Comp Clin Pathol
DOI 10.1007/s00580-012-1535-1

ORIGINAL ARTICLE

The effect of water-soluble fraction of crude oil on serum biochemical changes in the great sturgeon *Huso huso*

Abdolreza Jahanbakhshi · Aliakbar Hedayati

Received: 6 February 2012 / Accepted: 8 June 2012

Abstract Caspian Sea has oil treasure as well as fisheries resources. One the most precious resources of fisheries are sturgeons. Crude oil is a very dangerous pollutant of water ecosystems which are in possession of oil resources. In the present study, juveniles beluga were exposed to four indirect doses (0, 10, 100, 500, and 1,000 ppm) of water-soluble fraction (WSF) of crude oil for 0 h, 48 h, and 7 days. The results showed that after 48 h of exposure, all biochemical indices (glucose, triglyceride, calcium, and protein) significantly increased ($P \leq 0.05$). Furthermore, all biochemical indices showed significantly elevation after exposure to WSF of oil for 7 days ($P \leq 0.01$). Among enzymatic parameters in both sampling times, alkaline phosphatase decreased and the aminotransferase and alanine aminotransferase increased along with different doses of WSF of crude oil exposure.

Keywords Biochemical · Biomarkers · Caspian Sea · Beliga · Oil pollution

Introduction

Among the different types of aquatic pollutants, petroleum products are contributed to be the most related to the water toxicology (Pacheco and Santos 2001). In Caspian Sea ecosystem, one of the largest oil spills occurred in sturgeon stocks area. However, these types of oil spills are widely present in the media; it is believed that the main source of inland waters contamination from oil and its derivatives is due to small and continuous leakages from underground bulk storage tanks, thereby reaching groundwater and later rivers (Tiburtius et al. 2005). Altogether, little research has been done on the effects of oil products on aquatic organisms.

Acute and chronic exposure to water-soluble fraction (WSF) of crude oil and its derivatives can induce a variety of toxic symptoms in aquatic animals. Petroleum hydrocarbons can produce free radical as a mediator in aquatic animals (Achuba and Osakwe 2003) and this modulation can lead to the biochemical changes.

Studies with the valuable fishes have indicated that exposure to the oil derivatives can causes different effects in serum biochemical concentrations (Pacheco and Santos 2001), suggesting that these contaminants might interfere in the fish stress response. Despite the previous research done on the effect of oil derivatives on fishes, some toxicological response levels in fish poorly understood, revealing the lack of data regarding the stress mechanism, as well as many biochemical responses (Pacheco and Santos 2001). In addition, there are only few papers concerning the effects of crude oil exposure on physiological indices in sturgeons and there is a real need of information about the effects of this fuel oil on these fish species.

Beluga, which is known as Great sturgeon (*Huso huso*), is native to the Caspian Sea and a valuable species for caviar production. Considering the growing environmental pollution, such as spills of oil distillate products into continental waters in the recent years in Caspian sea, the aim of the present study was to investigate the biochemical indices of *H. huso* exposed to crude oil, as potential biomarkers, in order to assess pollution through these petroleum products and getting information on the threat imposed by these spills to this valuable fish species.

A. Jahanbakhshi (✉) · A. Hedayati
Department of Fishery, Faculty of Fisheries and Environment, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
e-mail: abdolreza.jahanbakhshi@yahoo.com

