Original Article

The Biological Effects of Imatinib on Male Fertility of Wistar Rats

Parichehre Yaghmaei, Ph.D.[1*], Kazem Parivar, Ph.D.[1], Aghdas Gharibi, M.Sc.[2], Mohammad Nabiuni, Ph.D.[3]

1. Biology Department, Faculty of Sciences, Islamic Azad University, Science and Research Campus, Tehran, Iran
2. Islamic Azad University, Science and Research Campus, Tehran, Iran
3. Cell and Developmental Biology, Biology Department, Tarbiat Moallem University, Tehran, Iran

Abstract

Background: Imatinib is used in chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. The major aim of this study was to investigate the effects of Imatinib on male fertility in Wistar rats.

Materials and Methods: Three groups of rats were gavaged with 6, 9, and 12 mg/kg Imatinib dissolved in dH_2O for 30 consecutive days. On days 7, 14 and 30, blood samples were collected and LH, FSH, and testosterone levels were measured by the ELISA method. The numbers of sperm located in the epididymis were counted by staining with aqueous Eosin Y. Other sections of the testes were stained with H & E, investigated histologically, and the results were statistically analyzed.

Results: On day 7 of the experiment, testosterone concentrations in the experimental groups were decreased ($p \leq 0.01$), LH and FSH increased significantly, and the number of sperm in both the epididymis and sertoli cells decreased ($p \leq 0.01$). There was an increase in tunica albuginea thickness ($p \leq 0.05$) but the diameter of the seminiferous tubules showed a significant decrease ($p \leq 0.01$). There was also a decrease in the number of Leydig cells, spermatogonia, stem cells, primary spermatocyte and spermatid. In the second and third samples (14 and 30 days after treatment), the testosterone levels, numbers of spermatogenic cells, Sertoli and Leydig cells showed an increase when compared to the first sample.

Conclusion: These findings suggest that a dose dependent administration of Imatinib has a profound effect on spermatogenesis.

Keywords: Imatinib, Spermatogenesis, Testis, Rat

Introduction

Imatinib is used in the treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), a number of other malignancies (1) and hypereosinophilic syndrome (2). Imatinib is known as a specific inhibitor of tyrosine kinases c-kit, platelet-derived growth factor receptor (PDGF-R), abl (the Abelson proto-oncogene) and breakpoint cluster region (bcr) (3).

Tyrosine kinases (TKs) are one of the most important groups of signaling molecules in the cellular regulation of proliferation, differentiation, survival, function, motility and various tumors. TK overexpression leads to uncontrolled mitogenic signals to the neoplastic cells (4). Imatinib is teratogenic in mice and rats when administered during organogenesis at doses over 100 mg/kg, causing exencephaly or encephalocele, and absent or reduced frontal and parietal bones (5). The most critical period for teratogenicity is the first trimester of pregnancy in humans, which correlates with active organogenesis (6). One infant case had hypospadia and rotation of the small intestine (7).

Hypospadia occurs in the embryological period during urethral development, between the eighth and twentieth weeks of gestation, in approximately one in every 300 males (8). Imatinib effects on spermatogenesis appear to be dose-related (5).

C-kit is important for the development of Leydig cells and for migration, proliferation and survival of spermatogonia. Platelet-derived growth factor (PDGF) and PDGF-R are important for the development of both Leydig and myoid cells (9). On the basis of these data, the aim of this study was to examine the effect of Imatinib on luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone concentration, epididymal sperm count, the histological structure of the testis in adult male

Received: 28 Jun 2009, Accepted: 19 Sep 2009
*** Corresponding Address: Biology Department, Faculty of Sciences, Islamic Azad University, Science & Research Campus, Tehran, Iran**
Email: yaghmaei_p@yahoo.com

Royan Institue
International Journal of Fertility and Sterility
Vol 3, No 3, Nov-Dec 2009, Pages: 135-142

Wistar rats and accordingly investigate the biological effects of this compound on male fertility.

Materials and Methods

Animal care

Adult male Wistar rats were maintained at a temperature 23 ± 2°C, 50-55% humidity, and a lighting cycle of 12 hours light:12 hours dark. Commercial rat pellets and water were available ad libitum. Male Wistar rats weighting 200-250 g were used. The animals were randomly divided into four groups (control and three experimental groups, with eight animals in each group). The three experimental groups were gavaged Imatinib at doses of 6, 9 and 12 mg/kg in dH_2O, for 30 days and controls remained intact. The experimental samples were collected on days 7, 14 and 30 after the last day of treatment. The animals received humane care. The experiments were approved by the Research and Ethics Committee of Science and Research branch, Azad University, Tehran, and the experiments were performed according to international guidelines concerning the conduct of animal experimentation.

