INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 4, p. 55-60, 2018

RESEARCH PAPER OPEN ACCESS

Biochemical changes of serum Glutathione and Malondialdehyde by Paraqu at and Olive Oil on Adult Male Reproductive system of albino Rats

Dhuha A. Hussien, Zainab Jamal Mohammed Jawad*

Dept. of Pathology and Poultry Disease, College of Vet. Medicine, University of Baghdad , Iraq

Key words: *Paraquat, Olive Oil, Serum Glutathione, MDA, Rats.*

http://dx.doi.org/10.12692/ijb/13.4.55-60 Article published on October 14, 2018

Abstract

Paraquat (PQ) is a commonly used herbicide that induces oxidative stress via reactive oxygen species (ROS) generation. This study aimed to investigate the effects of the antioxidant (olive oil) against PQ induced oxidative stress in rats. A total of forty eight (48) male rats were randomly divided into 4 groups which administrated orally and daily for six weeks as follows: distilled water(C), 3mg PQ & 2ml Olive oil Kg B.wt(T1), 3mg /Kg B.wt PQ(T2) and 2ml/Kg B.wt Olive oil (T3)respectively. Blood samples were collected after 2, 4 and 6 weeks for estimation of the biochemical parameters (serum glutathione and MDA).The trends of glutathione were significantly different ($P < 0.05$) across the periods in all groups. In the T1, T2groups showed a significant decreasing at 6 week. On the other hand, the T3 showed a significant increasing ($P < 0.05$) within 4 and 6 weeks. The results showed the means of Glutathione were significantly lowered in T1 and T2 as compared with control. While the MDA shows a significant increase ($P < 0.05$)in theT1 and T2during the experiment periods as compared with control group, while group (T3) treated with olive oil only shows a significant decrease ($P <0.05$) in MDA concentration comparing with control group. In conclusion, MDA increased in rats treated with Paraquat more than other groups treated with olive oil as antioxidant, while the glutathione is decrease at same groups and increased at groups of olive oil.

* **Corresponding Author:** Zainab Jamal Mohammed Jawad ✉ zainab0jamal@yahoo.com

Introduction

Oxidative stress occurs when the dynamics of reduction and oxidation (REDOX) balance between oxidants and antioxidants is shifted towards oxidant potentials (Serafani and Del Rio ,2004).The mitochondria unlike other organelles, contains its own genome, mitochondria DNA (mtDNA) and is the largest supplier of ATP and the main source of reactive oxygen species (ROS) in aerobic organisms (Bindu*etal.*,2011,) . Due to the electron transport chain (ETC) in the inner mitochondrial membrane, there is a high endogenous ROS production that disrupts the homeostasis, cell signaling and also leads to mitochondrial genome instability (Devasagayam *et al.*, 2004). A compromised ETC increases the ROS levels introducing damage in lipids, DNA, and perturbs both the glutathione scavenging and antioxidant capacity (Harman, 1956). Cells are constantly challenged by exogenous stressors from environmental conditions such as chemicals, drugs, and UV light exposure as well as by endogenous stressors including ROS and quinones. The repetitive exposure to environmental agents such as pesticides/herbicides exacerbates the generation of ROS, and down regulates the levels of antioxidant defenses. For example, Paraquat (PQ; 1,1-dimethyl-4,4-bipyridium dichloride), is a redox cycling compound that selectively produce ROS by uncoupling the ETC complex in the mitochondria (Castello *et al.,* 2007)This causes an increase in the intracellular oxidative microenvironment leading to the decline in the supply of cellular energy, inducing cell death, and depleting glutathione (GSH) (Chen, *et al.,* 2010). It is a delicate process and highly sensitive to toxic damages (Mirhoseini *et al.,* 2012).Olive oil is a strong antioxidant, its Hydrophilic phenols are the most abundant natural antioxidants protecting against damage from free radicals and against the formation of cancer (Servili *et al.*, 2009).

