AN EVALUATION OF WATER POLLUTION IN THE WADI HANEFAH, A NATURAL RESERVOIR IN SAUDI ARABIA USING OXIDATIVE STRESS INDICATORS IN *Poecilia latipinna*

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ABSTRACT

Fish samples were collected at Wadi Hanefah (WH) and in an unpolluted commercial fish farm designated as the control site (CS). Several antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione (GSH)) and the oxidant malondialdehyde (MDA) were selected as bioindicators, and their concentrations were assessed in *Poecilia latipinna* from WH and CS. SOD activity was increased by 52.8% in the kidney, 61.3% in the liver and 18.2% in the heart of *P. latipinna* from WH, whereas a significant decrease (39.4%) was observed in the gills compared to the control fish, *P. latipinna*. In contrast, CAT activity was decreased by 33.2%, 49.2%, 52.1% and 56.2% in the gills, kidney, heart and liver, respectively in *P. latipinna* from WH. GST activity was also increased in the kidney, heart and liver of *P. latipinna* from WH by 34.4%, 42.2% and 54%. However, GST activity was decreased (55%) in the gills of fish from WH compared to the fish from CS. GSH concentrations were increased by 44.8%, 35.3% and 32.7% in the kidney, heart and liver but were decreased by 33.6% in the gills. MDA levels in *P. latipinna* from WH were increased in the gills, kidney, heart and liver by 83.3%, 167.3%, 161.4% and 178.7%.

Key words: *P. latipinna*, biomarkers, oxidative stress, pollution, biomonitoring

INTRODUCTION

Many industrial, agricultural and urbanization processes cause environmental pollution and contribute to the contamination of water ecosystems, thereby threatening the health of aquatic biota and humans. The health of all living organisms in an aquatic ecosystem is also affected as a result of the deterioration of water quality (Doherty *et al*., 2010). The biological integrity of an ecosystem can often be assessed based on the health of its fauna (Robinson, 1986). Heavy metals cannot be destroyed through biological degradation and therefore tend to bioaccumulate in aquatic animals, potentially having deleterious effects not only on the health of these animals but also on human beings (Kalay and Canil, 1999). Thus, the identification, monitoring and reduction (as much as possible) of the effects of pollution on the health of aquatic animals through management programs are vital.

Fish are sensitive to changes in their habitat, including increases in water pollution. Examining the health of fish may therefore reveal changes in the aquatic ecosystem. The presence of trace metals in fresh and marine waters has been observed to disturb the delicate balance present in aquatic ecosystems (Asalou and Olaife, 2005). Early toxic effects of pollution may be used as an indicator at the cellular or tissue level, before prominent changes appear in the behavior or external appearance of fish (Summarwar and Verma, 2012).

Fish are widely used as model organisms for understanding oxidative stress in aquatic ecosystems and can be employed as a bioindicator of environmental pollution because they may concentrate pollutants in their tissues directly from water through their diet and respiration (Dautremepuits *et al.,* 2004; Lopes *et al.,* 2001). Water pollution is a major contributor to oxidative stress in fish due to the redox cycling of pollution. Xenobiotic metabolism causes continuous production of reactive oxygen species (ROS), even without pollution (Ahmad *et al.,* 2000). Heavy metals accumulated in the tissues of fish may catalyze reactions that generate ROS, which may lead to environmental oxidative stress. To cope with the continuous generation of ROS from normal aerobic metabolism, cells and tissues contain a series of cellular antioxidants that show both enzymatic and non-enzymatic activity (Nordberg and Arner, 2001).

Like mammals, fish exhibit well-developed antioxidant defense systems for neutralizing the toxic effects of ROS (Almeida *et al.,* 2002; Pandey *et al.,* 2003). Fish display various antioxidant defense enzymes, such as superoxide dismutases and glutathione S-transferase, that show detoxifying activity towards lipid hydroperoxides produced by heavy metals (Tjalkens *et al.,* 1998; Farombi *et al.,* 2007). Several other low molecular weight antioxidants, such as glutathione, beta-carotene (precursor to vitamin A), ascorbate (vitamin C) and tocopherol (vitamin E), may participate in the process of oxyradical removal as well (Van Der Oost *et al.,* 2003; Yildirim and Asma, 2010), and

malondialdehyde (MDA) levels have also been used to detect the oxidative status of fishes (Sole *et al.,* 1996). The levels of antioxidant enzymes in fish have been successfully applied as an early indicator of water pollution (Lin *et al.,* 2001). Fish are often subjected to the pro-oxidant effects of different pollutants that are commonly present in water bodies (Velkova-Jordanoska *et al.,* 2008). Thus, the activity of SOD, CAT, GST and glutathione (GSH) and the formation of MDA may be considered indicators of water pollution in the African catfish (*Clarias gariepinus)* (Velkova-Jordanoska *et al.,* 2008; Yildirim *et al.,* 2011).

