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Histopathology and Morphometry of Brain and Seminiferous Tubules of Male Albino Mice (*Mus musculus)* Treated with Female Hormonal Contraceptive Pills

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Abstract

Female hormonal contraceptive pills (Ethinyl estradiol levonorgestrel) are used to prevent pregnancy in women. However, it is also being used by the male homosexuals for them to develop female secondary sexual characteristics. There is a paucity of studies in the effect of female birth control pills on the histology and morphometry of their brain and testes, as well as on their behavior and physical changes, thus, this study was undertaken to determine the effects of these pills on male albino mice (Mus musculus, L.). As regards behavior and physical changes in male mice, restlessness, irritability, hair loss in their body and increased body weight were observed in mice treated with the contraceptive pills. However, mice with over dosed pills exhibited slow growth and development. Histological alterations in brain of male albino mouse were observed, such as increase in microscopic holes, vacuolated neurons, necrosis, increase incidence of shrunken eosinophilic neurons with either pyknotic or karyorrhectic nuclei, number of neuron swelling and vacuolation and gradual decrease in the number of healthy neurons as the dose of pills increased. Likewise, histological changes in testicular tissue were also observed such as decrease in the normal architecture and abnormal distance of seminiferous tubules, a number of large spermatogonia, increase in number of necrotic cells, disintegration and atrophy of seminiferous tubules with widening of lumen and reduction of germ cell and somatic cell population, hyperplasia, and increase in the diameter of lumen. Cell counts in selected seminiferous tubules also decreased, such as, in the number of spermatocytes, spermatids and sertoli cells, however, there is no significant difference among the treatment groups with respect to the number of spermatogonia. An incidence of necrosis or cell death in the seminiferous tubules increased as the dose of pills increased, however, there is a decreased in length and diameter of seminiferous tubules as the dosage of pills increased. The study will provide information on the male communities with regards the effects of contraceptive pills on the brain and testis of an individual.

Keywords: Female hormonal pills, seminiferous tubules, cerebral cortex, brain, apoptosis, spermatogonia, spermatocytes, spermatids, sertoli cells, neurons, necrosis, pyknosis, histopathology and morphometry.

Introduction

Female birth control pills are used to prevent pregnancy in women. They are usually made of synthetic forms of estrogen and progesterone. Even though rarely used, female hormones are used or taken by men for some medical reasons such as treatment to prostate enlargement, testicular cancer and aromatase deficiencies. Because female hormonal pills are not for men, side effects have not been well studied; however, they may cause a number of adverse effects in men comparable to those seen with the use of other female hormonal pills[1]. In the Philippines and other countries, oral contraceptive pills for women are being used by the male homosexuals for them to develop characteristics of women such as decrease in sizes of legs, chest and arm muscles and also develop gynecomastia, a benign proliferation of male breast glandular tissue, develop breast tissue, also known as gynecomastia, along with enlargement and darkening of the nipples. In addition, there is no studies conducted yet as to the effects of these pills on the central nervous system, particularly, the brain of the male mice. Hence, this study will provide information on the effects of female birth control pills on the nervous and reproductive systems of males taking this type of pills.

Specifically, the study was carried out to assess the occurrence of histological abnormalities, number of neurons and necrotic neurons in the cerebral cortex, number of spermatocytes, spermatogonia, spermatids, sertoli cells, necrotic cells and morphometrics of randomly selected seminiferous tubules in male mice treated with varying dosage of female hormonal contraceptive pills (Ethinyl estradiol levonorgestrel) as well as on their behavior and physical changes.

Methodology

Research Design: This experimental study tried to investigate the occurrence of histological abnormality in brain and testicular tissue among treatment groups of male mice after having been treated with varying dosage of female hormonal contraceptive pills (ethinyl estradiol levonorgestrel) and on the number of

neuron and necrotic neuron cells in the cerebral cortex and the necrotic cells, germ cells and somatic cell counts in randomly selected seminiferous tubules. Seminiferous tubules' diameter and length were also evaluated which include the diameter of lumen.

The experimental mice were grouped into four treatments. The following treatments were replicated three times, with three mice per treatment. T0- Control (distilled water), T1- recommended dose (10mg/kg), T2- underdosed (5mg/kg), T3- overdosed (20mg/kg).

