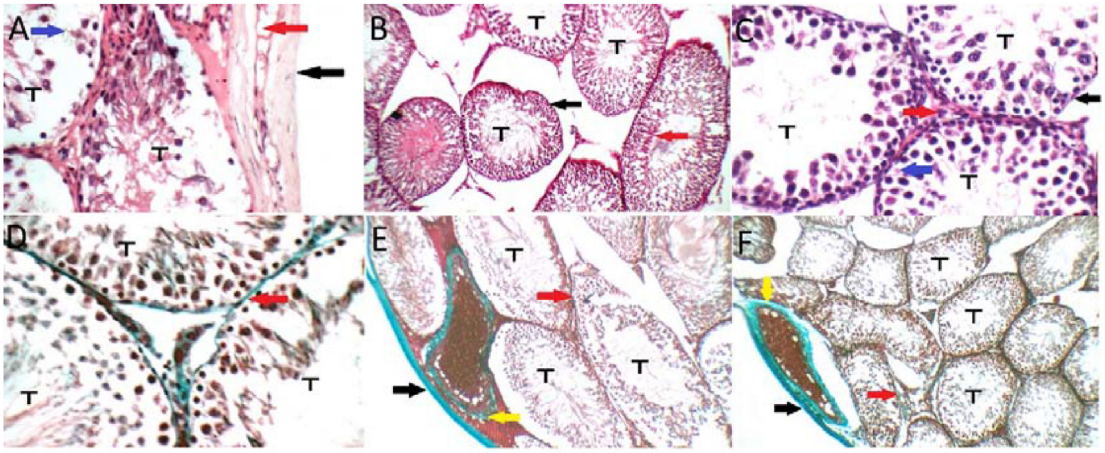

Fig. 3. Sections of testis of group III showing (a) thick tunica albuginea (black arrow) with average subcapsular blood vessels (red arrow) and average-sized tubules (T) with average germinal lining (blue arrow). (b) Average-sized tubules (T) with average germinal lining (black arrow) and complete spermatogenesis (blue arrow). (c) Average-sized tubules (T) with average tunica propria (black arrow), scattered apoptotic germinal lining (blue arrow), and average interstitium with average Leydig cells (red arrow). (d) Average collagen tubular wall (red arrow). (e) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (f) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (a, c, hematoxylin and eosin stain, × 400 and b, × 200 and e, f, Masson trichrome stain, × 200 and d, × 400).