Published online: 28 June 2012

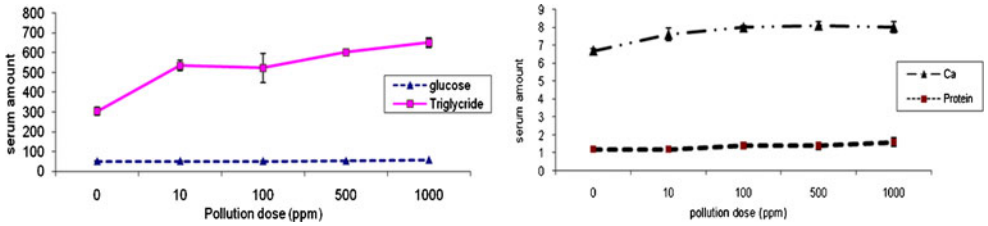

Fig. 1 Serum biochemical indices of beluga after 48 h exposure to WSF diesel oil

Materials and methods

Fish culture

Great sturgeons with average weight of 200 g, were supplied by fisheries research laboratory, Gorgan, Iran. Before toxicological tests, fish were acclimated to laboratory conditions (T, 20.1 °C; pH 7.44; DO, 7.89 mgO_2L^{-1}) for a minimum of 1 week in a 300-L tank with urban tap water. During acclimatization, fish were fed with commercial pellet twice a day.

Preparation of WSF

A part of commercial crude oil (purchase from Gas station, Gorgan, Iran), four parts of water in a glass container was added. The mixture was then exposed to intense sunlight for 6 h, simulating a diesel spill in tropical conditions. Then, the upper insoluble phase was discharged and the remaining water phase was collected (Simonato et al. 2008).

Crude oil exposure

Fish were submitted to different WSF doses (10, 100, 500, and 1,000 ppm) for 0 h, 48 h, and 7 days static exposure tests, performed in tanks of 300 L, each containing four fish. One control group, consisting of seven fish exposed only to water (the same as that used for acclimation), was sampled at each experimental interval along with the experimental groups, which were exposed to water plus WSF diesel oil. Three replicates were carried out for each experimental duration. During the experiment, water was continuously monitored for temperature, dissolved oxygen, pH, and conductivity (Hedayati et al. 2010).

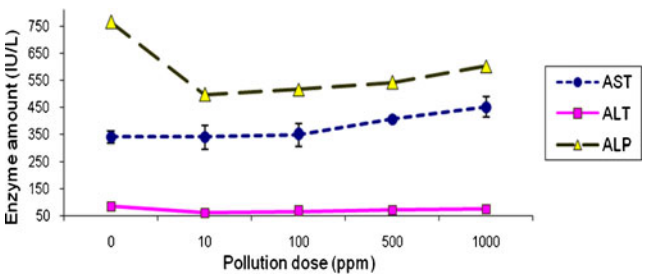

Fig. 2 Serum enzymatic indices of beluga after 48 h exposure to WSF diesel oil

Fish sampling

Fish were sampled for 0 h, 48 h and 7 days, in the morning before noon. Immediately after removing the fish from the tank, anesthetized with clove powder, and blood samples were taken from the caudal vein by means of heparinized plastic syringes. Blood was then centrifuged for 5 min at 3,000×*g* and serum samples were stored frozen until subsequent analysis (Hedayati and Safahieh 2011).

Biochemical assays

Serum samples were assayed in duplicate. Ultrapure water was used for all serum dilutions and standard preparations and duplicate readings were recorded for standards and serum samples. The quantitative determination of serum glucose and triglyceride were carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran, at 546 nm and 37 °C by the glucose oxidase method (Hedayati and Safahieh 2011). Serum total protein and calcium levels were determined using Pars Azmoon, Iran, kit, with bovine serum albumin serving as standard by the method of Canli (1996) at 546 nm and 37C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with Pars-Azmoon Diagnostics Infinity AST reagent kit and Sigma Diagnostics Infinity ALT reagent kit, respectively, by enzymatic methods with a Metrolab 2300 Plus auto-analyzer. Alkaline phosphatase (ALP) was determined by enzymatic method with the automate apparatus auto-analyzer, Metrolab 2300 plus, Argentina (Random Access Clinical Analyzer) with Darman Kave kit at 37C and 410 nm.

