POST TREATMENT ANALYSIS OF FLUOXETINE HYDROCHLORIDE ON TESTIS OF WISTAR RATS

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Abstract- Fluoxetine Hydrochloride (FH) is a second generation anti- depressant, commonly used in the treatment of depression, bulimia, PMS and obsessive compulsive disorders. Fluoxetine's metabolite Nor-Fluoxetine is also an equally potent and specific SSRI. FH is well absorbed following oral administration, and peak plasma FH concentrations usually occur within 4–8 hours and being approximately 15–55 ng/ml. The drug has a long half-life period of 4-6days. The present study is undertaken to carry out a post treatment analysis or reversal study to elucidate the long lasting effect of FH if any, based on the results obtained during the previous study titled "Sub Acute Toxicity of Fluoxetine Hydrochloride on Testis of Reserpine Depressed Wistar Rats". The study showed that irrespective of the dose levels, FH develops hypoglycemic effect, hypercholestremia and sexual dysfunction and alteration in the histology of the testis. The effect of FH and its metabolites is long lasting than the reported time and will remain for more time and can cause hypogonadism, impaired gametogenesis and reduction in sperm count in rats leading to infertility at higher doses.

Key Words- Fluoxetine Hydrochloride, reversal study, hypoglycemia, hypercholestremia,

I. INTRODUCTION

One of the most important health problems the world is going to face in the forth coming years is depression. According to the World Health Organization, unipolar depressive disorders were ranked as the third leading cause of the global burden of disease in 2004 and will move into the first place by 2030. More and more people are falling into the clutches of this disease due to the enormous tensions and pressure of the day-to- day life, especially the younger generation. As there is an increase in the reported cases of depression, the number of antidepressants in the market also shows an increasing trend, the major brand being the selective serotonin reuptake inhibitors, in this category the drug Prozac (Fluoxetine Hydrochloride) belongs.

After the drug is gone off patent in January 2001, also SSRIs being the primary drug for depression, all the major pharmaceuticals in India are manufacturing this drug under different commercial names like paroxetine, Nuzac, Fludac, Flunil, Sertraline to name a few.

Fluoxetine Hydrochloride (FH) is a second generation anti- depressant, commonly used in the treatment of depression, bulimia, PMS and obsessive compulsive disorders [1,2]. Fluoxetine's metabolite Nor-Fluoxetine is also an equally potent and specific SSRI. FH is well absorbed following oral administration, and peak plasma FH concentrations usually occur within 4–8 hours and being approximately 15–55 ng/ml. [3]. FH is extensively demethylated in the liver by CYP2C and 2D6 to the primary active metabolite norfluoxetine. The elimination half-life of the parent drug is 4 to 6 days, but it is increased to 4–16 days for norfluoxetine. On the other hand, FH inhibits isoenzyme CYP2D6 and thus its own metabolism. Due to extensive tissue distribution, FH has a high volume of distribution [4]. It is excreted by humans as 11% parent compound and 7% norfluoxetine, both of which are pharmacologically active, in urine, feces and breast milk [5]

The literature survey, related to this drug shows many side effects like hypoglycemia[6] –[7]), weight reduction, sexual dysfunction like anorgasmia, erectile problems, involuntary sperm emission, etc .in human beings [8]-[9]- [10]. The result of our previous study (Table I) titled "Sub Acute Toxicity of Fluoxetine Hydrochloride on Testis of Reserpine depressed Wistar rats" [11] showed that F H induce hypoglycemic condition irrespective of the dose levels. Exposure of depressed rats to higher doses of FH also induced hypercholestremia and decreased the serum testosterone, testicular proteins and cholesterol, reduced the number of epididymal sperms and altered the histo- architecture of testis suggesting its adverse effect on reproduction. Another study on "Sub Acute Toxicity of Fluoxetine Hydrochloride on fertility of male wistar rats" [12] showed that FH treatment increases the mating time and the fertility index showed a dose related linear decrease. Mating behaviour, physical activity, sexual motivation and sleep pattern did not change in lower doses of FH but as the dose level increased the rats were showing decreased physical activities, self grooming activities were predominant (anxiogenic behaviour)., showed decreased libido and piloerection was predominant.

