*Asian Journal of Research in Animal and Veterinary Sciences*

*7(2): 15-24, 2021; Article no.AJRAVS.65383*

# Reproductive Toxicity of Paraquat in Male Guinea Pig (*Cavia porcellus*)

**Boufack Judith Laure[1*], Guiekep Nounamo Arthénice Jemina[2], Nantia Akono Edouard[3], Vemo Bertin Narcisse[1], Kenfack Augustave[1], Menkem Brice[1], Ngaleu Njieudeu Claude Cédric[1], Yidjeu Nana Ferry[1]**

*[1]Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 188, Dschang, Cameroon.*
*[2]College of Technology, University of Bamenda P.O. Box 39, Bambili, Cameroon.*
*[3]Department of Biochemistry, Faculty of Sciences, University of Bamenda, P.O. Box 39, Bambili, Cameroon.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors BJL, KA, NAE and VBN contributed substantially to conception and design of the study, data analysis and interpretation. Authors GNAJ, MB, NNCC and YNF contributed in data acquisition. Authors BJL, KA and VBN contributed in drafting the article or revising it critically for important intellectual content. All the authors read and approved the final manuscript.*

***Article Information***

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar, Iraq.
Reviewers:
(1) Marcello Ruberti, University of Salento, Italy.
(2) Tarique Mahmood, Integral University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65383

***Original Research Article***

***Received 05 December 2020***
***Accepted 13 February 2021***
***Published 02 March 2021***

## ABSTRACT

**Aims:** This study was undertaken in order to evaluate the effects of paraquat (pesticide) on some reproductive parameters in male guinea pig.
**Methodology:** Forty adult male guinea pigs averagely weighing 370.31 ± 39.43 g were distributed into 4 groups of 10 animals each, comparable in terms of body weight. During 42 days, each group of guinea pigs received by force-feeding either distilled water (control) or one of the following three doses of paraquat: 1.33, 2, 4 mg/kg of body weight. The effects of this pesticide were evaluted on some reproductive parameters (total cholesterol, libido, serum concentration of testosterone, weight of genital organs and characteristics of epididymal spermatozoa) and oxidative stress makers (testicular levels of MDA, SOD, CAT and GSH).

**Corresponding author: E-mail: boufackjudith@gmail.com;*

**Results:** Results showed that the total cholesterol increased significantly (*P*<.05) in guinea pigs exposed to paraquat relatively to the control. Animals forced-fed with the pesticide showed a significant (*P*<.05) decrease in the serum concentration of testosterone and an increase in the reaction time. A significant (*P*<.05) rise in abnormal spermatozoa as well as a significant (*P*<.05) drop in sperm mobility (65.00 ± 19.58) and spermatozoa plasma membrane integrity (52.88 ± 8.22) was observed in pesticide-treated animals compared to control. The testicular level of MDA (5.35 ± 1.89) and the activities of SOD (10.13 ± 2.03) and CAT (15.26 ± 4.28) increased significantly (*P*<.05) in paraquat treated guinea pigs compared to control animals. The reverse was observed concerning the testicular concentration of GSH (126.11 ± 48.94).
**Conclusion:** Paraquat has been shown to be toxic to male guinea pig, from 2 mg /kg bw through the induction of oxidative stress, thus imparing reproductive parameters.

*Keywords: Male guinea pig; oxidative stress; paraquat; reproduction; toxicity.*

## 1. INTRODUCTION

Paraquat is a contact herbicide, particularly effective for weed control [1]. It is one of the most widely used herbicides in the world [2], sold in more than 120 countries [1,3]. It is used mainly in the tropics, where weed growth is extremely fast and makes manual weeding techniques very inefficient, or even the use of a large number of other phytosanitary products [4]. It is used in floriculture and in certain woodlots to weed or prepare the soil for a hundred crops of cereals (corn, wheat, barley, rye, rice, etc.), soybeans, potatoes, fruits (apple, orange, banana), plants intended for the manufacture of beverages (coffee, tea, cocoa) and crops such as those of cotton, oil palm, sugar cane and rubber [5,6]. It helps protect seedlings against competition from a wide variety of perennial plants, weeds, which reduce yield and crop quality by competing for space, water, nutrients and light [7,8].

Paraquat is however extremely toxic to those who use it [9-13]. In fact, [9] showed the toxicity of this herbicide on human kidneys, liver and lungs [14-16,8,17]. In aqueous solution, this herbicide has an irritant action on the skin, pancreas, mucous membranes and conjunctiva [18-21]. It is absorbed through the skin, as well as through the lungs where it tends to accumulate [22-25].