Glutathione is equipped for counteracting harm to essential cell parts caused by responsive oxygen species, for example, free radicals, peroxides, lipid peroxides, and substantial metals (Pompella *et al.,* 2003), Glutathione (GSH), tripeptide containing cysteine, plays a role as a cellular defense factor against reactive oxygen species generated in tissues (Meister,1991) Glutathione has been found to tie to and initiate ionotropic receptors that are not the same as some other excitatory amino corrosive receptor, and which may constitute glutathione receptors, possibly making it a neurotransmitter (Oja*et al.,* 2000).

Malondialdehyde (MDA) is the organic compound with the nominal formula $CH_2(CHO)_2$. A colorless liquid, malondialdehyde is a highly reactive compound that occurs as the enol (Nair *et al.,* 2008).Direct measurement of ROS formation in cells is very difficult in the clinical setting. Instead, several indirect methods have been developed to measure free radicals and their metabolites, such as malondialdehyde (MDA; the final product of lipid peroxidation) (Lykkesfeldt ,2007) .

Materials and methods

Preparation of Paraquat (PQ) doses

Three mg PQ was dissolved in 100ml distilled water to prepare stock solution and prepare other doses The doses were administered daily to male rats using gastric intubation(Chen *et al.,* 2017).

Preparation of olive oil doses

The olive oil dosage was calculated by the following equation:

$\frac{V^1}{W^1} = \frac{V^2}{W^1}$ = $\frac{2\ ml}{1000\ g} = \frac{V^2}{100\ g}$ = V^2 = $\frac{200\ ml/g}{1000\ g}$ = 0.2 ml injected dose

The doses were administered daily to male rats using gastric intubation (Banihani, 2017).

Blood collection

Blood samples at different intervals of the experiment were collected via cardiac puncture by using disposable medical syringes (5ml). Blood from each rat was kept in disposable tubes which held for not more than four hours before serum isolation. Samples were centrifuged at a speed of 2500 rpm for 15 minutes and then serum samples were stored in freezer at -18°C until used for biochemical test (MDA and Glutathione).

Experimental design

A total number of forty eight (48) male Albino Wistar rats weighting (180-220 g) were used in this experiment. Their ages ranged between (2.5-3.5) months. Experimental animals were housed in plastic cages at (22-25°C) in the animal house of department of Physiology and Pharmacology / College of Medicine -University of Maysan, with controlled lightening and the air of room was changed continuously by using ventilation vacuum. They were left for two weeks for acclimatization with the experimental conditions. Animals had free access to water and standard pellet di *et al* ong the experimental period (Hafez, 1970). Forty eight adult male rats were used in this experiment. After acclimatization for two weeks they were divided equally into four groups as follows:

Group one control (C): This group received distilled water daily for 6 weeks

Group two (T1): This group received (3mg & 2ml/Kg B.wt) PQ & Olive oil daily for 6 weeks.

Group three (T2): This group received (2ml/Kg B.wt) Olive oil daily for 6 weeks.

Control Four (T3): This group received (3mg/Kg B.wt) PQ daily for 6 weeks.

The experiment was lasted for 6 weeks. Blood samples were collected after 2, 4, 6 weeks of the experiment for Testosterone, MDA and Glutathione concentrations. Body weight was measured at 0,2,4,6 weeks of the experiment.

At the during of the experiment, three animals from each group were anesthetized and killed for the histological study of testes, epididymis and vas deferens.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. The results were expressed as mean ± standard errors (SE) and $P < 0.05$ is considered statistically significant (SAS, 2010).

Results

Serum Glutathione concentration

Glutathione concentration in response to oral administration of Paraquat and olive oil, Paraquat only and Olive oil only at Paraquat 3 mg/Kg B.wt. and Olive oil 2 mg/Kg B.wt.in adult male rats for six weeks is shown in Table 1.