Because of the long half-life of Fluoxetine and Nor-Fluoxetine, it may take up to 1–2 months for the drug substance to disappear from the body. The present work is undertaken carry out a post treatment analysis or reversal study to elucidate the long lasting effect of FH if any considering the results of the study titled, "Sub Acute Toxicity of Fluoxetine

Hydrochlioride on Testis of Reserpine Depreesed Wistar rats"

II. METHODS AND MATERIALS

Drugs used:

Reserpine was used for inducing depression. 3mg/ kg stock solution was prepared daily by dissolving reserpine in distilled water. The use of reserpine induced depression is one of the basic models for assessing antidepressants in studies of new pharmaceuticals and is still used in many studies which require depression induction in animal models [13]-[14]

Fluoxetine hydrochloride (FH) is the other drug used in this study. NUZAC capsules (Manufactured by Protec, Division of Cipla Ltd, Mumbai) containing FH were procured from market. Each 20 mg capsule contained Fluoxetine Hydrochloride equivalent to 20 mg, fluoxetine, non- medicinal ingredients like silicone and starch. Stock solutions of different concentrations in mg/ml were prepared freshly in distilled water. The volume of the drug to be administered is calculated according to the body weight of the animal as mg/ml.

40 healthy male albino rats (Rattus norvegicus) of Wistar strain weighing 190-210g (procured from Haffkine Bio-Pharma Corporation Ltd., Parel, Mumbai) were housed in polyurethane cages with wire mesh tops and rice husk bedding and maintained on standard pellet diet (Amrut feeds, supplied by Excel Agencies, Mumbai) with water ad-libitum. Animals were acclimatized at a room temperature of 28 ^{0}C. Artificial lighting at a sequence of 12 hrs light and dark cycles and humidity at 50-77% RH was maintained during the study period.

Study plan and Treatment procedure:

Forty rats were grouped into 4 (10 rats in each group). First group (control I) was injected with saline for 19 days. Second group of animals were injected with reserpine to induce depression for 5 days and considered as control for group III and IV. The group III and IV were given daily 20mg/kg and 80mg/kg Fluoxetine Hydrochloride respectively for 14 days after inducing depression by giving reserpine (3mg/kg) for 5 days. All injections were given intra-peritoneal in the morning hour. After the treatment period rats were kept for 60 days and sacrificed for the Post treatment analysis.

Parameters studied:

General behavior, body weight, food and water intake were noted daily during the study period. All the rats were sacrificed after 60 days for further studies. Blood was collected by cardiac puncture under light anesthesia for the estimation of sugar, blood cholesterol and serum testosterone. Weight of the testis was noted. Excised pieces of testis were fixed in neutral formalin for 48 h for histological analysis.

A known amount of cauda epididymal tissue (100 mg in 2ml) was taken in physiological saline and teased gently to release the spermatozoa. This suspension was used for counting the number of sperms.

Biochemical estimations:

Estimation of blood sugar [15] and blood cholesterol [17] was carried out by using standard diagnostic reagent kits procured from Span Diagnostics Pvt. Ltd. Serum testosterone level was estimated by radioimmunoassay using antibody coated test tube assay kits. The sperm count was determined from the cauda epididymis using a Haemocytometer Neubauers chamber [17]

Histological methods:

After fixation, the testis was washed and paraffin blocks were prepared. 5µm sections of the tissues mounted on clean glass slides, stained with haematoxylin and eosin, were examined under light microscope [18].

Statistical analysis:

For all the quantitative data generated by the experiments, differences between individual groups were analyzed for statistical significance using the appropriate test. Inter-graph difference was statistically assessed using student's t-test. The results were expressed as Mean ± SE. Any difference with a probability value less than 0.05 were considered statistically significant.[19]

III. RESULT AND DISCUSSION

Post Treatment Analysis

No toxic symptoms were observed in Control I. Reserpine depressed group (Control II) did not recover completely even after 60 days. The physical activity was reduced and sleeping pattern was altered. Group III rats started showing recovery from depression after one week of treatment. Food and water intake was normal. During the non-treatment period all of them were healthy, sleeping pattern and physical activity was normal. Weekly exposure to sexually mature females showed normal mating behavior. Upon sacrifice the general viscera showed no toxic symptoms. The Group IV rats on weekly exposure to sexually mature females, neither elicited sexual urge nor showed any copulatory behavior. Many of them showed hair loss and red rimmed eyes. The morphology of the reproductive organs did not show any alterations in 20mg/kg group but the size of testis in the group treated with 80mg/kg showed highly significant reduction in size and weight.

The body weight of the experimental animals before and after the treatment, gives the drug's effect on anabolism and catabolism. Body weight gain of the reserpine treated animals became normal during the post treatment period where as Group III animals did not show any significant changes during the reversal period. Fluoxetine in higher doses did not improve the depressed condition of the animal, also produced toxic symptoms in rats.

