The Egyptian Journal of Hospital Medicine (January 2019) Vol. 74 (8), Page 1698-1701

Histopathological Study of the Chronic Toxic Effects of Dapoxetine Administration on Testes of Male Albino Rats

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ABSTRACT

Background: Dapoxetine is a short acting oral SSRI, purely created for the on-demand treatment of premature ejaculation**.** The present study was carried out to see the histopathological effects of Dapoxetine on the testis of adult albino rats. **Materials and methods:** Dapoxetine was administered orally by gavage to rats for consecutive 70 days, Eighty apparently healthy male albino rats weighing 125–150 g were used throughout the experiments .The rats were divided randomly into four groups (n=6). Each group 20 rats: Group (1) Control group, Group (2) receiving 1 mg of dapoxetine /70 days, Group (3) receiving 5 mg of dapoxetine /70 days and Group (4) receiving 10 mg of dapoxetine /70 days. Histological slides were prepared and stained with H and E stain. On examination, distortion of seminiferous tubules, decreased thickness of germinal epithelium, decreased diameter of seminiferous tubules and decreased counts of germinal cell lineage were found in treated groups.

Keywords*:* dapoxtine; histopathological effects; ssri.

INTRODUCTION

Dapoxetine, a selective serotonin reuptake inhibitor, can be considered as an antidepressant drug and has been used for the treatment of premature ejaculation, in males. Dapoxetine hydrochloride is a rapidly absorbed short-acting SSRI being investigated specifically for on-demand treatment of premature ejaculation, in males. Side effects include the following: allergy, G.I.T disturbances in the form of nausea, vomiting, withdrawal of the drug leads to premature ejaculation, headache and infertility **(1).**

Mechanism of action of chronic dapoxetine effects on the Reproductive system. Its action occurred by decrease of testosterone production, due to deficits in neuroendocrine cells of hypothalamic pituitary axis, decrease of both FSH and LH secretion and increased prolactin secretion **(2).** Spermatogenesis at all levels is affected. It includes increase percentage of teratozoospermia, decrease normal formed sperms, sperm motility affected, decrease of percentage of viable sperms and daily sperm production is decreased **(3).** The chronic dapoxetine treatment has a detrimental effects on the spermatogenesis, impairs sperms **(4).**

AIM OF THE WORK

The aim of the current study is to see the Chronic Toxic Effects of dapoxetine administration on testes of male albino rats.

MATERIALS AND METHODS

This study was carried out at Animal House, Faculty of Pharmacy, Al-Azhar University. Approval of the ethical committee was obtained. Eighty apparently healthy male albino rats weighing 125–150 g were used throughout the experiments. The animals were housed in metal cages in a conditioned room and were provided with a standard laboratory diet and water ad libitum. Rats had received dapoxetine hydrochloride orally by gavage for 70 consecutive days.

The rats were divided randomly into four groups (n=6). Each group 20 rats:

- Group (1) Control group on water freely and normal ordinary diet.
- Group (2) receiving 1 mg of dapoxetine /70 days.
  Maximal therapeutically equivalent dose (Max.T.E.D).
- Group (3) receiving 5 mg of dapoxetine /70 days.
  5 times maximal therapeutically equivalent dose (5 times Max.T.E.D).
- Group (4) receiving 10 mg of dapoxetine /70 days.
  10 times maximal therapeutically equivalent dose (10 times Max.T.E.D). At the end of the experiment (at end of 70 days) the animals were sacrificed and male sex organs (testes) were dissected and preserved in suitable fixative and prepared for histopathological examination.

1. The testes were dissected out from the rats under ether anesthesia. The tissues were fixed in 10% formalin, processed and blocks were made in paraffin wax. 4-5 µm thick sections were cut and stained with hematoxylin and eosin. The sections were examined in the light

Received:26/10/2018
Accepted:15/11/2018

microscope under high magnification (X 400). Student's t test was used for statistical purpose.

2. The dose selection was done on the body surface area ratio by referring to the standard table of Paget and Barnes [5] which convert human dose to rat dose i.e. for rats, Human dose × 0.018 for rats.

Histopathological studies:

Histopathological studies of the testes were done.

Statistical Methods

- Data were analyzed by Sigma Plot version 12.5.
- Data was summarized as mean ± SD.
- Differences between groups were analyzed by (Kruskal-Wallis test) and (Shapiro Wilk test) and t-test. Post-hoc testing was performed by the Tukey test to compare the difference among the groups.
- Simple linear correlation (Pearson correlation coefficient test) (r) was also done to test for linear relations between lead and cadmium and other variables.
- P-value is considered significant if < 0.05.

RESULTS

It was observed that albino rats where dapoxetine was administered for 70 days showed a decrease in the diameter of seminiferous tubules and thickness of its germinal epithelium in experimental groups 2 and 3 (fig.2&3).