Statistical analysis

For each index, the data were tested for normality and homogeneity. One-way analysis of variance with Duncan's post hoc was used to determine significant differences to evaluate the effect of crude oil on parameters. The differences between means were analyzed at the 5 % probability level. Data are reported as means±standard deviation. The software

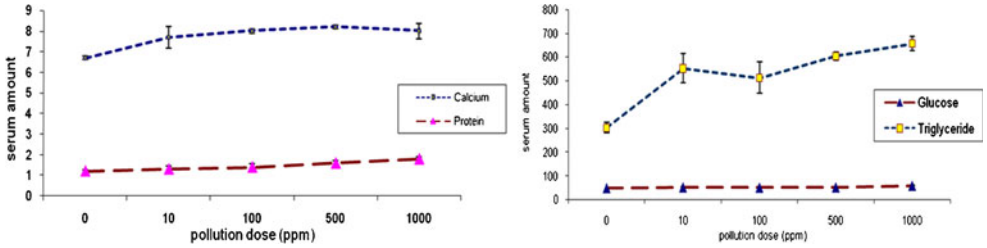

Fig. 3 Serum biochemical indices of beluga after 7 days exposure to WSF diesel oil

SPSS, version 11.5 (SPSS, Richmond, VA, USA) was used as described by Dytham (1999).

Results

Animals exposed to WSF crude oil for 48 h showed a significant elevation in all serum biochemical indices, in relation to the control, whereas serum glucose, triglyceride, calcium, and protein in fish exposed to WSF crude oil for 48 h was significantly greater compared to the control groups ($P \leq 0.05$; Fig. 1). Enzymatic indices do not have the same progress during 48-h exposure to WSF; however, AST significantly increased and ALT as well as ALP significantly decreased in both acute exposure to WSF (Fig. 2).

Furthermore, the results confirmed similar change after exposure on day 7. It means all serum biochemical indices showed significant increase in relation to the control treatment ($P \leq 0.01$). Whereas serum glucose, triglyceride, calcium, and protein was significantly higher by comparison with the control groups ($P \leq 0.05$; Fig. 3). Enzymatic indices also showed same progress on 48 h exposure to WSF, however all enzymes did not significantly change. AST significantly increased and ALP significantly decreased, but ALT did not significantly decrease on day 7 exposure to WSF (Fig. 4). On the other hand, serum triglyceride, total protein, and ALT showed a time-dependent increase and ALP revealed a time-dependent decrease during WSF crude oil exposure.

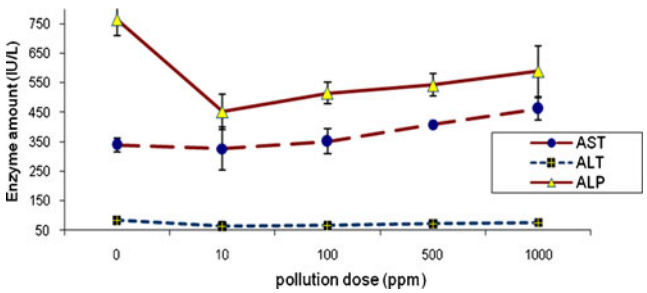

Fig. 4 Serum biochemical indices of beluga after 7 days exposure to WSF diesel oil

Discussion

AST, ALT, and ALP are the enzymes that have been applied for evaluating hepatocellular damage (Gad 2007). In the present study, this enzyme was used as antioxidant indices and indices of physiological response to WSF oil.

Aminotrasferase can leak from hepatocytes into serum in any condition and alters membrane permeability to a sufficient degree. The magnitude of serum activity elevation is proportional to the number of affected hepatocytes and is not indicative of the reversibility of the lesion (Gad 2007). Therefore, elevation of ALT and AST is probably due to reversibility of the lesion, or reversible cellular hypoxia secondary to hypoolemic shock than might occur with focal necrosis caused by localized hepatic abscess. Increased ALT is regarded as a specific enzyme indicator of hepatocellular injury in most species (Hedayati et al. 2010).

In the present study, WSF may affect intestinal functions by inhibiting the enzyme activity during absorption processes; especially serum alkaline phosphatase is mainly originated from intestine, liver, and bones. Oil is one of the agents which disturb cell lipid membranes, leading to the release of hydrolases. It seems that WSF has a bouncing effect on alkaline phosphatase activity. ALP is basically a membrane-bound enzyme, and any perturbation in the membrane property caused by interaction with pollutants could lead to alteration in ALP activity (Hedayati et al. 2010).

