THE EFFECT OF PARAQUAT HERBICIDE ON *IN VIVO* SPERMATOGENESIS IN MICE

Nguyen Nhut Vu, Nguyen Thi Thuy Hang,
Nguyen Thi Bich Thao, Trinh Huu Phuoc
Ho Chi Minh City Open University
Email: trinhhuuphuoc.ou@gmail.com

(Received: 23/01/2015; Revised: 26/01/2015; Accepted: 03/02/2015)

ABSTRACT

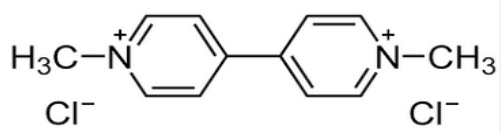
Paraquat (PQ) herbicide (1,1-dimethyl 4,4'-bipiridillium) is the most widely used in agricultural area on more than 100 crops in about 100 countries. PQ is highly acutely toxic with an acute oral LD_{50} for rat of 157 mg/kg. For aims to evaluate the toxic effects of low concentration of Paraquat on mouse spermatogenesis, mice (5 groups, n =7) were inter-dermal injected to the scrotal sac with the dose of PQ with four concentration as T1 (10 mg/kg), T2 (15 mg/kg), T3 (20 mg/kg), T4 (25 mg/kg) and a control group with PBS treated. The evaluation of spermatogenesis were carried out by behavioral changes observation, weight body assessments, sperm counting and histological testis HE stained. The results indicated that PQ has toxic effects on spermatogenesis, especially, in the dose of 25 mg/kg, PQ significantly effect on the structure of testis leads to the failure of sperm producing.

Keywords: *Paraquat, spermatogenesis, testes, sperm counting, HE staining.*

1. Introduction

In recent year, there is a significant increase in using Paraquat (PQ), 1,1-dimethyl 4,4'-bipiridillium, which is a chemical component in herbicide (chemical weed killer). In agriculture, PQ is used to control a wide range of agricultural weeds in more than 100 crops including cereals, fruit and vegetables (Hauck SJ et al, 2002; Ashoka Deepananda and De Silva, 2013). PQ has wide spectrum weedicide properties and hence popular among farmer for variety of weed and pest control (Odenkirchen and Eisler, 1998; Ashoka Deepananda and De Silva, 2013). It is fact that toxic PQ, capable of entering and its distribution over the body via the food chain and prolongs contact, is harmful to the human physiology, for examples, the main target organ of PQ distribution to the heart, liver, kidney, etc. (Isenring, 2006; Ashoka Deepananda and De Silva, 2013; Wesseling et al, 2001a). According to WHO, (2006), it is classified as a solid and with an acute oral LD50 for rats of 157 mg/kg.

Figure 1. Molecular structure of Paraquat



Spermatogenesis is a process of germ cell proliferation and differentiation which leads to produce spermatozoa from testis. In addition, the major change spermatozoa are the reduction of DNA material from diploid to haploid via a series of mitotic and meiotic divisions. It is a delicate process and highly sensitive to toxic damages (Mirhoseini et al, 2012; Ashoka Deepananda and De Silva, 2013). Moreover, the incitrate regulation and cellular interactions that occur in the testis provide multiple distinct targets by which toxicants can disrupt spermatogenesis (Boekelheide et al, 2000). The effects of toxic chemical exposures on the male reproductive system have been demonstrated by using *in vivo* model testing. A previous study suggested that free radicals which generated by PQ, are highly vulnerable to sperm membrane and

mammalian epididymis, subsequently, leading to reduce sperm density (Ashoka Deepananda and De Silva, 2013). Therefore, this study was designed to investigate the effects of PQ toxic low concentration on the spermatogenesis using the laboratory mammals.

2. Materials and methods

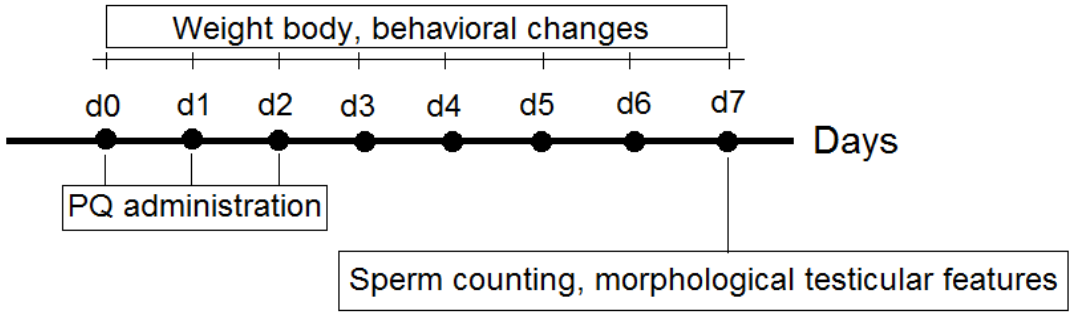
Animals and chemicals

In this study, thirty five healthy and male mice (*Mus musculus var. Albino*), weight 25 – 30 gram, were purchased from Pasteur Institute of Ho Chi Minh City. They were acclimatized in house conditions for further three weeks before experiments were carried out. Gramoxone®SL (30.1% Paraquat dichloride) (*Syngenta*), most common herbicide used in agriculture, was used.