Experimental Procedure: Acclimatization of animals: A total of 12 (6 week-old) sexually matured male albino mice (*Mus musculus* L.), weighing 20-25 grams, were used in this study. There was one mouse in each cage, each cage was provided with a clay feeder and two plastic bottle containers. Commercial pigeon pellet supplements were given to the experimental animals *ad libitum.* The animals were maintained under proper temperature (25-30°C), ventilation and hygienic conditions. Mice were acclimatized for two weeks before the administration of pills[2].

Preparation and administration of treatments: Commercially available female hormonal contraceptive pills were crushed and dissolved in 75 ml olive oil per tablet. The mixture was administered orally to the mice using the intragastric gavage technique. The weight of the mice and the treatment group to which the mice belong were taken into consideration. Administration was done every 11 o'clock in the morning for 30 days. A recommended dose of 10 mg/kg of contraceptive pills was used in the experiment[3]. During the administration of treatments, weight and behavior of the male mice were strictly monitored on weekly basis.

Preparation of tissue samples for histopathology and morphometric study: After the 30-day treatment, male albino mice were sacrificed through cervical dislocation. The brains and testes were carefully collected and fixed in 10% formalin. Samples were sent to High-Precision Diagnostic for embedding and staining using hematoxylin and eosin. A total of 108 sections were prepared for histopathological examination.

Histopathological observation of the brain and testes of male albino mice: All of the sections were studied using light microscopy, with multiple magnifications (100x, 400x and 1000x). It analyzed the alteration of tissue structures on each particular organ and the correlation of these changes with clinical signs and symptoms.

Neurons and necrotic neurons were counted in ten (10) randomly selected fields of cerebral cortex per brain tissue samples under 400x magnifications were used in this study[4, 5, 6]. Germ cells and somatic cells were also counted in five (5) randomly selected seminiferous tubules per testicular tissue sample. Spermatogonia, spermatocytes, spermatids, sertoli cells and necrotic cells were counted using point counting approach under 1000x magnification[7].

Morphometric study of the seminiferous tubules: The diameter and length of seminiferous tubules and diameter of lumen were measured from twenty five (25) randomly selected tubules in each prepared tissue sample with a total of seventy five (75) tubules per replication.

Statistical Analysis: Data were presented as means (SD) and ranges (minimum-maximum) and between-group comparisons were made using the One- Way Anova and Tukey's Multiple Comparison Test. Statistical analysis was performed using version 5 of the Graphpad prism.

Results and Discussion

Weight and Behavior of Male Albino Mice: Figure 1 shows the mean body weight of mice from the different treatments. Statistical analysis revealed no significant difference among the treatment means. However, treatments 1 (recommended dose) and 2 (underdosed) have higher mean body weight of male mice than in treatments 3 (overdosed) and 0 (control group- distilled water). This can be attributed to the increase in female hormones in male mice. Estrogen and progesterone dominance in males can cause increase in body weight as well as development of pot belly[8]. In addition, testosterone deficiency in men can cause high cholesterol and triglycerides level in body that may contribute to the increase of body weight and abdominal weight gain or pot belly. However, a treatment group with overdose pills had a slow weight development because of the toxic effects of pills. Chemicals that are at high doses, can induce abnormal development[9]. With respect to the behavior and physical changes in mice that were monitored every week, restlessness and irritability in mice were observed as well as hair loss in their body. Such observation is supported by the findings of Perkins, wherein estrogen dominance in males may result to outburst of anger, hair loss, mood swings and impotence.

Histopathological observation in the brain of male albino mice (*Mus musculus,* L.): Histological alterations in the brain of male albino mouse were observed, such as increase in microscopic holes, vacuolated neurons, necrosis, increase incidence of shrunken eosinophilic neurons with either pyknotic or karyorrhectic nuclei, number of neuron swelling and vacuolation and gradual decrease in the number of healthy neurons as the dose of pills increased. An increase in necrotic cell counts may result to organ failure[9,10].