In reproduction it has been shown that paraquat caused the diminution of the female fertility by impairing the nuclear and cytoplasmic maturation of oocytes in mice. [26]. Sperm count, viability and motility were negatively affected in adult male rat exposed to paraquat [27]. The negative effects of this pesticide were observed in the reproduction of many other animal species [28-30]. However, to the best of our knowledge, no such study has yet been carried out in guinea pig reproduction, despite the high probability that it has to consume the fodder contaminated by this pesticide, thus the aim of the present study.

## 2. MATERIALS AND METHODS

### 2.1 Animals and Lodging

Forty adult male guinea pigs (*Cavia porcellus*) aged 4 months and averagely weighing 370.31 ± 39.43 g were used. The lodges were equipped with feeders and drinkers. Their floors were covered with white woodchip litter, replaced every 7 days.

### 2.2 Feeding and Pesticide

During the whole trial, animals received drinking water, fresh pre-wilted forage (*Pennisetum purpureum*) and provender *ad libitum*.

The studied pesticide was paraquat, commercially named almoxone super. It is a dark green solution containing 276 g of paraquat and 200 g of paraquat ions per liter produced by Agro one. In guinea pigs, the oral LD50 is 20-40 mg / kg bw.

### 2.3 Experimental Trial

The 40 adult male guinea pigs were weighed, identified using earrings and divided into 4 groups of 10 animals each, comparable in terms of body weight. Animals of the control group daily ingested and by gavage 1 ml / kg bw of distilled water, while those of the other groups received in 1 ml of solution, 1.33; 2 or 4 mg / kg bw of paraquat. The animals were weighed individually each week and the volume of distilled water or paraquat solution to be administered was readjusted accordingly. After 42 days of treatment, all the animals were sacrificed.

## 2.4 Studied Parameters and Data Collection

### 2.4.1 Serum testosterone concentration and serum total cholesterol

Blood was collected by cardiac puncture and put into test tubes without anticoagulant. 8 hours later, serum was collected and distributed in labeled microtubes, then stored at -20° C for the dosage. The serum concentrations of cholesterol and testosterone were determined according to the guide in CHRONOLAB kits (Barcelona, Spain) and the Omega Diagnostic ELISA Kit (Scotland, Ingland) respectively.

### 2.4.2 Reaction time and percentage of males reacting in the presence of a female

Prior to sacrifice, a female was presented to each of the experimental males. The stopwatch was started as soon as the male and female were placed together and stopped when a reaction from the male was observed. The time recorded was considered as the reaction time. The maximum waiting time was 5 minutes. The proportion of males that reacted in the presence of a female was then calculated in each lot, using the following formula:

$$\%\ \text{of males} = \frac{\text{Number of males having reacted}}{\text{Total number of males presented to females}} X100$$

### 2.4.3 Sexual organs weight and s $_{X100}$ characteristics

Animals were weighed using a scale of capacity 160 g and precision $10^{-3}$ g. At the end of the treatment they were anesthetised using ether vapour and then dissected. The testes, epididymides, vas deferens, and sex accessory glands were removed, freed from fat and weighed.

The cauda epididymides were minced in 5 ml of 0.9% NaCl solution (at 37°C) for sperm count, mobility, plasma membrane integrity and morphology evaluation.

A drop of the obtained solution was placed on a slide and observed under the microscope and a mobility score was attributed according to [31], using a scale from 0 to 5. The sperm count was done using the Thoma haemocytometer, while sperm morphological abnormalities (small and big heads, coiled tails) and the integrity of the plasma membrane were evaluated using an eosin-nigrosin solution and the hypo-osmotic test [32] respectively.

## 2.5 Concentration of MDA, SOD, CAT and GSH

One of the testes was ground in a 0.9% NaCl solution so as to obtain 15% homogenates. This latter was centrifuged at 3000 rpm for 30 minutes, and the supernatant removed and stored at -20° C, and later used to determine the concentration of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (Cat) and total glutathion (GHS). These concentrations were respectively determined according to [33-36].

## 2.6 Statistical Analysis

One-way analysis of variance was performed to test the effect of the dose of paraquat on the studied characteristics. Duncan's test was used to separate means when there was a significant difference. Results were expressed as the mean ± standard deviation. The significance limit was 5%.

# 3. RESULTS

## 3.1 Serum Total Cholesterol

The concentration serum total cholesterol significantly increased with the dose of paraquat (Fig. 1).

## 3.2 Reaction Time and Percentage of Males Reacting in the Presence of a Male

The reaction time was higher in paraquat fed animals compared to the control. However, it was significantly ($P$<.05) longer in guinea pigs given 2 or 4 mg/kg bw of the pesticide compared to the control (Fig. 2).

