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EFFECTS OF CHRONIC EXPOSURE TO 2G AND 3G CELL PHONE RADIATION ON MICE TESTIS – A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Objective: The aim of our study is to evaluate possible effects of chronic exposure to 900 - 1800 MHz radiation emitted from 2G cell phone and 1900 -2200 MHz from 3G cell phone on the testis of mice and to compare the effects of 2G and 3G radiation on testis at the histological level.

Methods: Mice were exposed to 2G and 3G ultra-high frequency radiation, 48 minutes per day for a period of 30 to 180 days. The sham control mice were exposed to similar conditions without 2G or 3G exposure. Animal's weight of 2G and 3G cell phone exposed group were recorded before sacrificing at the end of 30, 60, 90,120,150 and 180 days. Same numbers of control animals were sacrificed on the same period. Blood samples were collected to measure plasma testosterone. Both the testes were dissected and its size, weight and volume were measured. The testes were processed for histomorphometric study.

Results: Following chronic exposure of 2G and 3G cell phone radiation in mice, there was significant reduction of animal weight at first, second and fourth month. The mean testis weight and volume of 2G and 3G radiation exposed mice were significantly reduced in the first three months. The comparison between 2G and 3G exposed groups, showed no significant changes in mean body weight, mean testis weight and mean testis volume. The mean density of seminiferous tubule, mean seminiferous tubule diameter, mean number of Sertoli and Leydig cells of 2G and 3G exposed groups had significantly lower value than the control. The following microscopic changes were observed in the 2G and 3G radiation exposed mice testis over control. 1. Wide interstitium 2. Detachment of Sertoli cells and spermatogonia from the basal lamina. 3. Vacuolar degeneration and desquamation of seminiferous epithelium. 4. Peripheral tubules showed reduced thickness of seminiferous epithelium and maturation arrest in the spermatogenesis. 5. Seminiferous tubules scored 7 to 9 using Johnson testicular biopsy score count. The mean total serum testosterone level of first, second, third, fourth and sixth month 2G and 3G exposed mice had significantly lower serum testosterone level than control. However, comparison between 2G and 3G showed no significant difference in the mean serum testosterone level.

Conclusion: Chronic exposure to ultra-high frequency radiation emitted from 2G and 3G cell phone could cause microscopic changes in the seminiferous epithelium, reduction of serum testosterone level, reduction in the number of Sertoli cells and Leydig cells.

Key Words: 2G cell phone, 3G cell phone, Mice testis, Testosterone, Ultra-high frequency radiation

INTRODUCTION

The increasing use of cell phone and handset devices emitting radiofrequency electromagnetic fields, particularly by children and teenagers, raises a great concern about the interactions of radiofrequency radiation on 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

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(2G) network operates in the 900-1800 MHz frequency and third generation cell phone (3G) network operates in the 1900-2200 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-Ionising 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°C)[1].

Review of literature 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 number in the adult female rat cerebellum[8], and disturbance of short term memory in mice[9]. Authors have 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 semen to cell phone radiation[16]. Long term exposure to mobile phone radiation could lead to reducing sperm motility, serum testosterone levels[17-20], increased ROS (reactive oxygen species) [21-24], reduction in seminiferous tubule diameter and thickness of epithelium[25] and vacuolisation in the cytoplasm of Sertoli cell [26]

In contrary to above findings 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[27], no effect on mouse embryonic lens development[28], psychomotor performance was not influenced by brief repeated exposures to mobile phones[29].The lack of histological changes on rat testis[30, 31] and no alterations in serum testosterone[32] were cited.

The present study is undertaken because of the contradictory findings on the effects of exposure to non-ionizing radiation emitted from the 2G and 3G cell phone on testis. The aim of our study is to evaluate possible effects of chronic exposure to 900 - 1800 MHz radiation emitted from 2G cell phone and 1900 -2200 MHz from 3G cell phone on the testis of mice; and to compare the effects of 2G and 3G radiation on testis at microstructure level.

MATERIALS AND METHODS

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

New born mice (with the mother for twenty one days) were randomly divided into three independent groups; control, 2G exposed and 3G exposed. Animals were kept in mice cages at the temperature of 22 ± 1°C, 60% relative humidity and housed in the central animal house 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 900-1800 MHz frequency radiation emitted from 2G cell phone and eighteen mice were exposed to 1900-2200 MHz frequency radiation emitted from 3G (video call) cell phone. Eighteen mice were sham control. The roof of the mice cage was designed to hang the 2G and 3G (video call) cell phone from the distance of five centimetres from the floor; which allow the mice to move freely and to avoid direct thermal injury in mice. 2G and 3G (video call) mobile phone in non-vibrating, silent, do not disturb (DND) and auto answer mode activated was kept hanging inside the mice cage. EMF emitted from a 2G and 3G standard handset with a frequency bandwidth of 900-1800 MHz and 1900 – 2200MHz respectively with the power of 2W/Kg. The highest specific absorption rate (SAR) value for this standard handset was 1.69 W/Kg (10gm).The mobile phone which was kept inside the mouse's cage was rung upon from other 2G and 3G (video call) cell phone for every half an hour, each call lasting for two minutes. Mice were exposed 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 used to measure the amount of radiation exposed in 2G and 3G experimental groups. The sham control group of eighteen mice was kept under similar conditions without 2G or 3G exposure. Before sacrificing, we measured the body weights of mice in all three groups.