Imatinib administration

The animals were firmly restrained (the animal was grasped by the loose skin of the neck and back) to immobilize the head and maintained in an upright (vertical) position. The gavage needle was passed through the side of the mouth, followed the roof of the mouth, and advanced into the esophagus toward the stomach. After the needle was passed to the correct length, Imatinib was injected (10).

Biochemical assays

After 30 days of treatment, blood samples were collected from the heart. LH, FSH and testosterone concentrations were measured by the ELISA method. Testosterone: [DRG Instruments GmbH, Germany, ELISA Reader: Stat Fax-2100(AWARENESS)], LH and FSH: [Diaplus Inc. USA, ELISA Reader: Stat Fax-2100(AWARENESS)].

Tissue preparation and histotechniques

Laparatomy was performed and the testis and epididymis were carefully removed and separated. Testes were fixed in fixator for 24 hours, embedded in paraffin, after which 6 µm sections were cut and H & E stained. The sections were observed under light microscope (Lica-1100) for qualitative and quantitative changes in testis structure. The thickness of tunica albuginea (TTA), diameter of seminiferous tubules (DST) and number of spermatogenic cells were determined.

Sperm count

The epididymis was minced in 1 ml phosphate buffered saline (pH 7.2) and a suspension was made. Subsequently, 50 µl of the sample was removed and mixed with 1% aqueous Eosin Y (10:1) and kept for 30 minutes based on the protocol. An aliquot of the epididymal sperm suspension was used for spermatozoa count, using a Neubauer hemocytometer (11).

Statistical analysis

Data were analyzed with SPSS software and all values were calculated from standard errors of means (Mean ± SEM). Statistical analysis was done by one way ANOVA. $p \leq 0.05$ was considered significant.

Results

After drug administration, epididymis samples were taken on days 7, 14 and 30. These days were chosen because the process of spermatogenesis is 14 days in mice and it repeats every 28 days. The primary indicator was calculated on day 7. The results are as follows:

Type A spermatogonia

The numbers of type A spermatogonia in the first, second and third samples of experimental group II ($p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.05$, respectively) and experimental group III ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.01$, respectively) decreased in comparison to the control group (Fig 1).

Type B spermatogonia

A decrease in type B spermatogonia was seen with histological evaluation, however the decrease was not statistically significant (Fig 2).

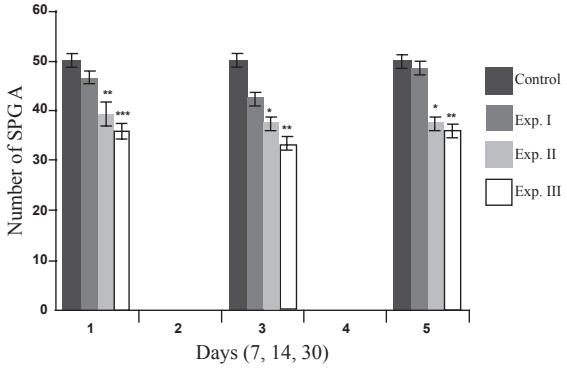

Fig 1: Comparison of results obtained from counting the numbers of type A spermatogonia cells sampled on days 7, 14 and 30. Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with the control.

Primary spermatocyte

In experimental group III, the number of primary

spermatocyte in the first sample was significant ($p \leq 0.01$) and a decrease was seen in the second and third samples (Fig 3).

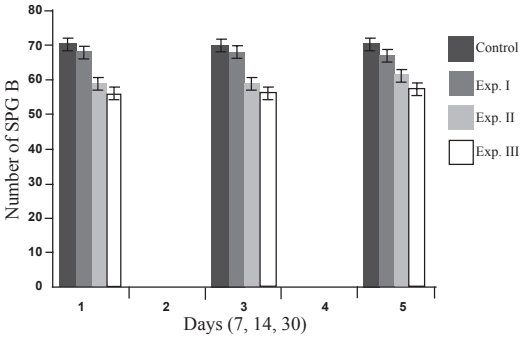

Fig 2: Comparison of results obtained from counting the number of type B spermatogonia cells in three periods of samples (7, 14, 30 days).

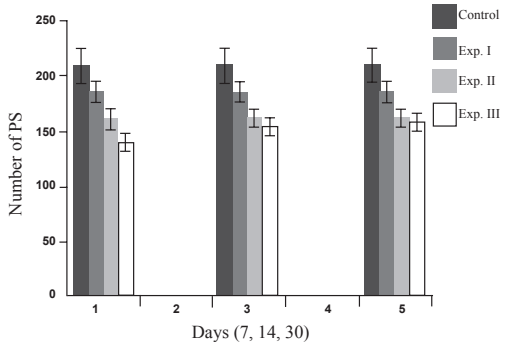

Fig 3: Comparison of results obtained from counting the number of primary spermatocyte cells in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at ** $p \leq 0.01$, compared with control.

