A Comparison of the Effects of Crude Oil on Testis of two Teleosts (*Poecilia sphenops* and *Xiphophorus helleri* [1]

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***Abstract*—**Fish can suffer serious impacts to their physical health and reproductive success when exposed to crude oil. Gonadal histology can provide some important understandings for the effects of various chemicals. The aim of this study is to determine the effects of water soluble fractions of crude oil (WSF) on the testis of *Poecilia sphenops* and *Xiphophorus helleri* comparatively.

Tissue samples of experimental animals of each species was exposed to 40% concentrations of WSF for 96 h, dissected, and investigated with a comparison to negative controls. The testis of the experimental fish were expressed some marked histological changes identified as widespread hemorrhage and vasodilation in testicular tissue, disorganized and atrophied seminiferous tubules and germinal epithelium cells, pycnosis and necrosis in Leydig cells and widely necrosis in interstitial area between seminiferous tubules were viewed.

According to the levels of these alterations, the most resistant species was recorded as *Poecilia sphenops*. The results are clearly pointed that, oil spill is a serious environmental problem not only for seawater fish, but also for freshwater ones. All of the enhanced technical researches use advanced techniques can be performed on the basis of fundamental data on the histological level; and histopathology is the golden key of the researches in specific areas. In terms of reproductive success of fish, it can be concluded that more researches, both field and laboratory, is needed to reveal the importance of these histopathological alterations.

***Keywords*—**Crude oil, Histopathology, *Poecilia sphenops*, Testis, *Xiphophorus helleri*.

I. Introduction

WHEN remembered the well-known tanker ship accidents of Amoco Cadiz (1978), Exxon Valdez (1989), Prestige (2002) and Cosco Busan (2007); everybody was reached a consensus that oil spill is a global environmental problem [1,2]. Destructive effects of crude oil on aquatic environment were more recently seen in Gulf of Mexico spilling (2010) [3]. Due to it is not only released accidentally, but also invaded continuously by the intense activities of production, transportation and consumption; both of the marine and freshwater ecosystems is under a great threat of the crude oil [4].

The WSF is consist of toxic mixtures of hydrocarbons and heterocyclic compounds; and affects all of the aquatic organisms included fish, which are widely used for biomonitoring about water pollution [5-8]. These substances can be significantly adversely affect aquatic vertebrate reproductive success and population dynamics and reduce biodiversity in long term [9, 10].

Morphological, especially histological, studies were constituted a rapid, reliable and efficient method for the detection of acute and chronic effect due to chemical exposure to aquatic organisms [11-13]. Additionally, when associated with other biomarkers, the results are more efficient [2-11]. By the way, histopathological changes of the testes are concluded as a common indicator for exposition to chemicals. From this point of this study, in order to determine the reproductive problems arising from crude oil, it was aimed at comparing the acute effects of WSF on the testis of *Poecilia sphenops* and *Xiphophorus helleri*, tropical freshwater teleosts, histologically.

II. Materials and Methods

This study was carried out in accordance with the Animal Ethics Committee Report (No. 2008-49) based on Decisions of Ethical Committee for Experimental Animals prepared by Faculty of Pharmacy, Ege University.

A. Animal Groups and Experimental Design

Specimens of the *Poecilia sphenops* (molly) (n=20), with an average body weight of 1.13±0,06g and length of 4-6cm and specimens of the *Xiphophorus helleri* (swordtail) (n=20), with an average body weight of 2.85±0,5g and length of 6±1cm were obtained commercially. During the acclimatization period of 15 days, each group were kept in 10L aquaria, with aerated tap water at 22±2˚C (pH 7,5±0,4). Water quality parameters were measured daily; and calculated as follows: French hardness value= 31.0±1; salinity= 0,1%; dissolved oxygen 3.5±0.5 mg/L; free chlorine= 0,6 mg/L; sodium= 18 mg/L; aluminum= 0.030 ± 0.008 mg/L; and iron=0.08 ± 0.055 m/L.