Sub acute toxicity Analysis

The highly significant reduction in the body weight gain in Group IV rats can be correlated to the anorectic effect of fluoxetine. Though the blood sugar level has improved in reversal study (GP III and IV) , the significant reduction in the blood sugar level in the entire experimental groups shows that the FH's hypoglycemic effect prevails even after the treatment period. Fluoxetine causes hypoglycemia irrespective of the dose levels, and this effect is independent of insulin levels [6]- [7]. and food intake.

Many researchers have shown that hypercholestremia has a detrimental effect on Leydig and sertoli cell's secretory function, spermatogenesis, epididymal sperm maturation process and the overall sperm fertilizing capacity [20] - [21] Fluoxetine causes an increase in steroid concentration in plasma and brain in humans. Scientists have already established the effect of fluoxetine on brain-pituitary-adrenal-axis [22]- [23]. The hypercholestremia observed in the rats treated with 20mg/kg FH during sub acute toxicity study has reversed during the present study, indicating normal cholesterol metabolism in Group III rats.

Circulatory androgens maintain the structural and functional integrity of reproductive organs [24] and any change or alteration in the androgen level results in general decrease in testicular weight [23].-[24] The highly significant decrease in serum testosterone level observed in Group IV rats during the 14- day sub acute toxicity study has not improved during the reversal period, indicating the possible effect of FH on hypothalamic-pituitary-testis axis. The highly significant reduction in the weight of testis in Group IV rats can be correlated to the highly significant decrease in testosterone level. Long- term effect of androgen depletion will cause testicular injury, that results in changes in spermatogenesis and eventually testicular atrophy [Fig.IV] [25] .

Table: I. Denotes the results of the study titled "Sub Acute Toxicity of Fluoxetine Hydrochloride on Testis of Reserpine depressed Wistar rats"

Parameters	Control I	Control II	Group III 20 mg/kg	Group. IV 80 mg/kg
Food intake (gms/day)	21.414 ±0.453	18.400± 0.340***	22.080 ±0.515	5.171±0.943***
Water intake (ml/day)	45.465 ±1.094	48.800 ±3.199	48.880 ±3.184	34.727 ±1.515 ***
Blood Sugar (mg/100ml)	86.742 ± 1.667***	69.712 ±1.336***	48.113 ± 0.637	31.026 ± 0.977 ***
Blood Cholesterol (mg/100 ml)	109.004 ± 18.345	127.138 ±5.082	37.679±4.774 ***	185.256 ± 21.820**
Weight of Testis (gms)	2.203 ± 0.070	1.981 ±0.150	2.503 ± 0.042 ***	2.099 ± 0.129
Serum Testosterone (ng/dl)	370 ± 53.787	261.00 ± 54.147***	152.7± 47.307 □□□	35.95 ± 3.672 ***
Sperm Count (millions/cu mm)	12.375 ± 0.577	4.270 ±1.050 ***	11.400 ± 0.857	8.180 ± 0.878 **
Body Weight (gms) Initial wt	204	212	203.5	200
Final wt	236	235	250.5	199
Combined SE	±0.934	±0.542***	±4.002**	±3.841***

All values are expressed as mean ± SE. *** indicates P ≤ 0.001 (Highly significant), ** indicates, P ≤0.01 (significant)

Table: II. Table denotes the fluctuations of various parameters in Reserpine and FH treated groups as compared to control I (Post Treatment Analysis).

Parameters Expressed.	Control I	Control II	Group. III 20 mg/kg	Group.IV 80 mg/kg
Food intake (gms/day)	24.127 ± 0.421	16.230 ±0.211***	20.461 ± 0.324	15.171 ±0.728***
Water intake (ml/day)	48.278 ± 0.074	49.110 ±3.199	48.880 ±2.135	30.727±1. 348 ***
Blood Sugar (mg/100ml)	87.143 ± 4.199	69.899 ±0.822	78.573 ± 3.102***	46.371 ± 2.338 ***
Blood Cholesterol (mg/100 ml)	129.163 ± 8.233	110.462 ±10.25	110.73 ± 5.74	75.283 ± 7.962***
Weight of Testis (gms)	2.538 ± 0.029	2.662 ±0.058	2.789 ± 0.046 ***	1.32 ± 0.128***
Serum Testosterone (ng/dl)	418 ± 17.714	239.00 ± 54.060	242.7± 17.789 ***	69.00 ± 5.319 ***
Sperm Count (millions/cu mm)	12.32 ± 0.262	9.2 ±0.289 ***	12.01 ± 0.303	2.246 ± 1.005 ***
Body Weight (gms) Initial wt	207	200	206	215
Final wt	290	282	304	274.6
Combined SE	±0.867	±4.694	±7.675	±11.44 **