Signs of cellular degeneration were observed in the tubules accompanied with distortion and loss of alignment in group 4 (Tables 1 and 2). Leydig and Sertoli cells were found to be decreased in number (Table 3).

The cells of the spermatogenic lineage also showed a similar decrease in number (Fig.3 and Tables 4-7).

Loss of alignment with decreased diameter of seminiferous tubules and thickness of its germinal epithelium were also observed in experimental rats of group 3 and 4 (Fig.4a&4b and Tables 1 and 2). A similar decrease in Leydig, Sertoli and spermatogenic cells were also observed (Tables 3-7).

Table (1) Changes in the mean diameter (µm) of the seminiferous tubules

Groups	70 days (Mean ± SD)
Group1	63.40±6.96
Group2	54.44±11.89 p=0.002
Group3	55.44±9.55 p=0.060
Group4	62.20±8.24 p=0.02

Table (2) Changes in the mean thickness (µm) of the germinal epithelium.

Groups	70 days (Mean ± SD)
Group1	20.60 ±1.90
Group2	16.30 ± 3.74 p=0.002
Group3	18.60 ± 2.32 p=0.036
Group4	15.30 ± 5.25 P<0.001

Table (3) Changes in the mean number of Sertoli cells

Groups	70 days (Mean ± SD)
Group1	21
Group2	19.5 ± 1.20 P=0.002
Group3	20.2 ± 0.6 P=0.05
Group4	15.83 ± 9.57 P=0.02

Table (4) Changes in the mean number of Spermatogonia A (Pale type)

Groups	2 weeks (Mean ± SD)
Group1	16.4 ± 3.64
Group2	10.4 ± 1.96 P<0.001
Group3	13.7 ± 2.97 P=0.05
Group4	12.8 ± 2.99 P=0.02

Table (5) Changes in the mean number of Spermatogonia A (Dark type)

Groups	70 days (Mean ± SD)
Group1	21.1 ± 3.42
Group2	16.7 ± 2.72 P=0.017
Group3	16.2 ± 2.65 P=0.13
Group4	15.1 ± 4.99 P=0.25

Table (6) Changes in the mean number of Spermatogonia B

Groups	70 days (Mean ± SD)
Group1	37.2 ± 6.69
Group2	37.1 ± 6.41 P=0.049
Group3	35.2 ± 3.28 P=0.14
Group4	32.6 ± 11.59 P=0.18

Table (7) Changes in the mean number of Primary Spermatocytes

Groups	70 days (Mean ± SD)
Group1	71.3 ± 13.59
Group2	49.9 ± 6.99 P=0.003
Group3	44.7 ± 10.71 P=0.01
Group4	19.8 ± 3.16 P<0.001

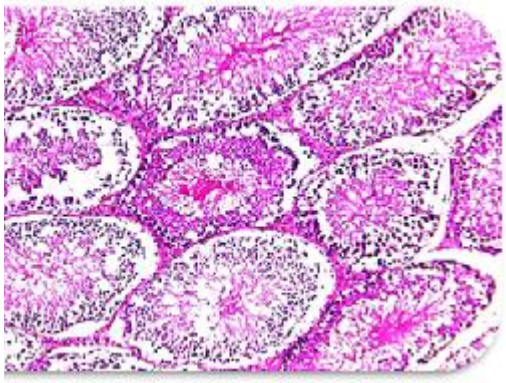

Fig. 1 A normal rat testicular tissue showing seminiferous tubules with normal pattern and diameter (H&E x100)

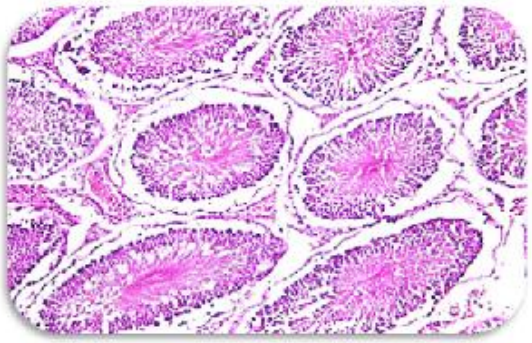

Fig. 2: A testicular tissue of rat receiving (1mg /day) showing loose arrangement of seminiferous tubules with decrease in thickness of germinal epithelium and decrease in the number of spermatogonia (H&E x100)

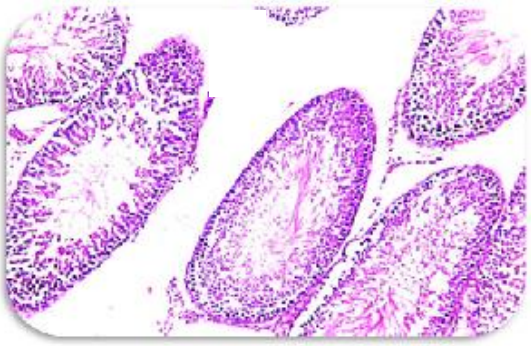

Fig. 3: A testicular tissue of rat receiving (5mg /day) showing signs of degeneration with distortion & loss of alignment of seminiferous tubules. There are decrease in thickness of germinal epithelium and decrease in the number of spermatogonia, Leydig cells & Sertoli cells (H&E x100)