Spermatid

The number of spermatid in experimental groups I, II, and III showed a significant decrease when compared to the control group ($p \leq 0.001$). The same result was obtained from the second sample ($p \leq 0.05$), but the decrease was not statistically significant in the third sample (Fig 4).

Sperm

The number of sperm in the epididymis of the first and second samples (days 7 and 14) showed a significant decrease in experimental group III in comparison to the control group ($p \leq 0.01$; Fig 5).

Stem cells

The number of stem cells of the first sample of experimental groups I, II and III showed a decrease when compared to the control group ($p \leq 0.001$). The decrease was not statistically significant in the third sample of experimental group I, however it was significant in experimental groups II ($p \leq 0.05$) and III ($p \leq 0.01$; Fig 6).

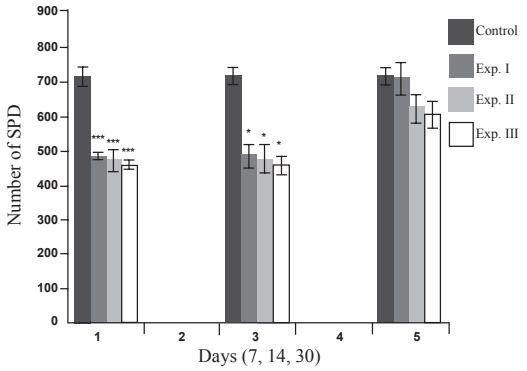

Fig 4: Comparison of results obtained from counting the number of spermatid cells in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at *$p \leq 0.05$, *** $p \leq 0.001$ compared with control.

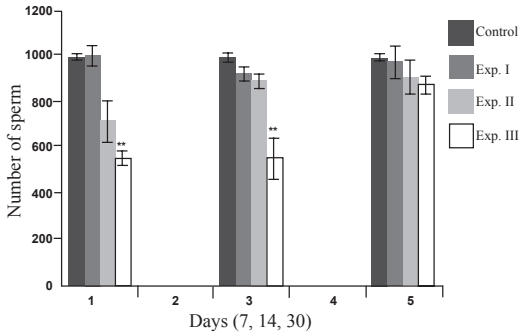

Fig 5: Comparison of results obtained from counting on the sperm of epididmyis in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at ** $p \leq 0.01$, compared with control.

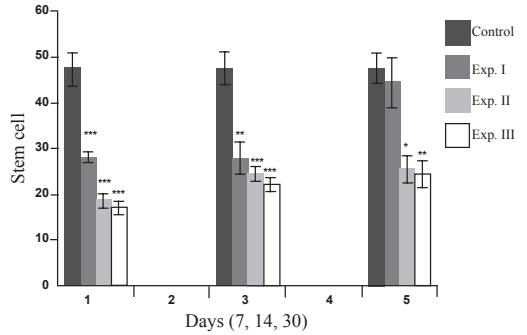

Fig 6: Comparison of results obtained from counting the number of stem cells in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with control.

Leydig cells

The number of Leydig cells of the first experimental group showed a significant decrease ($p \leq 0.01$). As shown in Figure 7, a statistically insignificant decrease was seen in both the second and third samples.

Sertoli cells

The number of sertoli cells in the first and second samples showed a significant decrease in experimental groups I, II and III ($p \leq 0.01$), and the third sample

was decreased in groups II and III ($p \leq 0.05$; Fig 8). One way ANOVA showed a significant difference between the numbers of cells in the seminiferous tubules but there was not a significant relation amongst the numbers of type B spermatogonia cells (Table 1). Figures 18 and 19 show a comparison between the experimental and control groups. The numbers of Leydig cells, type B spermatogonia, primary spermatocyte, spermatid and spermatozoa were less than the control group.

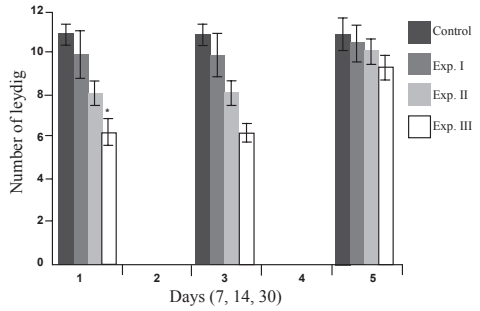

Fig 7: Comparison of results obtained from counting the number of leydig cells in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, compared with control.