Table 1. Glutathione concentration in response to oral administration of T1 Paraquat & olive oil, T2 Paraquat only and T3 olive oil only. (at Paraquat 3 mg/Kg B.wt. and olive oil 2 ml/Kg B.wt. in adult male rats for six weeks)

Weeks	Groups			
	C Control	T 1	T 2	T 3
2	A15.47 ± 0.1a	A11.66 ± 0.25bc	A12.03 ± 0.31b	B11.24 ± 0.34c
4	A14.21 ± 0.29b	A11.98 ± 0.23c	B10.70 ± 0.19d	A15.05 ± 0.34a
6	A14.61 ± 0.33a	B10.90 ± 0.14b	B10.95 ± 0.25b	A14.89 ± 0.13a

Mean ± SE (n=12 rats/ group). LSD:0.7439

Capital letters indicate a significant (P< 0.05) difference within group.

Small letters denote a significant (P< 0.05) difference between groups.

The results revealed that the trends of glutathione significantly different ($P < 0.05$) across the periods in all groups. In the T1 it was shown a significant decreasing at 6 week. In the T1 it was shown a significant decreasing at 6 week, while the T2 showed significant decreasing in the 4 and 6 weeks. On the other hand, the T3 showed a significant increasing ($P < 0.05$) within 4 and 6 weeks.

The results showed that the means of Glutathione in T1 and T2 were significantly ($P<0.05$) lowered than control.

Table 2. Serum MDA level (pg/ml) in response to oral administration of T1 Paraquat & olive oil, T2 Paraquat only and T3 olive oil only. (at Paraquat 3mg/Kg B.wt. and olive oil 2 ml/Kg B.wt. in adult male rats for six weeks).

Weeks	Groups			
	C Control	T 1	T2	T 3
2	A3.32 ± 0.17b	A3.58 ± 0.17ab	B3.93 ± 0.09a	A2.83 ± 0.29c
4	A3.51±0.15ab	A3.41±0.21b	B4.00 ± 0.10a	A2.89 ± 0.25c
6	A3.43 ± 0.12b	A3.72 ± 0.12ab	A5.07 ± 0.15a	A2.56 ± 0.15c

Mean ± SE (n=12 rats/ group). LSD: 0.5049

Capital letters indicate a significant (P< 0.05) difference within group.

Small letters denote a significant (P< 0.05) difference between groups.

Malondialdehyde (MDA) concentration

The effect of different doses of Paraquat+ olive oil, Paraquat only and olive oil only on mean values of MDA concentration is shown in Table 2. This Table shows a significant increase ($P < 0.05$) in MDA concentration in treated groups (T1) and (T2) along the experimental periods comparing with control group while group (T3) treated with olive oil only shows a significant decrease ($P < 0.05$) in MDA concentration comparing with control group. At the meantime, the highest concentration of MDA is shown in (T2) as compared to other groups. Within the time, the concentration of MDA reveal no significant difference ($P < 0.05$) between the second, fourth and sixth weeks in control, T1 and T3 groups. However, this difference is significant in T2 i.e. the table showed significant increase ($P > 0.05$) in MDA level after the 6th week in comparison to the 2nd and 4th week in T2.

Discussion

Glutathione can be diminished back by glutathione reductase after the oxidization, utilizing NADPH as an electron contributor (Couto *et al.,* 2013). So, as we reported a significant decrease in dosed rats after 6 weeks and while increased in olive oil group of rats.

MDA occurs naturally and is a marker for oxidative stress. (Nair *et al.,* 2008) and that pointed at groups treated with Paraquat and olive oil which reported increase of MDA. Wither rat groups treated with olive oil only showed significant decrease of MDA .Oxidative stress contributes to chronic inflammation of tissues (Gheita and Kenawy, 2014).

Malondialdehyde (MDA) is an advanced oxidation product and a recognized oxidative stress biomarker (Melisa*etal.,* 2016). Yamamoto (1993) reported that Paraquat decreased GSH contents in the liver of mice.

Conclusion

Markers of oxidative damage include malondialdehyde (MDA), and glutathione. Paraquat side effect on Glutathione serum concentration and MDA reveled from this study by notice low level of glutathione peroxidase and high level of MDA)on the other hands, olive oil as antioxidant showed a good result at this study. The animals are exposed to stressful conditions (Paraquat, Paraquat & olive oil) these have been associated to the increase of reactive oxygen species that attack cell membrane unsaturated lipids and other biomolecules and thus, inducing oxidation.

