

Effects of Long Term Exposure to a 2G Cell Phone Radiation (900 - 1900 MHz) on Mouse Testis

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Abstract: ***Objective:** The aim of our study was to evaluate possible effects of chronic exposure to 900 - 1900 MHz radiations emitted from 2G cell phone on the testis of mice at the histological level. **Methods:** Mice were exposed to 2G ultra-high frequency radiation, 48 minutes per day for a period of 30 to 180 days. The amount of electromagnetic field (EMF) exposed was calculated by the radiation frequency meter. The sham control mice were exposed to similar conditions without 2G exposure. Each animal's weight was recorded before sacrifice. Three animals each were sacrificed at the end of 30, 60, 90, 120, 150 and 180 days of exposure in the experimental group after 24 hours of last exposure. Same numbers of control animals were sacrificed on similar period. We collected blood samples to measure plasma testosterone. We measured and analyzed the size, weight and volume of the testis. Testis sections were analyzed under the light microscope for structural changes. **Results:** In 2G exposed group animal weight was lower at first, second and fourth month (p value ≤ 0.05). The mean testis weight of 2G exposed mice was significantly reduced in all months except fourth month (p value < 0.05) and the mean testis volume was significantly reduced in the first three months (p value 0.02). The mean seminiferous tubule density per unit area was significantly lower (p value < 0.001) in the 2G exposed testis. The mean seminiferous tubule diameter was significantly reduced in 2G exposed testis (p value is highly significant < 0.001) except the second month. The mean number of Sertoli cells and Leydig cells were significantly reduced in 2G radiation exposed mice (p value is highly significant < 0.001). While compared with control group, mean serum testosterone level of 2G exposed mice were significantly lower (p value 0.004). The following microscopic changes were found in the testis of 2G cell phone radiation exposed mice. 1. The interstitium appeared wide 2. Sertoli cells and spermatogonia were detached from the basal lamina. 3. Vacuolar degeneration and desquamation of seminiferous epithelium. Most of the peripheral tubules showed maturation arrest in the spermatogenesis. Seminiferous tubules scored between 8 and 9 using Johnson testicular biopsy score count. **Conclusion:** Chronic exposure to ultra-high frequency radiation emitted from a 2G cell phone could cause microscopic changes in the seminiferous tubules, reduction in the number of Sertoli and Leydig cells and decreased serum testosterone level. Long term use of cell phones could cause male infertility.*

Keywords: 2G cell phone, Leydig cells, mice testis, testosterone, ultra-high frequency radiation

1. Introduction

The electromagnetic fields (EMF) emitted by mobile phones and towers are a major public concern today. The increasing use of cell phone and handset devices, particularly by children and teenagers, has increased worldwide concern about the interactions of radiofrequency radiation with the male reproductive organs. Electromagnetic radiation emitted from the cell phone could be absorbed by testis when they are carried in belts. Most of the cellular phones work on the ultra-high frequency bandwidth of 900-2200 MHz's. Ultra high frequency (UHF) electromagnetic radiation or radiofrequency radiation (RFR) with a frequency range of 300- 3000 MHz is "non-ionizing". The present inquest is concerned this form of radiation either to incriminate it as potentially hazardous or absolve it as absolutely harmless. The second generation cell phone (2G) network operates in the 900-1900 MHz frequency for GSM (Global System for Mobile Communications) [1]. Mobile phone in operation emits a pulsed radiofrequency electromagnetic field (RF-EMF). Most of the energy is found to be absorbed into user's body particularly in the head region, which can

produce heat stress and non-thermal stress in the form of releasing free radicals, alter the enzyme reaction and thereby compromises immune system [2]. Specific absorption rate (SAR) is a unit of Watt per kilogram to measure the amount of electromagnetic radiation absorbed by body tissue whilst using a mobile phone [3], [4]. The higher the SAR the more radiation is absorbed. International Commission on Non-Ionizing Radiation Protection (ICNIRP Guidelines 1998) recommendations has set a SAR limit of 2.0 W/Kg in 10 grams of tissue. Whole body average SAR of 0.4W/Kg is widely adopted in most guidelines, which were based on the threshold of the observed effects due to whole-body heating to cause significant elevation of core temperature ($>1^{\circ}\text{C}$) [1].

