Damanhour Journal of Veterinary Sciences

Journal homepage: www.damanhourjvetsci.com

E-ISSN 2636-3003 | ISSN 2636-2996

Effects of Potassium Permanganate on vitellogenin gene expression in male Nile tilapia (*Oreochromis niloticus*) exposed to Phenol pollution

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A B S T R A C T

Vitellogenin is a large serum phospholipoglycoprotein normally produced in the liver of female oviparous vertebrates in response to circulating endogenous estrogen. It is taken up by the ovary as a precursor of egg yolk proteins. Vitellogenin is normally undetectable in the plasma of males as they lack circulating estrogen, although its gene can be induced by estrogen exposure. The present study was carried out on fifty male Nile tilapia (Oreochromis niloticus). Fish divided into equal five groups(n=10), control group (Group1) and four treated groups; Group 2 exposed to 10mg phenol/L, Group 3 exposed to 5ppm potassium permanganate/L, Group 4 and Group 5 exposed to mixture of potassium permanganate 2.5 ppm and 5ppm respectively with phenol 10mg/L. After four weeks of exposure, tissues and blood samples were taken and the results showed that plasma testosterone level was decreased in the Group2 treated with phenol while plasma vitellogenin and vitellogenin gene expression were increased. In the Group exposed to potassium permanganate only Group3 or exposed to a mixture of potassium permanganate in addition to phenol Group 4,5 resulted in lowering of plasma vitellogenin and vitellogenin gene expression also increased of testosterone hormone compared to group2. The results of the present study reinforce the efficiency of potassium permanganate as an oxidative agent in the amelioration of the toxic effects of phenol pollution in aquaculture.

Key words: Gene expression, Phenol pollution, potassium permanganate, Tilapia fish, Vitellogenin.

1. Introduction

Vitellogenin is a large serum phospholipoglycoprotein normally produced in the liver of female oviparous vertebrates in response to circulating endogenous estrogen and is taken up by the ovary as a precursor of egg yolk proteins (Tyler et al., 1988). Vitellogenin is normally undetectable in the plasma of males as they lack circulating estrogen, although the vitellogenin gene in male can be induced by estrogen exposure (Kidd et al., 2007). As a result, the presence of notable vitellogenin in the plasma of male animals is considered evidence that they have been exposed to exogenous estrogens or estrogen mimics (Flores et al., 2010). Endocrine disruptors (EDCs) are exogenous agents that interfere with the synthesis of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development and behavior (Gültekin and Ince 2007).

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P ISSN: 2636-3003 EISSN: 2636-2996
Doi: 10.5455/DJVS.23053
Received: November 25, 2018; Received in revised form: December 26, 2018; accepted: December 29, 2018.

Various natural and synthetic chemicals have been identified to induce estrogen-like responses including pharmaceuticals, pesticides, industrial chemicals and phytoestrogens (Giesy et al., 2002). Among the environmental estrogens, several phenolic compounds have received special concerns due to their ubiquitous distribution in the environment (Staples et al., 2011). The effluents of municipal wastewater treatment plants have been identified as one major source of EDCs released into the aquatic environments (Ternes and Joss 2006). As a result, many EDCs have been frequently detected in effluent-affected surface waters (Koplin et al., 2002). Chemical oxidation can convert risk pollutants to less toxic compounds, and may be a good choice to eliminate EDCs in wastewaters (Shao et al., 2010). Permanganate compared to other oxidants is sometimes preferred because of several advantages, it has a relatively low cost, ease of handling, comparative stability and effectiveness over a wide pH range (Zhang et al., 2013) and a wide range of temperature (Shao et al., 2010). Permanganate oxidation is an effective method for clearing waters containing phenolic EDCs. Some researchers reported the kinetics and mechanism of EDCs degradation using permanganate in aqueous solution (Jiang et al., 2010).

The present study aims to determine VTG level in blood of male fish and using as a biomarker of water pollution, also the effect of phenol on VTG gene expression in male Nile tilapia (*Oreochromis niloticus*) as model test species. Moreover, the efficiency of potassium permanganate to minimize the adverse effect of phenol pollution on VTG gene expression in male Nile tilapia (*Oreochromis niloticus*) was evaluated.

2. Material and methods

2.1. Fish

Fifty sexually mature male Nile tilapia (*Oreochromis niloticus*) with an average initial body weight of 140±3 g were purchased from private fish farm and transported to Department of Physiology, faculty of veterinary medicine, Damanhour university and acclimatized in aquaria under laboratory conditions (temperature 25±2°C and pH range of 7.5 to 8.5) for 2 weeks prior to the experiment. During the experimental periods which extended for 4 weeks they were fed twice daily on artificial ration (Tropical Tadeusz Ogrodnik™) contain protein 28% and raw fat1% the feeding was daily at 3% of the body weight (Gad and Saad 2008).

