EROD & GST Responses in Liver of Mudskipper Periophthalmus waltoni at Oil Polluted Areas

Mehrnooosh Shirani, Alireza Mirvaghefi, Hamid Farahmand and Mohammad Abdollahi

Abstract—Current annual amounts of oil entering the Persian Gulf region are over 150000 tones and due to polycyclic aromatic hydrocarbons (PAHs) toxicity, there is a need to evaluate the induction capacity of PAH biomarkers on its marine organism. So two biochemical biomarker responses were measured in mudskipper Periophthalmus waltoni from Soltani inlet with high and Ameri port with low pollution. The selected biomarkers were ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST). EROD and GST activities were significantly elevated in Soltani inlet and the highest elevation was up to 4 and 2 times for EROD and GST activity (respectively), comparing with Ameri Port (P<0.05). In male mudskippers enzymatic activity was greatest at both stations but it wasn’t significant between different sexes in the same site. These biomarker assessments can be used in this region to detect the toxic effects of chronic oil pollution even in low concentrations as early warning responses.

Keywords— EROD, GST, mudskipper, oil pollution, PAHs, Persian Gulf.

I. INTRODUCTION

Oil spills is one of the most important hazards in the estuarine and coastal water, which often exhibit long-term impacts [1]. The Persian Gulf has many characteristics and it’s under pressure from industrialization, and particularly, oil and petroleum pollution. Two different types of hydrocarbons exist in crude oil which are aliphatic and aromatic, among them the second type have attracted more concerns because of its adverse effects on aquatic organisms. Polycyclic aromatic hydrocarbons (PAHs) constitute about 0.2 to 7% of the total hydrocarbons in the oil. Among this The Persian Gulf have high amounts of PAHs compounds and every 16 of them that are reported by US Environmental Protection Agency (EPA) and World Health Organization (WHO) as priority pollutants that are present in this water body [2&3].

The biomarker approach is widely used for in vivo and in vitro studies to evaluate the effects of xenobiotics on the selected biological models [4]. In this study 2 biomarkers from detoxification process in biochemical level responses of biological organization were selected for analyzing; the enzymes that are involved in this process, can biotransform the xenobiotics with activation and conjugation reactions (Phase I & II), between them, ethoxy resorufin O-deethylase (EROD), has been used to determine phase I responses, because EROD activity especially in fish liver has been used as a biomarker for the effects of PAHs and some other organic contaminants and a number of field studies have established [5,6&7]. Phase II involves in the deactivation synthesis of xenobiotic or a phase I activated metabolite by covalent linkage to an endogenous hydrophilic molecule, like glutathione resulting in a non toxic or less toxic compound [5], in this phase GST activity is an important marker that is commonly assessed [8], and in this case was assayed too.

Mudskippers Periophthalmus waltoni (Gobiidae) are completely amphibious fish, they are uniquely adapted to intertidal habitats. These fish are air-breathing species [9&10] and have a suitable distribution in northern and southern coastal area of the Persian Gulf [11]. They have a high density on tidal mudflats that are formed in creeks and estuaries and mangrove forest floors which are important in supporting the fish population by providing food sources (like organic matter and detritus) [12].

In this study we were assessed EROD and GST in mudskipper’s liver to evaluate the differences between selected sites with different amounts of petroleum compounds.

II. MATERIALS & METHODS

Mudskippers were collected from 2 stations along the Persian Gulf in Bushehr coastal area. Sampling was performed with survival fishing technique. PAHs have low vapor pressure and solubility in seawater, they tend to be absorbed by suspended organic matter and finally deposit in sediment [3]. Ameri Port and Soltani Inlet were selected with low and high amounts of organic matter (OM) in their mud which were analyzed for total organic matter and total organic carbon. Sampling took place between April and June 2011 (it’s their reproduction time so sex was determined in all individuals).

The fish were collected from both stations and killed and
EROD and GST activity were assayed from 2 sampling sites. For this purpose all steps were performed at 4°C or on ice.

In brief the livers were removed and after rinsing with ice cold serum physiologique, homogenized in phosphate buffer (100mM, pH=7.5) containing 1mM EDTA, 150mM KCl and 1mM dithiothreitol, 25% w/v. Homogenates were centrifuged at 12000 xg for 20 min then microsomal and cytosolic fractions were prepared by centrifuging the supernatant at 100000 xg for 60 min. The supernatant containing cytosol and used for GST activity assay and the microsomal pellets were resuspended in 100mM phosphate buffer pH=8 containing 150mM KCl and 1mM DTT, that was used for EROD assay. Microsomal and cytosolic proteins were measured by the method of Bradford as described in [13], using bovine serum albumin (BSA) as standard. The assays were done in triplicate with using of microplate reader (Synergy HT multi-mode biotech, USA).

EROD activity was assayed in microsomes according to the modified method of Martinez-Gomez et al. [14] adapted to a microplate reader. Final concentrations in the well (350 μl) were 100 mM phosphate buffer pH 8.0, 7-ethoxyresorufin and 0.25 mM NADPH. Fluorescence was determined at the 530/585nm excitation/emission wavelengths (pmol min⁻¹ mg⁻¹ protein).