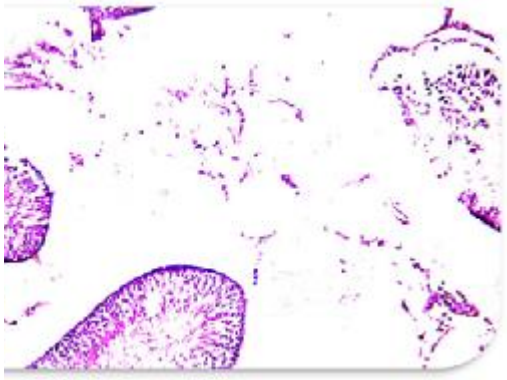

Fig. 4a: A testicular tissue of rat receiving (10mg /day) showing advanced signs of degeneration with marked distortion, loss of alignment and

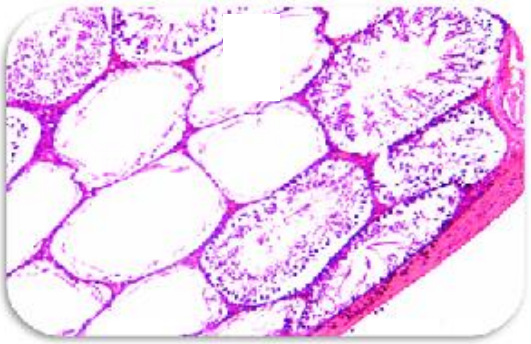

Fig. 4b: A testicular tissue of rat receiving (10mg /day) showing advanced signs of degeneration in spermatogenic cells with complete loss of spermatogenic cells in some seminiferous tubules at the periphery of the testis. (H&E x100)

DISCUSSION

The most conspicuous observation noted in this study was that most of the experimental rats of Group 4.

This study showed that disruption of the seminiferous tubular epithelium with focal damages ranging in severity from increased degeneration of spermatogenic cell profiles to complete loss of the germinal epithelium. In the present study it was observed that as the duration and dose of dapoxetine increased the entire cell lineage (Sertoli cells, Spermatogonia A - pale and dark type, Spermatogonia B, Primary spermatocytes) of the germinal epithelium decreased in number.

The density of mature spermatozoa also reduced in the lumen of seminiferous tubules with a marked reduction in the sperm concentration and impaired sperm motility.

Elevation of the cerebral level of serotonin concentration causes decrease in HCG level, which leads to decrease secretion of gonadotrophic hormones (LH and FSH) which are essential for spermatogenesis and steroidogenesis. Serotonin acts on seminiferous tubules of testis, accessory reproductive organs and epithelial cells via 5-HT receptor type-2 (5-HT2R) and induces smooth muscle contraction. Sperm production and process of maturation are thereby affected.

In the periphery, excessive free serotonin continuously stimulates the smooth muscle of blood vessels directly via 5-HT2R or indirectly via Thromboxane A2. This action might induce vasoconstriction and smooth muscle proliferation due to microcirculation disturbances. 5-HT is also thought to be a powerful inflammatory mediator. Its high levels in the hypoxic conditions may leads to testicular interstitial tissue inflammation and fibrosis, leading to a decrease in blood supply and atrophy of Leydig cells. Leydig cell 5-HT2R stimulation by serotonin may induce the corticotrophin releasing factor (CRF). It has a negative effect on the HPG axis. CRF also exerts a local inhibitory role on androgen secretion of LH related interstitial endocrine cells. CRF could stimulate Leydig cell secretion of beta-endorphin, which might inhibit FSH regulatory action on Sertoli cells in spermatogenesis [(6)].

In the present study vasoconstriction with atrophy of Leydig cells were found without any inflammatory cells. Sertoli cell number and cells of germinal lineage also decreased as the dose and duration of the drug increased.

Silva JVA *et al.* observed increased diameter of the sex cords/seminiferous tubules, decreased number of sertoli cells with no significant changes in the total volume of Leydig cells [(7)].

Reduction of Spermatogonia A was observed in rats treated with 1, 5 and 10 mg/day of dapoxetine during its juvenile period. But no difference was observed in the number of Spermatogonia B [(7)].

The density of mature spermatozoa also reduced in the lumen of seminiferous tubules accompanied by a marked reduction in the sperm concentration and impaired sperm motility.

CONCLUSION

A further research is essential to know whether these changes in the male genital system are reversible or irreversible. Clinicians must take precautions in prescribing the dose and duration of Dapoxetine to their patients.

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