Effects of Fluoxetine on Testis of Albino rats – A Histological Assessment

Alka Aggarwal, S L Jethani, R K Rohatgi, Juhi Kalra*

Abstract – Fluoxetine is a long acting widely used antidepressant, prototype of Selective Serotonin Reuptake Inhibitors (SSRIs), with a plasma half-life of 2 days. The present study was carried out to see the histopathological effects of Fluoxetine on the testis of adult albino rats. Fluoxetine was administered intraperitoneally to rats for 2 weeks, 4 weeks and 12 weeks with mild (10mg/kg/day), moderate (20mg/kg/day) and severe doses (40mg/kg/day). 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.

Key words – Fluoxetine, Germinal epithelium, Leydig cells, Seminiferous tubules, Sertoli cells, Selective Serotonin Reuptake Inhibitors.

1 Introduction

Fluoxetine is a long acting widely used antidepressant, prototype of Selective Serotonin Reuptake Inhibitors (SSRIs), with a plasma half-life of 2 days. Its active demethylated metabolite has a half-life of 7-10 days. It is used in children above 7 years and in adults on the basis of its efficacy and lower side effect profile [1]. Fluoxetine inhibits the 5–hydroxytryptamine (5-HT) (Serotonin) reuptake. Increased synaptic availability of serotonin stimulates a large number of postsynaptic (5-HT) receptor subtypes which lead to complex secondary responses including gastrointestinal disturbances and sexual side effects which include loss of libido, delayed ejaculation, anorgasmia and impaired orgasm, decreased testicular development and decreased Sertoli cell population, which may lead to infertility in adults[2]. Depression may also be associated with sexual disturbances especially reduced libido [2],[3]. For psychiatric illnesses (Depression and anxiety) the SSRIs are used for a long duration. The aim of the present study was to evaluate the effect of Fluoxetine in short term and long term therapy by studying the histological changes in various cells of the testis in albino rats.

2 Materials and Methods

Before starting the experiment clearance from Institutional animal ethical committee were obtained. The study was conducted on 36 male adult Wistar albino rats weighing between 120-140 grams. The study was conducted in 3 Phases of 2, 4 and 12 weeks duration. For each phase 12 rats were taken. These 12 rats were further subdivided into Control and three Experimental groups of 3 rats each. The animals were group housed with ad libitum access to food and water. The Control group received equal amount of intraperitoneal(ip) injection of the vehicle (normal saline). Experimental Groups 1, 2 and 3 administered 10 mg, 20 mg and 40 mg/kg of body weight/day of ip injection of Fluoxetine respectively.

After completion of each of the three phases, the testes were dissected out from the rats under ether anaesthesia. 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 haematoxylin and eosin. The sections were examined in the light microscope under high magnification (X 400). Student's t test was used for statistical purpose.

3 Observations

It was observed that Phase I (2weeks) albino rats which were administered Fluoxetine for 2 weeks showed a decrease in the diameter of seminiferous tubules and thickness of its germinal epithelium in experimental groups 1(10mg/kg/day) and 2(20mg/kg/day). Signs of cellular degeneration were observed in the tubules accompanied with distortion and loss of alignment in group 3 (40mg/kg/day) (Tables 1 & 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.2 & Tables 4-7).

Loss of alignment with decreased diameter of seminiferous tubules and thickness of its germinal epithelium were also observed much earlier in Phase II (4weeks) and Phase III (12weeks) experimental rats of group 2 (Fig.3 &Tables 1 & 2). A similar decrease in Leydig, Sertoli and spermatogenic cells were also observed (Tables 3-7). The intensity of histological changes was more pronounced in Phase III than in Phase II experimental rats (Fig.4). Group 3 could not survive the toxicity of the drug beyond 2 weeks.

Table 1

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

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12 weeks (Mean ± SD)
Control	63.40±6.96	52.30±11.07	68.80±6.65
Group 1 (10mg/kg/day)	54.44±11.89 p=0.002	45.3±5.33 p=0.040	51.30±7.39 p=0.001
Group 2 (20mg/kg/day)	55.44±9.55 p=0.060	44.80±4.85 p=0.020	44.10±6.12 p=0.004
Group 3 (40mg/kg/day)	62.20±8.24 p=0.02	-	-