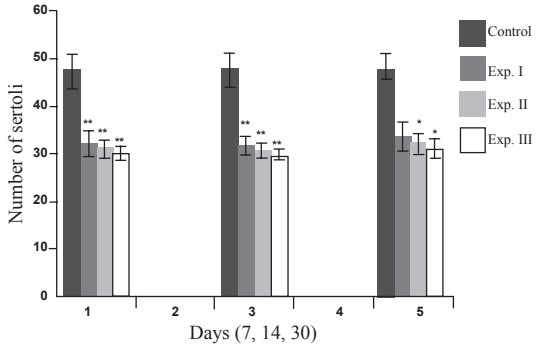

Fig 8: Comparison of results obtained from counting the number of sertoli cells in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, ** $p \leq 0.01$, compared with control.

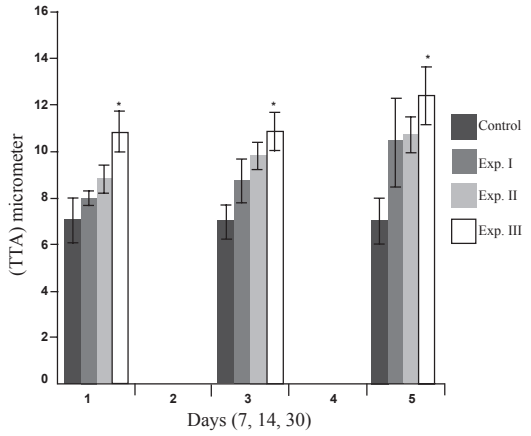

Fig 9: Effect of imatinib on the thickness of tunica albuginea in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, compared with control.

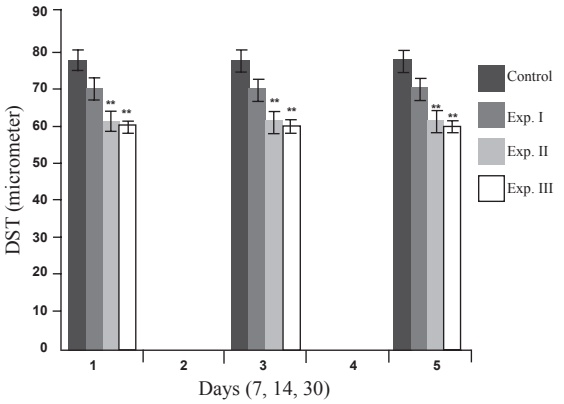

Fig 10: Effect of imatinib on the diameter of seminiferous tubules in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at ** $p \leq 0.01$, compared with control.

Thickness of tunica albuginea

The thickness of the tunica albuginea was increased in all three groups. It was significant in experimental group III with $p \leq 0.05$ as compared to the control group. In experimental groups I and II at days 7, 14 and 30, the thickness of the tunica albuginea showed an increase but it was not statistically significant (Fig 9). Any factor that causes a disorder in spermatogenesis, may cause increase in the tunica albuginea thickness. The effect of this drug (experimental group III) is shown in fig 15 where the thickness of this layer is more than the control group (Fig 14).

Diameter of seminiferous tubules

The diameter of seminiferous tubules in experimental groups II and III showed a significant decrease in the three samples ($p \leq 0.01$) when compared with the control group (Fig 10).

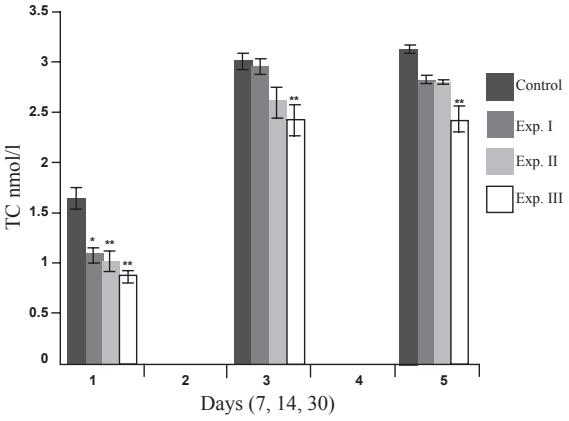

Fig 11: Comparison of results obtained from evaluating the concentration of testosterone hormone in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * $p \leq 0.05$, ** $p \leq 0.01$, compared with control.

Table 1: Effect of Imatinib in three experimental groups [Exp.I (6 mg/kg), Exp.II (9 mg/kg), Exp. III (12 mg/kg)] on the number of cells in the testis.

