



A histological study on the effect of imatinib on the rats' testis after early postnatal exposure

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Abstract

The safety region of imatinib, and markedly its role in testicular toxicity has been studied in a controversial manner in current years. This study was designed to address the repercussion of administration of groups of pups with imatinib mesylate (at neonatal or infantile periods) using histological analysis of their testes in several end points and in adulthood. Intact pups (albino-male) exposed to 200 mg/kg of oral imatinib once daily at neonatal and early infantile period on PND 1 to PND10 (for ten days). All experiments performed with age and weight matched control which administered with Distilled water. Pups were into categorized into 4 subgroups, according to the ages of euthanasia: 15 days postpartum (PND); 40days postpartum; 70 days postpartum and 140 days postpartum. The histological analysis was conducted in blind fashion after staining with (Harris Hematoxylin and Eosin stain. Ten randomly selected testicular sections from each rat were analyzed qualitatively and quantitatively. In addition, Johnsen' scores were used to analyze the effect of drug on spermatogenesis. Data were recorded and value is considered as significant when it $P < 0.05$. imatinib induced several alterations in testicular sections in comparison with those of control group including significant reduction of number of peripheral germ cells layers at PND 15. Different histological changes were more frequent in tissues obtained from rats euthanized at PND 40 and PND70. Quantitative changes were also noticed as diminishing of seminiferous tubular diameter of the, height of epithelial layer especially at PND70. Partial recovery was noticed in sections of rats euthanized at PND140. In conclusion, imatinib does affect the histology of rat testis markedly, but this effect is reversible once the drug administration is ceased. That may provide a benefit for the specialists in planning and addressing of the fertility associated issues in young patients - throughout their reproductive periods- who are being on imatinib treatment for gastrointestinal tumors and chronic myeloid leukemia (CML).

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Introduction

Malignancy is the second dominant reason of mortalities all over the world. Although cancer commonly affects people after they have completed their families, a serious minority are diagnosed at a younger age (1). In spite of the fact that the incidence of cancer in children all over the world is low, the age-optimized frequency is seventy to 160 newly diagnosed cases for each millions of children per year at the

age of zero to 14 (2). These patients have to complaint not only with the effect of having cancer and the immediate complications of treatment, but also with the possibility that surgery, radiotherapy or chemotherapy may lead to temporary or permanent squeal as gonadal damage, infertility, and organs toxicity (3,4). Imatinib (1st generation Tyrosine kinase inhibitors (TKIs)) is a recent anticancer agent that is strongly inhibited human BCR-ABL1-positive tumor (5,6), that make it a particularly suitable well planned

agent for the treatment CML and ALL. Imatinib also induced an inhibitory action on the expression of c-Kit -TK receptors in the alimentary tract which is contributed in the pathogenesis of gastrointestinal stromal tumor-GIST (7,8).

In addition, imatinib induces an inhibition to the PDGF- α and PDGF- β receptors (9) which may allow more therapeutic using. The major adverse effects enroll severe neutropenia and thrombocytopenia, oedema, fluid retention, nausea, mild diarrhoea, skin rashes, arthralgia, myalgia, bone pain, acute renal failure (10,11), and hepatotoxicity (12). The safety margin of imatinib, and liability to induce organ toxicity, has been studied in a conflicting manner in current years (13). There is a report of the GIST patient or CML patient (males) suffered from with gynaecomastia and hydrocele following exposure to imatinib mesylate (14). Little is known about the effect of early exposure to imatinib. This work aims to evaluate the repercussion of exposure (in vivo) of male albino rats to imatinib mesylate (at neonatal or infantile periods) using histological analysis of their testes in several end points and in adulthood using rat which is preferred as a suitable and an attractive alternate animal model for human.

Materials and methods

This experimental work was conducted on rats (male albino) purchased from Animal House of Veterinary College, university of Mosul, Mosul, Iraq.

Throughout the investigations the rats were breed under controlled normal environmental laboratory conditions and animal facility and were housed in an air-conditioned room with 12-hours light and dark cycles, where the temperature $23 \pm 2^\circ\text{C}$ and relative humidity 66-71% were kept stable. They were put in an individual manner in plastic cages (England) measuring $47 \times 34 \times 18$ cm lined with wood chips (15) provided from Animal House. Animals were let to be comfort for a ten days before any procedure was performed (16), and supplied with free access of water ad libitum and pelleted standardized food (commercial rodent chow) (17). All rats received humane care, and procedures enrolling animals and their care were conducted in conformity with international roles and programs and the articles on animals accepted. The experiments were performed during the light section (18,19).