Histopathological and morphometric observation in the testicular tissue of male albino mice (*Mus musculus,* L.): Shown in figure 5A is the normal testicular structure of mice. As observed in this figure, the seminiferous tubules have a distinct morphology that is circular with intact germ cells; the basement membrane of each tubule is vivid. Lumens are very distinct[11]. Figures 5B, C, D and 6 showed histological changes in testicular tissue such as decrease in the normal architecture

and abnormal distance of seminiferous tubules, disintegration and atrophy of seminiferous tubules with widening of lumen and reduction of germ cell and somatic cell population, and increase in the diameter of lumen as the dose of pills increased. Cell counts in selected seminiferous tubules also decreased, such as the number of spermatocytes, spermatids and sertoli cells, but, there is no significant difference among the treatment groups with respect to the number of spermatogonia. An incidence of necrosis or cell death in the seminiferous tubules increased as the dose of pills increased (figure 7).

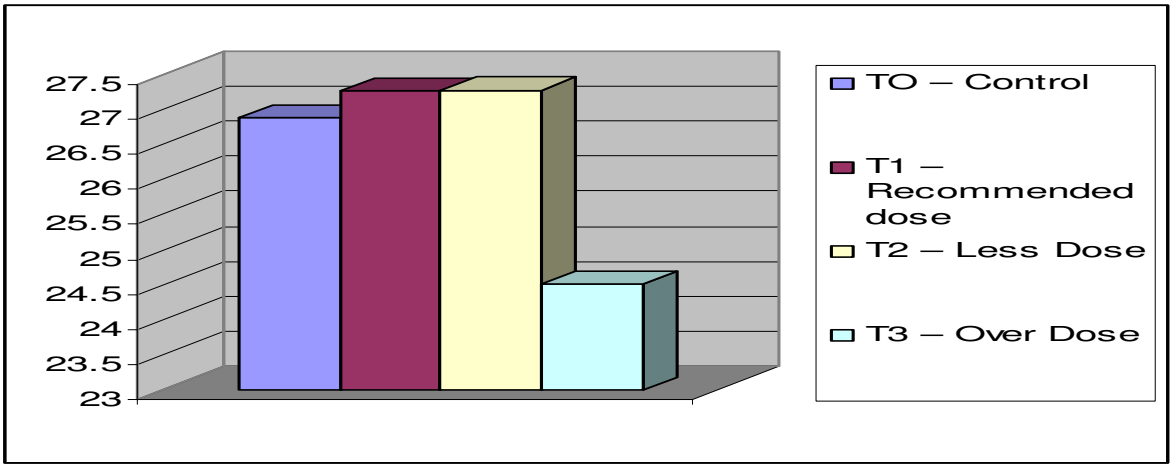

Figure-1
Mean body weights (g) of experimental male mice tested with different doses of female hormonal contraceptive pills