Three mice each were sacrificed at the end of 30, 60, 90, 120, 150 and 180 days of exposures in the experimental groups after 24 hours of last exposure. Equal numbers of control mice were sacrificed on a similar time points. We

sacrificed mice under anaesthesia and collected 1 ml of blood by cardiac puncture for total serum testosterone measurement and all samples were read in duplicate. Testes were dissected out and its weight and volume measured. We used Denver's digital weighing machine (0.001gm) for measuring weight and water displacement method to calculate volume. After the morphometric analysis, testes were fixed by 4% formalin solutions for a period of twenty four hours and then tissues processed and embedded in paraffin. Tissues were sectioned at five microns, stained with Haematoxylin and Eosin. We analysed testis sections from random slide, random sections and random field under the light microscope; for histomorphometric parameters and structural changes. Diameters of 50 randomly selected essentially round seminiferous tubules from each testis were measured using calibrated ocular micrometre. We measured the seminiferous tubule diameter in both horizontal and vertical axis and the mean average was taken. The mean seminiferous tubule density per unit area was calculated by square graticule which was mounted on an eyepiece. All the testis sections were blindly reviewed by the same investigator. Each seminiferous tubule was analysed and classified into one of 10 different grades utilizing Johnson testicular biopsy score count[33]. The total serum testosterone measured by enzyme linked fluorescent immunoassay (ELFA) method.

Statistical analysis

We used ANOVA and Kruskal–Wallis test to compare all three groups; independent t test and Mann Whitney U test for comparing 2G and 3G groups. P value ≤ 0.05 was considered statistically significant.

RESULTS

Morphometric study: The mean body weight of mice sacrificed during first, second and fourth month was significantly differing amongst three groups by ANOVA (p value <0.03); the 2G and 3G exposed mice showed significantly lower body weight than the control (Table.1) (Figure.1).The mean testis weight of first, second, third and fifth month mice were significantly differing in all three groups (p value <0.05) ; the 2G and 3G exposed mice had significantly lesser weight than the control in the first, second and third month but reverse in case of fifth month (Table.2) (Figure.2). The Mean testis volume of first, second and third month mice was significantly differing amongst three groups (p value <0.018); the 2G and 3G exposed mice had significantly reduced volume than the control (Table.3) (Figure.3).

A comparison between 2G and 3G exposed groups by independent t test, no significant changes were seen in mean body weight, mean testis weight and mean testis volume (p value > 0.05) (Table 1-3).

Histomorphometric study: The mean density of seminiferous tubule (per unit area of $578\mu^2$), mean seminiferous tubule diameter (in micron), mean number of Sertoli and Leydig cells of mice sacrificed every month were significantly differing amongst three groups by ANOVA (p value <0.001); 2G and 3G exposed groups had significantly lower value than the control (Table. 4-7).

A comparison between 2G and 3G exposed groups by independent t test; the mean seminiferous tubule density of 3G exposed mice was comparatively lesser than that of 2G exposed during the first, second, fifth and sixth month (p value <0.05) (Table. 4).The mean seminiferous tubule diameter of 3G exposed was comparatively lesser than that of 2G exposed mice in all months except the sixth month (p value <0.05) (Table. 5). Similarly the mean number of Sertoli cells in 3G exposed mice was lesser than that of 2G exposed mice in all months except fifth month (p value <0.05) (Table. 6). However, while comparing 2G and 3G exposed groups, statistically no significant changes were observed in the mean number of Leydig cells (p value >0.05) (Table. 7).

The following microscopic changes were seen in the 2G and 3G radiation exposed mice testis over control. 1. The interstitium between tubules appeared morewide 2. Sertoli and spermatogonial cells appeared detached from the basal lamina. 3. Vacuole degeneration and desquamation of seminiferous epithelium. 4. Most of the peripheral tubules showed reduced thickness of seminiferous epithelium and maturation arrest in the spermatogenesis 5. Seminiferous tubules scored 7 to 9 using Johnson testicular biopsy score count (Table. 8) (Figure. 4 and 5).

Biochemical Study: Mean serum testosterone (ng/ml) of first, second, third, fourth and sixth month mice were significantly differed amongst three groups by ANOVA (p value <0.05); the 2G and 3G exposed mice showed lower serum testosterone level than the control. However, comparison of 2G and 3G by independent t test showed no significant difference in the mean serum testosterone level of both groups (p value >0.05) (Table.9) (Figure.6).