Animals were randomly assigned. Intact pups exposed to oral imatinib (Glivec[®], STI 571; Novartis- purchased from Ibn-Sena Teaching Hospital, Mosul) at neonatal and early infantile period on PND 1 to PND10 (n=32) using gavage with 24 gage needle and they were represented the treated group. They were received imatinib 200 mg/kg once daily for 10 days starting from PND 1 to PND10. While control group includes age and weight matched pup which received D. W following the same program applied to imatinib group (n=4 for each). Each treated pup was observed for clear signs

of toxicity for the next four hours, and mortality throughout the next 24hours (20).

Imatinib doses choosen were justified to be in accordance with those used in clinical treatment regimens (21) (400-800 mg/d or 340-590 mg/m² as the weight is 70 kg) dose surface area according to body-weight, $f \times \text{mg/kg} = \text{mg/m}^2$, f is a factor equaling to 6. 0 in rats (22).

Pups were categorized into 4 subgroups, according to the ages of euthanasia: 15 days postpartum (n=8); 40days postpartum (n=8); 70 days postpartum (n=8) and 140 days postpartum (n=8) (18).

Euthanization of rat with ether (19,23) was done 24 h after the final dose was given at laboratory of postgraduate studies of Department of Anatomy, Nineveh College of Medicine, University of Nineveh, Mosul, Northern Iraq.

After longitudinal incision gonads were excised and fixed in Bouin's solution (24). The histological analysis was conducted after processes of fixation, dehydration, clearing, impregnation and embedding in paraffin. Then tissues were submitted to sectioning and staining (Harris Hematoxylin and Eosin stain (H&E) Ten randomly selected testicular sections from each rat were analyzed blindly for any disorganization of cytoarchitecture of seminiferous tubules, evidence of vacuoles, gaps and abnormal cells in the seminiferous epithelium and sloughed cells, degeneration of seminiferous epithelium and interstitial spaces in order to achieve qualitative assessment (25).

On the other hand, sections were examined quantitatively (via visopan Reichert, Austria) where the diameter of seminiferous tubules (STD), epithelial height of seminiferous tubules (SEH), mean number of Sertoli cells and Leydig cell were obtained using eyepiece (16), and counted in fifty tubules of each sample at 400X magnification (26,27).

In addition, Johnsen' scores were used to analyze the effect of drug on spermatogenesis (28,29) The photomicrographs will be captured via Leica microscope with digital camera installed using planapochromatic objectives.

Data were recorded using excel program. Data were analyzed using Microsoft SPSS software version 17. Value is considered as significant when it $P < 0.05$.

Results

All animals remain alive during the experimental work. At necropsy, no clear gross tissue abnormalities were observed of any animal. The histopathological analysis of the testes of pups that received repeated doses of imatinib at neonatal period or at neonatal /infantile periods showed that the by PND15, loss of differentiated germ cells was shown in testicular sections from treated animals (Figure 1).

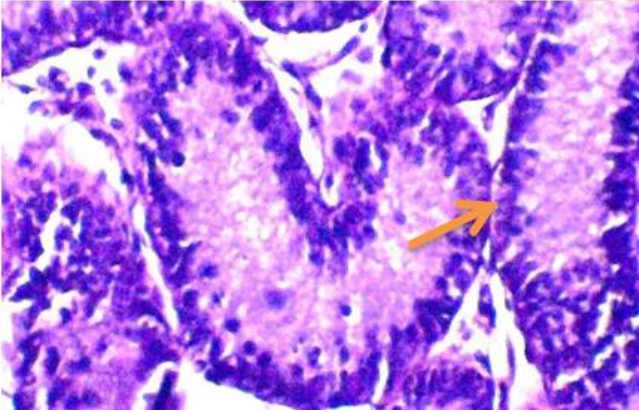


Figure 1: A photomicrograph of a section obtained from rats at PND15 after treatment with 200mg/kg/day of imatinib on PND1-PND10, proliferation of spermatogonia is noticed (red arrow) (H&E×250).