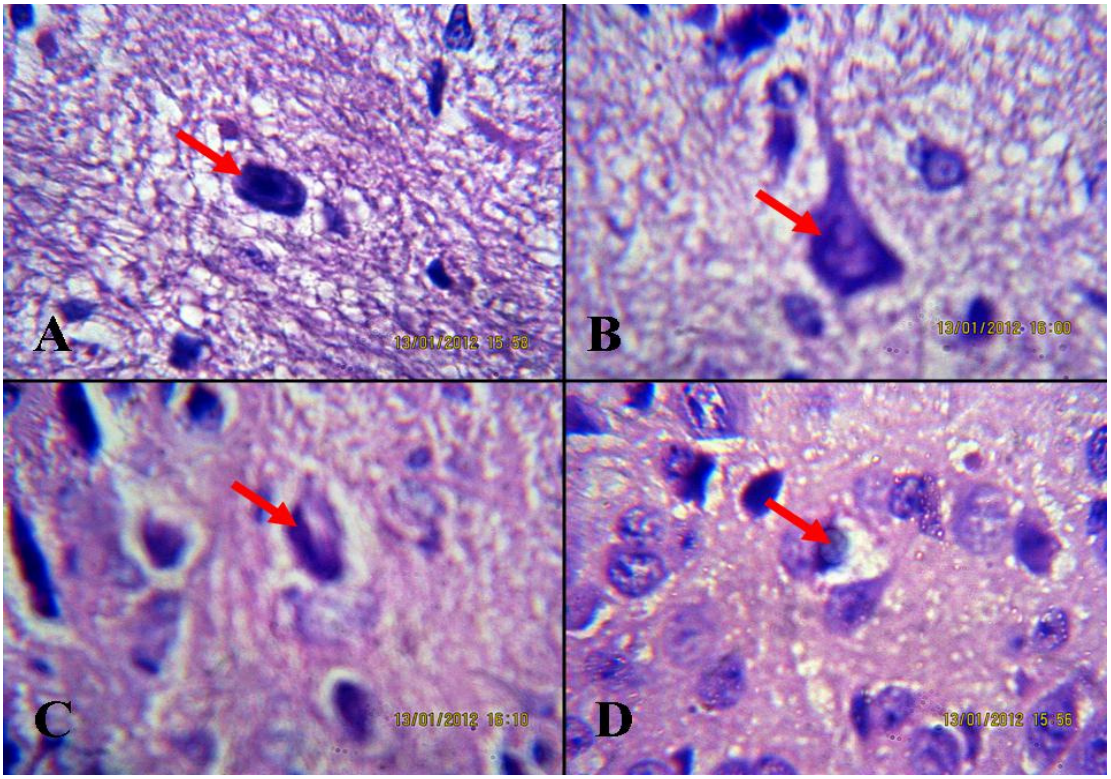

Figure-2
Histopathological observation in the brain of male albino mice (A) Normal neuronal morphology from control group;(B) Representative neuron from T1 (recommended dose) showing further cell shrinkage with condensation of chromatin and nucleus; (C) Representative neuron from T2 (under dosed) showing nucleus and cellular condensation;(D) Representative neuron from T3 (overdosed) with loss of nuclear membrane, circular chromatin, dense, and small size (HE x 1000 magnification)

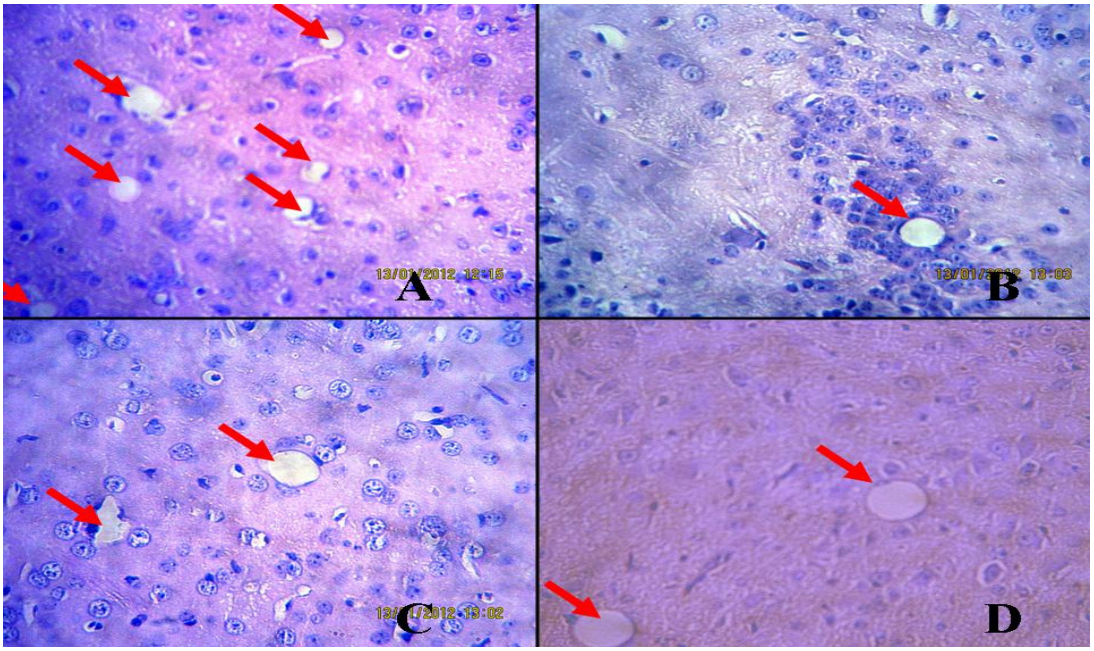

Figure-3
Representative tissues from male mice overdosed with female hormonal contraceptive pills showing a number of microscopic hole or vacuoles in the neurons (HE x 400 magnification)

