Biological Forum – An International Journal **7**(2): 573-575(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effects of paraquat on testicular histomorphometry of male rats

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(Received 22 June, 2015, Accepted 13 September, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Paraquat (PQ) is a herbicide which is used all around the world. The aim of this study was to evaluate the effect of high dose of PQ on spermatogenic cells. This study was performed on 14 male wistar rats that divided into 2 groups. For experimental groups was regularly fed PQ for 7 days in 50 mg/kg concentration. Treatment was carried out for one week. Histological evaluation on testis section was performed by using tissue processing and hematoxylin-eosin staining and parameters of seminiferous tubules in testes. There was significant decrease in spematocyte, spermatid and leydig cells, but there were no significant differences in spermatogonia and sertoli cells. And also we showed significant increase in parameters of seminiferous tubules. It concluded that PQ produce free radicals which have toxic and significant effects on number of spermatogenic cells and parameters of seminiferous tubules.

Keyword: Paraquat, Spermatocyte, Spermatogonia, Spermatid, Leydig, Sertoli

INTRODUCTION

Paraquat (PQ) has chemical formula as N1, N paraquat-dimethyl 4,4 dipyridine that is very toxic to humans and animals with a delay mechanism that this mechanism produce anion super oxide (Lambert and Bondy 1989). PQ is consumed and produced very much in the world because it has unique characteristics and is a very powerful herbicide (Hamay *et al.,* 2012, Jafarinejad and Ghazi-khansari).

Free radicals toxicity are highly that can synthesize with macromolecules and produce a lot of oxygen species (ROS) such as hydrogen peroxide and superoxide onion and this two free radicals can cause severe damages in various organ (Taylor *et al.,* 2002, Najafiyan *et al.,* 2012, Abdollahi *et al.,* 2004). Studies showed that the free radicals have harmful in liver and kidneys in pesticide exposure (Rashid, 2008, Ranjbar, 2014, Ranjbar *et al.,* 2002). It was shown that PQ can caused a decrease in the weight of the male reproductive organs in rat such as testes, epididymis, prostate and seminal vesicle and the number of sperms, cell and also decrease in spermatogonia cells (Zain, 2007). Studies showed that PQ does not have mutagenic effects and it can weaken the immunity system in bacteria, rat and human. PQ causes decrease in the quantity of sexual cells among Bul/B Syrian rats and also it can wrinkle sexual cells (Ranjbar *et al.,* 2014). The purpose of this research is to study the effects of PQ herbicide on testicular histomorphometry in rat.

MATERIALS AND METHODS

A. Animals

Male Wistar rats (180-250g) were obtained from the animal colony of the Pastor Institute, Iran. Animals were maintained under standard conditions of temperature (22±1°C), humidity (45-55%) and light (12/12-h light/dark cycle). The rats in control group (n = 5) were treated with the saline solution. The rats in PQ-treated group (n = 5) were orally given solution of PQ (5 mg/kg/day) by gastric gavage for 7 consecutive days (Gromadzka-Ostrowska *et al.* (2012). The experiments were conducted according to the ethical rules approved by Institutional Review Board (IRB).

B. Histological investigation

Right testes were fixed in a 10% formalin solution and embedded in paraffin. The paraffin blocks were cut in slices (3µm) and mounted on silanized microscope slides. Sections (3 per animal) were stained with hematoxylin and eosin, and examined under light microscopy. For each animal 25 tubules with visible circular cross section were randomly chosen and spermatogonia, spermatocyte , spermatid, leydig, sertoli cells were counted. In addition, for each tubule the following parameters were determined under the light microscope equipped with motic camera 2.0m pixel: area, circumference and diameter (Balford and Anderson 1991).

C. Statistical analysis

Statistical analysis was performed with SPSS V:11 and Statistical Evaluation was performed by Mann - Whitney test and the level of statistical significance was P value <0.05.

RESULT

A. The effect of concentration of paraquat on spermatogenic cells

In mann-whitney test, microscopic studies showed a significant reduction in number of spermatocyte (P<0.001) and spermatid (P<0.001) and leydig (P=0.015) cells (Table 1). But there were no significant differences for spermatogonia, sertoli cells number (Fig. 1).

B. Histological assessment

The result indicated a significant increase (P<0.001) in diameter, area and circumference of seminiferous tubules in animals treated with PQ (Table 2).

Table 1: The effect of paraquat on testicular histomorphomertic.