DISCUSSION

The present study has been undertaken to investigate the effects of chronic exposure of 2G and 3G cell phone radiations on mice testis; and to compare the effects of 2G and 3G radiations on testis at the histological level. Chronic exposure of 2G and 3G cell phone radiation to mice, resulted in reduction of animal weight at first, second and fourth month. The mean testis weight of 2G and 3G radiation exposed mice was significantly reduced

in the first three months, however in fifth month mean testis weight was significantly increased. Similarly mean testis volume of 2G and 3G radiation exposed mice was significantly reduced in the first three months. The mean density of seminiferous tubule, mean seminiferous tubule diameter, mean number of Sertoli and Leydig cells of 2G and 3G exposed groups were significantly lower than control group.

When compared to control group mean serum testosterone level of 2G and 3G exposed mice was significantly lower. Sections of 2G and 3G 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 reduced thickness of seminiferous epithelium and maturation arrest in the spermatogenesis. Seminiferous tubules scored 7 to 9 in Johnson testicular biopsy score count.

In earlier studies of Ozguner M et al (2005)[34] and Hanci H et al (2013)[25], rat was exposed to 900MHz cell phone radiation and found there was a significant decrease in seminiferous tubular diameter, mean height of the seminiferous epithelium and serum total testosterone level. Our study agreed with Ozguner M et al and Hanci H et al study with the above mentioned parameters in mice testis indicating that there was no species difference. Our study agreed with S Dasdag et al study (1999)[35] on rat exposed to microwaves emitted by cell phone The author reported significant reduction of mean seminiferous tubular diameter and Johnson testicular biopsy score count was between 8 to 10. In the study of LatifaIshaqKhayyat (2011)[12] and Pradeep Kumar (2014)[36], the 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 conducted with mice was in agreement with Latifa Ishaq Khayyat[12] and Pradeep Kumar study[36]. Our study agreed with the findings of Ali H.M.Omer et al (2009)[37] who observed reduction of serum testosterone level inthe rat after exposure of 900MHz electromagnetic radiation. Similar reduction in serum testosterone level have been cited by Salem Amara et al (2006)[38], Mugunthan et al (2014)[39] and Wang S M et al (2003)[20].

H.OzlemNisbet et al (2011)[40] found that exposure of the rat to 900 to 1800 MHz radiations produced severe vacuolar degeneration, necrosis and desquamation of the seminiferous epithelium; they also reported high level of mean plasma testosterone in experimental group than the sham control group. Our study showed significant reductions in mean serum total testosterone level in mice. Study conducted by ZsoltForgacs et al (2006)[41] on mice exposed to 1800 MHz GSM like microwave observed significant increase in serum testosterone without any structural changes in testis. The present study showed structural changes in mice seminiferous epithelium and lower serum testosterone level.The present study disagreed with Ji Yoon Kim et al (2007)[42] who 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.

Leydig cells are most susceptible to electromagnetic radiation. Radiation might be detrimental to the structure and function of Leydig cells and thereby reduce the serum testosterone level[20]. This could be responsible for the significant reduction in the mean number of Leydig cells and serum testosterone level of 2G and 3G exposed mice in our study. Cell phone radiation could cause increased vascular permeability and thereby interstitial oedema[43]. We observed wide interstitium in the sections of 2G and 3G radiation exposed mice testis and it could be the reason for the significantly low mean density of seminiferous tubules per unit area in 2G and 3G radiation exposed mice testis. The surface organ such as testis could be more affected by the radiation emitted from the cell phone. Even though mice testis movesto abdomen through the inguinal canal (abdomino-scrotal), energy absorbed (SAR) by testis could be more as it is predominantly surface organ. This could be probable reason for the predominant damages observed on the peripheral tubules of testis exposed to 2G and 3G cell phone radiations.

CONCLUSION

Chronic exposure of mice to ultra-high frequency radiation emitted from 2G and 3G cell phone could cause a reduction in body weight, testis weight and volume. Microscopic changes in the testis such as reduction in mean seminiferous tubule density, seminiferous tubule diameter, vacuolar degeneration and desquamation of the seminiferous epithelium; reduction in the thickness of seminiferous epithelium and maturation arrest in the spermatogenesis of the peripheral tubules could occur. Decreased serum testosterone level, reduction in the number of Sertoli and Leydig cells could also occur following chronic exposure to 2G and 3G cell phone radiation. Thus long term exposure of cell phone radiation could cause male infertility in mice.

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Table 1: Comparison of control, 2G exposed and 3G exposed Mice in terms of body weight.