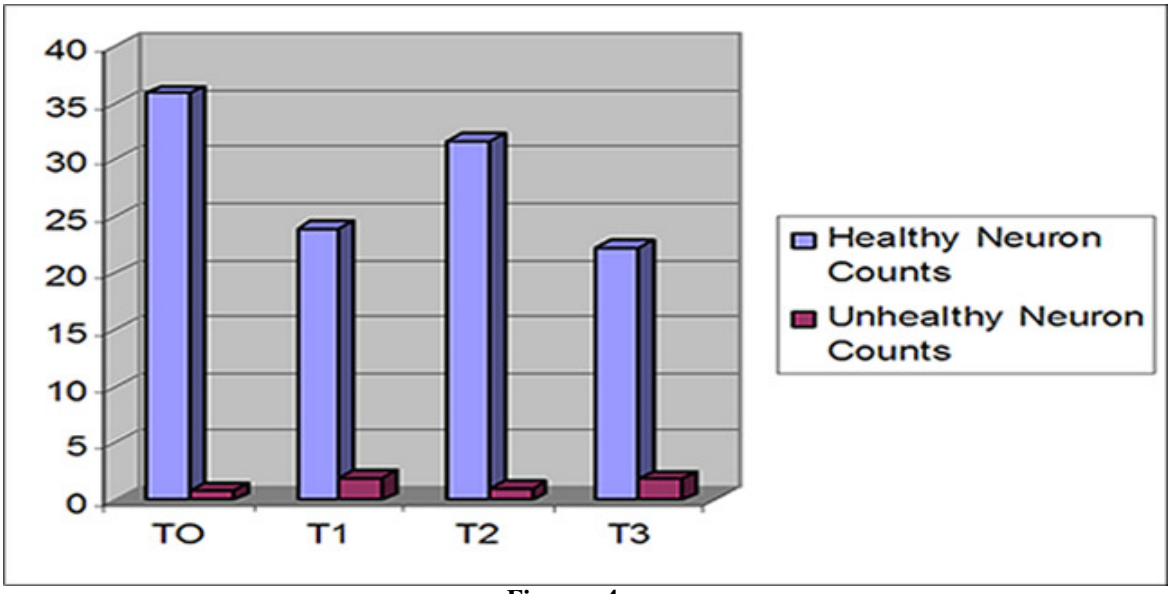

Figure - 4
Mean healthy and unhealthy neuron counts in randomly selected fields in cerebral cortex per treatment group

Since all the germ cells at various stages of maturation and their supporting sertoli cells develop within seminiferous tubules, a decrease in size or disorganization and hypoplasia with widening of lumen affect the germ cell population resulting to low number of germ cells that may lead to infertility. Destruction of testicular tubules is due to the subsequent elevation of only FSH or high levels of estrogen and progesterone levels[12]. The dominance of these hormones in male mice inhibit the process of spermatogenesis because they alter the delicate balance of male hormone and shuts down receptors for all the hormones crashing a man's system due to the lost of androgen dominance[7,13,14]. A decrease in the sertoli cells also indicates a decrease in testosterone level, because sertoli cells are the ones carrying testosterone intracellularly and it serves as testosterone reservoir within the seminiferous tubule[15]. The overdose group of mice showed a great number of necrotic cells indicating that over accumulation of female hormonal pills may contribute to cell death or necrosis of germ cells and increase in the number of necrotic cells is a major cause of organ dysfunction.