	Control	**50 mg/kg**
Spermatogonia	43.17±.35	42.45±0.34
Spermatocytes	121.2±0.54	114.8±0.74*
Spermatid	106.4±0.63	95.0±0.60**
Sertoli	11.68±0.19	11.19±0.17
Leydig	13.15±0.22	12.39±0.20***

*Significant increase (P<0.001) between experimental and control group in mean±SE.

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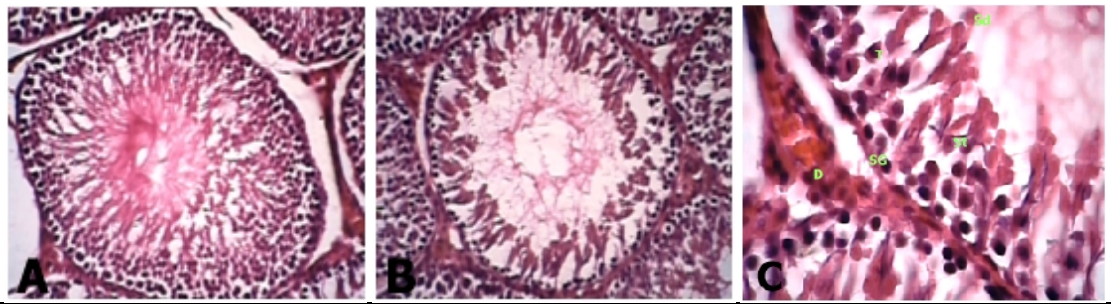

Fig. 1. A) Seminiferous tubules in control group. B) The effect of paraquat on seminiferous tubules (magnification 40×). C) spermatogonia cell (SG), spermatocyte cell (St), spermatid cell (Sd), sertoli cell (T), leydig cell(D) (magnification100×).

Table 2: Seminiferous tubule parameters in testes.

Feature	**Control**	**50 mg/kg**
Area ($\times 10^{-8} m^2$)	10.58±0.20	*11.56±0.21
Circumference	1.19±0.05	*1.24±0.04 ($\times 10^{-3}$m)
Diameter mean	0.35±0.00	*0.36±0.00 ($\times 10^{-3}$m)

Significant increase (P<0.001) between experimental and control group in mean SE.

DISCUSSION

Many investigation, on the effect of different doses of herbicides, on different organs in different animals have been done. The studies have shown that PQ have changes on histopathology in rats and especially in testicle and ovary atrophy (Deepananda and De Silva).

Our study about PQ effects on rat testes, can complete the effects of herbicides in different species which were studied previously. Our study showed that spermatocyte, spermatid, leydig cells have significant decrease, in agreement to our result, hemayatkhah et al showed that paraquat in concentrations 10, 15 and 20 mg/kg for two weeks intraperitoneally was decreased in treated groups in cells kinds of seminiferous tubules.

It was shown that when PQ was orally administrated at 4 mg/kg to male rats for 60 days in rabbit, there were multinucleated giant cells in testicular tubules and but there were no significant deviations in the spermatozoa count or motility, no significant changes in histological examination of the testicular tubule cells (Kan *et al.*, 2010). A decrease in route of spermatogenesis was also observed in rabbits that were given a single toxic dermal dose of 70 to 500 mg/kg PQ during 24 hours (Halliwell, 2012).

Studies showed that oxidative stress plays an main role in pathogens of different disease such as cancer, diabetes, vascular and heart disease and cataract (Lin and Beal 2006). Free radicals cause oxidative stress and mitochondria are places for production free radicals. Mitochondria are the main places for PQ toxin in body tissues (Ranjbar, 2014). Therefore, we have concluded that this significant reduction is because of the produced free radicals in mitochondria which are in PQ exposure and also PQ have destructive and harmeul effects in reproductive in rat.

Our previous study showed PQ reduced lung injury with *Matricaria chamomilla* L. (Gromadzka-Ostrowska *et al.* (2012). They concluded that these toxins shrinked cell cytoplasm by damage to cell proteins. Reduction of diameter may be due to either reduction in cell numbers or their cytoplasm shrinkage. But our study indicated significant increase in diameter of seminiferous tubules in rat, it seems that PQ have acute and significant effects in increase of diameter. More experimental investigation are necessary to elucidate better conclusion regarding it. Our study showed that high dose of PQ have significant effect on spermatocyt, spermatid, leydig cells and parameters of seminiferous tubules (Fig. 1) because of free radicals in PQ which have toxic and significant effects on number of spermatogenic cells and parameters of seminiferous tubules.

ACKNOWLEDGMENTS

The authors are very grateful Dr. Hajilui for his help in use of his lab for some stainings and also his staff, Mis Give.

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