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean body weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Mean body weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Mean body weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	14.6	0.99	12.7 to 16.6	9.8	0.06	9.7 to 9.9	9.5	0.20	9.1 to 9.9	0.03*	0.10
2	25.4	0.32	24.7 to 26.0	23.7	0.64	22.4 to 25.0	22.1	0.47	21.2 to 23.1	0.03*	0.10
3	26.2	0.40	25.4 to 27.0	26.8	0.55	25.7 to 27.9	25.7	0.72	24.2 to 27.1	0.17	0.20
4	31.0	1.00	29.0 to 33.0	26.3	0.35	25.6 to 27.0	24.6	0.42	23.8 to 25.5	0.03*	0.10
5	30.7	0.58	29.5 to 31.8	31.7	3.14	25.4 to 38.0	29.4	0.98	27.4 to 31.4	0.35	0.40
6	31.7	0.31	31.1 to 32.3	31.3	0.66	30.0 to 32.6	29.7	0.91	27.9 to 31.5	0.05	0.10

n = 18 in each group, * p value statistically significant (≤0.05)

Table 2: Comparison of control, 2G exposed and 3G exposed Mice in terms of testis weight

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean testis weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Mean testis weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Mean testis weight (in gram)	Standard Deviation (in gram)	95% Confidence Interval (Mean ± 2SD) (in gram)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	0.70	0.09	0.53 to 0.87	0.02	0.00	0.02 to 0.02	0.02	0.00	0.02 to 0.02	0.021*	1.00
2	0.12	0.00	0.12 to 0.12	0.07	0.00	0.06 to 0.07	0.06	0.00	0.06 to 0.06	0.020*	0.10
3	0.14	0.01	0.12 to 0.15	0.07	0.01	0.06 to 0.08	0.06	0.00	0.06 to 0.06	0.033*	0.20
4	0.15	0.00	0.15 to 0.16	0.09	0.01	0.08 to 0.11	0.09	0.01	0.06 to 0.11	0.052	0.40
5	0.11	0.00	0.11 to 0.11	0.13	0.00	0.12 to 0.13	0.13	0.01	0.12 to 0.14	0.046*	1.00
6	0.12	0.00	0.12 to 0.12	0.08	0.01	0.07 to 0.10	0.08	0.01	0.06 to 0.10	0.058	1.00

n = 18 in each group, * p value statistically significant (≤0.05)

Table 3: Comparison of control, 2G exposed and 3G exposed Mice in terms of testis volume

	Control Mice		2G exposed Mice		3G exposed Mice		Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
Month	Mean testis volume (in ml)	Standard Deviation (in ml)	Mean testis volume (in ml)	Standard Deviation (in ml)	Mean testis volume (in ml)	Standard Deviation (in ml)		
1	0.10	0.00	0.05	0.00	0.05	0.00	0.018*	1.000
2	0.20	0.00	0.10	0.00	0.10	0.00	0.018*	1.000
3	0.20	0.00	0.10	0.00	0.10	0.00	0.018*	1.000
4	0.10	0.00	0.10	0.00	0.10	0.00	1.000	1.000
5	0.10	0.00	0.10	0.00	0.10	0.00	1.000	1.000
6	0.10	0.00	0.10	0.00	0.10	0.00	1.000	1.000

n = 18 in each group, * p value statistically significant (≤0.05)

Table 4: Comparison of control, 2G exposed and 3G exposed Mice in terms of seminiferous tubule density

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean Seminiferous Tubule Density/ unit area of 578 μ^2	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean Seminiferous Tubule Density/ unit area of 578 μ^2	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean Seminiferous Tubule Density/ unit area of 578 μ^2	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	14.18	2.48	9.2 to 19.1	13.34	2.08	9.2 to 17.5	12.10	1.84	8.4 to 15.8	0.000*	0.002*
2	15.56	2.35	10.9 to 20.3	14.44	2.41	9.6 to 19.3	12.44	1.91	8.6 to 16.3	0.000*	0.000*
3	16.92	2.65	11.6 to 22.2	10.34	1.84	6.7 to 14.0	10.26	1.71	6.8 to 13.7	0.000*	0.822
4	16.20	2.61	11.0 to 21.4	9.98	2.33	5.3 to 14.6	9.34	1.33	6.7 to 12.0	0.000*	0.095
5	17.48	2.43	12.6 to 22.3	15.00	3.57	7.9 to 22.1	11.98	2.34	7.3 to 16.7	0.000*	0.000*
6	17.08	2.72	11.6 to 22.5	15.64	2.74	10.2 to 21.1	13.16	2.75	7.7 to 18.7	0.000*	0.000*

n = 150 observations in each group, * p value statistically significant (≤0.05)

Table 5: Comparison of control, 2G exposed and 3G exposed Mice in terms of seminiferous tubule diameter