Number of	Days	Control	Exp.I	Exp.II	Exp.III
			M±SD		
SPGA	7	50.83 ± 1.75	46.16±1.70	39.16 ± 2.83**	35.3 ± 1.08***
	14	50.83 ± 1.75	47.6 ± 2.48	39.6 ± 2.29*	36.8 ± 2.89**
	30	50.83 ± 1.75	50.16 ± 3.13	40.6 ± 2.31*	38.5 ± 1.47**
SPGB	7	70.6 ± 5.4	68.3 ± 4.16	58.16 ± 5.08	56.16 ± 6.01
	14	70.6 ± 5.4	68.3 ± 3.92	59.5 ± 4.68	56.6 ± 4.40
	30	70.6 ± 5.4	67 ± 3.52	61 ± 5.31	58.83 ± 2.19
Primary spermatocyte	7	209 ± 22.5	180.3 ± 9.14	166.3 ± 6.94	132.8 ± 3.6**
	14	209 ± 22.5	180.6 ± 9.23	170.5 ± 17.2	165.16 ± 11.3
	30	209 ± 22.5	186.83 ± 7.79	168.16 ± 9.3	166.16 ± 10.04
Spermatid	7	717.5 ± 34.41	503 ± 7.43***	486.33 ± 36.72*	458.5 ± 17.35
	14	717.5 ± 34.41	506.3 ± 75.05***	499.5 ± 61.14*	485.16 ± 33
	30	717.5 ± 34.41	712 ± 65.09***	605 ± 58.5*	575 ± 54.9
Sperm	7	1002.83 ± 14.7	1005.16 ± 60.3	703.3 ± 119.2	564 ± 31**
	14	892.3 ± 38.8	849.1 ± 30.9	556.8 ± 83.1**	1002.83 ± 14.7
	30	1002.83 ± 14.7	1005.16 ± 60.3	730.3 ± 119.2	564 ± 31
Stem cell	7	47.16 ± 4.46	26.83 ± 1.68***	19.3 ± 1.83***	16.6 ± 1.28***
	14	47.16 ± 4.46	25.6 ± 3.69**	21.83 ± 1.6***	18.83 ± 2.1***
	30	47.16 ± 4.46	44 ± 6.16	24 ± 4.47*	22.5 ± 3.68**
Leydig	7	10.83 ± 0.83	9.16 ± 1.2	8.3 ± 0.71	6.8 ± 0.79*
	14	10.83 ± 0.83	10.16 ± 1.07	8.6 ± 1.25	7.16 ± 0.54
	30	10.83 ± 0.83	10.3 ± 0.98	10 ± 1.06	8.83 ± 0.7
Sertoli	7	47.83 ± 3.52	33 ± 3.1**	31.83 ± 2.3**	30.6 ± 1.9**
	14	47.83 ± 3.52	31.83 ± 2.4**	30.66 ± 2.72**	29.5 ± 2.84**
	30	47.83 ± 3.52	35.16 ± 4.02	33.5 ± 3.14*	32.6 ± 2.1*

Values are expressed as Mean ± SEM, significant at *: p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, as compared with the control.

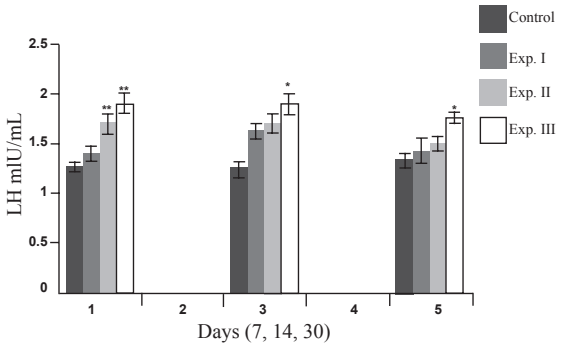

Fig 12: Comparison of results obtained from evaluating the concentration LH in three periods of samples (7, 14, 30 days). Values are expressed as Mean ± SEM. Significant at * p ≤ 0.05, ** p ≤ 0.01, compared with control.

Testosterone

The concentration of testosterone in the first sample of experimental group I was significant ($p \leq 0.05$) as well as in the second and third samples of experimental group III with $p \leq 0.01$ (Fig 11).

Table 2 shows the thickness of the tunica albuginea and diameter of seminiferous tubules.

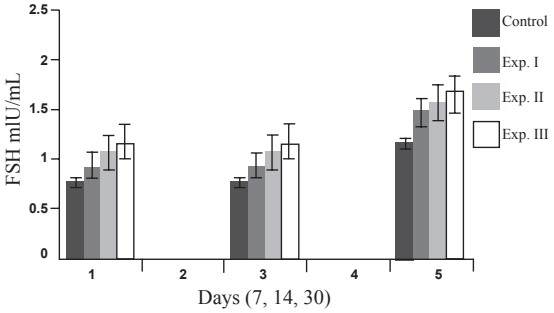

Fig 13: Comparison of results obtained from evaluating the concentration FSH in three periods of samples (7, 14, 30 days).