In contrast, all the males reacted in all groups (Fig. 3).

## 3.3 Blood Testosterone Concentration

The serum testosterone concentration diminished continuously with increasing dose of the herbicide. Yet, no significant difference ($P$>.05) was recorded among treatments (Fig. 4).

## 3.4 Reproductive Organs Weight

The weight of the genitals organs reduced with the augmentation of the dose of paraquat. However, this decrease was significant (P<.05) only for the weight of the epididymis (Table 1).

### 3.5 Epididymal Sperm Characteristics

The sperm mobility and percentage of spermatozoa with intact plasma membrane were significantly (*P*<.05) lower in pesticide-treated guinea pigs compared to controls. The sperm count, be it per cauda epididymis or per gram of cauda epidimdymis did not significantly (P> .05) changed no matter the dose of paraquat ingested. The percentages of micro- and macrocephalic and coiled-tailed spermatozoa increased in paraquat treated animals with reference to controls, although the significant difference was only observed in males submitted to the highest dose (4 mg / kg bw) for tail windings (Table 2).

### 3.6 Concentrations of MDA, SOD, CAT and GSH

The testis level of MDA and the activity of SOD were significantly (p<0.05) higher in pesticide-treated guinea pigs compared to controls. The reverse was observed for the concentration of GSH. Catalase activity increased with an increasing dose of the pesticide. However, only guinea pigs exposed to the highest dose of paraquat showed a significant (p<0.05) increase compared to the controls (Table 3).

## 4. DISCUSSION

The increase in reaction time in guinea pigs given paraquat in the present study is in coherence with the result reported by [37] in rats exposed to lambda cyhalothrin. This result might explain the inhibition of sexual desire by paraquat in male guinea pigs. This anti-libido effect of paraquat could be due to the diminution in serum testosterone concentration probably linked to the decrease in the level of cholesterol in the testis [38]. On the other hand, paraquat through its neurotoxic action [39] could directly inhibit the sex center in the hypothalamus [40] and reduce libido. The relation between the increase of the reaction time with the dose of the pesticide and the decrease in blood testosterone concentration is similar to the result of [41] in male guinea pig treated with cypermethrin.

The decrease in the weights of sexual organs could be explained by the drop of testosterone concentration in the blood. Indeed testosterone is an androgen responsible for the development of reproductive organs [42]. This results is in agreement to those of [43] in rats submitted to carbaryl, [44] in rats exposed to lindane, [45] in rats treated with propoxur and [46] in male guinea pigs treated with acetamiprid. However, is in contrast with the findings of [47] in rats receiving chlorpiryphos-ethyl. Paraquat could also have direct effects on the structure of the epididymis.

The diminution of sperm mobility, count and the increase in the percentage of micro- and macrocephalie and coiled-tailed sperm cells were observed in paraquat-treated animals. These observations are similar to those reported by [47,48,45] while exposing rats respectively to pirimiphos-methyl, acephate and chlorpiryphos-ethyl.

The decrease in sperm mobility observed in paraquat-treated guinea pigs could be due to the change in the properties of the plasma membrane of the spermatozoa following the induction of reactive oxygen species [49,50,18]. In fact, pesticides might have induced the production of reactive oxygen species which could have detrimental effects on the viability, mobility and morphology of spermatozoa [51-53]. The negative effects of this herbicide on the characteristics of epididymal sperm could also be due to reduced testosterone levels [54,55].

**Table 1. Variation in the weight of the genital organs with the dose of paraquat in male guinea pigs**

| Weigth of sexual organs (g/100g bw) | Dose of paraquat (mg/kg bw) | | | | *P* |
|---|---|---|---|---|---|
| | 0 (n = 8) | 1.33 (n = 8) | 2 (n = 8) | 4 (n = 8) | |
| Testes | 0.48±0.11 | 0.47±0.10 | 0.46±0.10 | 0.45±0.13 | .93 |
| Epididymis | 0.11±0.07[a] | 0.07±0.01[b] | 0.07±0.02[b] | 0.07±0.03[b] | .03 |
| Vas deferens | 0.05±0.02 | 0.04±0.01 | 0.04±0.01 | 0.04±0.01 | .45 |
| Accessory glands | 0.35±0.06 | 0.26±0.11 | 0.31±0.12 | 0.31±0.14 | .54 |

*n: number of animals; (a, b) on the same line, the values assigned to the same letter do not differ significantly (P> .05); P: probability; bw: body weight*