Crude oil [API: 31.25, density: 0,8690 kg/L, total sulfur: 1,98 (% weight), pour point: -35°C, water: 410 ppm] was

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maintained from Tüpraş Petroleum Refinery. Slow-stirring method [14, 15] was used for providing WSF of crude oil. Experimental concentrations of WSF (10%, 20% and 40%, respectively) were prepared by mixing of stock solutions and water.

Animals were randomly divided into one control and one experimental groups of 10 fish each. No treatment was applied to controls. Experimental groups were exposed to 40% concentration of WSF for 96h. All of the specimens were euthanized in MS222 (Sigma-Aldrich).

B. Histological Analyses

For light microscopic examination, the testes were collected fixed in Bouin's fixative for 24h at room temperature; dehydrated in alcohol series, cleared in xylol and embedded in paraffin. Thus, fixed tissues were then serially sectioned at 5 µm sagitally and stained with Mayer's Haematoxylin-Eosin (H&E) procedure; entirely examined and photographed light microscopically (Olympus BX-51; Altra20 Soft Imaging System).

III. Results

A. Xiphophorus helleri

Control Group

Testicular parenchyma in *X. helleri* is surrounded by a capsule consisting of connective tissue and tunika albuginea. Parenchyma consists of seminiferous tubules in the form of cysts embedded in interstitial space. Interstitial space contains interstitial cells producing male sex hormone in connective tissue rich in terms of fibroblasts and collagen fibrils. Primary and secondary spermatogonia are closely located in seminiferous tubules surrounded by a thin epithelium. Primary and secondary spermatocytes, spermatids, mature spermatozoa and sertoli cells are located in seminiferous tubules. Sertoli cells support, protect, feed the developing spermatozoa spermatogenic cells and phagocyte the residual cytoplasmic particles during formation of spermatozoa. Sertoli cells in squamous appearance displays increase in the numbers in the periphery of seminiferous tubules called spermatozeugma and include spermatozoa (Fig.1).

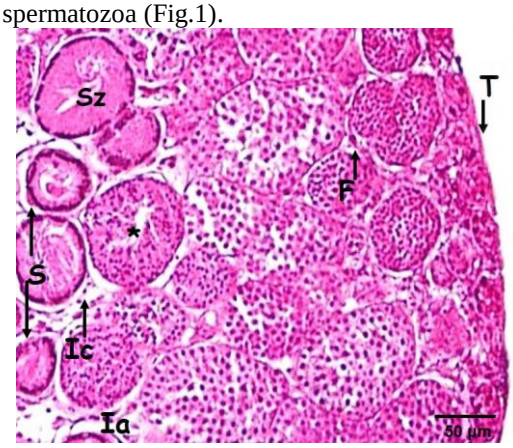

Fig. 1 *X. helleri*, control group; T: tunica albuginea, Ia: interstitial area, Ic: interstitial cell, *: seminiferous tubule, F: fibroblast; Sz: spermatozeugma, S: sertoli cell, H&E.

Experimental Group

Necrosis in the general structure of the testes and atrophy of tubules especially called as spermatozeugma are most advanced. Due to the atrophy the number of tubules have decreased significantly. Oedema observed in some places of sections spread over very large areas in other sections. It was observed that the number and size of sertoli cells dramatically increased (Fig.2).

There was a significant decrease in the number of tubules due to atrophy and some accumulations observed in the center of these atrophic tubules. The incidences of sloughing and disorganized tubules significantly increased in treatment group compared to the control group. Oedema observed in various places of some sections spread over very large areas in other sections. It was observed that the number and the size of Sertoli cells increased greatly (Fig.2).