Earlier reports have shown that exposure to mobile phone radiation could induce damage to tissues which include an increase in single and double strand DNA breakages [5], increased risk of acoustic neuroma associated with mobile phone use of at least ten years duration [6], genotoxic effects in human peripheral blood leukocytes [7], reduction of Purkinje cell population in the adult female rat cerebellum

[8], and disturbance of short term memory in mice [9]. Researchers have also reported that short term exposure to mobile phone radiation induced damage to kidney [10]-[14]. Keeping a cell phone on or close to the waist can decrease sperm concentration [15], decrease in sperm viability and motility due to direct exposure of the semen to cell phone radiation [16]. Long term exposure to mobile phone radiation could lead to reduced sperm motility, serum testosterone levels [17]-[20] and increased ROS (reactive oxygen species) [21]-[23].

In contrary to the above reports, some researchers reported that no adverse biological effects of exposure to non-ionizing radiation emitted from the cell phone, such as no double stranded DNA breaks or effects on chromatin of rat brain [24], no effect on mouse embryonic lens development [25], psychomotor performance was not influenced by brief repeated exposures to mobile phones [26], the lack of histological changes on rat testis [27] and no alterations in serum testosterone [28]. The present study was undertaken because of the contradictory findings on the effects of exposure to non-ionizing radiation emitted from the 2G cell phone on testis.

2. Materials and Methods

Our study was approved by the Institutional Animal Ethics Committee of Mahatma Gandhi Medical College and Research Institute, Puducherry. Thirty six male neonatal albino mice were obtained from the King Institute of Preventive Medicine and Research, animal section, Guindy, Chennai.

New born mice were kept with the mother for twenty one days followed by randomly divided into two independent groups and housed in mice cages at the temperature of $22 \pm 1^\circ\text{C}$ and 60% relative humidity. Animals were housed in the central animal house and provided with adequate ventilation, twelve hours of illumination alternated with twelve hours of darkness. During the study, all the animals received appropriate animal care and were fed with laboratory diet and water ad libitum.

Eighteen mice were exposed to 900-1900 MHz frequency radiation emitted from 2G cell phone and eighteen mice were sham control. The roof of the mice cage was designed to hang the 2G cell phone from a distance of five centimeters from the floor which allow the mice to move freely and to avoid direct thermal injury. A 2G mobile phone in non-vibrating, silent, do not disturb (DND) and auto answer activated mode was kept hanging inside the mice cage. A standard 2G handset with a frequency bandwidth of 900-1900 MHz and power of 2W/Kg was used. The highest specific absorption rate (SAR) value for this standard handset was 1.69 W/Kg (10gm) and this SAR value was within the limit of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) recommendation. The mobile phone which was kept inside the mouse's cage was rung upon from other cell phone for every half an hour, each call lasting for two minutes. Exposure time was forty eight minutes per day for a twelve hour periods (from 8.00AM to 8.00PM) and total duration

of exposure was 30 to 180 days. RF meter was kept inside the mice cage in switch on mode to measure the amount of radiation exposed (Fig.1.a). The sham control group of eighteen mice was kept under similar conditions without 2G exposure. 2G cell phone in switch off mode and RF meter was kept inside the cage of control mice.

Before sacrificing the mice, we measured the weights of mice in both groups. Three mice each were sacrificed at the end of 30, 60, 90, 120, 150 and 180 days of exposures in the experimental group after 24 hours of last exposure. One ml of blood was collected through cardiac puncture for serum testosterone assay. Equal numbers of control mice were sacrificed on the same period. We dissected out both testes and their weight was measured using Denver's digital weighing machine (0.001gm) and the volume by water displacement method. The testes were immediately fixed in 4% formalin solutions for twenty four hours and then tissues processed and embedded in paraffin. Tissues were sectioned at five microns, stained with Haematoxylin and Eosin. We analyzed testis sections from random slide, random sections and random field under the light microscope; studied histomorphometric parameters and structural changes. Diameters of 50 randomly selected essentially rounded seminiferous tubules from each testis were measured by using the micrometer mounted eyepiece (Fig.1.b).