**Table 2. Variation in sperm characteristics with the dose of paraquat in male guinea pigs**

| Characteristics of cauda epididymal sperm | Dose of paraquat (mg/kg bw) | | | | *P* |
|---|---|---|---|---|---|
| | 0 (n = 8) | 1.33 (n = 8) | 2 (n = 8) | 4 (n = 8) | |
| Mobility (%) | 87.00±4.83[a] | 68.00±13.98[b] | 75.00±12.69[ab] | 65.00±19.58[b] | .01 |
| Number/cauda epididymis (x $10^6$) | 28.75±7.75 | 30.50±8.18 | 22.08±8.43 | 21.00±11.40 | .40 |
| Number /g of cauda (x $10^6$) | 164.12±48.60 | 203.75±38.41 | 148.33±99.32 | 132.51±84.78 | .55 |
| IPMS (%) | 74.33±10.31[a] | 58.29±11.46[b] | 60.67±16.22[b] | 52.88±8.22[b] | .02 |
| Micro- and macrocéphalies (%) | 7.38±1.60[b] | 11.50±2.67[a] | 10.67±3.50[a] | 9.33±2.94[a] | .04 |
| Coiled tail (%) | 0.88±0.64[b] | 1.33±0.52[ab] | 1.17±0.75[ab] | 1.83±0.75[a] | .05 |

*n: number of animals; (a, b) on the same line, the values assigned to the same letter do not differ significantly (p> .05); p: probability; bw: body weight; IPMS: Integrated Plasma Membrane Spermatozoa*

**Table 3. Variation of testicular levels of some oxidative stress biomarkers with the dose of paraquat in male guinea pigs**

| Oxidatives stress biomarkers | Dose of paraquat (mg/kg bw) | | | | *P* |
|---|---|---|---|---|---|
| | 0 (n = 8) | 1.33 (n = 8) | 2 (n = 8) | 4 (n = 8) | |
| CAT (nM/g) | 8.70±3.40[b] | 9.80±3.83[b] | 13.04±4.15[ab] | 15.26±4.28[a] | .03 |
| SOD(nM/g) | 3.13±1.00[b] | 11.64±2.84[a] | 9.82±1.83[a] | 10.13±2.03[a] | .00 |
| GSH(nM/g) | 187.83±36.25[a] | 125.74±32.11[b] | 126.19±48.94[b] | 151.05±31.31[ab] | .04 |
| MDA (nM/g) | 314±0.42[b] | 4.62±1.00[a] | 4.92±1.21[a] | 5.35±1.89[a] | .00 |

*n: number of animals; (a, b) on the same line, the values assigned to the same letter do not differ significantly (P> .05); p:probability; bw: body weight; CAT: Catalase; SOD: Superoxyde-dismutase; GSH: Glutathion; MDA: Malondialdehyde*