Table 2: Effect of Imatinib in three experimental groups [Exp.I (6mg/kg), Exp.II (9mg/kg), Exp.III (12 mg/kg)] on the thickness of tunica albuginea and the diameter of seminiferous tubules.

Number of	Days	Control	Exp.I	Exp.II	Exp.III
			M ± SD		
TTA (μμ)	7	7 ± 1	8.05 ± 0.39	9.03 ± 0.78	10.8 ± 1.07*
	14	7 ± 1	8.8 ± 0.82	9.8 ± 0.57	11 ± 0.96*
	30	7 ± 1	10.76 ± 1.9	11 ± 1.06	12.8 ± 1.5*
DST (μμ)	7	77.1 ± 2.7	69.8 ± 2.8	62.8 ± 2.7**	62 ± 1.9**
	14	77.1 ± 2.7	69.3 ± 3.02	63.1 ± 2.7**	61.8 ± 2.3**
	30	77.1 ± 2.7	69.4 ± 1.3	63.5 ± 2.8**	62.8 ± 2.7**

*Values are expressed as Mean ± SEM, significant at * $p \leq 0.05$, ** $p \leq 0.01$, as compared with the control.*

Table 3: Effect of Imatinib in three experimental groups [Exp. I (6 mg/kg), Exp. II (9mg/kg), Exp. III (12 mg/kg)] on T (Testostrone), LH, FSH.

Number of	Days	Control	Exp.I	Exp.II	Exp.III
			M ± SD		
T (nmol/l)	7	1.63 ± 0.12	1.06 ± 0.08*	1 ± 0.11**	0.76 ± 0.06**
	14	2.93 ± 0.08	2.83 ± 0.08	2.43 ± 0.12	2.23 ± 0.14**
	30	3.2 ± 0.05	2.8 ± 0.11	2.76 ± 12	2.33 ± 0.14**
LH (mlU/mL)	7	1.2 ± 0.05	1.4 ± 0.05	1.6 ± 0.08**	1.83 ± 0.06**
	14	1.2 ± 0.2	1.6 ± 0.11	1.6 ± 0.08	1.9 ± 0.05*
	30	1.2 ± 0.05	1.4 ± 0.05	1.6 ± 0.08	1.83 ± 0.06*
FSH (mlU/mL)	7	0.8 ± 0.03	0.93 ± 0.08	1.06 ± 0.14	1.16 ± 0.17
	14	0.76 ± 0.06	0.9 ± 0.15	1 ± 0.15	1.13 ± 0.14
	30	1.1 ± 0.05	1.5 ± 0.15	1.6 ± 0.2	1.8 ± 0.25

*Values are expressed Mean ± SEM, significant at * $p \leq 0.05$, ** $p \leq 0.01$, as compared with the control.*

Luteinizing hormone

The concentrations of LH in experimental groups II and III increased ($p \leq 0.01$). It was also statistically significant in second and third samples of experimental group III ($p \leq 0.05$; Fig 12).

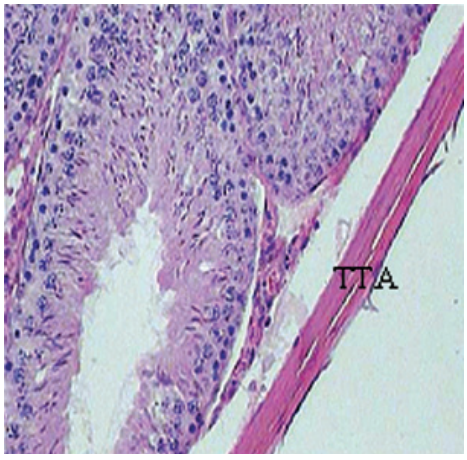

Fig 14: Effect of imatinib on thickness of tunica albuginea in the control group (H&E, × 400).

Follicle stimulating hormone

The concentration of FSH in all experimental groups (I, II, III) increased but it was not statistically significant (Fig 13). One-way ANOVA showed a significant concentration difference in testosterone and LH levels between experimental groups and control group, however there was no statistically significant difference in FSH concentration (Table 3).

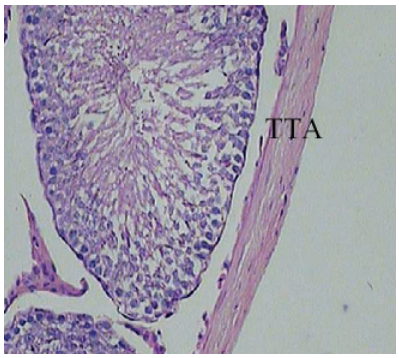

Fig 15: Effect of imatinib on the thickness of tunica albuginea in the group which received 12 mg/kg (Exp III; H&E, × 400).