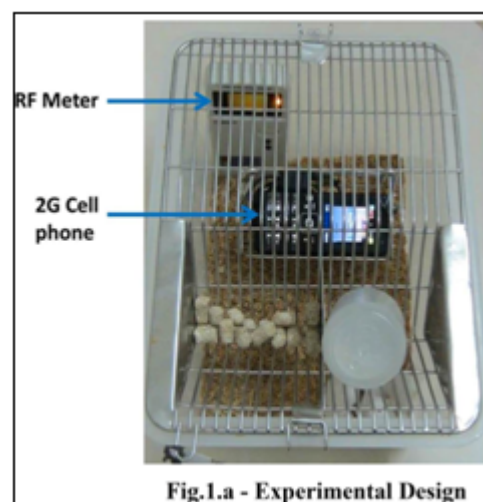


Fig.1.a - Experimental Design

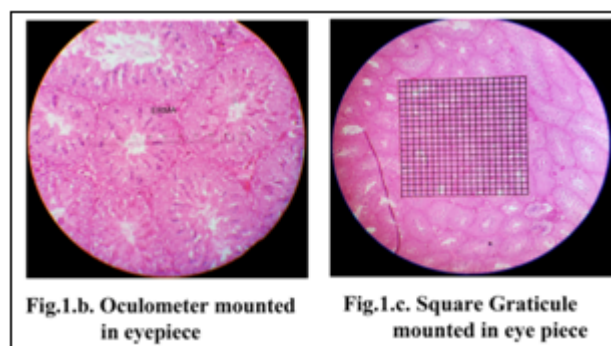


Fig.1.b. Oculometer mounted in eyepiece

Fig.1.c. Square Graticule mounted in eye piece

The seminiferous tubule diameter were measured both in horizontal and vertical axis and the mean average was calculated. Each seminiferous tubule was analyzed and classified into one of 10 different grades using Johnson testicular biopsy score count [29]: Grade 10 – complete

spermatogenesis with many spermatozoa. Grade 9 – much spermatogenesis, but the germinal epithelium disorganized with marked sloughing or obliteration of lumen; Grade 8 – only few spermatozoa present (< 5 to 10); Grade 7 –no spermatozoa but many spermatids present; Grade 6 - no spermatozoa and only few spermatid presents; Grade 5 –no spermatozoa, no spermatids but several and many spermatocytes present; Grade 4 – only few spermatocytes (<5) and no spermatids or spermatozoa; Grade 3 – only spermatogonia; Grade 2- no germ cells, but Sertoli cells present; Grade 1-no cells in tubular section. The seminiferous tubule density per unit area was calculated using eyepiece square graticule with grids. (Fig.1.c). All the testis sections were blindly reviewed by the same investigator. Total serum testosterone was measured by fully automated enzyme linked fluorescent immunoassay (ELFA), Biomerieux, France.

3.Statistical Analysis

We applied non-parametric Mann Whitney U test for comparing the morphometric data and t test for comparing histomorphometric data of testis. *p* value <0.05 was taken statistically significant.

4.Results

4.1 Morphometric Study

In 2G exposed group animals, weight was reduced from the first, second and fourth month (*p* value 0.05). The mean testis weight of 2G exposed mice was significantly lower in

all months except fourth month (*p* value <0.05); mean testis volume was significantly reduced in the first three months (*p* value 0.02) (Table.1).

4.2 Histomorphometric Study

The mean seminiferous tubule density per unit area of 30 square millimeters was significantly decreased (*p* value <0.001) in all 2G exposed testis. The mean seminiferous tubule diameter was significantly reduced in 2G exposed testes. *p* value is highly significant <0.001, except second month. The mean number of Sertoli cells and Leydig cells was significantly reduced in 2G radiation exposed mice. *p* value is highly significant <0.001 (Table. 2). The following microscopic changes were observed in the radiation exposed mice testis: Interstitium appeared wide; detachment of Sertoli and spermatogonia cells from the basal lamina; vacuolar degeneration and desquamation of the seminiferous epithelium. Most of the peripheral tubules showed maturation arrest in the spermatogenesis (Fig.2). Seminiferous tubules scored between 8 and 9 using Johnson testicular biopsy score count (Table.3).