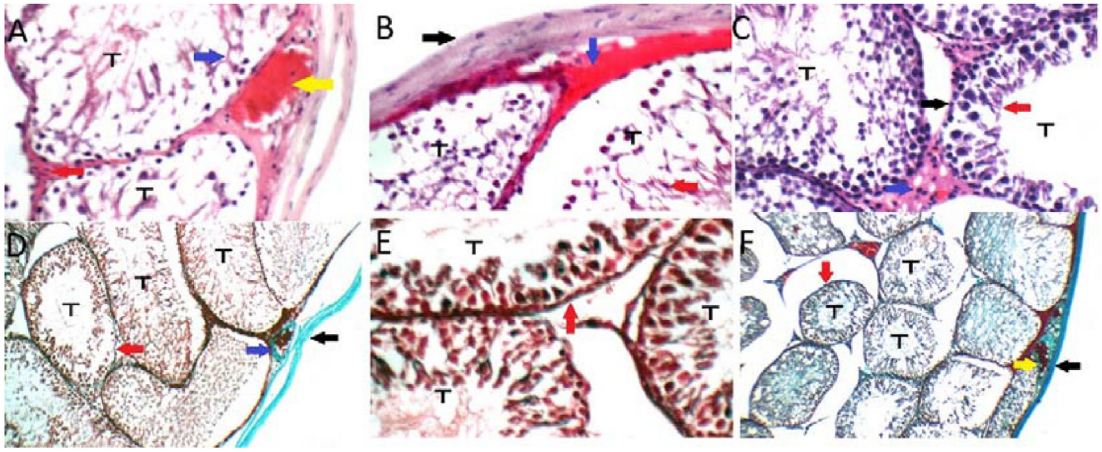

Fig. 4. Sections of testis of group IV showing (a) average tunica albuginea (black arrow) with mildly congested subcapsular blood vessels (yellow arrow), average-sized tubules (T) with average germinal lining (blue arrow), and average interstitium (red arrow). (b) Average tunica albuginea (black arrow), average-sized tubules (T) with average germinal lining (red arrow), and average interstitium (blue arrow). (c) Average-sized tubules (T) with average tunica propria (black arrow), average germinal lining (red arrow), and average interstitium with average Leydig cells (blue arrow). (d) Average collagen in tunica albuginea (black arrow), in blood vessel wall (blue arrow), and in tubular wall (red arrow). (e) Average collagen in tubular wall (red arrow). (f) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow) (a, b, and c, hematoxylin and eosin stain, × 400 and d, f, Masson trichrome stain, × 200 and e, × 400).

for 40 min daily for 17 days Fathi et al.,[8] and exposure to 50 Hz, 5 mT magnetic field for periods of 1,2 and 4 weeks Moustafa et al.,[9] represented that have no effects on testosterone level of male rats significantly. In contrast with our results, Bahaodini et al.[10] reported that long-term exposure to low-frequency EMF increased testosterone levels. Amara et al.[11] showed that exposure to static magnetic field (128 mT; 1 h/day for 30 days) decreased rat testosterone levels.

Avocado possibly induced this protection through the antioxidant effects of its bioactive compounds.[3] In the present study, comparison between studied groups regarding semen analysis showed that there was a statistically significant increased spermatic count in group II when compared with the other groups.

There were statistically significant increased abnormal forms of sperms in group V when compared with the other groups. No statistically significant difference was found between studied