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

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12 weeks (Mean ± SD)
Control	20.60 ±1.90	17.50 ± 3.57	17.20 ± 2.90
Group 1 (10mg/kg/day)	16.30 ± 3.74 p=0.002	13.20 ±3.39 p=0.018	13.30 ± 3.13 p=0.008
Group 2 (20mg/kg/day)	18.60 ± 2.32 p=0.036	15.20 ± 6.71 p=0.186	12.20 ± 3.29 p=0.006
Group 3 (40mg/kg/day)	15.30 ± 5.25 P<0.001	-	-

Table 3
Changes in the mean number of Sertoli cells

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12 weeks (Mean ± SD)
Control	21	20.3	19.1
Group 1 (10mg/kg/day)	19.5 ± 1.20 P=0.002	19.0 ± 1.34 P<0.001	17.2 ±0.75 P=0.002
Group 2 (20mg/kg/day)	20.2 ± 0.6 P=0.05	15.9 ± 0.7 P<0.001	14 ± 0.63 P<0.001
Group 3 (40mg/kg/day)	15.83 ± 9.57 P=0.02	-	-

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

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12 weeks (Mean ± SD)
Control	16.4 ± 3.64	15.9 ± 2.91	15.6 ± 3.14
Group 1 (10mg/kg/day)	10.4 ± 1.96 P<0.001	11.2 ± 2.23 P=0.003	10.7 ± 1.42 P=0.001
Group 2 (20mg/kg/day)	13.7 ± 2.97 P=0.05	11.8 ± 2.04 P=0.001	14.6 ± 2.42 P=0.22
Group 3 (40mg/kg/day)	12.8 ± 2.99 P=0.02	-	-

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

Groups	2weeks (Mean ± SD)	4weeks (Mean ± SD)	12weeks (Mean ± SD)
Control	21.1 ± 3.42	19.9 ± 2.74	19 ± 2.49
Group1 (10mg/kg/day)	16.7 ± 2.72 P=0.017	15.9 ± 1.22 P=0.001	13.7 ± 3.41 P=0.002
Group2 (20mg/kg/day)	16.2 ± 2.65 P=0.13	15.5 ± 2.20 P=0.03	12.7 ± 3.1 P=0.42
Group3 (40mg/kg/day)	15.1 ± 4.99 P=0.25	-	-

Table 6
Changes in the mean number of Spermatogonia B

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12weeks (Mean ± SD)
Control	37.2 ± 6.69	37.3 ± 6.57	36.5 ± 6.05
Group 1 (10mg/kg/day)	37.1 ± 6.41 P=0.049	33.2 ± 3.12 P<0.001	26.1 ± 6.66 P=0.002
Group 2 (20mg/kg/day)	35.2 ± 3.28 P=0.14	32.1 ± 7.20 P=0.03	25.3 ± 9.20 P=0.34
Group 3 (40mg/kg/day)	32.6 ± 11.59 P=0.18	-	-

Table 7
Changes in the mean number of Primary Spermatocytes

Groups	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	12 weeks (Mean ± SD)
Control	71.3 ±13.59	69.3 ± 12.34	66.5 ± 9.44
Group 1 (10mg/kg/day)	49.9 ± 6.99 P=0.003	44.7 ± 4.6122 P<0.001	35.9 ± 6.62 P<0.001
Group 2 (20mg/kg/day)	44.7 ± 10.71 P=0.01	41.9 ± 7.09 P=0.01	37.3 ± 7.44 P<0.001
Group 3 (40mg/kg/day)	19.8 ± 3.16 P<0.001	-	-

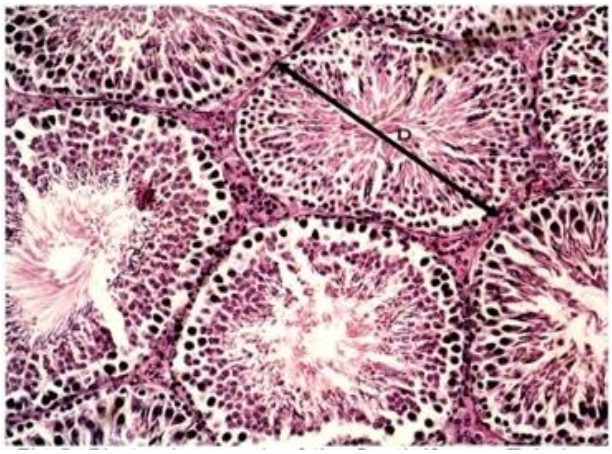

Fig 1: Photomicrograph of the seminiferous tubules showing the normal pattern and diameter in the control group of albino rats H & E ; X 400.