GST activity was assayed in triplicate on cytosolic fraction by a modification of the method described in Nahrgang et al. [15] and expressed as nmol min⁻¹ mg⁻¹ of total cytosolic protein. The reaction mixture contained 10μl of cytosolic fraction, 10μl GSH (20 mM), 10μl CDNB (20 mM), 170 μl phosphate buffer (100mM, pH=7.5) in microplate by following the conjugation of GSH with CDNB at 340 nm (extinction coefficient: e = 9.6 mM⁻¹ cm⁻¹).

### A. Statistical analysis

Responses were determined individually in 10 organisms for each enzyme. Values are presented as mean±SE. Statistical significance of biomarkers responses across stations were assessed by t-test (P<0.05), using the software SPSS version 16.0. Assumption of normality of data was verified.

### III. Results

By analyzing sediments from stations, both of total organic matter and total organic carbon respectively was higher in Soltani Inlet (0.67% and 1.15%) while these factors were 0.39% and 0.67% in Ameri Port.

The enzymes activity in microsomal and cytosolic fractions of Ameri Port and Soltani Inlet’s fish is shown in Table 1 & Figure 1. Mean±SE hepatic EROD activities in Ameri Port and Soltani Inlet’s fish were 1.488±0.132 and 5.79±0.496 pmol resorufin min⁻¹ mg⁻¹ protein, respectively. That was about 4 times higher in Soltani Inlet than Ameri Port’s samples. Again GST activity in cytosolic fractions was showed a significant difference with higher mean in fish from Soltani Inlet than Ameri Port’s samples. Both of enzymes in male mudskippers were higher than female from the same site; however it wasn’t significant (Table 1).

<table>
<thead>
<tr>
<th>SEX</th>
<th>EROD pmol min⁻¹ mg⁻¹ protein</th>
<th>GST nmol min⁻¹ mg⁻¹ protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7.47±0.09</td>
<td>20.81±1.14</td>
</tr>
<tr>
<td>Female</td>
<td>4.21±0.64</td>
<td>15.36±1.95</td>
</tr>
</tbody>
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<td>2.12±0.11</td>
<td>8.38±0.18</td>
</tr>
<tr>
<td>Female</td>
<td>0.83±0.14</td>
<td>7.00±0.68</td>
</tr>
</tbody>
</table>

* showed significant differences (P<0.05)

### IV. Discussion

Significant increase of EROD in mudskippers from Soltani Estuary which have high exposure to oil pollution was supported the idea of sensitive tools for aquatic environment monitoring that was admitted in many field and laboratory studies. For instance Trembley et al. [16], were confirmed the reliability of this biochemical biomarkers. Damasio et al. [17] have seen significant differences between fish from impacted site’s EROD activity and the other stations in Mediterranean river (Spain). A comparison between sexes is showed differences between male and female from the same station and it was higher in males that is similar with some studies like Ramsak et al. [18] and Tuvikene, [19]: the suppression of EROD activity in females seems to be mediated by estradiol [18] though it wasn’t significant between fish in the same site.

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**TABLE I**

EROD & GST/activity in liver sample of mudskippers from different sites.

<table>
<thead>
<tr>
<th>N=10</th>
<th>EROD pmol min⁻¹ mg⁻¹ protein</th>
<th>GST nmol min⁻¹ mg⁻¹ protein</th>
<th>SEX</th>
<th>EROD pmol min⁻¹ mg⁻¹ protein</th>
<th>GST nmol min⁻¹ mg⁻¹ protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soltani Inlet</td>
<td>5.79±0.5*</td>
<td>18.1±1.40*</td>
<td>Male</td>
<td>7.47±0.09</td>
<td>20.81±1.14</td>
</tr>
<tr>
<td>Female</td>
<td>4.21±0.64</td>
<td>15.36±1.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ameri Port</td>
<td>1.488±0.132</td>
<td>7.69±0.41</td>
<td>Male</td>
<td>2.12±0.11</td>
<td>8.38±0.18</td>
</tr>
<tr>
<td>Female</td>
<td>0.83±0.14</td>
<td>7.00±0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Soltani inlet  Ameri port

![GST activity](image)

Soltani inlet  Ameri port

![EROD activity](image)

Fig.1. EROD and GST activity in liver of mudskippers were collected from Soltani Inlet and Ameri Port.

Recently assay of GST activity as a biomarker in aquatic organism is noteworthy. In agreement with present study GST shows a good sensitivity in several studies, though the affect of sex differentiation is not distinct: in this study GST in males was higher than females in the same site. Also Yuanyuan et al. [20], said GST is a good biomarker for early monitoring index of oil pollution, their results showed GST is sensitive and has significant differences between fish exposed to soil that was polluted by crude oil and control group.

These biomarkers assays showed this potential that they can be used in Persian Gulf as the early warning responses for biomonitoring the pollution. It’s a totally serious point that marine life of Persian Gulf with high biodiversity and special traits must get under control for conserving and preserving it for next generations.

REFERENCES