At PND40, sections of control rats showed normal architecture of seminiferous tubule and interstitial tissue (Figure 2). While disturbance in the arrangement of the seminiferous tubules, germ cells depletion, epithelial cells sloughing, intraepithelial cells spaces, an appearance of some multinucleated cells and some apoptotic cells, and interstitial edema were the most evident features of testicular sections of treated animals. Sertoli cell also showed alterations in their morphology in these rats. Their nuclei showed abnormal shape and some of them were far from the basal membrane or even in the tubular lumen. Finally, Sertoli cell only tubular sections were seen (Figure 2) while an appearance of eosinophilic bodies was also noticed in some sections (Figures 2-5) (Table 1).

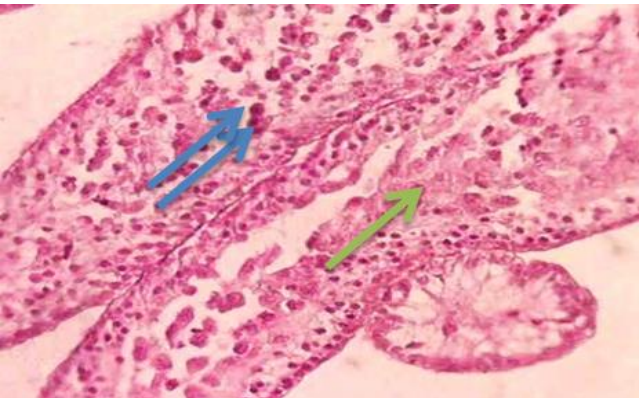


Figure 2: A photomicrograph of a testicular section obtained from rats at PND40 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with disturbed seminiferous tubules, depleted germ cells, presence of vacuolated and detached Sertoli cells (green arrow), multinucleated cells (double blue arrow). Sertoli only cells feature is seen in one of the tubules (H&E×250).

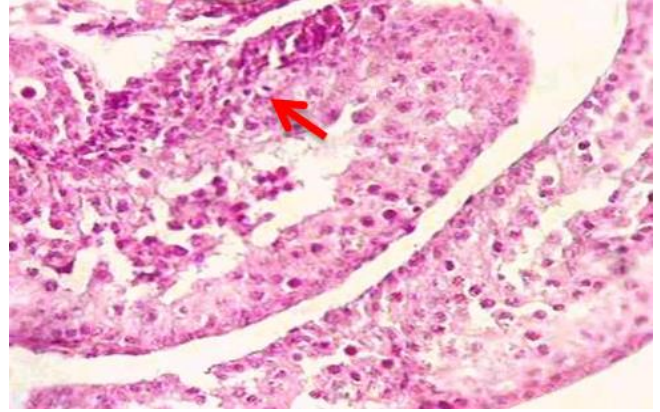


Figure 3: A photomicrograph of testicular section obtained from rats at PND40 after treatment with imatinib on PND1-PND10 with disorganized cells and apoptosis (arrow) (H&E×250).

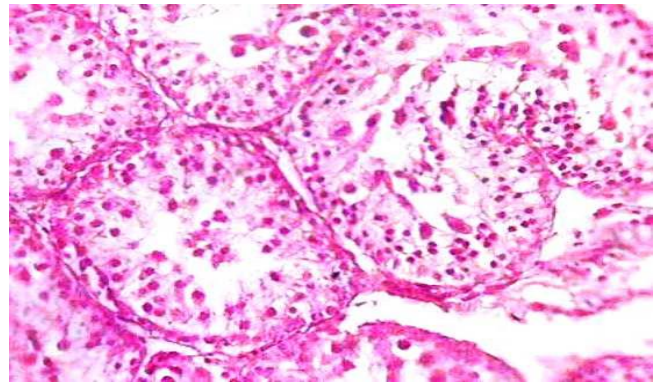


Figure 4: A photomicrograph of a testicular section obtained from rats at PND40 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with germ cells depletion (H&E×250).