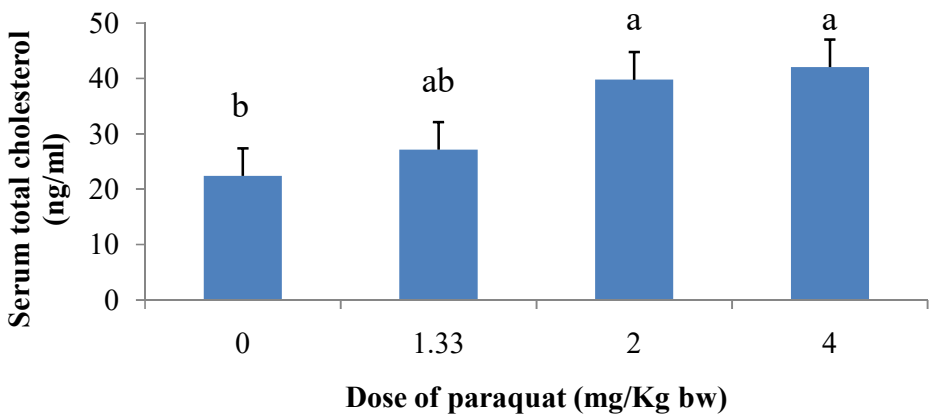


**Fig. 1. Variation of serum total cholesterol concentration according to the dose of paraquat in male guinea pigs**

*a,b: Bars with the same letters are not significantly (p >.05) different*

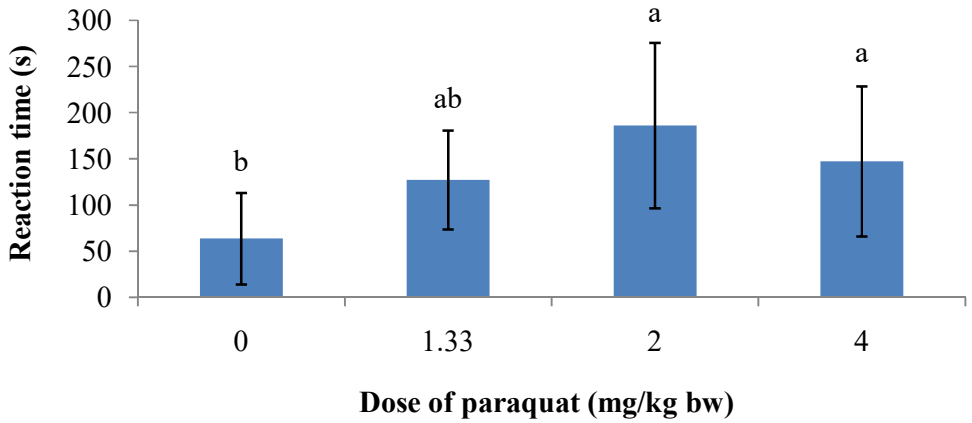


**Fig. 2. Variation of reaction time according to the dose of paraquat in male guinea pigs**

*a,b: Bars with the same letters are not significantly (P >.05) different*

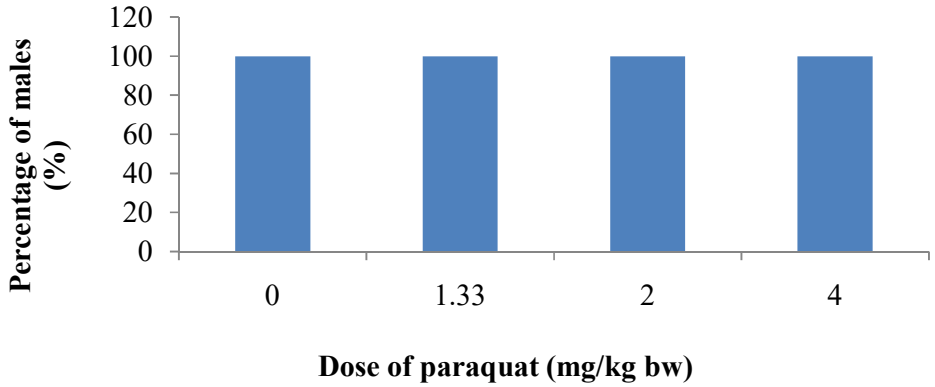


**Fig. 3. Variation of the percentage of males reacting in the presence of female according to the dose of paraquat**

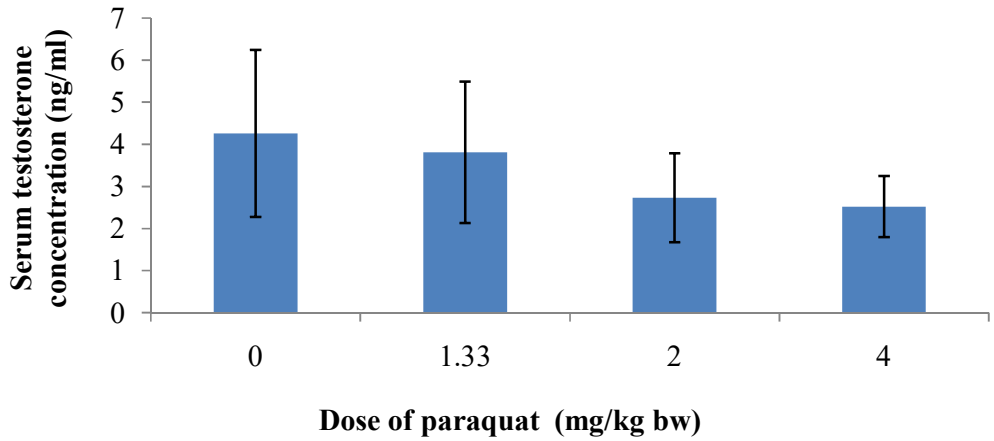


**Fig. 4. Variation of serum testosterone concentration according to the dose of paraquat in male guinea pigs**

The testicular activities of CAT and SOD and the level of MDA increased in guinea pigs exposed to paraquat. The increase in the concentration of MDA is believed to be due to a strong lipid peroxidation caused by the pesticide, since MDA is a product of lipid peroxidation [56,8]. This could probably be the cause of the increase in the number of abnormal sperm cells, and the decrease in the number of sperm cells with intact plasma membrane. SOD and CAT are considered as antioxidant enzymes and their increase might be a response to that of free radicals [28,57,58]. The decrease in testis GSH level in treated guinea pigs is similar to that observed by [59] in male Wistar rats gavaged with 25 mg/kg bw of carbendazim for 48 days and [60] in rats orally exposed to 2.5 mg/kg bw of DDVP for 28 days. This result could therefore prove the existence of oxidative stress, with the consumption of antioxidants in guinea pigs exposed to the pesticide.