4.3 Biochemical Study

Mean total serum testosterone in the control group was 2.41 ± 1.664 ng/ml and 2G exposed group was 0.12 ± 0.021 ng/ml. In comparison to the control group, the mean serum testosterone level of 2G exposed mice was significantly lower (*p* value is 0.004).

Table 1: Morphometric parameters of mice testis: 2G exposed versus control

| Months | Mean animal weight (gram) | | | Mean testis weight (gram) | | | Mean testis volume (milliliter) | | |
|--------|---------------------------|--------------|-----------------------------|---------------------------|-------------|-----------------------------|---------------------------------|-------------|-----------------------------|
| | Control | 2G | P value for mean difference | Control | 2G | P value for mean difference | Control | 2G | P value for mean difference |
| 1 | 14.63 ± 0.98 | 9.76 ± 0.05 | 0.04* | 0.70 ± 0.08 | 0.02 ± 0.00 | 0.03* | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.02* |
| 2 | 25.36 ± 0.32 | 23.73 ± 0.64 | 0.05* | 0.06 ± 0.00 | 0.03 ± 0.00 | 0.02* | 0.20 ± 0.00 | 0.10 ± 0.00 | 0.02* |
| 3 | 26.23 ± 0.40 | 26.83 ± 0.55 | 0.18 | 0.13 ± 0.00 | 0.06 ± 0.00 | 0.04* | 0.20 ± 0.00 | 0.10 ± 0.00 | 0.02* |
| 4 | 31.00 ± 1.00 | 26.33 ± 0.35 | 0.05* | 0.15 ± 0.00 | 0.09 ± 0.00 | 0.04* | 0.10 ± 0.00 | 0.10 ± 0.00 | 1.00 |
| 5 | 30.67 ± 0.57 | 31.70 ± 3.14 | 0.50* | 0.11 ± 0.00 | 0.12 ± 0.00 | 0.03* | 0.10 ± 0.00 | 0.10 ± 0.00 | 1.00 |
| 6 | 31.73 ± 0.30 | 31.30 ± 0.65 | 0.37 | 0.12 ± 0.00 | 0.08 ± 0.00 | 0.03* | 0.10 ± 0.00 | 0.10 ± 0.00 | 1.00 |

* P value statistically significant (<0.05)

Table 2: Histomorphometric parameters of mice testis: 2G exposed versus control

| Months | Mean Seminiferous Tubule Density / unit area (30 square millimeter) | | | | Mean Seminiferous Tubule Diameter (in micron) | | | | Mean Number of Sertoli cell / Tubule | | | | Mean Number of Leydig cell | | | |
|--------|---------------------------------------------------------------------|--------------|---------|-----------------|-----------------------------------------------|----------------|---------|-----------------|--------------------------------------|---------------|---------|-----------------|----------------------------|-------------|---------|-----------------|
| | Control | 2G | P value | Mean Difference | Control | 2G | P value | Mean Difference | Control | 2G | P value | Mean Difference | Control | 2G | P value | Mean Difference |
| 1 | 14.18 ± 2.48 | 13.34 ± 2.08 | 0.069 | 0.84 | 124.05 ± 11.80 | 110.62 ± 10.52 | <0.001* | 13.42 | 33.24 ± 3.79 | 26.16 ± 5.14 | <0.001* | 7.08 | 12.06 ± 3.19 | 7.60 ± 2.71 | <0.001* | 4.46 |
| 2 | 15.56 ± 2.35 | 14.44 ± 2.41 | 0.021* | 1.12 | 147.55 ± 20.11 | 143.45 ± 9.42 | 0.195 | 4.10 | 40.26 ± 7.65 | 26.80 ± 4.046 | <0.001* | 13.46 | 12.20 ± 2.58 | 8.76 ± 2.74 | <0.001* | 3.44 |
| 3 | 16.92 | 10.34 | <0.001* | 6.58 | 159.02 | 139.60 | <0.001* | 19.42 | 44.80 | 29.02 | <0.001* | 15.78 | 14.86 | 7.64 | <0.001* | 7.22 |