References

Bindu M, Guha I, Mallika T, Behera P, Chakrabarti S. 2011"Age-related oxidative decline of mitochondrial functions in rat brain is prevented by long term oral antioxidant supplementation." Bio gerontology **12,** 119-131.

Castello P, Drechsel D, Patel M. 2007 "Mitochondria are a major source of Paraquat-induced reactive oxygen species in the brain." The journal of biological chemistry **282,** 14186-14193.

Chen CW, Wu, YH, Chien SC, M Lin JC. 2018. A survival case of intravenous paraquat intoxication: A case report. Medicine **97(30),** 11669-11684.

Chen Q, Niu Y, Zhang R, Guo H, Gao Y, Li Y, 2010. "The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stress." Neurotoxic ology **31,** 310-316.

Chen Q, Xin Z, JinoYan Z, Xiao-Ning L, Peng-Sheng ZH, Xiang X. 2017. Oxidative damage of the male reproductive system induced by paraquat. Journal of Biochemical and Molecular Toxicology **31(3).**
http://dx.doi.org/10.1002/jbt.21870

Couto N, Malys N, Gaskell SJ, Barber J. 2013. Partition and turnover of glutathione reductase from Saccharomyces cerevisiae: a proteomic approach. Journal of Proteome Research **12(6),** 2885–94.

Devasagayam T, Tilak JC, Boloor KK, Sane Ketaki S, Ghaskadbi Saroj S, Lele RD. 2004. "Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects." Journal of Association of Physicians of India (JAPI) **52,** p 796-797.

Gheita TA, Kenawy SA. 2014. Measurement of malondialdehyde, glutathione, and glutathione peroxidase in SLE patients. Methodsin Molecular Biology **1134,** 193-9.
http://dx.doi.org/10.1007/978-1-4939-0326-9_14.

Hafez E. 1970. Reproduction and Breeding Techniques for Laboratory Animals. Lea and Fibiger, Philadelphia:, London.

Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology **11,** 298-300.

Lykkesfeldt J. 2007. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clinical Chimes try Acta. **380**, 50–58.

Meister A. 1991. Glutathione deficiency produced by inhibition of its synthesis, and its reversal: applications in research and therapy. Pharmacology and Therapeutics Journal **51**, 155-194.

Mirhoseini M, Mohamadpour M, Khorsandi L.2012. Toxic effects of Carthamus tinctorius L. (Safflower) extract on mouse spermatogenesis. Journal of Assisted Reproduction and Genetics **29,** 457–461.

Nair V, Crystal L, O'Neil PGW.2008. Malondialdehyde", Encyclopedia of Reagents for Organic Synthesis. John Wiley & Sons, New York.

Nguyen NV, Nguyen NTT, Thao NTB, Phuoc TH. 2015. The effect of paraquat herbicide on in vivo spermatogenesis in mice. Journal of Science Ho Chi Minh City Open University **1(13),** 3-7.

Oja S, Janáky R, Varga V, Saransaari P. 2000. Modulation of glutamate receptor functions by glutathione. Neurochemistry International **37(2–3),** 299–306.

Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. 2003. The changing faces of glutathione, a cellular protagonist. Biochemical Pharmacology **66(8),** 1499–503.

SAS. 2010. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

Serafani Mauro, Del Rio Daniele. 2004 "Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity is the right tool?" Redox Report, **9,** 145-152.

Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF. 2009. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities

according to their chemical structure Inflammopharmacology **17(2),** 76-84.

Yamamoto H. 1993. Protection against paraquat induced toxicity with sulfite or thiosulfate in mice. Toxicology **79,** 37-43.

Yonny ME, García EM, López A, JoséI A, Nazareno MA. 2016. Measurement of malondialdehyde as oxidative stress biomarker in goat plasma by HPLC-DAD. Micro chemical Journal **129,** 281-28.