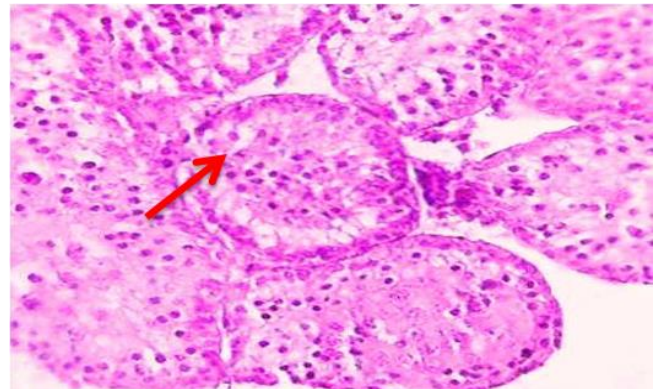


Figure 5: A photomicrograph of a testicular section obtained from rats at PND40 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with vacuolation (arrow) of Sertoli cells (H&E×250).

In addition, The Seminiferous tubular diameter of this group was significantly reduced mean $117.2 \pm 2.5 \mu\text{m}$. The epithelial height of the tubules was significantly reduced at this group mean $17.3 \pm 3.1 \mu\text{m}$. These sections revealed that mean number of Sertoli cells/seminiferous tubule was 10.5 ± 2.2 , while that of Leydig cells was 3.0 ± 1.6 ., however, some sections exhibited Sertoli only cells feature (Figure 2) (Table 1 and 2).

Qualitative Analysis of Testicular sections obtained from rats euthanized at PND70 reveals intense several alterations (Table 1). The most obvious features were germ cells loss and sloughing, disorganized appearance of the seminiferous tubules, detachment of some seminiferous tubules from their basement membrane, presence of vacuolated and detached Sertoli cells, some multinucleated cells were noticed, some apoptotic cells were seen, interstitial edema, and retention of elongated spermatid (Figures 6-9). Moreover, histological analysis demonstrated that these animals presented decreased Johnsen's scores in relation to those in control animals (Table 1).

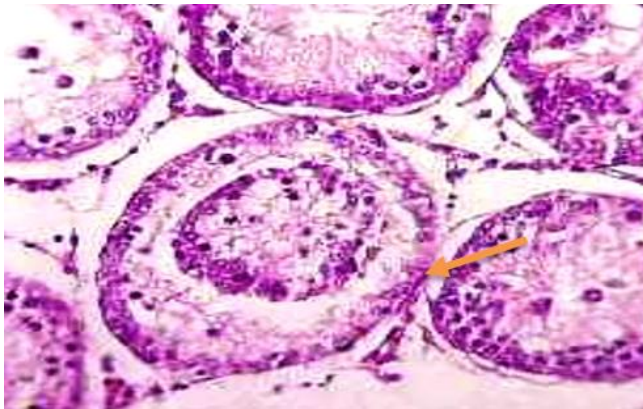


Figure 6: A photomicrograph of a testicular section obtained from rats at PND70 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with disorganized appearance of the seminiferous tubules and some multinucleated cells (red arrow). (H&E \times 250).

Quantitative analysis

The seminiferous tubular diameter and their epithelial height of this subgroup at PND70 were diminished in comparison to those in the control rats, with mean of $125 \pm 4.1 \mu\text{m}$ and $7.0 \pm 1.1 \mu\text{m}$ respectively. Moreover, the number of Sertoli cells per seminiferous tubule was raised with mean of 18.2 ± 0.2 . Mean Leydig cells number was 7.0 ± 0.1 (Table 2).

At PND140 the Qualitative Analysis of testicular sections revealed presence of intraepithelial spaces, sloughing of epithelial cells, arrest at spermatid stage and disorganized appearance of the seminiferous tubules. Johnsen's scores of 5.1 ± 0.9 indicated incomplete recovery of spermatogenesis (Figure 10).

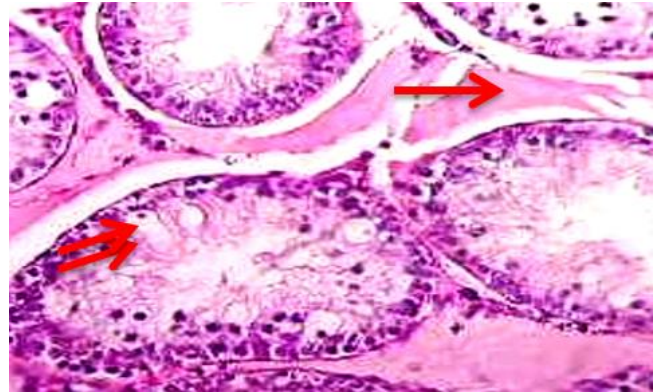


Figure 7: A photomicrograph of a testicular section of rat at PND70 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with germ cells depletion, interstitial edema (arrow), and vacuolation (double arrow) (H&E \times 250).