Experiment designed

All experimental animals were divided into five groups. Five groups were named T1, T2, T3, T4 for the toxic PQ treatment groups and C for negative control. Dosing was done by way of an inter-dermal injection to the scrotal sac. Animals were administrated with concentration of PQ/weight body including 10 mg/Kg, 15 mg/Kg, 20 mg/Kg, and 25 mg/Kg for T1, T2, T3 and T4, respectively. As the control group, it was given distilled PBS (Phosphate-Buffered Saline) only. The Schedule of PQ administration and several criteria for evaluation (including the body weight, behavioral changes, sperm counting, morphological testicular features) were described in Figure 2.

Figure 2. Schedule of experiment designed. In the control group, PBS was administrated in the first – three days replaces the PQ administrated.



Behavioral changes

Animals were observed daily at an exact period time (from 9.30 am to 11.30 am) for any behavioral changes throughout the experiment. In the first three days, it was observed after treatment by PQ or PBS for 6 hours. In addition, any external changes in major organs such as eyes, ears, testicular sac, digestive, etc. were also checked (Ashoka Deepananda and De Silva, 2013).

Sperm counting and morphological testicular feature

At the day 7 after initial PQ/PBS treatment, all animals were sacrificed. Testes and epididymis were surgically removed, then, it were cleaned and removed blood tissues in PBS solution. Regions of epididymis were separated and put it into the PBS solution. Tissue minced by using a fine pair of scissors and an aliquot of sperm was centrifuged to collect the residues, then, resolved in 1 ml sperm counting solution. Sperm counting was counted by using Neubeauer Haemocytometer. Concerning to evaluate morphology of testis, samples were fixed in 4% PFA, then, using hematoxylin and eosin (HE) stained section.

Statistical analysis

The data were statistically analyzed using one way ANOVA followed StatGraphic Plus 3.0 and results were presented as the mean ± SD. The p value was under 0.05 was considered significant.

3. Results and discussion

Behavioral changes and weight body

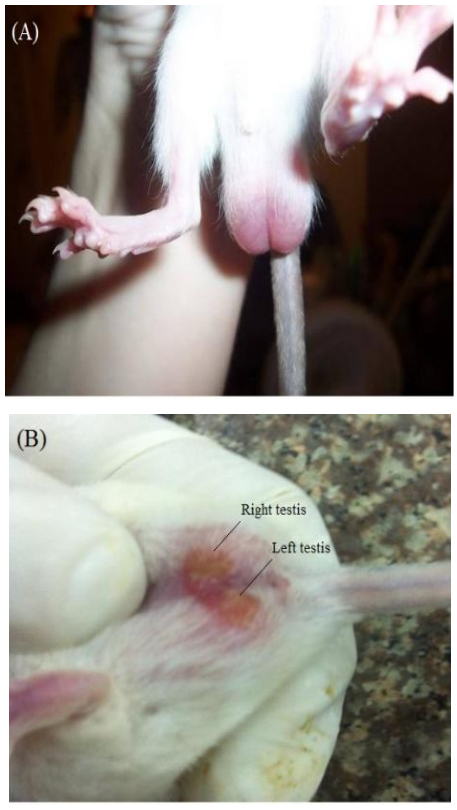
assessments.

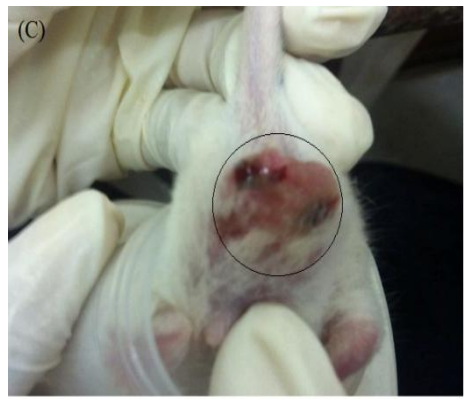
Behavioral changes were observed during experiments, in control group, there were no any significant changes. In contract, in PQ treatment groups, animals' mobility was significant decreased, eating disorder leading to the loss of weight body (Table 1). In comparison with the control group, weight body in PQ group was significant reduced after seven-day experiment ($p<0.05$). Moreover, concerning to external change in testes was considerable modified during period time for PQ exposure. In the region of PQ injection, after three days PQ administration, at day 4, both left and right testis were getting burnt, inflammatory phenomenon and the skin of scrotal sac become drier and atrophied than in the control group (Figure 3A, 3B). At the end of experiment, both testes were seemed to be atrophied and necrosis (Figure 3C). Its phenomena will be confirmed by HE staining of both testis (Figure 5).