2.2. Experimental design

After fish had been acclimated, the Fish were divided into five groups 10 fish in each as following;

Group1 kept under standard condition and considered as control.

Group 2 exposed to 10mg/L of phenol dissolved in water of aquaria (Demoraes et al., 2015).

Group 3 exposed to 5ppm of potassium permanganate (Xiao-Yan et al., 2015).

Group 4 exposed to 10mg/L of phenol with 2.5ppm potassium permanganate and group5 exposed to 10mg/L of phenol with 5ppm potassium permanganate (Xiao-Yan et al., 2015).

Seven fish from each aquarium were transferred live to a genetics lab. Tissue samples (liver and testes) were collected and stored at -80º C for RNA isolation, also Blood samples were collected from caudal vessels for

obtaining of plasma which were used to determine vitellogenin and testosterone level.

2.3. mRNA extraction and real time PCR

Total RNA was extracted using RNase mini kit (QIAGEN) according to manufactures protocol.

All primers of real time PCR were synthesized by (Bio Basic Canada Inc). oligonucleotide primers used in SYBR Green real time PCR (Gröner et al., 2015) were illustrated in table 1.

Preparation of PCR Master Mix was carried out according to Quanti Tect SYBR green PCR kit (QIAGEN). Cycling conditions for Elongation Factor 1α (EF-1α) and VTG gene were illustrated in table 2. Amplification curves and ct values were determined.

Real time PCR data were analyzed using Graph pad prism 6 software. EF-1α was used as a house keeping gene. Expressions of target gene were normalized to the corresponding level of EF1-α mRNA.

Table 1: Primer sequences used for gene expression quantification of reference gene elongation factor 1α (EF-1α) and VTG gene of *O. niloticus*;

Gene	Primer sequence(5'-3')	Reference
EF-1α	Forward GCTTCAACGCTCAGGTCATC	**Gröner *et al*., 2015**
	Reverse TGTGGGCAGTGTGGCAATC	
VTG	Forward CTTTCCATCCAGCCACCAAG	
	Reverse CTGCAGGAGGTTGATGATGC	

Table 2. Cycling conditions for EF-1α and VTG genes by real time PCR

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing Optimum	Extension	Secondary denaturation	Annealing	Final denaturation
EF1 alpha	50°C 30 min.	94°C 15 min.	94°C 15 sec.	62°C 30 sec.	72°C 30 sec.	94°C 1 min.	62°C 1 min.	94°C 1 min.
VTG	50°C 30 min.	94°C 15 min.	94°C 15 sec.	60°C 30 sec.	72°C 30 sec.	94°C 1 min.	60°C 1 min.	94°C 1 min.

3. Results

Figure (1) show fold changes (means± SD) in mRNA expression of VTG gene in *O. niloticus* livers relative to Elongation Factor 1-α (EF1-α) gene. In comparison with the control group, addition of 2.5and 5ppm potassium permanganate to aquarium water caused significant down regulation in expression of VTG gene in *O. niloticus* livers fish in group 4 and group 5. Treatment by 2.5ppm resulted in a decrease of 12.11± 0.712 fold while 5ppm leads to a 9.13± 0.227 fold decrease.

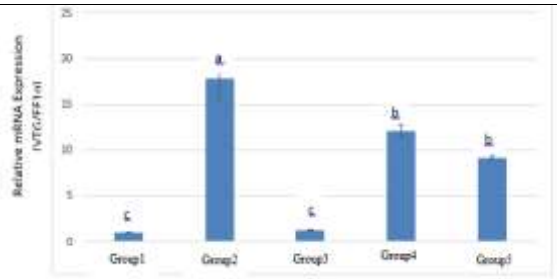

Figure1: Vitellogenin (VTG) gene expression in the fish liver after 4 weeks exposure to phenol, potassium permanganate and mixture of phenol and potassium permanganate.

The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between this two groups at p <0.05.

Group.1= control group, Group.2= 10mg/L of phenol, Group.3= 5ppm of potassium permanganate, Group.4= 10mg/L of phenol with 2.5ppm potassium permanganate and Group.5= 10mg/L of phenol with 5ppm potassium permanganate.