| | | | | | | | | | | | | | | | | |
|---|-----------|-----------|---------|------|------------|------------|---------|-------|-----------|-----------|---------|-------|-----------|-----------|---------|------|
| | ± 2.65 | ± 1.84 | | | ± 12.71 | ± 12.08 | | | ± 4.76 | ± 5.03 | | | ± 3.43 | ± 2.23 | | |
| 4 | 16.20 | 9.98 | | 6.22 | 161.12 | 150.38 | | 10.75 | 44.52 | 31.42 | | 13.10 | 14.16 | 7.86 | | 6.30 |
| | ± 2.61 | ± 2.33 | <0.001* | | ± 15.36 | ± 13.39 | <0.001* | | ± 5.37 | ± 5.24 | <0.001* | | ± 5.04 | ± 2.84 | <0.001* | |
| 5 | 17.48 | 15 | | 2.48 | 157.95 | 141.30 | | 16.65 | 44.44 | 31.48 | | 12.96 | 15.34 | 8.32 | | 7.02 |
| | ± 2.43 | ± 3.57 | <0.001* | | ± 14.31 | ± 13.75 | <0.001* | | ± 5.43 | ± 7.83 | <0.001* | | ± 4.12 | ± 2.65 | <0.001* | |
| 6 | 17.08 | 15.64 | | 1.44 | 153.30 | 133.40 | | 19.90 | 44.06 | 32.90 | | 11.16 | 15.66 | 9 | | 6.66 |
| | ± 2.72 | ± 2.74 | 0.010* | | ± 10.78 | ± 16.10 | <0.001* | | ± 4.34 | ± 6.42 | <0.001* | | ± 4.78 | ± 3.21 | <0.001* | |

n = 50, * P value statistically significant (<0.05)

Table 3: Johnson Testicular Biopsy Score Count

| | | | | | | | | | | |
|---------------------------|---|---|---|---|---|---|---|----|----|----|
| Score no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Control mice | - | - | - | - | - | - | - | - | - | 18 |
| 2G Radiation exposed mice | - | - | - | - | - | - | - | 05 | 13 | - |

n=18

Grade 10 – complete spermatogenesis with many spermatozoa. Grade 9 – much spermatogenesis, but germinal epithelium disorganized with marked sloughing or obliteration of lumen. Grade 8 – only few spermatozoa present (< 5 to 10). Grade 7 –no spermatozoa but many spermatids present. Grade 6 - no spermatozoa and only few spermatid present. Grade 5 –no spermatozoa, no spermatids but several and many spermatocytes present. Grade 4 – only few spermatocytes (<5), no spermatids or spermatozoa. Grade 3 – spermatozoa are the only germ cells. Grade 2- no germ cells, but sertoli cells present. Grade 1-no cells in tubular section.

5. Discussion

The present study was undertaken to investigate the effects of chronic exposure of 2G cell phone radiation on mice testis at the histological level. Following chronic exposure of 2G cell phone radiation in mice, there was significant reduction of animal weight at first, second and fourth month. The mean testis weight of 2G exposed mice was significantly reduced in all period except the fourth month. Mean testis volume was significantly reduced in the first three months. The mean seminiferous tubule densities per unit area was significantly lower and mean seminiferous tubule diameters were significantly reduced in all 2G exposed testis except second month. The mean number of Sertoli cells and Leydig cells was significantly reduced in 2G radiation exposed mice. In comparison to control group mean serum testosterone level of 2G exposed mice was significantly lower. Sections of radiation exposed mice testis showed wide interstitium, detachment of Sertoli cells and spermatogonia from the basal lamina, vacuolar degeneration and desquamation of the seminiferous epithelium. Most of the peripheral tubules showed maturation arrest in the spermatogenesis. (Fig.2) Seminiferous tubules scored between 8 and 9 in Johnson testicular biopsy score count showed that peripheral tubules were affected and deeper tubules were in functional status.

