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Research Article

Assessing testicular function in paraquat poisoning and Vitamin E and C Amelioration in Rats

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Abstract:
Paraquat is a poisonous chemical used for weed control. This study showed the impact of vitamin E and C on fasting lipid profile in paraquat prompted toxicity in rats. Exactly 180 rats were divided into four groups—A, B, C, and D— each group having three subgroups (0, VE, and VEC) of 15 rats each. B, C, and D were test groups, while A the control group. For three months, B0, C0, and D0 received treatments of 0.02g, 0.04g, and 0.06g of paraquat, twice every two weeks, A0 did not. BVE, CVE and DVE were treated in increasing dosages of paraquat and treated with 500mg of vitamin E weekly for three months. AVE had vitamin-E treatment. BVEC, VEC and DVEC were treated in rising dosages of paraquat, following 500mg of vitamin-E and 2000mg/dl of L-ascorbic acid weekly for three months. Vitamin E and C were treatments for AVEC. Oral treatment was used for all treatments. B0, C0, A0 and D0 subgroups were sacrificed and samples collected for male gonadal hormones. The findings demonstrated that there was no significant effect on male gonadal hormone levels after paraquat dose-dependent poisoning and no significant difference in male gonadal hormone levels after the vitamin treatment.

Keywords: hormones, paraquat, rat, testis, vitamins

1. Introduction

Due to rising cancer cases, pesticide production and improper waste disposal, exploitation of environmental substances, poor environmental protection or safety policy and implementation (Okolonkwo et al., 2022; Ibama and Amadi,2018; Amadi et al., 2022; Okafor et al., 2022), studies on the toxicology of environmental pollutants on human health have gained significant interest. The Centre for Disease Control and Prevention (CDC) described paraquat (PQ) as a harmful herbicide utilized in crop

cultivating to control weed (Centers for Disease Control and Prevention (CDC), 2018). Due to its potential toxicity, it is considered "restricted used" in the United States because it can only be used by licensed individuals. However, farmers worldwide continue to use paraquat as one of the most effective herbicides. Ingestion is the primary method of exposure to paraquat that results in paraquat poisoning. However, there have also been reports of severe skin exposure, especially when the skin is damaged, such as from cuts or sores. The skin injury allows the toxic substance to easily enter the circulatory system. On the other hand, if paraquat is inhaled, it may damage the lungs, particularly if the dose and duration of the exposure are high (Centers for Disease Control and Prevention (CDC), 2018).

Paraquat's severity is influenced by the dose and duration of exposure; consequently, individuals who are exposed for shorter periods of time will experience a milder effect than those who are exposed frequently or at high doses (Okolonkwo et al., 2022). According to some studies, paraquat poisoning may result in the following organ failure: heart, kidney, liver and lungs (Okolonkwo et al., 2022; Okolonkwo et al., 2022). There is no known remedy for paraquat poisoning, according to a report released on April 4, 2018, by CDC (Centers for Disease Control and Prevention (CDC), 2018). To fill in this research gap identified, this study was centered on evaluating the impact of vitamin E and C treatments in testis of rats poisoned with various concentrations of paraquat as a way to determine the potential candidacy of vitamin E and C as antidotal therapy paraquat testicular poisoning.

2. Materials and Methods

2.1. Study Design

The research was an experimental design consisting of 180 rats with a mean weight of 200g±20g. They were grouped according to their treatments as stated in the table below:

Table 1. Rat grouping

Main groups (45 rats per group)	Sub-groups (15 rats per sub-group)
A	A_0 = no treatment A_{VE} = 500mg of vitamin E weekly treatment for 3 months A_{VEC} = 500mg of vitamin E and 2000mg/dl vitamin C weekly treatment for 3 months
B	B_0 = 0.02g of paraquat treatment every 2 weeks for 3 months B_{VE} = 0.02g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E weekly for 3months B_{VEC} = 0.02g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months
C	C_0 = 0.04g of paraquat treatment every 2 weeks for 3 months

	C_{VE} = 0.04g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E weekly for 3months C_{VEC} = 0.04g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months
D	D_0 = 0.06g of paraquat treatment every 2 weeks for 3 months D_{VE} = 0.06g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E weekly for 3months D_{VEC} = 0.06g of paraquat treatment every 2 weeks for 3 months and then followed by 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months

From the Animal House of Department of Biology in Rivers State University, the rats were moved to Chemical Pathology Laboratory in Department of Medical Laboratory in the same institution where the animals were allowed for two weeks to acclimatize before commencing the study.