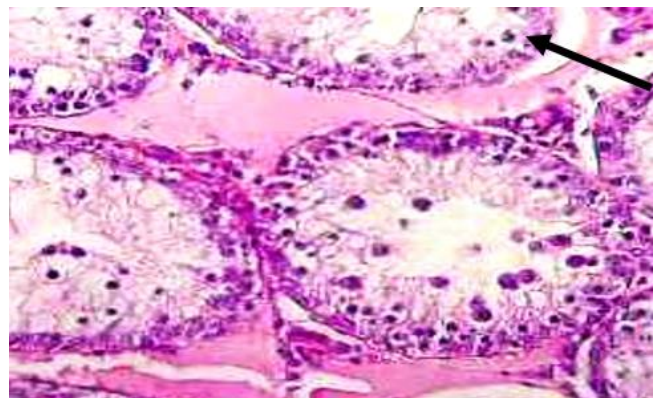


Figure 8: A photomicrograph of a testicular section of rat at PND70 after treatment with 200mg/kg/day of imatinib on PND1-PND10 with germ cells depletion, interstitial edema. Detached tubule from their basement membrane (arrow) (H&E \times 250).

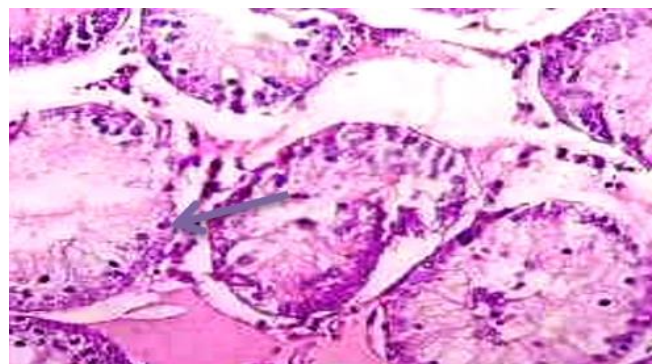


Figure 9: A photomicrograph of a section of rat at PND70 after treatment with of imatinib at PND1-PND10 with disorganization of the seminiferous tubules and germ cells depletion (arrow) (H&E \times 250).

Table 1: early and late term effects of imatinib on testicular histology

Histological changes	P40	P70	P140	P-Value
Disruption of normal cytoarchitecture	4 (50. 0%)	5 (62. 5%)	2 (25. 5%)	P<0.05
Depletion of germ cell layer	5 (62. 5%)	5 (62. 5%)	2 (25. 5%)	P<0.05
Detachment of germ cell layer	4 (50. 0%)	5 (62. 5%)	1 (12. 5%)	P<0.05
Sloughing of germ cells toward the lumen	4 (50. 0%)	5 (62. 5%)	2 (25. 5%)	P<0.05
Vacuoles in the germinal layer	4 (50. 0%)	5 (62. 5%)	1 (12. 5%)	P<0.05
Multinucleated giant cells	3 (37. 3%)	5 (62. 5%)	1 (12. 5%)	P<0.05
Apoptosis	3 (37. 3%)	3 (37. 3%)	1 (12. 5%)	P<0.05
Degenerated seminiferous tubules	1 (12. 5%)	2 (25. 5%)	1 (12. 5%)	P>0.05
Thick basement membrane	1 (12. 5%)	2 (25. 5%)	1 (12. 5%)	P>0.05
Vaculation	3 (37. 3%)	4 (50. 0%)	1 (12. 5%)	P<0.05
Sertoli cell Non-nucleated S cell abnormalities	3 (37. 3%)	4 (50. 0%)	1 (12. 5%)	P<0.05
Detached cell	3 (37. 3%)	4 (50. 0%)	1 (12. 5%)	P<0.05
Sertoli only cell syndrome	1 (12. 5%)	0 (0. 0%)	0 (0. 0%)	P>0.05
Thick Tunica albuginea	1 (12. 5%)	1 (12. 5%)	0 (0. 0%)	P<0.05
Dilated blood vessels	1 (12. 5%)	1 (12. 5%)	0 (0. 0%)	P<0.05
Interstitial edema	3 (37. 3%)	5 (62. 5%)	1 (12. 5%)	P<0.05
Inflammatory cells	0 (0. 0%)	0 (0. 0%)	0 (0. 0%)	P>0.05
Congested blood vessels	1 (12. 5%)	1 (12. 5%)	1 (12. 5%)	P>0.05
Hemorrhage	0 (0. 0%)	0 (0. 0%)	0 (0. 0%)	P>0.05
Johnsen's Scores		4. 5±0. 4	5. 1±0. 9	P>0.05

*P-value is considered as significant when P<0.05.