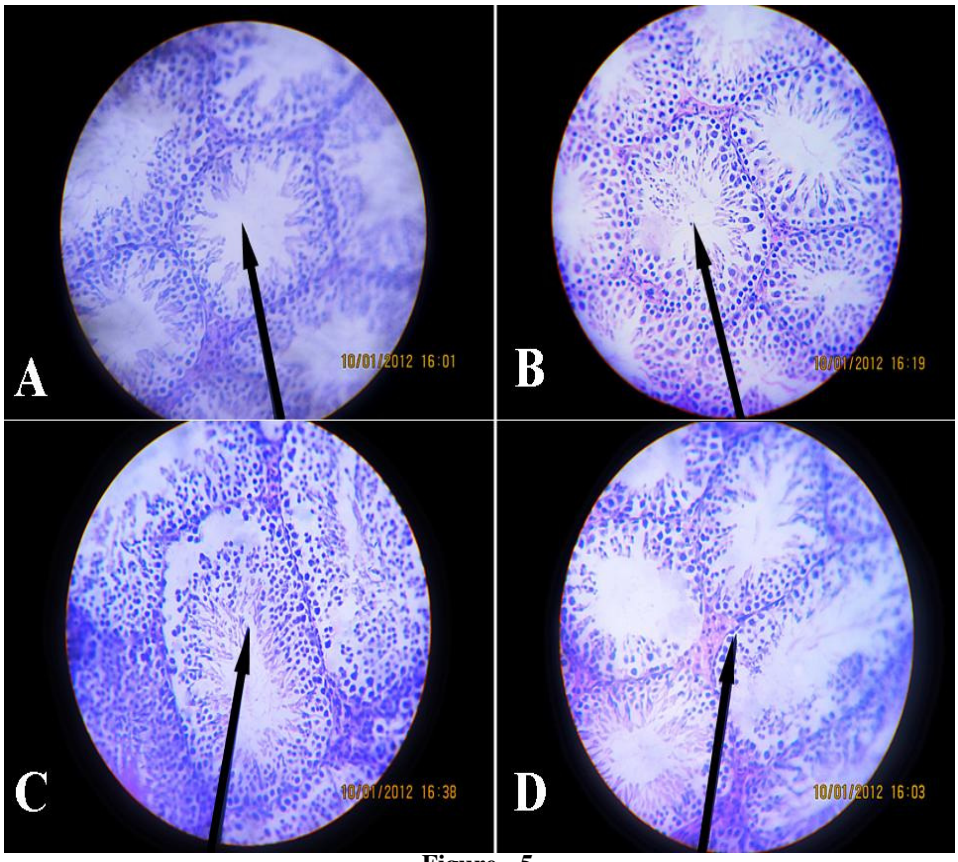

Figure - 5

Histopathological observation in the testicular tissue of male albino mice (A) Normal seminiferous tubules morphology from the control group; (B) Black arrow pointing out the representative seminiferous tubules from T1 (recommended dose) showing further sloughing of germ cells from basement membrane with the decrease of germ cell population; (C) Black arrow pointing out the representative seminiferous tubules from T2 (underdosed) with mild sloughing of germ cells from basement membrane; (D)Black arrow pointing out the representative seminiferous tubules from T3 (overdosed) with evidence of altered architecture of seminiferous tubules as well as widening of lumen and reduction of germ cell population (HE x 1000 magnification)