All values are expressed as mean ± SE. *** indicates P ≤ 0.001 (Highly significant), ** indicates, ≤0.01 (significant)

Pharmacological elevation of the cerebral serotonin concentration cause decrease in HCG level and leads to decrease in secretion of gonadotrophic hormones (LH and FSH), which are essential for spermatogenesis and steroidogenesis. The sperm count of Group IV rats of the reversal study showed highly significant reduction showing the adverse effect of FH on gametogenesis. These facts suggest that FH and its metabolites in lower dose levels do not affect gametogenesis. Sexual disorders and decreased germ count reported in patients on FH 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). [26] -[27]

Microphotograph of Testes - Reversal Study

Fig. I Control I – The histo-architecture of testis shows normal picture

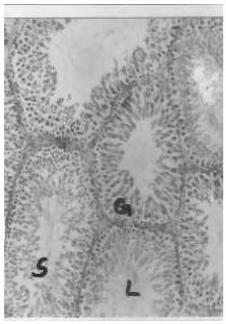


Fig. II Control I I– The histo-architecture of testis in reserpine treated rats remains unaltered

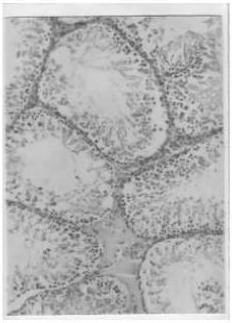


Fig. III Group III –The seminiferous tubules showing normal spermatogenesis.

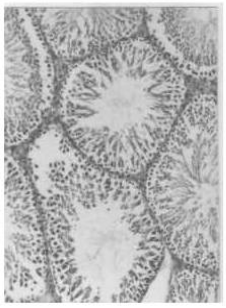
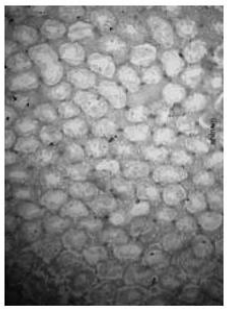


Fig. IV In Group IV, the semeniferous tubules have shrunk. . The stroma is highly vacuolated showing atrophy of the testis



From the overall study it is concluded that there is a dose related adverse effect on of FH on reproduction, specially on gametogenesis. Irrespective of the dose levels, FH causes hypoglycemia and hypercholestremia in rats. The reversal study shows that FH takes more time to get eliminated from the body and also the metabolites have a toxic effect on reproductive organs of male wistar rats leading to atrophy of testis and reduction in number of sperms. 20mg/kg or lesser doses are well tolerated by the reproductive system. An increase in dose level may not help to cure depression and also it causes many toxic symptoms and leads to infertility.

REFERENCES

[1] Altamura, A.C., Moro, A.R., and Perendam, M.: Clinican pharmacokinetics of Fluoxetine, Clin.Pharmacokinet., 26(3): 201-214 ,March ,1994.

[2] Schenker, S., Bergstrom, R.F., Wolen, R.L. et al.: Fluoxetine disposition and elimination in cirrhosis. Clin.Pharmacol. Ther., 44(3): 353-359, September1988.

[3] Eap CB and Baumann P: Analytical methods for the quantitative determination of selective serotonin reuptake inhibitors for therapeutic drug monitoring purposes in patients. J Chromatogr B Biomed Appl, 686: 51–63., November. 1996.

[4] Cheer SM and Goa KL: Fluoxetine—A review of its therapeutic potential in thetreatment of depression associated with physical illness. Drugs, 61: 81–110.,2001

[5] Van Harten J : Clinical pharmacokinetics of selective serotonin reuptake inhibitors. Clin Pharacokinet, 24: 203–220. 1993

[6] Post Introduction Report of Fluoxetine Hydrochloride: Irotec Laboratories , Ireland

[7] Eren Memisoglu, A., Ozdogan, U.K., Saraymen, R. and Tutus, A.: Effect of some antidepressants on glycemic and insulin levels of normoglycemic and alloxan- induced hypoglycemic mice. J. Pharm. Pharmacol., 51(6): 741-743 (1999).