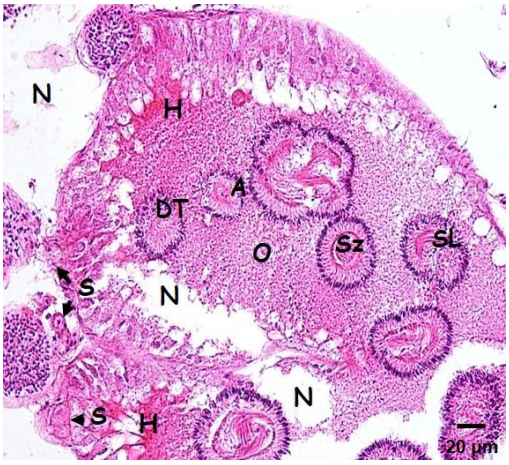

Fig. 2 *X.helleri*, experimental group; N: necrosis, Sz: spermatozeugma, H: hemorrhage, O: oedema, S: sertoli cell, DT: disorganized tubule, A: accumulations, SL: sloughing, H&E.

B. Poecilia sphenops

Control Group

Testis parenchyma surrounded by tunica albuginea consists of efferents ducts embedded in interstitial area comprising loose connective tissue and seminiferous tubules or cysts contain cells at various stages of spermatogenesis and supported by sertoli cells. Tubules are separated by a thin epithelium (Fig.3).

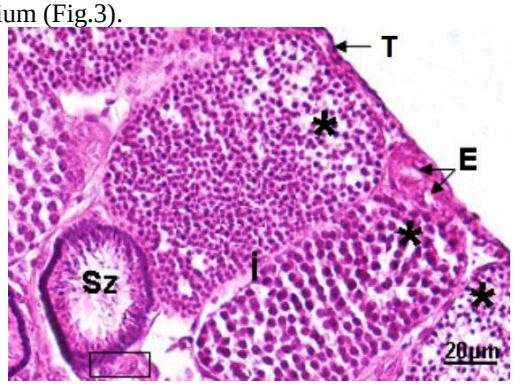

Fig. 3 *P.sphenops* control group; T: tunica albuginea, *: seminiferous tubule, İ: interstitial area, Sz: spermatozeugma, ▭: sertoli cell, E: efferent duct, H&E.

Experimental Group

A marked testicular damage caused by WSF was observed throughout the parenchyma. Oedema, hemorrhage and necrosis are frequent and obvious. Seminiferous tubules are in view of scattered, and leydig cells could not be distinguished (Fig.4).

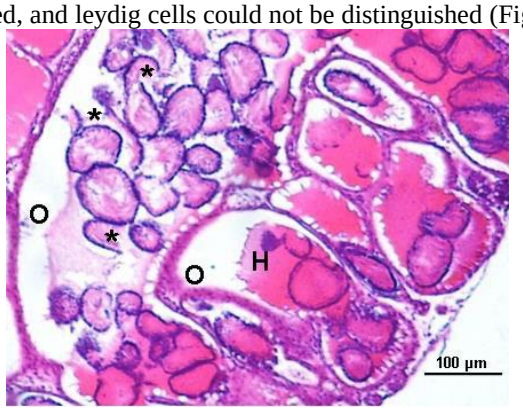

Fig. 4 *P.sphenops* experimental group; O: oedema, H: hemorrhage, *: disintegration in the seminiferous tubules, H&E.

IV. Discussion

It is known that the histological investigations are a powerful tools in studies on the reproductive health of fish and that the histological structure of the reproductive organs are sensitive, although not to chemicals, to pollution [16, 17]. The association between the changes in the reproductive organs and the amount of pollution plays a great role in determining the pollution level which threatens the fishery products. Pollutants that inhibit the endocrine system have attracted great attention in recent years, which pave the way for new studies investigating the effects of toxic chemicals on the teleost reproductive system [18-23].

The present study was conducted to examine histopathological changes in the testes of *X. helleri* and *P. sphenops* comparatively which were exposed to WSF. After exposure to WSF, a slowing down of the movements of animals in the experimental group *X.helleri* was observed more than the other experimental group (*P.sphenops*); on the other hand, death did not occur in any group during the experiment. Histopathological changes were more prominent in the experimental group *X.helleri* than in the experimental group of *P.sphenops*.