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean Seminiferous Tubule Diameter (in micron)	Standard Deviation (in micron)	95% Confidence Interval (Mean ± 2SD) (in micron)	Mean Seminiferous Tubule Diameter (in micron)	Standard Deviation (in micron)	95% Confidence Interval (Mean ± 2SD) (in micron)	Mean Seminiferous Tubule Diameter (in micron)	Standard Deviation (in micron)	95% Confidence Interval (Mean ± 2SD) (in micron)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	124.05	11.80	100.5 to 147.7	110.62	10.52	89.6 to 131.7	105.11	10.38	84.4 to 125.9	0.000*	0.010*
2	147.55	20.11	107.3 to 187.8	143.45	9.41	124.6 to 162.3	134.20	15.26	103.7 to 164.7	0.000*	0.000*
3	159.02	12.71	133.6 to 184.4	139.60	12.08	115.4 to 163.8	132.12	15.08	102.0 to 162.3	0.000*	0.007*
4	161.12	15.35	130.4 to 191.8	150.38	13.38	123.6 to 177.1	138.16	23.03	92.1 to 184.2	0.000*	0.002*
5	157.95	14.31	129.3 to 186.6	141.30	13.74	113.8 to 168.8	130.24	14.56	101.1 to 159.4	0.000*	0.000*
6	153.30	10.78	131.7 to 174.9	133.40	16.10	101.2 to 165.6	127.94	15.40	97.1 to 158.7	0.000*	0.086

n = 150 observations in each group, * p value statistically significant (≤0.05)

Table 6: Comparison of control, 2G exposed and 3G exposed Mice in terms of Sertoli cells number

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean of Sertoli cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean of Sertoli cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean of Sertoli cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	33.24	3.80	25.6 to 40.8	26.16	5.14	15.9 to 36.4	23.72	3.21	17.3 to 30.1	0.00*	0.01*
2	40.26	7.66	25.0 to 55.6	26.80	4.05	18.7 to 34.9	24.36	4.56	15.2 to 33.5	0.00*	0.01*
3	44.80	4.76	35.3 to 54.3	29.02	5.03	19.0 to 39.1	24.48	5.14	14.2 to 34.8	0.00*	0.00*
4	44.52	5.37	33.8 to 55.3	31.42	5.24	20.9 to 41.9	29.26	5.05	19.2 to 49.4	0.00*	0.04*
5	44.44	5.43	33.6 to 55.3	31.48	7.83	15.8 to 47.1	30.74	6.36	18.0 to 43.5	0.00*	0.61
6	44.06	4.34	35.4 to 52.7	32.90	6.42	20.1 to 45.7	28.58	5.84	16.9 to 40.3	0.00*	0.00*

n = 150 observations in each group, * p value statistically significant (≤0.05)

Table 7: Comparison of control, 2G exposed and 3G exposed Mice in terms of Leydig cells number

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean of Leydig cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean of Leydig cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Mean of Leydig cell number	Standard Deviation	95% Confidence Interval (Mean ± 2SD)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	12.06	3.20	5.7 to 18.5	7.60	2.71	2.2 to 13.0	7.20	2.08	3.0 to 11.4	0.00*	0.41
2	12.20	2.59	7.0 to 17.4	8.76	2.75	3.3 to 14.3	7.82	2.22	3.4 to 12.3	0.00*	0.06
3	14.86	3.43	8.0 to 21.7	7.64	2.23	3.2 to 12.1	7.38	1.79	3.8 to 11.0	0.00*	0.52
4	14.16	5.04	4.1 to 24.2	7.86	2.84	2.2 to 13.5	7.14	1.91	3.3 to 11.0	0.00*	0.14
5	15.34	4.12	7.1 to 23.6	8.32	2.65	3.0 to 13.6	7.88	2.23	3.4 to 12.3	0.00*	0.37
6	15.66	4.78	6.1 to 25.2	9.00	3.21	2.6 to 15.4	8.18	2.15	3.9 to 12.5	0.00*	0.14

n = 150 observations in each group, * p value statistically significant (≤0.05)

Table 8: 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	-
3G Radiation exposed mice	-	-	-	-	-	-	02	04	12	-

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 – spermatogonia are the only germ cells. Grade 2- no germ cells, but sertoli cells present. Grade 1-no cells in tubular section.

Table 9: Comparison of control, 2G exposed and 3G exposed Mice in terms of serum testosterone

	Control Mice			2G exposed Mice			3G exposed Mice				
Month	Mean serum testosterone (ng/ml)	Standard Deviation (ng/ml)	95% Confidence Interval (Mean ± 2SD) (ng/ml)	Mean serum testosterone (ng/ml)	Standard Deviation (ng/ml)	95% Confidence Interval (Mean ± 2SD) (ng/ml)	Mean serum testosterone (ng/ml)	Standard Deviation (ng/ml)	95% Confidence Interval (Mean ± 2SD) (ng/ml)	Comparison of Control, 2G & 3G by ANOVA (p value)	Comparison of 2G & 3G by Independent t test (p value)
1	0.98	0.26	0.46 to 1.50	0.123	0.012	0.10 to 0.15	0.097	0.01	0.09 to 0.11	0.026*	0.100
2	3.39	0.47	2.46 to 4.32	0.163	0.025	0.11 to 0.21	0.120	0.03	0.07 to 0.17	0.039*	0.200
3	4.07	0.14	3.79 to 4.35	0.136	0.006	0.12 to 0.15	0.117	0.02	0.07 to 0.16	0.046*	0.400
4	3.60	0.75	2.11 to 5.09	0.450	0.485	-0.52 to1.42	0.127	0.02	0.10 to 0.16	0.027*	0.100
5	3.46	0.52	2.42 to 4.49	0.150	0.020	0.11 to 0.19	0.140	0.02	0.11 to 0.17	0.055	0.700
6	1.03	0.66	-0.30 to2.35	0.150	0.020	0.11 to 0.19	0.107	0.01	0.08 to 0.13	0.027*	0.100