## 5. CONCLUSION

Paraquat has been shown to be toxic to the reproduction of male guinea pig since it negatively affected the reproductive characteristics.

## ETHICAL APPROVAL

Experimental protocols used in this study were strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Dinis-Oliveira RJ, Duarte JA, Sanchez-Navarro A. Paraquat poisonings: Mechanisms of lung toxicity, clinical features and treatment. Critical Rev Toxicol. 2008;38:13-71.
2. Lee EY, Hwang KY, Yang JO, et al. Predictors of survival after acute paraquat poisoning. Toxicol Ind Health. 2002;18(4):201–206.
3. Gao Y, Liu L, Li T, Yuan D, Wang Y, Xu Z et al. A novel simple risk model to predict the prognosis of patients with paraquat poisoning. 2021;11(1).
   Available:www.nature.com/scientificreports/.Scientific Reports
   DOI: 10.1038/s41598-020-80371-5
4. Nuber S, Tadros D, Fields J. Environmental neurotoxic challenge of conditional alpha-synuclein transgenic mice predicts a dopaminergic olfactory-striatal interplay in early PD. Acta Neuropathol. 2014;127:477-94.
5. Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. Br J Clin Pharmacol. 2011; 72:745-57.
6. Amizadeh M, Safari-Kamalabadi M, Askari-Saryazdi G, Amizadeh M, Reihani-Kermani H. Pesticide exposure and head and neck cancers: A case-control study in an

agricultural region. Iran J Otorhinolaryngol. 2017;29:275-85.
7. Li D, Urs AN, Allegood J, Leon A, Merrill AHJr, Sewer MB. Cyclic AMP-stimulated interaction between steroidogenic factor 1 and diacylglycerol kinase theta facilitates induction of CYP17. Mol Cell Biol. 2007;27:6669–6685.
8. Oghabian Z, Williams J, Mohajeri M, Nakhaee S, Shojaeepour S, Amirabadizadeh A, et al. Clinical features, treatment, prognosis, and mortality in paraquat poisonings: A hospital-based study in Iran. J Res Pharm Pract. 2019;8:129-36.
9. Berry C, La Vecchia C, Nicotera P. Paraquat and Parkinson's disease. Cell Death and Differenciation. 2010;17:1115-25.
10. Liu XW, Ma T, Li LL, et al. Predictive values of urine paraquat concentration,dose of poison, arterial blood lactate and APACHE II score in the prognosis of patients with acute paraquat poisoning. Exp Ther Med. 2017;14:79–86.
11. Jang YJ, Won JH, Back MJ, Fu Z, Jang JM, Ha HC, Hong SB, Chang M, Kim DK. Paraquat induces apoptosis through a mitochondria-dependent pathway in RAW 264.7 cells. Biomol Ther Seoul. 2015;23(5):407-13.
12. Lee Y, Lee JH, Seong AJ, et al. Arterial lactate as a predictor of mortality in emergency department patients with paraquat intoxication. Clin Toxicol (Phila). 2012;50:52–56.
13. Yang JO, Gil HW, Kang MS, et al. Serum total antioxidant statuses of survivors and nonsurvivors after acute paraquat poisoning. Clin Toxicol (Phila). 2009;47:226–229.
14. Chen HW, Tseng TK, Ding LW. Intravenous paraquat poisoning. J Chin Med Assoc. 2009;72:547-50.
15. Fathi N, Hossinipanah M, Hajihossini M, Ranjbar A, editors. Efects of Paraquat on Testicular Histomorphometry of Male Rats. Biological Forum– An International Journal. 2015;7:573-5.
16. Pavan M. Acute kidney injury following paraquat poisoning in India. Iran J Kidney Dis. 2013;7:64-6.
17. Su Y, Liu W, Dong G, Qian Z, et al. Investigation of simple, objective, and effective indicators for predicting acute paraquat poisoning outcomes. Toxicol Indust Health. 2020;36(6):417-426.
DOI: 10.1177/0748233720933522
18. Gao Y, Hou L, Wang Y, Guo S, Yuan D, Jiang Y, et al. Octreotide alleviates pancreatic damage caused by paraquat in rats by reducing inflammatory responses and oxidative stress, Environ. Toxicol. Pharmacol; 2020.
DOI: 10.1016/j.etap.2020.103456
19. Li Y, Wang M, Gao Y, et al. Abnormal pancreatic enzymes and their prognostic role after acute paraquat poisoning. Sci Rep. 2015;5:17299.
20. Wang WJ, Zhang LW, Feng SY, et al. Sequential organ failure assessment in pre-dicting mortality after paraquat poisoning: ameta-analysis. PLoS One. 2018;13:0207725.
21. Huang C, Bai L, Xue X, Peng L, Jiang J, Zhang X. Hyperamylasemia as an early predictor of mortality in patients with acute paraquat poisoning. Journal of International Medical Research. 2020;48(3)1–12.
DOI: 10.1177/0300060520910037.
22. Costa KM, Maciel IS, Kist LW, Campos MM, Bogo MR. Pharmacological inhibition of CXCR2 chemokine receptor modulates paraquat-induced intoxication in rats. PLoS ONE. 2014;9(8).
Available:http://dx.doi.org/10.1371/journal.pone.0105740
23. Goudarzi F, Armandeh J, Jamali K, Rahmati H, Meisami A, Abbasi H. Mortality analysis of patients with paraquat poisoning treated at two university hospitals in Shiraz, Iran. Asia Pac J Med Toxicol. 2014;3:141-5
24. Hedaiaty M, Sabzghabaee AM, Gheshlagh F, Eizadi-Mood N. Paraquat poisoning management in Iran (Isfahan): Devising a protocol. Journal of Advances in Medicine and Medical Research. 2016;16:1-10.
25. Wang L, Huang J. Paraquat induces acute respiratory distress syndrome by promoting the lung epithelial-mesenchymal transition. Int J Clin Exp Med. 2017;10:657-65.
26. Sun YL, Wang XL,Yang LL, Ge ZJ, Zhao Y, Luo SM, et al. Paraquat Reduces the Female Fertility by Impairing the Oocyte Maturation in Mice. Front. Cell Dev Biol; 2021.
Available:https://doi.org/10.3389/fcell.2020.631104 .
27. Parisotto EB, Nícolas GMS, Carolinne SW, Barbara R, Juliana TD, Vitória HC, et al. Effects of paraquat in the reproductive