Table 1. Weight body assessments in PQ treatment group and negative group.

	D0	D3	D7
C (Negative control)	37.05 ± 2.62 b	38.29 ± 2.49 b	40.23 ± 2.14 c
T1 (10 mg/kg)	35.65 ± 1.70 b	31.78 ± 1.32 a	29.88 ± 1.48 a
T2 (15 mg/kg)	38.48 ± 1.59 b	36.22 ± 1.61 ab	32.42 ± 1.42 ab
T3 (20 mg/kg)	39.62 ± 1.83 b	34.93 ± 1.71 ab	32.54 ± 1.12 ab
T4 (25 mg/kg)	43.24 ± 1.03 b	39.57 ± 1.32 b	36.21 ± 1.08 bc

Figure 3. The testes status in (A) control group, (B) day 4 and (C) day 7 after initial PQ administration (at the dose of 15 mg/kg).



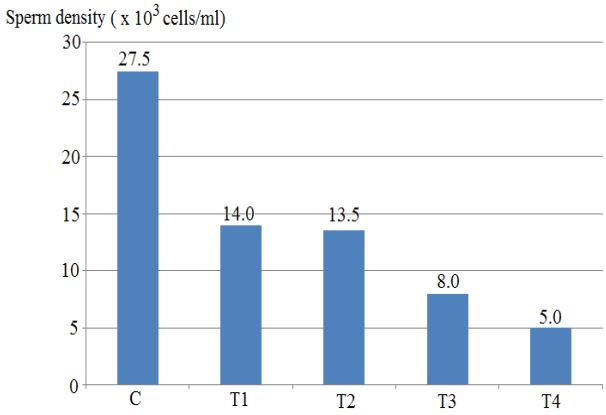


Assessment of spermatogenesis

At the day 7, all animals were scarified to carry out the assay for evaluating spermatogenesis which based on sperm counting. The results were showed at figure 4, total epididymal sperm counting in PQ treatment groups were significantly different from the control group ($p<0.05$). According to D'Souza (2006), without any drug treatment, the standard sperm density is 20 - 30 x 10^3 cells/ml. Making the comparison, in the control group, the sperm density in present study was 25.7 x 10^3 cells/ml which indicated that the process of spermatogenesis was not effected by PBS treatment. In contract, the

spermatogenesis of PQ treatment groups were abnormal, it were proved that the density of sperm producing were significantly reduced, subsequently, under the normal criterion. These results were similar to the study of D'Souza et al, (2006), Ashoka Deepananda et al (2013). In addition, the sperm density of T4 group was the lowest than other, it indicated that the high concentration of PQ was more harmful to the spermatogenesis. Moreover, the external morphology of testes in the dose of T4 (25 mg/kg) (Figure 3C) also supported the abnormal sperm density. In conclusion, the Paraquat herbicide was harmful to sperm density in *in vivo* mice model testing.

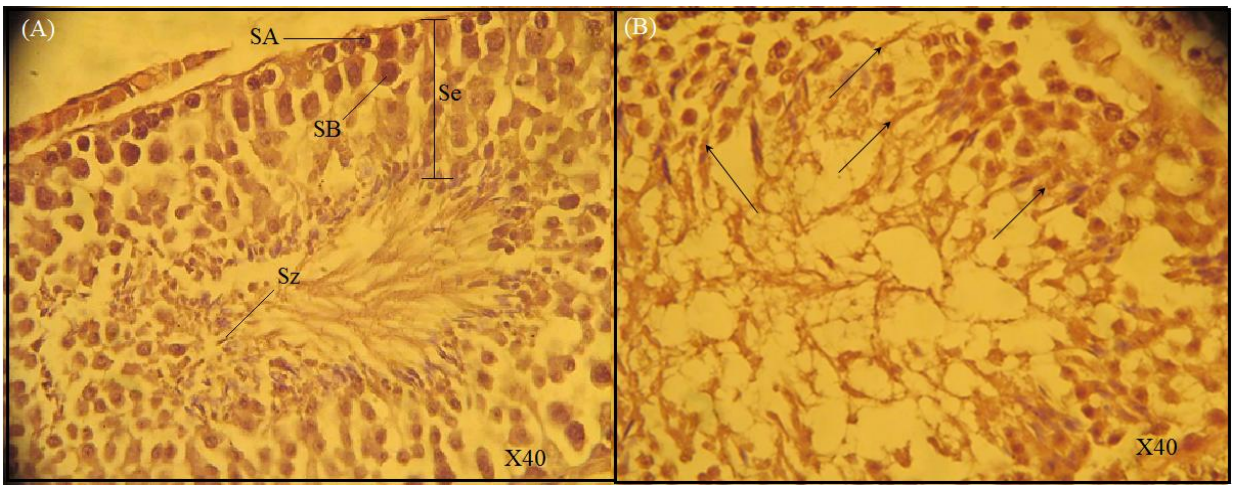
Figure 4. The density of sperm at day 7 after the initial administration