n = 18 in each group, * p value statistically significant (≤0.05)

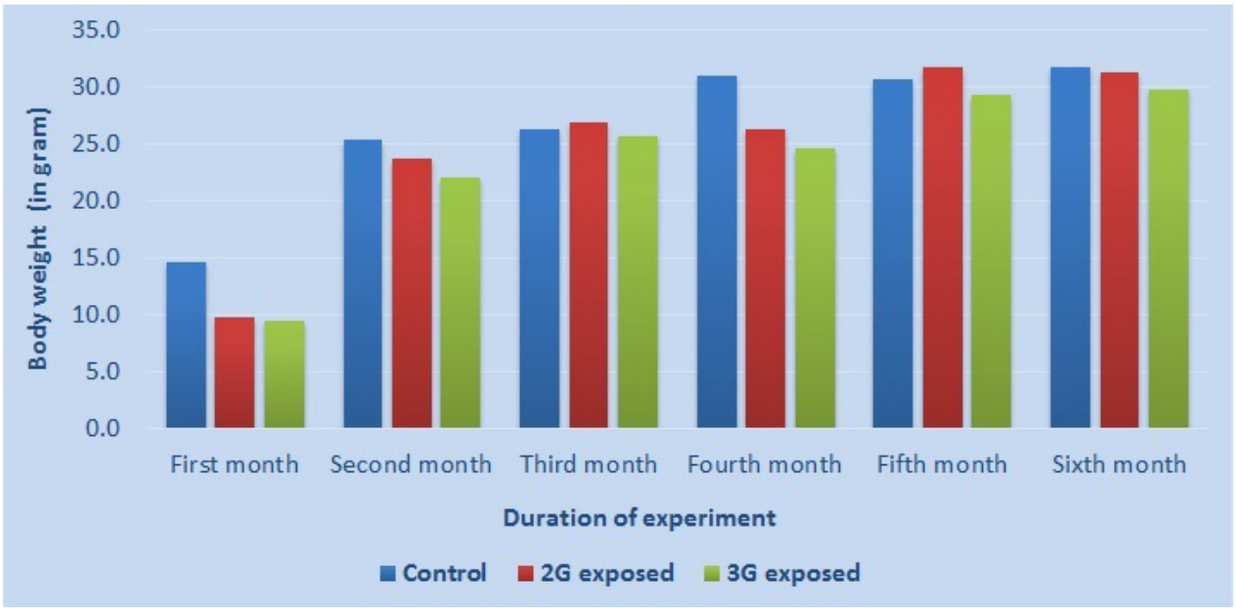

Figure 1: Comparison of control, ZG exposed and 3G exposed Mice in terms of body weight