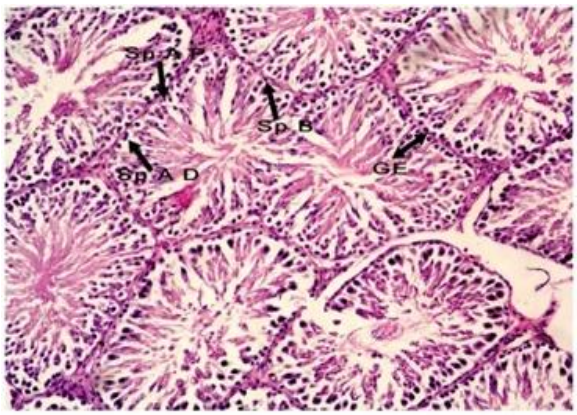

Fig 3: Photomicrograph of the seminiferous tubules in Phase II, Group 2 experimental rats showing its compact arrangement with decrease in thickness of germinal epithelium(GE), decrease in the number of Spermatogonia A- Pale type (Sp AP), dark type (SpA D) and Spermatogonia B(SpB) H & E ; X 400.

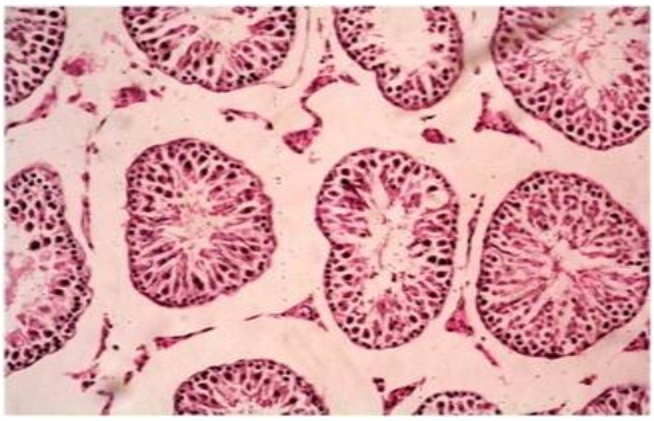

Fig 2: Photomicrograph of the seminiferous tubules in Phase I, Group 3 experimental rats showing signs of degeneration with distortion & loss of alignment. Leydig cells (LC) & Sertoli Cells (S) have decreased in number H & E ; X 400.

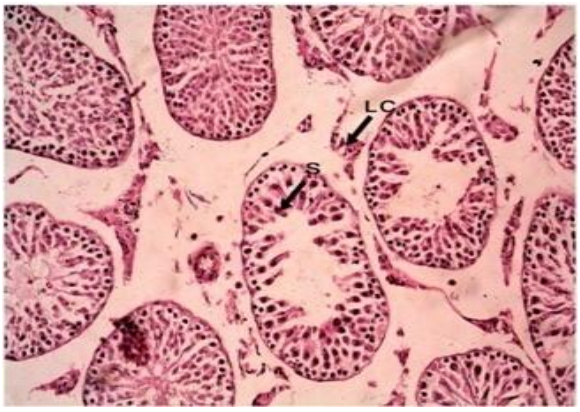

Fig 4: Photomicrograph of the seminiferous tubules in Phase III, Group 2 experimental rats showing advanced signs of degeneration in spermatogenic cells. Leydig (LC) & Sertoli Cells (S) have also decreased in number H & E ; X 400.

4 Discussion

The most conspicuous observation noted in this study was that most of the experimental rats of Group 3 (40 mg/kg/day) could not survive upto 4 weeks. Perhaps, this was a toxic dose to these animals which featured with muscle twitchings, sluggish movements and loose stools after 2 weeks of treatment. All the animals died during the 3rd week of administration of the drug.

Das et al [4], Xin-Min Z et al [5], and Meston CM [6], reported that pharmacological elevation of the cerebral

level of serotonin concentration cause 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-HT 2R) 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, contributing to microcirculation disturbances. 5-HT is also thought to be a powerful inflammatory mediator. Its high levels in the hypoxic conditions may induce testis 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. 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. The above observations in this study were thus similar to those reported by Das et al, Xin-Min Z et al and Meston CM.

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]. On the contrary, in our study loss of alignment with decreased diameter of seminiferous tubules and thickness of its germinal epithelium were observed much earlier in Phase II and III experimental rats of group 2.

Reduction of Spermatogonia A was observed in rats treated with 5, 10 and 20 mg/kg /day of Fluoxetine during its juvenile period. But no difference was observed in the number of Spermatogonia B [7]. However, observation in the reduction of the number of cells of Spermatogonia B in this study was contradictory to that of Silva JVA et al.