Both paraquat and vitamin E and C treatments were administered orally (oral gavage route) to ensure the rats were taking the right quantity of treatment required for the study.

Except in A, B0, C0 and D0 groups were the animals were sacrificed exactly after the third month treatment, other subgroups were sacrificed in a number of 5 rats per subgroup every month for 3 months. The rats were first anesthetized with chloroform and the sacrificed. Their blood samples were collected via cardiac puncture technique and assayed for male gonadal hormone profile.

Laboratory analyses of the hormones include FSH, LH and testosterone. The methods used for the determination of male gonadal hormone profile are methods described by Okolonkwo (Debe et al., 2007).

2.2. Statistical analysis

The collated data were analyzed using SPSS version 21.0 for descriptive statistics expressed as mean±SD and inferential statistics; Two Way ANOVA. The level of significance was set at $\alpha = 0.05$

3. Results

Table 1 to 3 showed that there were no significant ($p>0.05$) changes in the levels of LH, FSH and testosterone after paraquat poisoning and vitamin treatments in one to three months vitamin intervention plan.

Table 2: ***Changes in Male sex hormonal data after one-month treatment period.***

Sub-group	LH (mIU/L)	FSH (mIU/L)	Testosterone (ng/ml)
A_0	2.54 ± 0.04	1.11 ± 0.03	1.00 ± 0.06
A_{VE}	1.48 ± 0.04	0.90 ± 0.01	0.52 ± 0.02
A_{VEC}	2.28 ± 0.02	0.95 ± 0.02	0.83 ± 0.04
B_0	2.86 ± 0.04	0.69 ± 0.03	0.52 ± 0.02
BE	2.56 ± 0.09	0.93 ± 0.01	0.53 ± 0.03
B_{VEC}	2.00 ± 0.05	0.84 ± 0.02	0.71 ± 0.01
C_0	2.96 ± 0.09	1.01 ± 0.03	0.50 ± 0.02
C_{VE}	2.10 ± 0.04	1.10 ± 0.02	0.65 ± 0.02
C_{VEC}	1.88 ± 0.03	1.40 ± 0.03	0.55 ± 0.02
D_0	3.61 ± 0.07	1.71 ± 0.02	0.67 ± 0.02
D_{VE}	2.75 ± 0.04	1.84 ± 0.03	1.28 ± 0.02
D_{VEC}	1.79 ± 0.04	1.25 ± 0.03	1.41 ± 0.05

* means statistical significance

Table 3: ***Changes in Male sex hormonal data after two months treatment period***.

Sub-group	LH (mIU/L)	FSH (mIU/L)	Testosterone (ng/ml)
A_0	1.18 ± 0.04	1.61 ± 0.03	1.40 ± 0.05
A_{VE}	1.08 ± 0.04	1.78 ± 0.02	0.93 ± 0.03
A_{VEC}	1.16 ± 0.04	1.34 ± 0.03	0.59 ± 0.02
B_0	1.74 ± 0.03	1.35 ± 0.03	0.45 ± 0.02
BE	1.29 ± 0.03	1.32 ± 0.03	0.42 ± 0.02
B_{VEC}	1.55 ± 0.04	1.15 ± 0.01	0.74 ± 0.02
C_0	1.55 ± 0.03	1.62 ± 0.05	0.53 ± 0.04
C_{VE}	1.60 ± 0.03	1.63 ± 0.04	2.11 ± 0.11
C_{VEC}	1.33 ± 0.04	2.55 ± 0.04	0.99 ± 0.01
D_0	1.33 ± 0.02	1.97 ± 0.05	0.42 ± 0.02
D_{VE}	1.43 ± 0.04	1.77 ± 0.03	0.37 ± 0.02
D_{VEC}	1.39 ± 0.02	0.88 ± 0.01	0.43 ± 0.03

* means statistical significance

Table 4: ***Changes in Male sex hormonal data after three months treatment period.***