The African catfish is a commercially important fish because it is one of the most common freshwater fish that is cultured in Saudi Arabia. The species can therefore be used as a good model to study the responses to various environmental contaminants. The objective of the present study was to employ the physiological response of *P. latipinna* in terms of antioxidant enzyme activity and changes in lipid peroxidation *P. latipinna*as a biomarker of water pollution in the Wadi Hanefah (WH) and a control site in Saudi Arabia.

MATERIALS AND METHODS

Study area: The Wadi Hanefah is one of the major natural landmarks in the middle part of the Najd Plateau. The Wadi serves as a representative natural drain for surface water covering a wide area. It passes through the city of Riyadh, and approximately 70% of the city is situated within its catchment area. This valley extends from north of Al-Uyaynah to south of Al-Hair. The watershed of the Wadi Hanefah river is estimated to cover an area of approximately 4,400 km^2. The river flows towards the south from its source near Al-Uyaynah and ends in Wadi Sahba (Fig. 1). The main flood channel of the Wadi is situated slightly east of the center of its catchment area and flows northwest to southeast. Riyadh has a population of more than 4 million people and is located in the Wadi Hanefah catchment area.

Collection of fish: *Poecilia latipinna* were captured with a hand net from the Wadi Hanefah reservoir and from a commercial fish farm in Riyadh, Saudi Arabia, which served as the control site (CS). The captured fish were immediately anesthetized using 0.7 g L^{-1} benzocaine dissolved in ethyl alcohol (Sardella *et al.,* 2004). The effects of the anesthesia in the fish were deep sedation, loss of swimming activity and partial loss of equilibrium (Altun and Danabas, 2006).

The fish were then immediately transported to the laboratory in ice-cold containers (0-4°C). The sampled fish farm is free from any industrial input or any other source that could cause pollution. The fish samples weighed between 200 and 400 g. All fish samples were stored separately in a deep freezer at approximately -

10°C. The fish were allowed to thaw, after which the scales were removed, and the fish were washed with running water, prior to dissection with sterile scissors to remove the gills, heart, liver, kidney, muscles and skin. The post-mitochondrial fraction was prepared following Farombi *et al.* (2007).
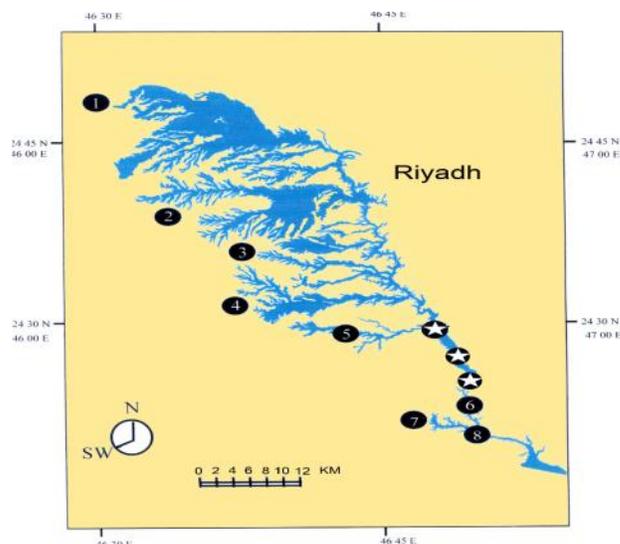

Figure 1. Map of the Wadi Hanefah, Saudi Arabia, showing the main stream and the sampling sites (stars).

Glutathione and glutathione S-transferase assays: The level of glutathione (GSH) was estimated in the 10,000 x *g* supernatant fraction from the *P. latipinna* heart, liver, kidney and gill homogenates following Jollow *et al.* (1974) at 412 nm using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The specific activity of glutathione S-transferase was expressed as the number of moles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1}cm^{-1}.

Antioxidant enzyme assays: Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the autoxidation of epinephrine at pH 10.2 at 30°C, as described by Magwere *et al.* (1997). The activity of catalase (CAT) was determined according to the procedure of Clairborne (1995).

Determination of malondialdehyde (MDA) levels: Lipid peroxidation was determined by measuring the levels of thiobarbituric acid-reactive substances (TBARs) as described by Farombi *et al.* (2000). Malondialdehyde (MDA) was quantified using the equation $= 1.56 \times 10^5$ M^{-1} cm^{-1}, following Buege and Aust (1978).

Statistical analysis: The obtained data were subjected to appropriate statistical analysis using Minitab software. Duncan's multiple range tests were applied to compare mean values and to detect significant differences (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The physicochemical characteristics of water samples collected from WH and the control site are presented in Table 1.