The results in figure (2) showed that, exposure of fish to phenol in aquaria water at concentration of 10mg/L (Group2) for four weeks resulted in significant increase in expression of VTG gene in *O. niloticus* testis 25.26±1.782 fold. Addition of 5 ppm of potassium permanganate in Group3 caused significant decrease in VTG expression (1.580±0.256 fold) when compared by Group 4 and 5 which showed VTG expression (15.00±1.302fold, 10.69±1.350 fold respectively). Treatment of aquaria water with potassium permanganate at a concentration of 2.5 ppm (Group4) reduced the concentrations to be 15±1.302 fold wherever increase of potassium permanganate concentration to 5ppm (Group 5) resulted in slight decrease in VTG gene to 10.69±1.35 fold.

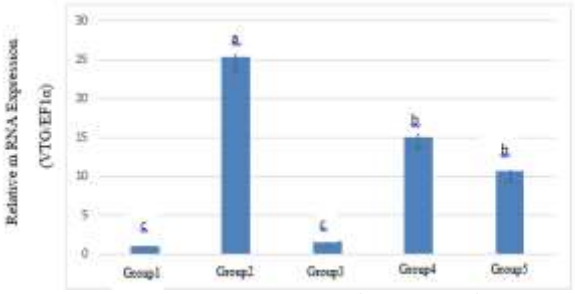

Figure 2: VTG gene expression in the fish testis after 4 weeks exposure to phenol, potassium permanganate and mixture of phenol and potassium permanganate.

The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between this two groups. Group.1= control group, Group.2= 10mg/L of phenol, Group.3= 5ppm of potassium permanganate, Group.4= 10mg/L of phenol with 2.5ppm potassium permanganate and Group.5= 10mg/L of phenol with 5ppm potassium permanganate.

From figure (3) it is evident that exposure of fish to phenol in aquaria water at concentration of 10mg/L for four weeks, resulted in significant increase in plasma VTG level 4.50±1.64µg/ml when compared with control group where plasma VTG level was 0.35±0.14 µg/ml. Addition of potassium permanganate at concentration of 5ppm resulted in non-significant increase in vitellogenin level 0.57±0.22 µg/ml as compared to control level 0.35±0.14 µg/ml. The addition of phenol and potassium permanganate mixture to aquaria water in Group 4 and 5 resulted in significant decrease in VTG level (1.71± 0.41 µg/ml, 0.61± 0.24 µg/ml) as compared with Group 2 (VTG level 4.5± 1.64 µg/ml) where the fish exposed to phenol alone.

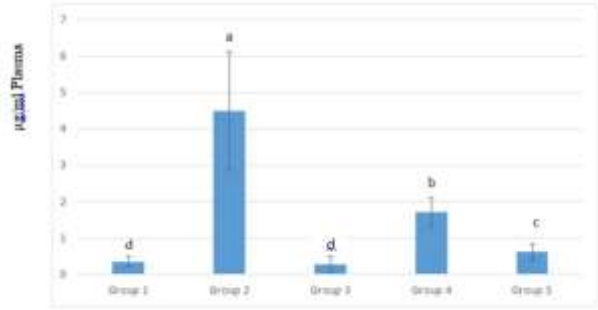

Figure (3): Vitellogenin level (µg/ml) in plasma of Nile tilapia fish after 4 weeks exposure to phenol, potassium permanganate and mixture of phenol and potassium permanganate.

The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between this two groups.

Group.1= control group, Group.2= 10mg/L of phenol, Group.3= 5ppm of potassium permanganate, Group.4= 10mg/L of phenol with 2.5ppm potassium permanganate and Group.5= 10mg/L of phenol with 5ppm potassium permanganate.

The results in figure (4) revealed that, in control group the testosterone level was 3.65± 1.13 ng/ml. However addition of phenol to aquaria water at10mg/L (Group2) resulted in significant reduction in testosterone level which reached a level of 0.72± 0.15 ng/ml. Addition of potassium permanganate at concentration of 5ppm (Group3) resulted in nonsignificant decrease in testosterone level as compared to control level.

The addition of phenol and potassium permanganate mixture to aquaria water in Group 4 and 5 resulted in significant increase in testosterone level(1.43±0.51 ng/ml, 3.57± 0.72 ng/ml) as compared to Group2 (testosterone concentration 0.72±0.15 ng/ml) where the fish exposed to phenol alone.