Taylor et al [8] did not notice any histopathological changes in the male reproductive organs. Drug information literature of SARAFEM [9] reported irreversible testicular degeneration and necrosis. Baines [10] and Martin–Du Pan RC [11] reported that Fluoxetine caused a marked reduction in the sperm concentration and impaired sperm motility (reversible) as it caused damage in the male genital organs. Hedger MP et al [12] found 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 Fluoxetine 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 accompanied by a marked reduction in the sperm concentration and impaired sperm motility. These findings were similar to those reported by Baines [10] and Martin–Du Pan [11] and Hedger MP et al [12].

Conclusion:

Sexual disorders and decreased germ count reported in patients on antidepressant Fluoxetine (SSRI) might be due to histological changes in the reproductive system and accessory reproductive organs via its indirect central and peripheral effects through the increased concentration of free Serotonin (5–HT). The present histological study results confirmed the previous reports about Fluoxetine induced male infertility and sexual disorders. In the present scenario every person of any age group and sex may suffer or pass from a period of anxiety and depression and therefore needs a prescription for use of these drugs. There is an essential need to know their safe dose and duration. 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 Fluoxetine to their patients.

Dr. Alka Aggarwal, *currently working as Assistant Professor in Anatomy Department in HIHT University, HIMS, Swami Ram Nagar, Dehradun (Uttarakhand) India.*
E-mail: alkadr2011@rediffmail.com

Dr. S L Jethani, *currently working as Head of Department in Anatomy Department in HIHT University, HIMS, Swami Ram Nagar, Dehradun (Uttarakhand) India.*
Dr. R K Rohatgi, *currently working as Professor in Anatomy Department in HIHT University, HIMS, Swami Ram Nagar, Dehradun (Uttarakhand) India.*
Dr. Juhi Kalra *currently working as Associate Professor in Anatomy Department in HIHT University, HIMS, Swami Ram Nagar, Dehradun (Uttarakhand) India.*

REFERENCES

1. Tripathi KD. Drugs Used in Mental Illness: Antidepressant and Antianxiety Drugs. In: Tripathi KD,Tripathi M editors. Essentials of Medical Pharmacology. 6th ed. New Delhi (India): JayPee Brothers Medical Publishers (P) Ltd. 2006: 439-449.
2. Ross JB. Drugs and the Treatment of Psychiatric Disorders in Depression and anxiety Disorders. In: Goodman and Gillman's The Pharmacological Basis of Therapeutics. 10th ed. New York: Mc Graw-Hill Companies. 2001: 447-483.
3. Fluoxetine official FDA information, side effects and uses. URL = http://www.drugs.com/pro/fluoxetine.html
4. Das TK, Mazumder R, Biswas NM. Spermatogenesis in rat: effect of L- tryptophan loading. Andrologia. 1982 May-Jun; 14(3): 242-249.
5. Xin-min Z, Traore S I, Wei-bin Z, Lu Qiang. Internal Spermatic and Peripheral Vein Plasma 5-Hydroxytryptamine Concentration Levels in Patients ith Varicocele. Urotoday International Journal. 2010Aug; 4(3):1944-1957. Www.urotodayinternationaljournal.com
6. Frohlich PF and Meston CM. Evidence that Serotonin affects female sexual functioning via peripheral mechanisms. Physiology and Behaviour. 2000; 71: 383-93.
7. Silva JVA, Lins AMJAA, Amorim JAA et al. Neonatal Administration of Fluoxetine Decreased Final Sertoli Cell Number in Wistar Rats. Int. J. Morphol. 2008; 26: 51- 62.
8. NTP-CERHR expert panel report on the reproductive and developmental toxicity of fluoxetine. April 2004; (http: //cerhr.niehs.nih.gov)
9. Drug Information for Fluoxetine (Mylan Pharmaceuticals Inc.): 8 Uses in Specific Populations. http://drug_information-for/fluoxetine-mylan-pharmaceuticals-inc-8-use-in-specific-populations/6841-496238.html
10. Can antidepressants cause male infertility? GP newspaper, 03 November 2006. (http://www.healthcarerepublic.com/news/603012).
11. C. Martin-Du Pan. Etiology of male infertility and Oligo-, Astheno-, Teratospermia (OAT), Arch Androl. 1997; 39: 197.
12. Hedger MP, Khatab S, Gonzales G, de Kretser DM. Acute and short-term actions of serotonin administration on the pituitary-testicular axis in the adult rat. Reprod Fertil Dev. 1995; 7(5): 1101-9.