Antioxidant and detoxification enzyme activities: The activities of antioxidant enzymes determined in the organs of *Poecilia latipinna* from WH and CS are presented in Tables 2-3. The oxidant and antioxidant status of tissues of C. trutta were investigated in gill, liver, kidney and heart. The biomarkers of oxidative stress analyzed showed sig-nificant variations (p<0.05) but no uniform trend was observed. SOD activity was increased by 52.8% in the kidney, 61.3% in the liver and 18.2% in the heart in *P. latipinna* from WH, whereas a decrease (39.4%) was observed in the gills of *P. latipinna* from WH compared to CS (Tables 2-3). SOD activity was found to be significantly (P<0.01) increased in the liver and kidney in fish from WH compared to the fish from CS, whereas it was significantly decreased in the gills (P<0.001). CAT activity was decreased by 33.2%, 49.8%, 52.1% and 56.2% in gills, kidney, heart and liver, respectively, in *P. latipinna* from WH, and these decreases in CAT activity were found to be statistically significant (P<0.001) (Tables 2-3). GST activity was increased in the kidney, liver and heart of *P. latipinna* from WH by 34.4%, 42.2% and 54.4%, respectively (P<0.05). A significant decrease (96.2%) in GST activity was observed in the gills of fish from WH compared to CS (Table 2). Additionally, the GSH concentration was increased by 44.8%, 35.3% and 32.7% in the kidney, liver and heart, whereas it was decreased by 33.6% in the gills of *P. latipinna* from WH compared to fish from CS (Tables 2-3). The level of MDA formation in *P. latipinna* was also found to be increased significantly, by 83.3%, 167.3%, 161.4% and 178.7% in gills, kidney, heart and liver, respectively (Table 3), in fish from WH.

Oxidative stress, which is a pathological process related to the overproduction of reactive oxygen species (ROS) in tissues, is one of the important general toxicity mechanisms for many xenobiotics. Oxidative stress has been shown to be induced by anthropogenic contaminants such as persistent organic pollutants (POPs) and heavy metals and by toxins produced during massive blooms of cyanobacteria (Ding *et al.,* 1998; Van Der Oost *et al.,* 2003). Many organisms, including fish, have evolved mechanisms to counteract the impact of ROS. Oxidative stress is an imbalance between the production of ROS and the ability of cells to reduce ROS, detoxify reactive intermediates and repair damage to cellular molecules. Such an imbalance may occur as a result of increased ROS production, decreases in defense mechanisms or both (Joseph *et al.,* 2012). ROS are produced endogenously within cells. However, many environmental parameters, including exposure to heavy metals, are known to induce oxidative stress (Almorth, 2008; Summerwar and Verma, 2012). In the present study, we assessed the response of *P. latipinna* to pollutants using various biomarkers that are indicative of oxidative stress as bioindicators of aquatic pollution *P. latipinna.* We observed a significant elevation of lipid peroxidation in all of the examined organs in the fish from WH. This increase in lipid peroxidation may be attributed to the accumulation of heavy metals in the organs of the fish. The significant increase in lipid oxidation markers may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. The clear increase in lipid oxidation and its markers may also be due to the decrease in antioxidant enzyme activities The metal-catalyzed formation of ROS capable of damaging tissues such as DNA, proteins and lipids is well documented (Pandey *et al.,* 2003; Farombi *et al.,* 2007).

Furthermore, the activities of SOD, GST and the redox-sensitive thiol compound GSH were elevated in all the organs except the gills. The significant increases observed in these organs may be a response to oxidative stress caused by heavy metals. The accumulation of heavy metals might have led to the production of superoxide anions, and thus, to the induction of SOD to convert the superoxide radicals to H_2O_2. SOD catalytically scavenges superoxide radicals, thereby providing a defense against these radicals, which appear to be an important agent of oxygen toxicity (Kadar *et al.,* 2006). GSH is a substrate for the activity of GST. An apparent increase in GSH levels and subsequent increase in the activity of GST in an organ indicates that this biomolecule is playing an adaptive and protective role against oxidative stress caused by the heavy metals. Due to change in glutathione redox ratio and followed by decrease in GSH levels, the glutathione-mediated detoxification process may also be affected. This might be a factor responsible for the lack of elimination of toxic compounds that enter the fish and thus result in their accumulation, aggravating oxidative stress (McCord, 1996). Our results are in agreement with the findings of Pandey *et al.* (2003) in the fish species *Wallgo attu* from the Panipat River in India. The decreased levels of antioxidant enzymes and GSH, together with reduced levels of GST in the gills could be the reason for the marked lipid peroxidation that was observed. The gills are more exposed to polluted waters compared to other organs, and metals can enter through their thin epithelial cells (Gul *et al.,* 2004). Under acute oxidative stress, the toxic effects of the pollutants may overwhelm the antioxidant defenses (Bebianno *et al.,* 2004). Stressful conditions lead to the formation of excessive free radicals, which represent a major internal threat to cellular homeostasis in aerobic organisms. Environmental stress has been demonstrated to cause increases in oxidative stress and imbalances in antioxidant status