Sub-group	LH (mIU/L)	FSH (mIU/L)	Testosterone (ng/ml)
A_0	1.46 ± 0.01	1.73 ± 0.04	1.50 ± 0.03
A_{VE}	2.74 ± 0.18	2.25 ± 0.15	2.08 ± 0.19
A_{VEC}	2.14 ± 0.12	1.90 ± 0.04	1.49 ± 0.13
B_0	1.55 ± 0.01	1.54 ± 0.06	1.51 ± 0.04
BE	1.35 ± 0.07	1.25 ± 0.03	1.98 ± 0.17
B_{VEC}	2.00 ± 0.09	2.90 ± 0.03	4.60 ± 0.04
C_0	2.55 ± 0.07	1.70 ± 0.07	1.98 ± 0.16
C_{VE}	2.85 ± 0.15	1.80 ± 0.08	1.62 ± 0.14
C_{VEC}	5.15 ± 0.07	4.00 ± 0.04	0.68 ± 0.03
D_0	3.50 ± 0.24	3.15 ± 0.05	1.35 ± 0.08
D_{VE}	2.20 ± 0.14	1.60 ± 0.02	1.56 ± 0.03
D_{VEC}	1.85 ± 0.01	2.00 ± 0.03	1.20 ± 0.10

* means statistical significance

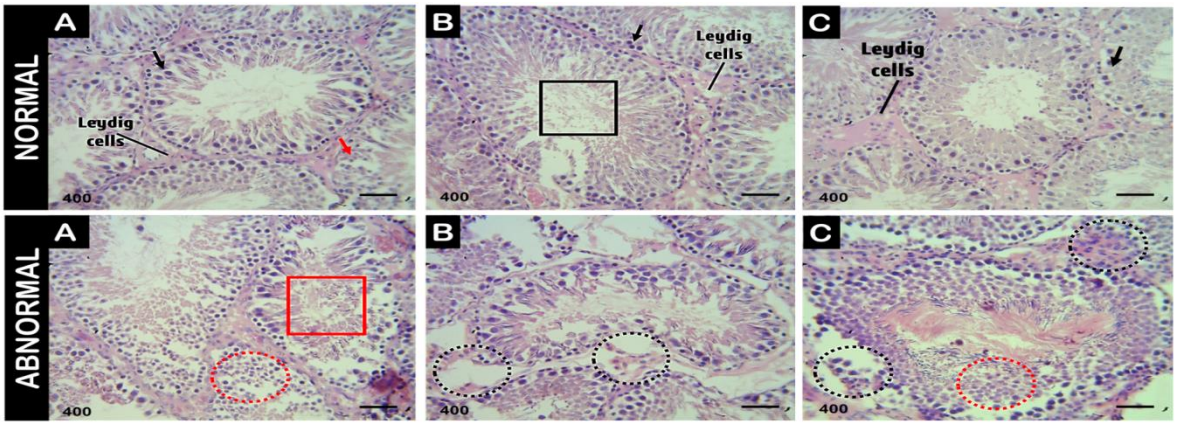

Figure 1. photomicrograph of the testis showing Normal (A, B and C) and abnormal (A, B and C) treatment groups [H&Ex400].

Indications: Black arrow (normal primary spermatocytes), black box (seminiferous tubules), red arrow (normal spermatid), red box (hypertrophy of seminiferous tubules), black circle (pyknotic leydig cells in interstitial tissue) and red circle (pyknotic primary spermatocytes).

"A" in the normal category represents group without any treatment or supplementation

"B and C" in the normal category represents groups treated with vitamins after paraquat poisoning

"A to C" in the abnormal category represents paraquat poisoned groups

3.1. Discussion

There existed little or no significant changes on the sex hormones of the PQ treated male rats within the period of study. The LH, FSH and testosterone mean values were insignificant ($P > 0.05$) when test groups were compared to the control groups. It could either mean that the duration of study was not enough; the PQ doses and route of administration was not proper or that PQ has no effect on the endocrine system. USA – EPA (United State of America – Environmental Protection Agency, 1987) in a long-term study of PQ toxicity at doses up to 5mg/kg/day, observed no adverse reproductive effects. So also, were so many other studies.