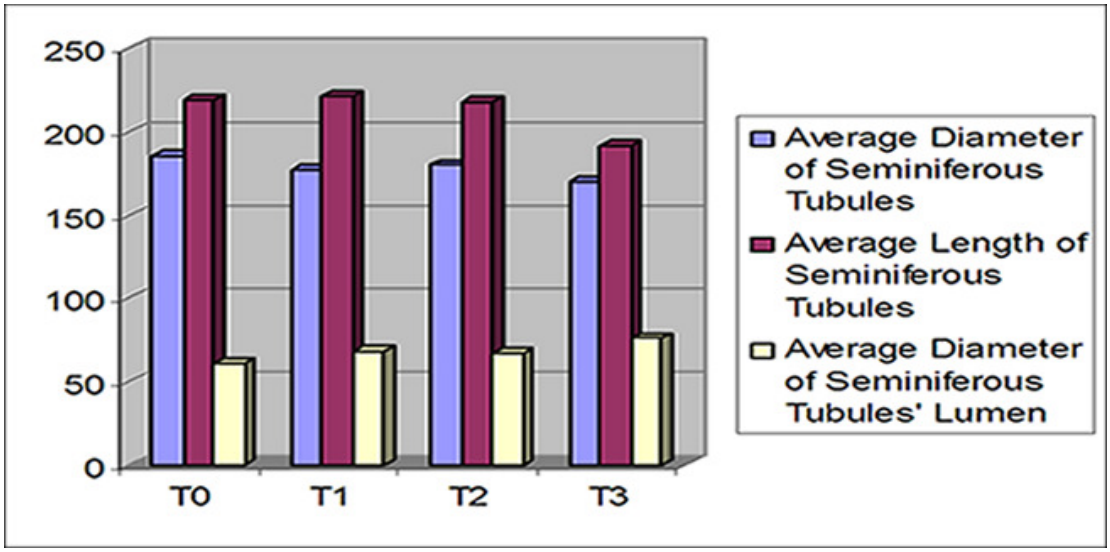

Figure-6

Average diameter and length in (µm) of randomly selected seminiferous tubules and average diameter of randomly selected seminiferous tubules' lumen per treatment group

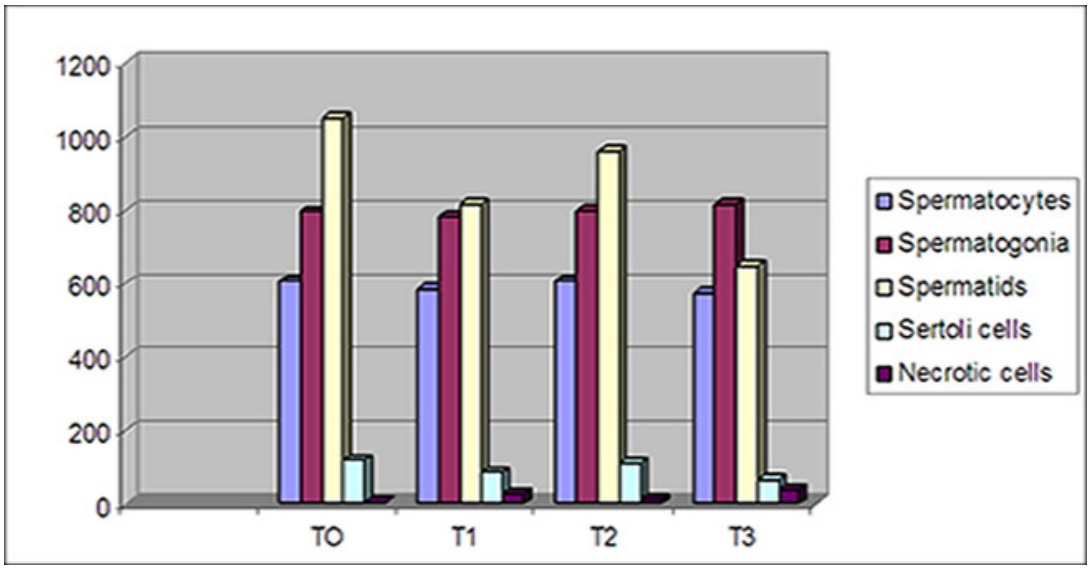

Figure-7
Mean spermatocyte counts, spermatogonium counts, spermatid counts, sertoli cell counts and necrotic cell counts in randomly selected seminiferous tubules per treatment group

Conclusion

Female hormonal contraceptive pills were found to have effect in the brain and seminiferous tubules of male albino mice. As regards behavior and physical changes in male mice, restlessness, irritability, hair loss in their body and increased body weight were observed in mice treated with the contraceptive pills. However, mice with over dosed pills exhibited slow growth and development.

Histological alterations in brain of male albino mouse were observed, such as increase in microscopic holes, vacuolated neurons, necrosis, increase incidence of shrunken eosinophilic neurons with either pyknotic or karyorrhectic nuclei, number of neuron swelling and vacuolation and gradual decrease in the number of healthy neurons as the dose of pills increased.

Likewise, histological changes in testicular tissue were also observed such as decrease in the normal architecture and abnormal distance of seminiferous tubules, increase in number of necrotic cells, disintegration and atrophy of seminiferous tubules with widening of lumen and reduction of germ cell and somatic cell population, hypoplasia and increase in the diameter of lumen. Cell counts in selected seminiferous tubules also decreased, such as, in the number of spermatocytes, spermatids and sertoli cells, however, there is no significant difference among the treatment groups with respect to the number of spermatogonia.

An incidence of necrosis or cell death in the seminiferous tubules increased as the dose of pills increased, however, there is a decreased in length and diameter of seminiferous tubules as the dosage of pills increased.

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