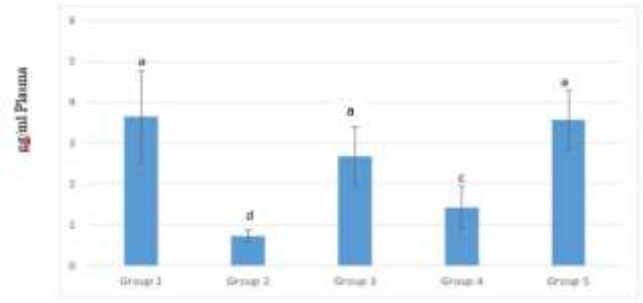

Figure 4: Testosterone level (ng/ml) in plasma of Nile tilapia fish after 4 weeks exposure to phenol, potassium permanganate and mixture of phenol and potassium permanganate.

The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between this two groups.

Group1= control group, Group 2= 10mg/L of phenol, Group 3= 5ppm of potassium permanganate, Group.4= 10mg/L of phenol with 2.5ppm potassium permanganate and Group 5= 10mg/L of phenol with 5ppm potassium permanganate.

4. Discussion

vitellogenin level (Blair et al., 2000: Laws et al., 2000). Accordingly vitellogenin level could be taken as a biomarker for phenol pollution (Isidori et al., 2010; Matozzo et al., 2008).

The induction of VTG gene expression in male O. niloticus by atrazine and endosulfan showed that these chemicals were capable of interfering with the normal function of endocrine system of male O. niloticus (Nwani, et al., 2011).

In the present investigation the control values of vitellogenin gene expression in liver and testicular tissues of male O. niloticus fish was within the same level range of Nile tilapia reported in previous studies (Elgaabary et al.,2016). Measurement of VTG gene expression in male fish liver is a rapid accurate method for detecting changes in VTG in fish exposed to estrogens and for monitoring estrogenic exposure (Barucca et al., 2006). Levels of VTG mRNA increase after exposure to phenolic EDCs (Scholz et al., 2004). VTG mRNA in fish continuously exposed to estrogens is up regulated in a dose dependent manner also VTG mRNA transcription is induced immediately and its half life is short as it is quickly degraded in the absence of estrogen (Bowman et al., 2000), these results in accordance with our results where Fish exposed to phenol exhibited marked significant increase in VTG gene expression in liver and testis. This finding was previously reported by (Elgaabary et al.,2016) who observed down regulation of the estrogenic biomarker VTG gene expression in livers of male O. niloticus exposed to phenol polluted water.

VTG in male fish is an ideal biomarker to study the estrogenicity of EDCs on fish (Gröner et al., 2015; Virk et al., 2014). The results of our study showed adown regulation of the estrogenic biomarker VTG gene expression in the livers and testis of male *O. niloticus* that exposed to potassium permanganate, this results could be explained by elimination of estrogenic potential via oxidation of estradiol, ethynylestradiol and bisphenol by potassium permanganate and decrease their adverse effect on fish.

In the present study exposure of fish to phenol resulted in significant increase in plasma vitellogenin. This result is in accordance with (Li et al., 2012) who reported that phenolic compounds showed positive induction of plasma vitellogenin.

Also our results showed that addition of potassium permanganate to aquaria water containing phenol resulted in lowering of vitellogenin concentration and down regulation of the estrogenic biomarker VTG gene expression in the livers and testis of male *O. niloticus* which was more evident in group 5 treated with phenol and high concentration of potassium permanganate.

These results agree with Guan et al., (2010) and Xiao-Yan et al., (2015) who reported that, Permanganate may have a high selectivity for EDCs oxidation in water also they stated estradiol concentration decreased with increasing permanganate dosage.

In the present study plasma testosterone levels of *O. niloticus* exposed to 10mg/liter of phenol significantly decreased compared to control group, this may be attributed to negative feedback of high dose of estrogen on hypothalamus or pituitary gland in secreting luteinizing hormone (Roepke et al., 2011) also it was reported by Mukherjee et al., (1990) that chronic, exposure of carp *(Cyprinus carpio)* to water-borne phenol caused significant accumulation of non-esterified cholesterol in tissues and serum by days 15 and 30 of exposure, respectively. This accumulation was due to the inability of the steroidogenic tissues to synthesize steroids.

Wherever in this study, addition of potassium permanganate at its two concentrations had a protective effect on the endocrine function of the testis proved by elevation of testosterone levels in fish exposed to phenol.

5. Conclusion

It may be concluded that, from the present work exposure of O. niloticus fish to phenol pollution lowered testosterone hormone production in exposed males. Also vitellogenin gene expression and vilellogenin plasma level were increased and this may be used as a biomarker for water pollution by phenol. These effects were ameliorated by addition of potassium permanganate. So we recommended with preventing the pollution of Nile River with factory effluents which polluted the water of the river with phenol product and resulted in decrease the fish reproduction and so decreasing fish production also we recommended with addition of potassium permanganate to the aquaria polluted with phenol product.

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