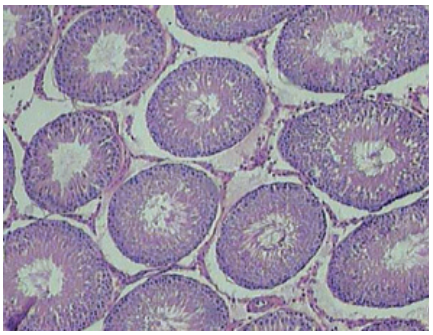

Fig 16: Effect of imatinib on the structure of seminiferous tubules in the control group (H&E, × 100).

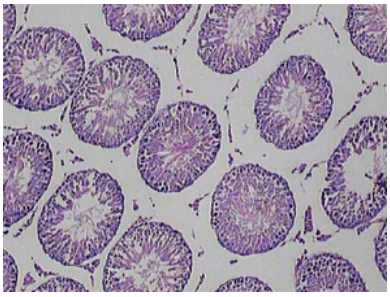

Fig 17: Effect of imatinib on the structure of seminiferous tubules in experimental group II (H & E, × 100).

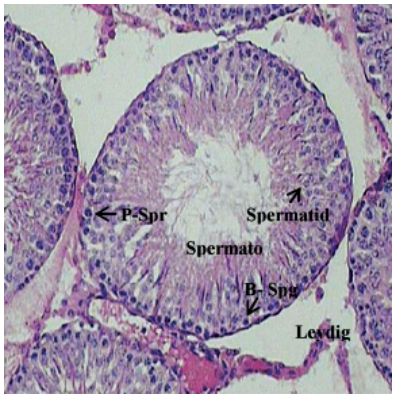

Fig 18: Effect of imatinib on the structure of seminiferous tubules in the control group (H & E, × 400).

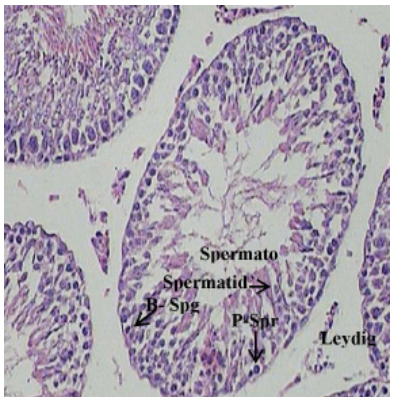

Fig 19: Effect of imatinib on the structure of seminiferous tubules in the group which received 9 mg/kg (Exp. II; H & E, × 400).

Discussion

Imatinib influences the quality and quantity of spermatogenesis and embryogenesis. According to our findings, the spermatogenesis of rats under treatment with Imatinib changed during the experimental period and returned to normal conditions after cessation of treatment. These results indicate a time and dose dependent relationship. The abovementioned results are similar to results presented by Senshardi (12). Those patients who take Imatinib and want to have a child should carefully consider the time and dose of this drug. Therefore, they should be advised by their consultants in order to determine the exact time which is safe to have a child.

Any malformation in spermatogenesis that is a result of Imatinib usage is related to drug dosage and duration of exposure. It is hoped that the adverse effects of Imatinib on patients' spermatogenesis would return to normal once they stop taking the drug.

Alut in 2006 reported the outcome of pregnancies involving eight male partners (median age 35 years; range: 26-38 years) who took a median dose of Imatinib 700 mg/d with an exposure period of 20 months. Of the eight reported pregnancies, there was one case of spontaneous abortion. Of the seven successful pregnancies, there was one baby born with gut malrotation who needed surgical intervention (7).

Hensley and Ford in 2003 reported four normal pregnancies, two therapeutic abortions, one spontaneous abortion and one death in-utero at 13 weeks; in eight of thirteen complete pregnancies involving male patients on Imatinib therapeutic trials (5, 13). Preliminary data from various reports of pregnancies among women whose male partners were taking Imatinib showed no impairment of spermatogenesis during Imatinib therapy (5), however this report does not support our findings in this research. Oligospermia has been reported in one patient on Imatinib at a dose of 800 mg/d for at least six months for chronic eosinophilic leukaemia (12). This report supports our investigations. Three days treatment of immature male rats with Imatinib (150 mg/kg) on postnatal days 5-7 delayed the formation of the germ-line stem cell pool, reduced proliferation of type A spermatogonia and induced germ cell apoptosis. PDGFR-mediated proliferation of mesenchymal myoid precursors also decreased and the length of the seminiferous cord was reduced (9), which also supported our findings.