In earlier studies of Ozguner M et al (2005) [30], rat was exposed to 900MHz cell phone radiation and reported a significant decrease in seminiferous tubular diameter, mean

height of the seminiferous epithelium and serum total testosterone level. The present morphometric study correlates with Ozguner M et al findings. Our study agreed with the findings of S Dasdag et al (1999) [31] where rats exposed to microwaves emitted by cell phone showed significant reduction of mean seminiferous tubular diameter and Johnson testicular biopsy score count was between 8 to 10. In the recent study of Latifa Ishaq Khayyat (2011) [12] and Pradeep Kumar (2014) [32], showed that electromagnetic field of cell phones induced Leydig cell hypoplasia, wide interstitium, atrophied seminiferous tubules, maturation arrest in the spermatogenesis, decreased germ cell population, pyknotic nuclei in germ cell and vacuolisation in spermatogenic cells. They also observed detachment of spermatogonia and Sertoli cells from the basal lamina, shrinkage, residual cytoplasm and debris of degenerating cells in the seminiferous tubules. The present study was consistent with Latifa Ishaq Khayyat [12] and Pradeep Kumar study [32] with above mentioned parameters. Our study agreed with the findings of Ali H.M.Omer (2009) [33], a very significant reduction in serum testosterone level of the rat after exposure of 900MHz electromagnetic radiation. Our study also observed lower serum testosterone level due to electromagnetic radiation similar to the reports of Salem Amara et al (2006) [34] and Wang S M et al (2003) [20].

H.Ozlem Nisbet et al (2011) [35] found that exposure of the rat to 900 to 1800 MHz electromagnetic radiations produced severe vacuolar degeneration, severe necrosis and desquamation of the seminiferous epithelium. They also found mean total plasma testosterone showed higher than the sham control group. Our study was in agreement with H.Ozlem Nisbet et al study [35] except for reductions in mean serum total testosterone level. Study conducted by Zsolt Forgacs et al (2006) [36] showed that mice exposed to 1800 MHz GSM like microwave had a significant increase in serum testosterone without any histopathological alterations in testis and our study showed structural changes. Ji Yoon Kim et al (2007) [37] study that they observed long term exposure of rats to 2.45 GHz radiations induced increase in the number of Leydig cells and increased serum total testosterone level and the present study showed a decreased level of serum testosterone.

Leydig cells are the most susceptible to electromagnetic radiation. Radiation might have caused injury to the structure and function of Leydig cells and thereby reduced the serum testosterone level [20]. This could be responsible for the significant reduction of mean serum testosterone

level of 2G exposed mice in our study. Cell phone radiation could cause increased vascular permeability and thereby interstitial oedema [38]. We observed wide interstitium in the sections of 2G radiation exposed mice testis. This could be the reason for the significantly low mean density of seminiferous tubules per unit area. The surface organ such as testis could be more affected by the radiation emitted from the cell phone. Even though mice testis can be moved through the inguinal canal to abdomen (abdomino-scrotal), energy absorbed (SAR) by testis could be more as it is a predominantly a surface organ. This could be the probable cause for structural damage met by the peripheral tubules by cell phone radiation.

6. Conclusion

Chronic exposure of mice to ultra-high frequency radiation emitted from 2G cell phone could cause a reduction in body weight, testis weight and volume. Microscopic changes such as reduction in mean seminiferous tubule density per unit area and seminiferous tubule diameter, vacuolar degeneration and desquamation of the seminiferous epithelium, maturation arrest in the spermatogenesis of the peripheral tubules, reduction in the number of Sertoli and

Leydig cells could occur. Serum testosterone level could be lower following exposure to chronic cell phone radiation. The long term use of cell phone may lead to male infertility.