There are many studies particularly on the adverse effects of heavy metals on the teleost reproductive system. For example, it is known that cadmium inhibits sexual maturation [18], slows down embryonic and larval development [24, 25], and alters hormone balance [20]. Histopathology observed in the present studies is consistent with the findings about the formation of extensive necrosis, tubular atrophy, and testicular deformation not affected by chemicals having been exposed to.

Concentration which increases as result of exposure to some toxic substances, the increase in the number of cells in the interstitial areas of the testis depending on the time, and oedema were interpreted as the biochemical response of the testicular secretory cells. But we don't practice with different concentrations in both species [21].

WSF caused tubular degeneration in each species. The incidences of disorganized tubules were significant in all treated groups. Mohamed (2003) investigated the effects of the polluted water from El-Salam Canal in *Oreochromis niloticus*, *Tilapia zillii* and *Synodoniis schall* and determined the seminiferous tubular epithelial degeneration in low dose group and significant tubular atrophy as well as germ cell degeneration in high dose group. As reported that in the testis, degenerative and necrotic changes in the cellular elements of seminiferous tubules, with inhibition of spermatogenesis (some seminiferous tubules appeared lucent or with a lesser number of sperms, indicating lack of active spermatogenesis), focal areas of necrosis and fibrous capsules around some seminiferous tubules were observed- Besides, malformation and distortion of the architecture of some seminiferous tubules were seen [26].

As reported that one of the most common morphological responses of Sertoli cells to harm was vacuolation and following this, germ cell degeneration, disorganization or exfoliation was generally seen [27]. It was reported that pesticides give rise to similar histologic abnormalities as sloughing, multinucleated giant cells, germ cell degenerations, vacuolization and the necrosis of spermatogenic cells in rats [28-31]. These findings are in accordance with our results. Similar findings such as seminiferous tubular degeneration, abnormal spermatogenesis and sloughing were observed in *S. occidentalis* [32]. Oedema and haemorrhage were also reported in the interstitial tissue of the testes of rats exposed to dimethoate [33], cadmium [34] like in *X.helleri* and *P.sphenops* exposed to WSF. The exact mechanism for these defects is still not clear. WSF has been reported to affect the function and morphology of reproductive organs in fishes [35-38]. Blazer (2002) determined sperm necrosis based on nuclear pleimorphism by pyknosis, karyorhexis and karyolysis stages in the samples of *Cyprinus carpio* ve *Micropterus salmoides* collected from different areas [16]. In our study, nuclei of spermatozoa couldn't distinguish in the spermatozeugma structure was observed the most atrophy. However, hyperplasia and hypertrophy observed in Sertoli cells is a typical sign would be phagocytes atrophic spermatozeugmas. The findings are confirmed this assessment. As reported that it was observed the histopathological changes including degeneration of spermatogenic cells in testes of male rats exposed to a high dose of pesticide [39]. It is suggested that the cause of testicular histopathology in rats exposed to carbaryl may be caused by the decreased testosterone level [40]. To disturb spermatogenesis, pesticides acts by hormonal or genotoxic pathways by passing through the blood-testis barrier [41, 42]. More detailed studies are necessary to elucidate the effects of the WSF on male reproductive system and physiologic mechanisms inducing these effects.

In the present study, accumulations were observed in *X.helleri* are thought to be the remnants of atrophic tissue. Accumulation regions observed in the tubules of the

experimental group of *X.helleri* were interpreted as the accumulation of solid matter by Çolakoğlu et al. (2004) [43]. We think that these accumulations are the remnants of necrotic germ cells. The increase in the number and size of sertoli cells in the periphery of necrotic spermatozeugma was interpreted the deformation of the spermatozoa [44, 45].

Our findings indicate that WSF affect the testicular tissue of *X. helleri* and *P.sphenops* adversely to a great extent and that the testicular tissue of *X. helleri* more affected by reason of WSF. Our study will provide the basis for the fact that the reproductive success and population dynamics of aquatic vertebrates should be carefully evaluated particularly after petroleum accidents likely to occur during transport despite the very great care taken.

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