Male rats exposed to 75% of the maximum human equivalent dose of 800 mg/d for about 70 days prior to mating had lower a epididymal and tes-

ticular weight along with reduction in the number of motile sperm. Summary product characteristics recommend strict avoidance in pregnant women (13). To date, there are limited data available on the outcome of pregnancies that occur when the male partner is receiving Imatinib (13). Information about the effects of drugs on the developing embryo is derived primarily from animal experiments and case reports describe the outcomes of pregnancies complicated by drug use. Some drugs can be teratogenic in certain animal species, but not in humans. In preclinical studies, Imatinib has been found to be teratogenic in rats, but not in rabbits. Impaired spermatogenesis was observed in rats, dogs, and monkeys; however, there was no evidence that Imatinib was genotoxic.

These observations lead to concerns that men treated with Imatinib may have reduced sperm counts. Clinical experience has not shown this to be true because male patients who were receiving Imatinib were partners in 18 successive pregnancies and four healthy infants. Owing to teratogenicity data in rats, it is recommended that women treated with Imatinib be aware of the potential teratogenicity of Imatinib, and effective contraception should be used during Imatinib therapy to prevent pregnancy (4). The previous study showed that, a period of two week exposure of female rats to Imatinib influenced the proliferation and ovarian follicular development, and in addition to sexual hormones levels and fertility (14). Imatinib has been used primarily for cancers such as CML, which demonstrates that tyrosine kinase inhibitors can have a wide therapeutic window and heralds the era of targeted cancer and noncancerous disease therapy (4).

Conclusion

The true incidence, possible dose dependence and reversibility of Imatinib-induced testis failure should be examined in future studies. Awareness of this potential complication will enable physicians to offer patients appropriate counseling and to consider strategies of preserving fertility and testis function before embarking on Imatinib therapy.

Acknowledgements

This work was supported by the Science and Research Center at Islamic Azad University. There is no conflict of interest in this article.

References

1. Droogendijk HJ, Kluin-Nelemans HJ, Van Doormaal JJ,Oranje AP, Van Delosdrecht AA, Van Daele PL. Imatinib mesylate in the treatment of systemic mastocy tosis a phase II trial. Cancer. 2006; 15: 107(2): 345-351.

2. Cools J, DeAngelo DJ, Gotlib J. A tyrosine kinase created by Fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl Med. 2003; 348: 1201-1214.

3. Krause DS, VanEtten RA. Tyrosine kinases as targets for cancer therapy. N Eng1 J Med. 2005; 353: 172-187.

4. Ali R, Ozkalemkas F, Ozcelik T, Ozkocaman V. Imatinib and pregnancy. J clin oncol. 2006; 24: 3812-3813.

5. Hensley ML, Ford JM. Imatinib treatment: Specific issurs related to safety, fertility, and pregnancy. Semin Hematol. 2003; 40: 21-25.

6. Choudhary DR, Mishra P, Kumar. Pregnancy on imatinib: Fatal outcome with meningocele. Ann oncol. 2006;17: 178-179.

7. Ault P, kantarjian H, O'Brien S, Faderl S, Beran M, Rios M, et al. Pregnancy among patients with chronic myeloid leukemia treated with imatinib. J Clin Oncol. 2006; 24: 1204-1208.

8. Duckett JW. Hypospadias. In: Walsh PC, Retik AB, Vaughn ED, Wein AJ, (eds). Campbell's Urology. 8th ed. Philadelphia, Pa: Elsevier; 1998; 2033- 2119.

9. Nurmio M, Toppari J, Zaman F, Andersson AM, Parankoj, Soder O, et al. In hibition of tyrosinc kinases PDGER and C-kit by imatinib mesylate interferes with postnatal testicular derelopment in the rat. Int J Androl. 2007; 30(4): 366-376.

10. Lu D, Qu C, Goussev A, Jiang H, Lu C, Schallert T, et al. Statin Increase Neurogenesis in the Dentate Gyrus, Reduce Delayed Neuronal Death in the Hippocampal CA3m Region, and Improve spatial Learning in Rat after Traumatic Brain Injury. J Neurotrauma. 2006; 24(7): 1132-1146.

11. D'sovza, Urban J.A . Toxic effects of 5-fluorouracil on sperm count in wistar rats. MJMS.2003; 10: 43-45.

12. Senshadri T, Seymour JF, McArthur GA. Oligospermia in a patient receiving imatinib therapy for the hypereosinophilic syndrome. N Engl J Med. 2006; 351:2134-2135.

13. Ramasamy K, Hayden J, Lim Z, Mmufti JG. Successful pregnancies involving men with chronic myeloid leu kaemia on imatinib therapy. Br J Haematol. 2007; 137: 374 383.

14. Yaghmaei P, Parivar K, Jalalvand F. Effect of imatinib on the Oogenesis and pituitary-ovary hormonal axis in female wistar rat. IJFS. 2009; 3: 11-16.