Morphological testicular feature evaluation

Testicular sections from control animals and PQ treatments were stained according to HE staining method. In the control group, several components of testis were clearly observed including epididymis, spermatogonia, sertoli cells. Moreover, histological of seminiferous tubules showed a spermatozoa forming during a varying stage of spermatozoa development. In contract, the histological of testes (T4 group) showed the disrupted structure including the lumen of seminiferous tubule and varying degree of degenerative changes were occurred (indicated by arrows), leads to loss of spermatid formation, spermatogonia degraded which lead to loss of sperm producing. This result was also support to the low sperm density counted in PQ treatment groups (Figure 4). Additionally, the histological testicular section structure in another PQ treatment groups (T1, T2 and T3) was also observed as the disorder and necrosis status (data not shown). Therefore, PQ herbicide has a toxic effects on mouse tissue. Based on these results, it could be explained that both testes were in the necrosis status, hence, seminiferous tubules and epididymis were also necrosis which led to the disruption of sperm producing.

Figure 5. Light microscopy of cross section of HE stained testis from (A) control group and (B) T4 PQ treated mice at day 7. SA: Spermatogonium A, SB: Spermatogonium B. Sz: spermatozoa, Se: Seminiferous epithelium.



4. Conclusion

The low concentration of Paraquat herbicide, at the dose of 10 mg/kg, 15 mg/kg, 20 mg/kg and 25 mg/kg, were demonstrated to be toxic to spermatogenesis using the laboratory mammals. Additionally, it was proved that PQ could change the histological structure of testes and lead to spermatogenesis failure. Moreover, it was found that paraquat caused some changes in external and behavioral changes during paraquat administration. Especially, at the dose of 25 mg/kg, both testes were in the necrosis status leading to the sperm density was significant reduced. However the mechanism, by which these abnormalities occur in the epididymis, is unclear, therefore, in further study, the study will be continuously carried out to evaluation of percentage fast moving, slow moving, twitching and immotile sperm as well as the understanding of the underlying mechanisms of PQ effects on spermatogenesis and the sperm maturation processes.

REFERENCES

Ashoka Deepananda KHM., De Silva WAJP (2013). Mechanism of Paraquat Action Shows Interference in Spermatogenesis and Epididymal Maturation of Sperm in Mice. *Journal of Biology, 1(3),* 67-76.

Boekelheide K., Fleming SL., Johnson KJ., Patel SR., Schoenfeld HA. (2000). Role of Sertoli cells in injury-associated testicular germ cell apoptosis. *Exp Biol Med., 225(2*), 105-115.

D'Souza UJ., Narayana K., Zain A., Raju S., Nizam HM., Noriah O. (2006). Dermal exposure to the herbicide-paraquat results in genotoxic and cytotoxic damage to germ cells in the male rat. *Folia Morphol (Warsz)., 65(1),* 6-10.

Hauck SJ., Aaron JM., Wright C., Kopchick JJ., Bartke A. (2002). Antioxidant enzymes, free-radical damage, and response to paraquat in liver and kidney of long-living growth hormone receptor/binding protein gene-disrupted mice, *Horm Metab Res., 34(9),* 481-486.

Isenring R. (2006). Paraquat: Unacceptable Heath Risks for Users'. Berne Declaration, PAN UK, PAN Asia and the Pacific. http://www.evb.ch/en/p10285.html.

Mirhoseini M., Mohamadpour M., Khorsandi L. (2012). Toxic effects of Carthamus tinctorius L. (Safflower) extract on mouse spermatogenesis. *J Assist Reprod Genet., 29(5),* 457-461.

Odenkirchen EW., Eisler R. (1998). Chlorpyrifos hazard to fish, wildlife and invertebrates: A synoptic review. Biological Report 85(1.13) in Contaminant Hazard Reviews. Report no. 13 Fish and Wildlife Service, US Department Interior.

Wesseling C., van Wendel de Joode B., Ruepert C., Leon C., Monge P., Hermosillo H., Partanen T. (2001a). Paraquat in developing countries. *Int J Occup Environ Health* **7**, 275-286.