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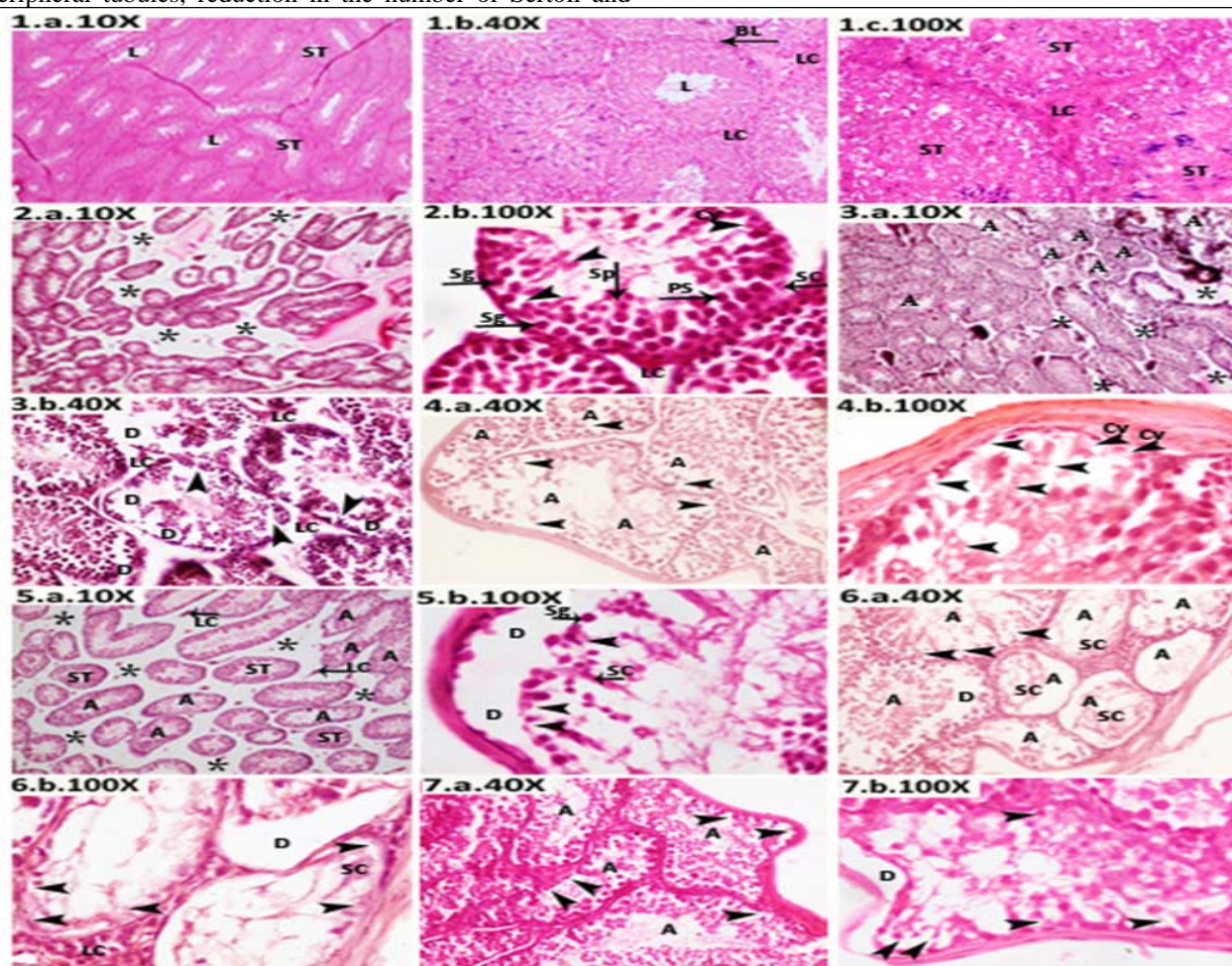


Fig.2. Haematoxylin & Eosin stain, 1a, 1b & 1c. Testis sections of control mice . 2a & 2b. Testis sections of 30 days 2G exposed. 3a & 3b. sections of 60 days 2G exposed. 4a & 4b. 90 days 2G exposed. 5a & 5b. 120 days 2G exposed. 6a & 6b 150 days 2G exposed. 7a & 7b. 180 days 2G exposed. 10X-100 times magnification, 40X-400 times magnification, 100X-1000 times magnification. A-affected tubules, BL-basal lamina, Cv-cytoplasmic vacuolation, D-detachment of cells from basal lamina, L-lumen, LC-Leydig cells, PS-primary spermatocytes, SC-Sertoli cells, Sg-spermatogonia, Sp-spermatid, ST-seminiferous tubules, Arrow head-vacuolar degeneration and desquamation of the seminiferous epithelium, *-wide interstitium.

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