[8] Murray, J.B. :Physiological mechanisms of sexual dysfunction side effect associated with antidepressant medication. J.psychol. 134 (4) : 407-16. July,1998.

[9] Seagraves, R.T.: Antidepressant induced sexual dysf unction. Clin. Psychiatry, 59(4): 48-54 (1998).

[10] Aizenberg,D. Zemishlany, Z. and Weizman, A. Cycloheptadine treatment of sexual disfunction Induced by serotonin reuptake inhibitors : Clin.Neuropharmacol 18 (4): 320-324, August, 1995.

[11] Unnikrishnan G, Shivabalan R, and Lillian J: Sub-acute toxicity of fluoxetine hydrochloride on fertility of male wistar rats. Journal of Herbal Medicine and Toxicology,3: 23-30. January,2009

[12] Unnikrishnan G, Shivabalan R, and Lillian J "Sub-Acute Toxicity of Fluoxetine Hydrochloride on Testis of Reserpine Depressed Wistar Rats": Journal of Cell and Tissue Research Vol.8 (1) 1339- 1345. December, 2008.

[13] Rein, H.J.: Prejudices in pharmacology and pharmacotherapy; reserpine as a model for experimental research in depression, pharmacopsychiatry. Neuropsychopharmacology, 11(6): 289-293. 1978

[14] Yamashita, K., Kitayama, I., Hamanaka, K.,and Nomura, J.: Effect of reserpine on 3-methoxy-4hydroxy phenyl ethylene glycol and 3-4 dihydroxy phenyl acetic acid in the hippocampus of depression model rat s: An in-vivo micro dialysis study . Brain Res., 785 (1): 10-17. August, 1998 .

[15] Cooper, G.R. and Mc Daniel, V: Estimation of blood sugar . In: Standard Methods of Clinical Chemistry. P 159.

[16] D.R.Wybenga, V.J.Pileggi, P.H.Dirsteine, and John. Di. Giorgio. Direct Manual Determination of Serum Total Cholesterol with a Single Stable Reagent. In: Clinical Chemistry. Vol. 16. No.12. 1970.

[17] Prasad M.R.N., Chinoy, N.J. and Kadam, K.M. Changes in succinate dehydrogenase levels in rat epididymis under normal and altered physiological condition, Fert. Sterilit. 23: 188- 190.1972

[18] Guyer, F.M. Practical exercises in zoological microtechniques. In: Animal Micrology. P.54. 1936

[19] Dr B.K. Mahajan In: Methods in Biostatistics for medical students and research workers, V edition. 1989

[20] Yamamoto, Y., Shimamoto, K., Sofikitis, N. and Miyagwa, I.: Effects of hypercholesterolaemia on Leydig and Sertoli cell secretory function and the overall sperm fertilizing capacity in the rabbit. Hum. Reprod., 14(6): 1516-1521 . June, 1999.

[21] Papakostas, G.I., Iosifescu, D.V., Petersen, T., Hamill, S.K., Alpert, J.E., Nierenberg, A.A., Rosenbaum, J.F., Fava, M.: J. Serum cholesterol in the continuation phase of pharmacotherapy with fluoxetine in remitted major depressive disorder. Clin. Psychopharmacol. 4: 467-469, August 2004.

[22] Bartke, A. : Concentration of free and esterified cholesterol in the testis of immature and Adult mice. J.Reprod. Fertil.25:153-156, April, 1971.

[23] Brooks, D.E. : Epididymal functions and their hormonal regulation. Aust J Biol Sci,36: 205-221. 1983

[24] Oko,R. and Hrudka, F. Comparison of the effects of gossypol, estradiol-17 beta and testosterone compensation on male rat reproductive organs. Biol Reprod, 30: 1198-1207. 1984

[25] Gary, R.Klinefelter., Rex,A.Hess. Toxicology of the male excurrent ducts and accessory glands: In:Reproductive and developmental Toxicology(ed. Kenneth,S.Korach) p.553-591

[26] Das T.K, Mazumder, R. Biswas N.M.Spermatogenesis in rat: effect of L- tryptophan loading. Andrologia. 1982 May-Jun; 14(3): 24

[27] Alka Aggarwal, S L Jethani, R K Rohatgi, Juhi Kalra:. Effects of Fluoxetine on Testis ofAlbino rats – A Histological Assessmen. International Journal of Scientific& Engineering Research. Vol.3, Issue.7.July 2012.

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