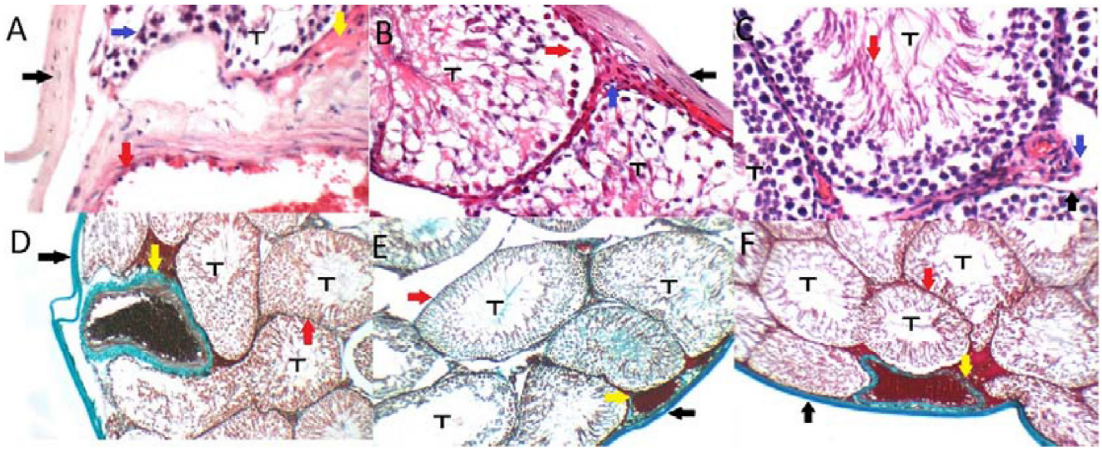

Fig. 5. Sections of testis of group V showing (a) average tunica albuginea (black arrow) with mildly congested subcapsular blood vessels (red arrow), average-sized tubules (T) with average germinal lining (blue arrow), and average interstitium (yellow arrow). (b) Average tunica albuginea (black arrow), average-sized tubules (T) (blue arrow) with scattered apoptotic germinal lining (red arrow), and average interstitium (blue arrow). (c) Average-sized tubules (T) with average tunica propria (black arrow), average germinal lining with complete spermatogenesis (red arrow), and average interstitium with average Leydig cells (blue arrow). (d) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (e) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow). (f) Average collagen in tunica albuginea (black arrow), in blood vessel wall (yellow arrow), and in tubular wall (red arrow) (a, b, and c, hematoxylin and eosin stain, × 400 and d, e, and f, Masson trichrome stain, × 200).