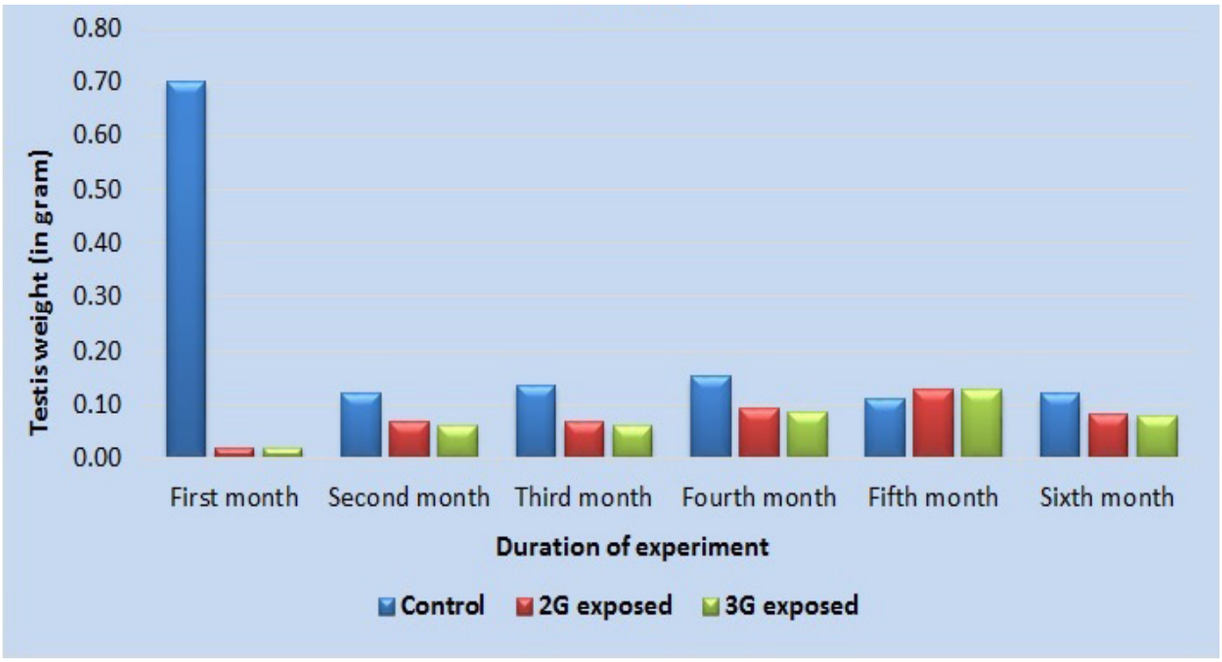

Figure 2: Comparison of control, 2G exposed and 3G exposed Mice in terms of testis weight

Figure 3: Comparison of control, 2G exposed and 3G exposed Mice in terms of testis volume

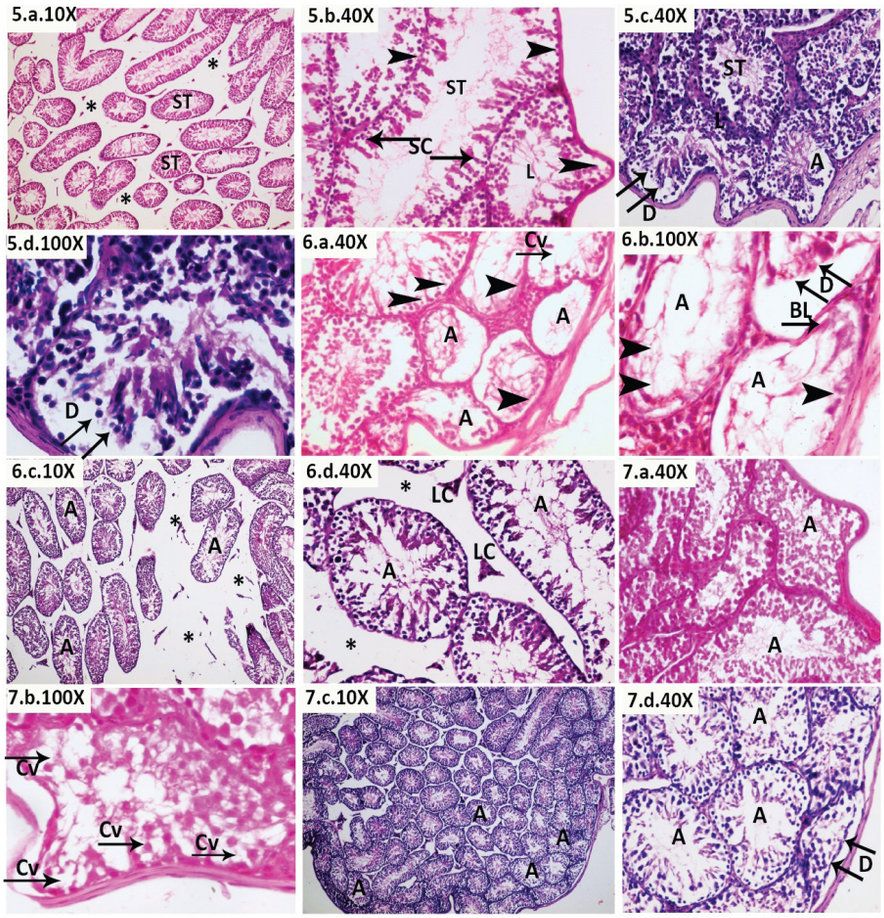

Figure 4: Haematoxylin & Eosin stain. 1a, 1b & 1c-Testis sections of control mice. 2a & 2b-Tests sections of 30 days 2G exposed, 2c & 2d-30 days 3G exposed. 3a & 3b-sections of 60 days 2G exposed, 3c & 3d- 60 days 3g exposed. 4a & 4b - 90 days 2G exposed. 4c & 4d - 90 days 3G exposed. 10X-100 times magnification, 40X-400 times magnification, 100X-1000 times magnification. A-affected tubules, Cv-cytoplasmic.