This finding revealed that histological architecture of the testis was impaired following paraquat poisoning in the studied rats. The spermatocytes were degenerating and because spermatocytes give rise to spermatids, they were structural alterations in the spermatid. Since these are processes needed for healthy sperm cell production, this implies that paraquat poisoning would have affect spermatogenesis which should give rise to poor semen quality. The hypertrophy of the seminiferous tubules may account to decrease in sperm cell count, although this parameter was not measured in this study. These findings were corroborated by Aitken (Aitken, 1995) and Dede *et al.* (Dede et al., 2007), when they observed that the generation of reactive oxygen species (ROS) in the male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function, and that most of the testicular lesions were caused by xenobiotics. Some histological changes in the testes have been reported in a few PQ toxicity studies. Butler and Kleinerman (Butler and Kleinerman, 1971) found multinuclear giant cells in rabbit's testicular tubules. However, this study demonstrated that treatment with the antioxidants (vitamin E and C) was able to repair the impaired testicular tissues within the treatment periods.

4. Conclusion

Although there was a report on changes in testicular architecture in the paraquat poisoned rats compared to the control group, there was no reported effect on the male gonadal hormone profile. This study also established that vitamin E and C treatment could restore impaired testicular damage inflicted by paraquat.

References

[1] Okolonkwo, B. N., Amadi, C. F. & Chimekagbe, O. E. (2022). Organophosphosphates toxicity: pathophysiology, diagnosis and treatment. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 12(2), 27-37

[2] Ibama, O. & Amadi, F. C. (2018). Assessment of serum levels of some heavy metals in carpenters residing in Port Harcourt in relation to their lifestyle. *Asian Journal of Research in medical and Pharmaceutical Sciences,* 4(4), 1-7

[3] Amadi, C. F., Aleruchi-Didia, T. N., Konne, F. E. & Nyenke, C. U. (2022). Environmental effect of kpo-fire in Niger Delta and future health implications. *Biotechnology Journal International*, 26(2), 18-24

[4] Okolonkwo, B. N. & Ikpeama, A. R. (2012). The Antioxidant Effects of Vitamin C on Liver Enzyme (SGPT) Activity in Rats under Paraquat Toxicity. *Annals. Food Science and Tech*, Volume 13, Issue 1, Pages 100 – 104. Available on-line at www.afst.valahia.ro.

[5] Centers for Disease Control and Prevention (CDC), (2018). Facts about paraquat. https://emergency.cdc.gov/agent/paraquat/basics/facts.asp

[6] Okolonkwo, B. N., Chukwubike, U. O., Amadi, C. F. & Nyenke, C. U. (2022). Therapeutic effects of vitamin E on paraquat induced liver toxicity in male albino rats (*Rattus norvegicus*). *Journal of Advances in Medicine and Medical Research*, 34(17), 1-7

[7] Okolonkwo, B. N., Amadi, C. F., Chukwubike, U. O. & Nyenke, C. U. (2022). The comparative effects of vitamin E + C on the chronic toxicity of paraquat in albino rats (*Rattus norvegicus*). European Journal of Medicinal Plants, 33(6), 7-13

[8] Debe, E. B., Okolonkwo, B. N. & Ngokere, A. A. (2007). Toxicological effects of paraquat on the histology of the stomach, small intestine and testis of male albino rats (*Rattus norvegicus*). Port Harcourt Medical Journal, 2(1), 51-55

[9] United State of America – Environmental Protection Agency (1987). Re-registration Eligibility Decision (RED). Paraquat dichloride office of Prevention, Pesticide and toxic substances. EPA 738 –F-96-018. Washington, D. C.

[10] Aitken, R. J. (1995). Free radicals, lipid peroxidation, sperm function. *Reproduction, Fertility and Development; 7, 659 – 668.*

[11] Dede, E. B., Okolonkwo, B. N. and Ngokere, A. A. (2007). Toxicological effects of paraquat on the histology of the stomach, small intestine and testis of male albino rats (Rattus rattus). Port Harcourt Medical Journal, 2, 51 – 55.

[12] Butler, C. and Kleinerman, J. (1971). Paraquat in the rabbit. British Journal of Industrial Medicine, 28, 67 – 71.