Table 2: Effects of imatinib on some testicular morphological parameters at various endpoints

Parameter	P40	P70	P 140	P-Value
Diameter of the seminiferous tubules (µm)	117. 5±2. 5	125±4. 1	185±2. 1	P<0.05
Height of the epithelial layer (µm)	17. 3±3. 1	7. 0±1. 1	60±2. 1	P<0.05
Sertoli cell /Seminiferous tubule	10. 5±2. 2	18. 2±0. 2	12. 9±0. 3	P<0.05
Leydig cell	3. 0±1. 6	7. 0±0. 1	5. 0±0. 9	P<0.05

*P-value is considered as significant when P<0.05.

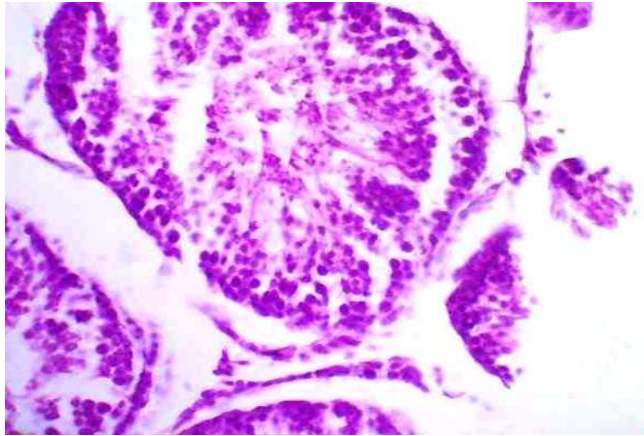


Figure 10: A photomicrograph of a testicular section of rat at PND140 treated with imatinib at PND1-PND10 with disorganization of the seminiferous tubules and germ cells depletion (H&E×400).

Quantitative analysis

The seminiferous tubular diameter and their epithelial height of this subgroup were reduced if compared with those in the control rats, with mean of 185±2.1 µm and 60.3±2.1 µm respectively. Moreover, the mean number of Sertoli cells/ seminiferous tubule is 12.9±0.3. Mean Leydig cells number was 5.0±0.9 (Table 2).

Discussion

Recently imatinib is the only TKI that is used for pediatric patients. It was chosen in treatment of children with Ph+ CML that labels for only for 2% of all leukemia in children. It is also accounted to manage pediatric GIST tumors (30). Answering the question if these new agents result harmful effects on the reproductive system at its development is a critical aspect of preclinical drug stages, especially if the agent is to be entitled for in patients with young. Ages. However, testing for a chronic term impact on testicular histology in some mammals in vivo is not simple,

even in rats, very frequently used animals to explore its toxic effects (6).

In this study morphological analysis of testicular sections was performed to clarify if imatinib makes a disturbance in the development of the spermatogonial stem cell pool. In control group, the centrally localized germ cells transfer to the basement membrane throughout the 1st postnatal days and forming a spermatogonial stem cell pool. The current work showed that repeated imatinib dosing starting on postnatal days 1-10 (200 mg/kg /once/day/10) prevented this migration and subsequently, significantly diminished number of peripheral germ cells. Further, germ cells localized at center underwent proliferation in adluminal parts and the number of centrally localized germ cells raised in sections of pups at PND15. These findings are similar to those of Basciani *et al.* (31) who attributed that to apoptosis in germ cells. Different histological changes were more frequent in sections obtained from rats euthanized at PND40 and at PND70, these findings are similar to those of other workers, who reported that prepubertal doxorubicin administration induce adverse effects on testicular histology at age of seventy postnatal days (32). This study showed that the persistence of harmful effects on rats after treatment at neonatal or early infantile period is similar to other studies which attributed that to the irreversible inhibition of TKR, c-kit and PDGFR which induces disturbance in migration (33), proliferations and the survival (34) of germ cells. Further, PDGFR-mediated proliferation of peritubular myoid cells was prevented leading to decreased growth of the seminiferous tubules longitudinally. Concerning that in humans gonocytes migration is mainly finished at birth, the impact of imatinib on somatic stem cells and spermatogonia, the liable or possible cellular galls for younger males during imatinib therapy, has remained a critical and challenging issue (32,35).