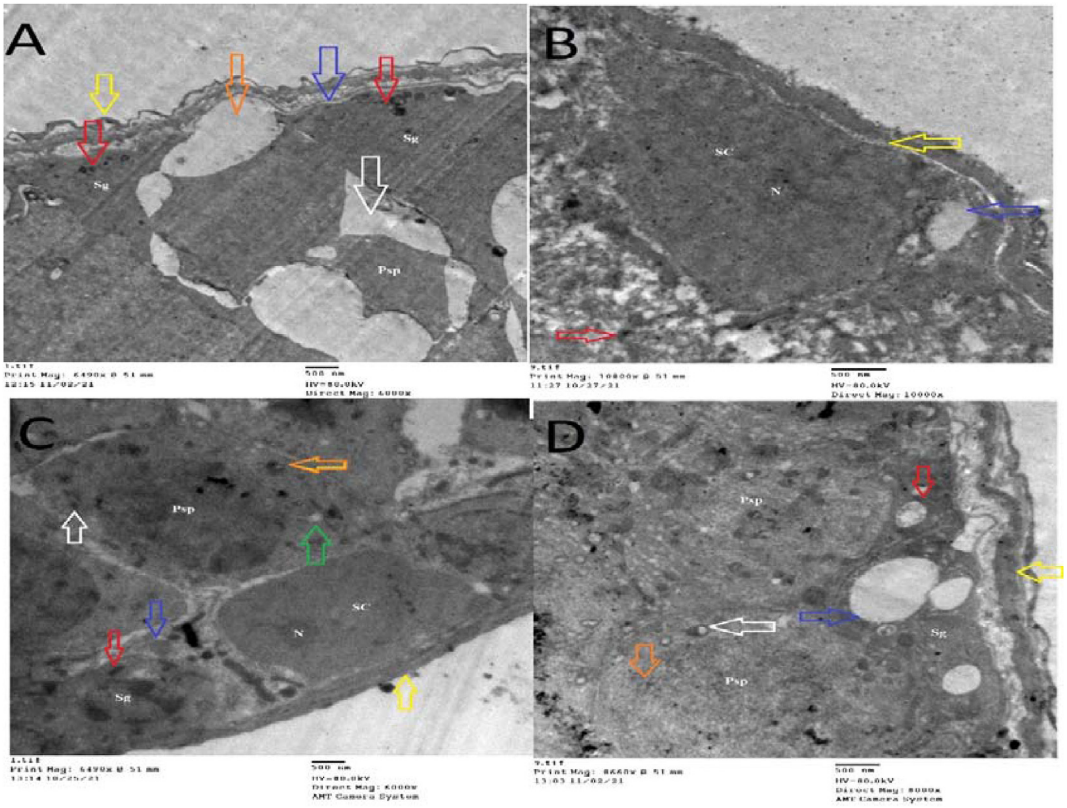

Fig. 6. Electron micrographs showing (a) seminiferous tubules with spermatogonia (Sp) resting on markedly irregular tunica propria (yellow arrow), markedly pyknotic nucleus with clumped chromatin (red arrow), swollen mitochondria (blue arrow) and large cytoplasmic vacuoles (orange arrow), and primary spermatocytes (Psp) with large cytoplasmic vacuoles (white arrow). (b) Seminiferous tubules with sertoli cell (SC) resting on average tunica propria (yellow arrow) with indented nucleus showing prominent nucleolus (N), average rounded mitochondria (red arrow) and small cytoplasmic vacuoles (blue arrow), and Sertoli cells. (c) Seminiferous tubules with spermatogonia (Sp) resting on average tunica propria (yellow arrow) with nucleus showing dispersed chromatin (red arrow) and average rounded mitochondria (blue arrow), primary spermatocytes (Psp) with nucleus showing dispersed chromatin (white arrow), small rounded mitochondria (orange arrow) and small cytoplasmic vacuoles (green arrow), and Sertoli cells (SC) with indented nucleus showing prominent nuclei (N). (d) Seminiferous tubules with spermatogonia (Sp) resting on thick irregular tunica propria (yellow arrow), swollen mitochondria (red arrow) and large cytoplasmic vacuoles (blue arrow), primary spermatocytes (Psp) with nucleus (N) showing dispersed chromatin (orange arrow), average mitochondria (green arrow) and small cytoplasmic vacuoles (white arrow).

groups regarding semen volume. Our results showed that avocado extract treatment significantly increased the spermatic count and abnormal forms of sperms, but EMF exposure significantly reduced the spermatic count and abnormal forms of sperms. Our results were supported by Bahaodini et al.,[10] who reported that regarding sperm quality evaluation, rats exposed to 50 Hz EMF for 24 h/day for 85 days had reduced sperm motility.

However, EMF did not affect the total sperm concentration and viability. Consistent with our findings, Erogul et al.[12] reported that cell phone waves decreased sperm parameters in human semen samples. Mailankot et al.[13] reported that rats exposed to mobile phone waves for 1 h/day for 28 days showed reduced percentage of motile sperm. Moreover, Mortazavi et al.[14] revealed that sperm count and motility in Wistar rats decreased as the magnetic field strength increased.

Kumar et al.[15] reported that radiofrequency EMW exposure from cell phones adversely affected male fertilizing potential of spermatozoa. Wdowiak et al.[16] reported significant harmful effects on male's semen parameters, including motility and morphology because of cell phone usage.

Our results showed that avocado extract had a positive effect of reducing aging imprint of male reproductive function. These results need to be confirmed by further studies. The age-related morphological changes as observed by Hussein et al.,[17] were as follows: in early adult rats, the testis was covered by a well-defined capsule formed of tunica albuginea and tunica vasculosa. The testicular parenchyma was composed of several seminiferous tubules with narrow interstitial spaces containing loose areolar tissue in between.

This can be supported by Owjfard and Fard,[18] who enrolled 80 male rats that were divided into neonatal

group ($n = 20$), immature group ($n = 30$), and mature group ($n = 30$). In this study, neonatal rats' long-term exposure to radiofrequency radiations emitted from a common mobile jammer decreased seminiferous tubule diameter, spermatid number, number of seminiferous tubules, and testosterone level, whereas the number of seminiferous tubules per unit area (per 0.5 $mm^2 \times 0.5$ mm^2) of testis increased. In immature rats, long-term exposure to radiofrequency radiations emitted from a common mobile jammer or stressed by being restrained had no significant effect on the diameter, number of spermatids, number of seminiferous tubules, and testosterone level. In mature rats, there were no significant differences in the diameter of seminiferous tubules, testosterone level, and sperm quality in the exposed rats when compared with the control group.

4.1. Conclusion

EMF emitted from cellular phones has potential adverse effects on the male reproductive functions. Avocado pear (*Persea americana*) seems to be promising in the treatment of EMF-related adverse effects and reducing the age-related adverse effects on male reproductive functions. These primary results need to be confirmed by larger studies.

Acknowledgment

Authors declare that no conflict of interest, no financial issues to be declared.

Conflict of interest

Authors declare that no conflict of interest.

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