(Yildirim *et al.,* 2010). The apparent decrease in the glutathione detoxification system in the gills, which is the first point of contact with environmental xenobiotics, indicates that this system can serve as a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982) in *P. latipinna.* An increase in the activity of CAT and SOD is usually observed in association with environmental pollutants (Dautremepuits *et al.,* 2004) because the SOD-CAT system represents the first line of defense against oxidative stress (McCord, 1996). In the present study, the activity of CAT was found to be decreased in all of the examined organs. This decrease in CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity (Stanic *et al.,* 2005). Similar findings regarding the activity of CAT have been reported in *Cyprinidae* fish from Seyhan Dam Lake in Turkey (Kono and Fridovich, 1982) and in sterlets (*Acipenser ruthenus* L.) from the Danube River in Serbia.

Here, we provide the first comprehensive report on the levels of antioxidants and MDA formation in four different tissues (gills, kidney, heart and liver) of *P. latipinna* from the Wadi Hanefah in Saudi Arabia. The unusual trends observed in oxidative defense systems in this study indicate the need for further research, especially to study environmental factors that affect the oxidative defense response of organisms to persistent high concentrations of heavy metals in their environment. There is also a need for an extensive evaluation and comparison of data obtained from field studies with those generated in laboratory studies, as there are environmental factors in the field that cannot be replicated under laboratory conditions that may also have significant effects on the oxidative defense response of organisms to persistent high concentrations of heavy metals.

Table 1. Physico-Chemical characteristics of water samples from Wadi Hanefah (WA) and Control site (CS)

Parameter	WH	CS
Water Temperature (^{o}C)	33.6	31.2
pH	6.9	7.2
Turbidity	24.3	16.2
Conductivity (μScm^{-1})	348.7	292.5
Total Ammonia (mg L^{-1})	1.7	0.6
Dissolved Oxygen (mg L^{-1})	5.2	7.4

Table 2: Level of activity of SOD, CAT, GST, and MD in gills, kidney, heart and liver tissues of *Poecilia latipinna* from Wadi Hanefah (WH) and control site (CS)

Tissues	SOD(nmol/mg rotein) n= 10		CAT (mg/ protein) n= 10		GST(nmol/mg protein) n= 10		GSH(nmol/mg rotein) n= 10		MDA (mg/protein) n=10	
	WH	CS	WH	CS	WH	CS	WH	CS	WH	CS
Gills	16.4±0.6d	27.1±1.7a	85.6±1.4b	128.2±2.1b	122.6±2.8d	240.6±2.2a	8.1±0.5c	12.2±0.8a	51.7±2.2c	28.2±1.6a
Kidney	32.6±1.9b	21.2±1.1c	78.6±1.9b	132.5±1.7b	288.3±2.2b	214.6±1.5b	11.3±0.6b	7.8±0.6c	55.6±1.8c	20.8±0.6c
Heart	26.6±1.7c	22.5±1.6c	60.2±1.1c	91.6± 3.3c	191.8±3.2c	134.9±2.8c	9.6±0.9c	6.8±0.4d	68.5±2.6a	26.2±2.1b
Liver	40.8±1.4a	25.3+1.5b	94.7+1.8a	147.9+2.8a	312.5+4.2a	202.8+2.4b	14.2+0.6a	10.7−0.9b	60.2+1.7b	21.6+1.5c

SOD (Superoxide dismutase), CAT (Catalase), GST (Glutathione S-transferase), GSH (Glutathione), MDA (Melanoaldehyde)
Means in the same column followed by different letters are significantly different according to Ducan's test ($P \leq 0.05$)

Table 33 Result of percentage difference in enzymatic activities of changes in various tissues of *Poecilia latipinna* from Wadi Hanefah

Tissues	% SOD	% CAT	% GST	% GSH	% MDA
Gills	-39.4	-33.2	-96.2	-33.6	83.3
Kidney	52.8	-49.8	34.4	44.8	167.3
Heart	18.2	-52.1	42.2	35.3	161.4
Liver	61.3	-56.2	54.0	32.7	178.7

Conclusions: The alterations in antioxidant enzyme activities and other biomarkers of oxidative stress observed in *P. latipinna* were found to be caused by the high concentrations of pollutants in the examined water body. The biochemical dysfunction detected in this species might have been induced by these pollutants. In addition, the results provide evidence that enzymatic and nonenzymatic biomarkers of oxidative stress can serve as sensitive indicators of aquatic pollution.

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