In the present work, fish were exposed to an indirect solution of WSF diesel oil. Levels of glucose were measured as conventional stress biomarkers to assess the reliability of stress response triggered under WSF crude oil stress. Serum glucose levels in control fish did not significantly difference, but in two times glucose increased along with WSF crude oil exposure. Levels of glucose often increase during the first phase of the stress response due to an elevated breakdown of glycogen (Wendelaar-Bonga 1997).

Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas 1996). In suboptimum or stressful conditions (internal or external), the chromaffin cells release catecholamine hormones, adrenaline, and noradrenaline toward blood circulation (Reid et al. 1998). Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through

glucogenesis and glycogenolysis pathways to cope with the energy demand produced by the stressor for the "fight of flight" reaction (Wendelaar-Bonga 1997). Glucose is then released (from liver and muscle) toward blood circulation and enters into cells through the insulin action (Nelson and Cox 2005).

Proteins are a major constituent in the metabolism of animals, and oil pollutions may be involved in the normal working of these molecules; therefore, it is important to detect the alterations in protein metabolism induced by oil exposure for further information. Probable alterations are the increased synthesis or breakdown of proteins and the inhibition or activation of certain enzymes (Canli 1996). The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of WSF exposure (Shakoori et al. 1994).

Biochemical properties of fish are poorly understood and there is no adequate knowledge concerning the metabolism of reference toxicants. More knowledge of these biochemical activities in the beluga is necessary before they can be employed as biochemical indicators of stress due to oil pollutions. Finally, the results obtained in this work exactly showed that WSF crude oil can cause serious biochemical alterations in *H. huso* at the physiological level.

Funding The authors thank the aquaculture research center and Fishery group for the supply of research material. This work was supported by the Gorgan University of Agricultural Sciences and Natural resources.

References

Achuba FI, Osakwe SA (2003) Petroleum-induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol Biochem 29:97–103

Canli M (1996) Effects of mercury, chromium, and nickel on glycogen reserves and protein levels in tissues of *Cyprinus carpio*. J Zool 20:161–168

Dytham C (1999) Choosing and using statistics: a biologist's guide. Blackwell, London

Gad SC (2007) Animal models in toxicology. CRC, New York, p 950

Hedayati A, Safahieh A (2011) Serum hormone and biochemical activity as biomarkers of mercury pollution in the Yellowfin seabream *Acanthopagrus latus*. Toxicol Ind Health. doi:10.1177/0748233711410916

Hedayati A, Safahieh A, Savar A, Ghofleh Marammazi J (2010) Assessment of aminotransferase enzymes in Yellowfin sea bream (*Acanthopagrus latus*) under experimental condition as biomarkers of mercury pollution. World J Fish Mar Sci 2(3):186–192

Lucas A (1996) Physical concepts of bioenergetics. In: Lucas A (ed) Bioenergetics of aquatic animals, Englishth edn. Taylor and Francis, France

Nelson DL, Cox MM (2005) Lehninger principles of biochemistry, 4th edn. Freeman, New York, 1013p

Pacheco M, Santos MA (2001) Biotransformation, endocrine, and genetic responses of *Anguilla anguilla* L. to petroleum distillate products and environmentally contaminated waters. Ecotoxicol Environ Saf 49:64–75

Reid SG, Bernier NJ, Perry SF (1998) The adrenergic stress response in fish: control of catecholamine storage and release. Comp Biochem Physiol C 120:1–27

Shakoori AR, Iqbal MJ, Mughal AL, Ali SS (1994) Biochemical changes induced by in organic mercury on the blood, liver and muscles of freshwater Chinese grass carp, *Ctenopharyngodon idella*. J Ecotoxicol Environ Monit 4:81–92

Simonato JD, Guedesb CLB, Martinez CBR (2008) Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicol Environ Saf 69:112–120

Tiburtius ERL, Peralta-Zamora P, Emmet A, Leal ES (2005) Degradac-ão de BTXs via processos oxidativos avanc-ados. Quim Nova 28:61–64

Wendelaar-Bonga SE (1997) The stress response in fish. Physiol Rev 77:591–625