function and on redox state of adult male rats. Free Radical Bio Med. 2018;20:88-89.
28. Zhang Z, Yang J, Liu X, Qin Z, Li S and Li J. The protective effect of aspirin eugenol ester on paraquat-induced acute liver injury rats. Front Med; 2020.
Available:https://doi.org/10.3389/fmed.2020.589011
29. Chen F, Liu ZL, Li W, Li D, Yan BL. The significance of serum HMGB1 level in humans with acute paraquat poisoning. Sci Rep. 2019;9:1.
DOI: 10.1038/s41598-019-43877-1
30. Cousin M, Silva-Zacarin E, Kretzschmar A, El Maataoui M, Brunet JL, Belzunces LP. Size changes in honey bee larvae oenocytes induced by exposure to Paraquat at very low concentrations. PLoS One. 2013;8(5):65693.
31. Baril G, Chemineau P, Cognie Y, Guerin Y, Leboeuf B, Orgeur P, Vallet CJ. Manuel de formation pour l'insémination artificielle chez les ovins et les caprins. FAO. Station de la physiologie de la reproduction institut national de la recherche agronomique (INRA). 1993;83:125. French
32. Kumi-Diaka J. Subjecting canine semen to the hypoosmotic test. Theriogenology. 1993;39(6):1279-1289.
33. Nilsson UA, Olsson LI, Carlin G, & Bylund-Fellenius AC. Inhibition of lipid peroxidation by spin labels. The J Biol Chem. 1989;264:11131-5
34. Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. The J Biol Chem. 1972;247:6960-6962.
35. Sinha AK. Colorimetric assay of catalase. Anal. biochem. 1972;47:389-394.
36. Habbu PV, Shastry RA, Mahadevan KM, Hanumanthachar J, Das SK. Hepatoprotective and antioxidant effects of argyreia speciosa in rats. Afr J Tradit Complement Altern Med. 2008;5(2):158-164
37. Ratnasooriya WD, Ratnayake SSK and Jayatunya YNA. Effects of a pyrethroid insecticide ICON (Lambda cyhalothrin) on reproductive competence of male rats. Asian J androl. 2002;4(1):35-41.
38. Stephanie T, Beth AM, Carol LL, Amy BO, William JB and John BM. Higher testosterone levels are associated with increased high-density lipoprotein cholesterol in men with cardiovascular disease: Results from the Massachusetts Male Aging Study. Asian J Androl; 2007.
DOI:10.1111/j.1745-7262.2008.00332.x
39. Clark JM. Insecticides as tools in probing vital receptors and enzymes in excitable membranes. Pest Biochem Physiol. 1997;57:235-54.
40. Ganong WF. Review of medical physiology. 17th edition, London: Prentice-Hall International Inc. 1995;214.
41. Vemo BN, Kenfack A, Ngoula F, Akono NE, Ngaleu NCC, Guiekep NAJ, et al. Toxicity and reproductive parameters impairment of cypermethrin in male guinea pig (*Cavia porcellus*) TJAF. 2018;6(2):130-135.
42. Nassar GN, Leslie Sw. Physiology, Testosterone. Stat Pearls. 2020;1-9.
43. Pant N, Shankar R, Srivastava SP. Spermatotoxic effects of carbaryl in rats. HET. 1996;15:736-738.
44. Sujatha R, Chitra KC, Latchoumycandane C and Mathur PP. Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. Asian J Androl. 2001;3:135-138.
45. Kenfack A, Ngoula F, Womeni PDZ, Ngouateu OB, Tsambou MAM, Chombong KJ, et al. Persistence of the reproductive toxicity of chlorpiryphos-ethyl in male Wistar rat. Asian Pac J Reprod. 2015;4(1):37-40.
46. Kenfack A, Guiekep AJN, Ngoula F, Vemo BN, Bouli FP, Pamo TE. Reproductive toxicity of acetamiprid in male guinea pig. JASVM. 2018;3(4):105-111.
Available:https://doi.org/10.31248/JASVM2018.101
47. Ngoula F, Watcho P, Dongmo MC, Kenfack A, Kamtchouing P, Tchoumboué J. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. Afr Health Sci. 2007;7(1):3-9.
48. Jasuja ND, Preeti S, Suresh C, Joshi. A comprehensive effect of acephate on cauda epididymis and accessory sex organs of male rats. Afr J Pharm and Pharmacol. 2013:7(23): 1560-1567.
49. Lenzi A, Cualosso F, Gandini L, Lombardo F. and Dondero F. Placebo controlled, double-blind, cross-over trial of glutathione therapy, in male infertility. Hum Reprod. 1993;9:2044-2050.
50. Armstrong IS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy

metabolism. Free Radical Biol Med. 1999;26:869-80.
51. El-Demerdash FM, Attia AA, Elmazoudy RH. Biochemical and histopathological changes induced by different time intervals of methomyl treatment in mice liver. J Environ Science Health. 2004;47(12):1948-1954.
52. Kumar S, Ajeet KBV, Meena K. Effect of heat stress in tropical livestock and different strategies for its amelioration. J Stress Physiol Biochem. 2010;7:45-54.
53. Joshi SC, Bansal B, Jasuja ND. Evaluation of reproductive and developmental toxicity of cypermethrin in male male albino rats. Toxicol and Environ Chemistry. 2011;93:593-602.
54. Aly Okab. Semen characteristics and plasma testosterone of New-Zealand male rabbits as affected by environmental temperatures. Slovak J. Anim. Sci. 2007;40(4):161-167.
55. Luiz WOS, André FCA, Eneiva CCC, João A, Rubens PA. Correlation between sperm characteristics and testosterone in bovine seminal plasma by direct radioimmunoassay. R Bras Zootec; 2011. DOI.org/10.1590/S1516-35982011001200015
56. Antonio A, Mario F, Muñoz and Sandro A. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal Hindawi Publishing Corporation. Oxid Med Cell Longev. 2014;31. DOI: org/10.1155/2014/360438
57. Shao Y, Zhao Y, Zhu T, Zhang F, Chang X, Zhang Y, et al. Paraquat preferentially induces apoptosis of late stage effector lymphocyte and impairs memory immune response in mice. Int J Environ Res Public Health. 2019;16:60. DOI: 10.3390/ijerph16112060
58. Xu CW, Luo JJ, He L, Montell C, Perrimon N. Oxidative stress induces stem cell proliferation via TRPA1/RyR-mediated Ca2+signaling in the Drosophila midgut. Elife. 2017;6:40. DOI: 10.7554/eLife.22441.040.
59. Rajeswary S, Kumaran B, Ilangovan R, Yuvaraj S, Sridhar M, Venkataraman P, et al. Modulation of antioxidant defense system by the environmental fungicide carbendazim in Leydig cells of rats. Reprod Toxicol. 2007;24(3-4):371-380.
60. Ajiboye TO. Redox status of the liver and kidney of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) treated rats. Chem Biol Interacts. 2010;185(3):202-207.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/65383*