Irreversible damage happens if Sertoli cells cannot provide a support for stem cell spermatogonia or if the whole stem cell spermatogonia number is reduced. While the impacts of cancer therapy become immediately identifiable in the testes of adults, the sequel of such treatments in immature males are remaining not obvious till puberty. Recently, exploration of gonadal damages cannot be done prior to this (6,35).

One of the changes associated with imatinib treatment is the appearance of multinucleated cells which are present in sections of 3 (37. 3%), 5 (62. 5%) rats at PND40, PND70 respectively. These findings are in accordance with those of another author (17) and those of Cerebasi *et al* (36), who considered formation of multinucleated cells in testicular sections of rats after administration with 5-fluorouracil and cyclophosphamide respectively as a marker of testicular injury (36,37).

To our knowledge the mechanisms of formation, shape and fate of abnormal germ cells have not been previously demonstrated in imatinib treated animals. Authors discussed

the pathogenesis of these cells in rat testis exposed to 5-FU, and they reported that multinucleated cells were present in the seminiferous epithelium, tubular lumen and exhibited a positive correlation between the number these cells and epithelial sloughing. The present work showed the appearance of fusion of nuclei, which may represent the continuous process of cell fusion in the epithelium, these findings are in accordance with those of other authors (36) who exposed rats to cyclophosphamide. On the other hand, eosinophilic bodies in some testicular section from imatinib treated rats were shown. These findings are similar to those of other researchers (17,37). They revealed that some of multinucleated cells appeared as empty spaces after treatment with 5-FU as this agent induced cytotoxicity by inducing effects on RNA or DNA and cell cycle arrest, leading to development of abnormal germ cell and disorientation of spermatids in the testis of rats and sloughing of epithelium, while others reported the presence of similar lesion after treatment of rats with doxorubicin treatment during pre-pubertal phase (33).

Imatinib induced epithelial sloughing in testicular sections of rats when administered at peri-puberty (19), this work revealed that 4 (50%) out of 8 rats, 5 (62%) out of 8 rats exhibited the same findings in rats treated with imatinib at pre-puberty and euthanized at PND40, PND70 respectively. This sloughing as may be due to various factors (21). Authors suggested that it is greatly due to the damage of Sertoli cell and blocking of intercellular bridges and reported that sloughed spermatids may fuse together to form the multinucleated cells (19). The appearance of eosinophilic bodies in a section from imatinib treated rats in the present work, may be explained as the multinucleated cells may suffer from degeneration of their nuclei and gradually disappeared and left PAS positive bodies with well identified borders. In addition, Imatinib has expected alterations and speculative influences cell cycle progression by Cdc₂ inhibition as reported by others (38) that may explain its role in formation of multinucleated cells and epithelial sloughing in the rat testis.

On the other hand, the changes observed in sertoli cells were more evident in PND70. A similar observation was reported by others (32). Their data showed that doxorubicin exposure throughout prepuberty causes morphological long term damage to Sertoli cells; such effect happened after the germ cell primary injury and shared in enhancement of the spermatogenic deleterious effects induced by drug. In addition, there was a slightly raising number of elongated spermatids in the rats administered with imatinib comparing to those of controls. These findings are in accordance with those of others (32,35). Regarding the quantitative analysis of testicular sections after administration with imatinib at neonatal and early infantile period, this study revealed a diminishing in the diameter of the seminiferous tubules, height of the epithelial layer especially at PND70.

There is one study on the histomorphometric analysis after treatment with imatinib at the neonatal period (33) which reported absence of significant alterations in the seminiferous tubular diameter or in epithelial thickness and this difference may be due to the individual variation. However, similar observations were noticed in that study of Nurmio regarding the number of Sertoli cells as the number of Sertoli cells /cord cross section increased at PND70 as in our study as Sertoli cells do not show an expressing of c-kit or PDGFR receptors, so, Sertoli cells undergo proliferation as in normal states (35). Although the present study used the rat as an animal model, immature human testes is also susceptible to the impact of imatinib since c-kit is reported to show an expression in human testis at prepuberty (39) and the mode of expression of the PDGF receptor in human fetus and adult testicular section is similar to that in rats (31).