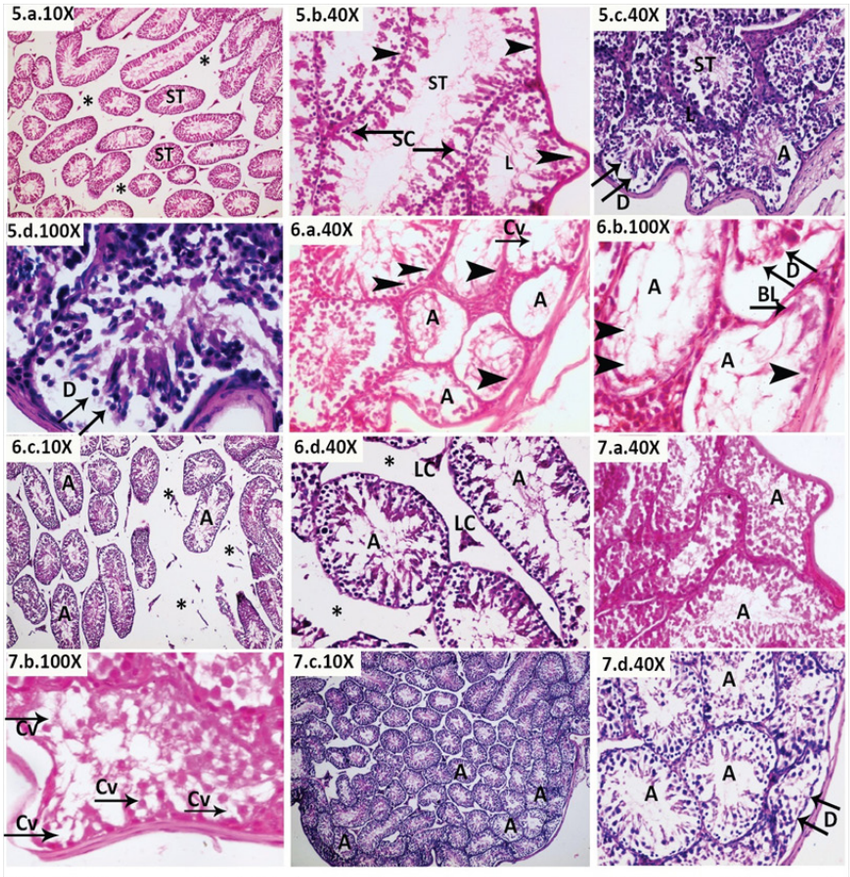

Figure 5: Haematoxylin & Eosin stain, 5a & 5b - testis sections of 120 days 2G exposed mice, 5c & 5d - 120 days 3G exposed, 6a & 6b - sections of 150 days 2G exposed, 6c & 6d - 150 days 3G exposed, 7a & 7b - sections of 180 days 2G exposed, 7c & 7d - 180 days 3G exposed, 10X-100 times magnification, 40X-400 times magnification, 100X-1000 times magnification. A-affected tubules, BL-basal lamina Cv-cytoplasmic vacuolation, D-detachment of seminiferous epithelium from basal lamina, L-lumen, LC-Leydig cells, SC-Sertoli cells, ST-seminiferous tubules, Arrow head-vacuolar degeneration and desuamation of the seminiferous epithelium. *-wide interstitium.

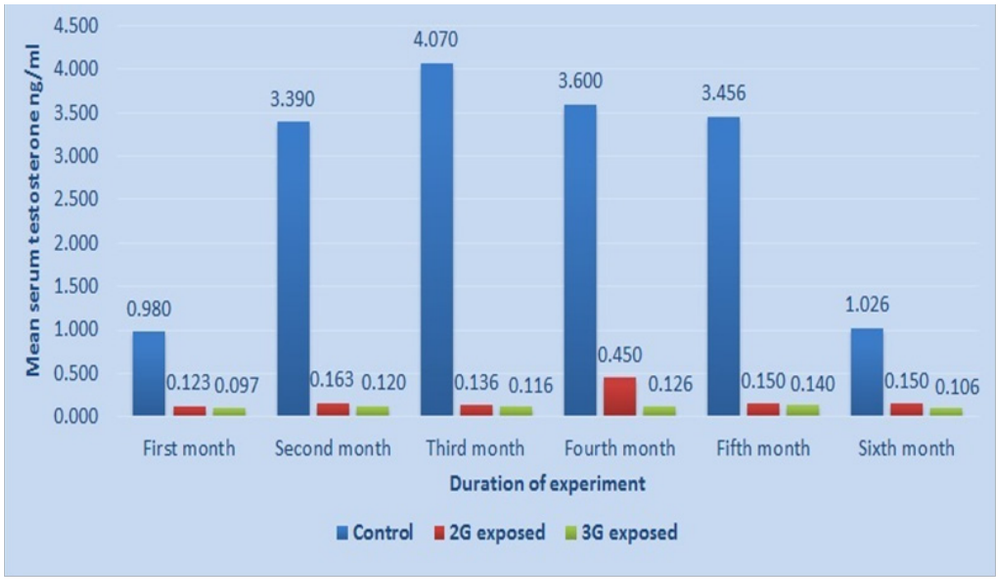

Figure 6: Comparison of control, 2G exposed and 3G exposed Mice in terms of serum testosterone