It has been reported (in our previous work) that the recovery of rat testes after administration of imatinib at peripuberty is better than that in those treated with imatinib in prepubertal phase (19). As shown in this study, partial recovery was noticed at PND140. These observations are similar to those of previous studies which concluded that the prepubertal testes are more susceptible to the effects of imatinib and doxorubicin respectively (40,41) Our observations showed that imatinib induced (to some extent) late term adverse effect on testicular histology.

Conclusion

Imatinib has short and long term effects on testicular histology after exposure at pre-pubertal period and the induced histological changes were more obviously seen in sections of rats euthanized at P40 and at P70. A milder recovery was noticed at P140 indicating that the pre-pubertal testes are more vulnerable to the effects of imatinib and reflects its age dependency. Future studies should be recommended regarding the exploring effects of this drug on other components (Sertoli and Leydig cells) using immunohistochemistry and TEM. Late -term evaluation of the agents at clinically relevant doses and applying valid experimental models are necessary especially for novel ones.

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Conflict of interest

Authors declared that there is no conflict of interest.

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دراسة نسيجية لتأثير الایماتنب علی خصیة الجرذان بعد التعرض المبكر لما بعد الولادة

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الخلاصة

إن الحيز الأيمن للایماتنب وخاصة إمكانية حدوث سمية في الخصيتين قد نوقشت بطريقة متناقضة في السنوات الأخيرة. تهدف هذه الدراسة إلى تقييم انعكاس تعرض ذكور الجرذان البيض للایماتنب في مرحلة حديثي الولادة والرضع باستعمال التحليل النسيجي للخصية في مراحل عدة وعند البلوغ. جراء سليمين تم تعريضهم إلى ٢٠٠ غرام من الایماتنب لكل كيلوغرام من الوزن مرة واحدة عن طريق الفم في مرحلة ما بعد الولادة مرحلتها ما بعد الولادة والرضاعة (بعمر اليوم الأول إلى اليوم العاشر). كل التجارب أجريت مع مجموعة سيطرة مع الانسجام بالعمر والوزن جرعو بماء مقطر. تم تقسيم الجراء إلى أربعة أقسام حسب العمر الذي تم به القتل الرحيم: ١٥ يوم بعد الولادة و ٤٠ يوم بعد الولادة و ٧٠ يوم بعد الولادة و ١٤٠ يوم بعد الولادة. تم التحليل النسيجي بطريقة التعمية بعد الصبغ بهاريس هيماتوكسيلين. عشر مقاطع للخصية تم اختيارها عشوائياً للتحليل النوعي والكمي. بالإضافة إلى معايير جونسين لتحليل تأثير الدواء على تكوين النطف. سجلت البيانات واعتبرت قيمة ب معنوية عندما كانت أقل من ٠,٠٥. التجريع بالایماتنب قد أحدث بضع تحويرات في شرائح الخصية بالمقارنة مع مجموعة السيطرة تشمل تغييرات معنوية في عدد الخلايا الجرثومية المحيطة عند اليوم الخامس عشر بعد الولادة. تغييرات نسيجية مختلفة كانت الأكثر حدوثاً في الشرائح المأخوذة من الجرذان الذين تم قتلهم بشكل رحيم في اليوم الأربعين والسبعين بعد الولادة. تغيرات كمية أيضاً لوحظت كقلة في قطر النبيبات الناقلة للمني وكقلة في ارتفاع الطبقة الظهارية خاصة في اليوم والسبعين بعد الولادة. تحسن جزئي لوحظ في الشرائح المأخوذة من الجرذان المقتولة رحيماً في اليوم المائة والأربعين بعد الولادة. نستنتج أن الایماتنب أثر على نسيج الخصية بصورة معنوية ولكن هذا التأثير انعكاسي بعد سحب الدواء. هذه الاستنتاجات ممكن أن تساعد الطبيب أن يخطط وينتبه لقضايا الخصوبة في المرضى من صغار العمر والذين يعالجون بالایماتنب لعلاج أورام القناة المعدية والهضمية وبيضاض الدم المزمن.