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Impact of Zinc and Selenium on Reproduction Profile of Hybrid Red Tilapia Treated with Sub-lethal Concentration of Water-Soluble Fractions (WSFs) of Petroleum Crude Oil

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ABSTRACT

After determination of LC_{50}, sublethal concentration of water soluble fractions (WSFs) of petroleum crude oil -**quarter of LC_{50}-** and same concentrations of zinc and selenium '1.3 mg/l were used to study their effects on reproduction profile of hybrid red tilapia. The experiment was continued for 2 months and the groups were "control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil". Triplicates of each treatment were established and fish was fed twice/daily on commercial diet 30% protein. By experiment's end, sex ratio was calculated, gonads were obtained for Gonado-somatic index 'GSI', biochemical and histological analysis. Sex ratio was around the normal, 3 females :1 male; results of female GSI showed no significant different between treatments while male GSI showed significant different between treatments compared to control. Steroid's hormones 'estradiol, testosterone and progesterone' showed significant differences between treatments compared to control which has no significant differences with other treatments. The histological analysis showed changes in reproduction organs (testes and ovary) tissues in WSFs of petroleum crude oil treatments, while these changes were eliminated in treatments with WSFs of petroleum crude oil treatment + zinc or selenium; in zinc and selenium treatments the tissue structure closed to be normal compared to the control group.

INTRODUCTION

Oreochromis hybrids including red tilapia had been used extensively, since tilapia culture is considered as the most popular because it is relatively easy for culture in different aquaculture systems and a favorable selection fish as food **(Siddiqui and Al-Harbi, 1995)**.

Red tilapias are hybrid that selected from tilapia species in the genus *Oreochromis. Oreochromis* sp. is taxonomically distinct from other tilapia genera because females that incubate fertilized eggs in their mouths.

Thus, red tilapia breeds similarly to Nile tilapia, blue tilapia, *O. aureus,* Mozambique tilapia, *O. mossambicus*, and Zanzibar tilapia, *O. hornorum*, and the first documented of hybrid red tilapia was produced in Taiwan by the late 1960s; it was a result

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of crossing between a normal male *O. niloticus* and mutant reddish-orange female *O. mossambicus* and this strain had been called 'the Taiwanese red tilapia' **(Watanabe *et al.,* 1989)**.

Environmental pollution including water pollution considered as a serious issue in all world countries, because it is not only affecting the survivability and reproduction processes of aquatic organisms, but also adverse many impacts on human health through bioaccumulation **(Dai *et al.,* 2014)**.

Water soluble fractions (WSFs) of petroleum crude oil are a toxic and complex mixture of hydrocarbons that all aquatic organisms directly confronted oil spills; it plays an important role in the petroleum crude oil's toxicity to aquatic organisms **(Lari *et al*., 2015)**. Petroleum crude oil contains high levels of PAHs, but the relative concentration of each compound depends on both type and source of oil. Most of polyaromatic hydrocarbons 'PAHs' have toxic, mutagenic and/or carcinogenic properties. PAHs considered as highly lipid soluble and this clearly make its absorption easily from the gastrointestinal tract of organisms and rapidly distributed in different kind of tissues with oblivious tendency for localization in body fat. Metabolism of PAHs occurs via the cytochrome peroxidase (P450)-mediated mixed function oxidase system with oxidation or hydroxylation as the first step **(Abdel-Shafy and Mansour, 2015)**.

The terms of both antioxidant and free radical are known as popular expressions that used by nutrition scientists and other healthcare professionals. Recently, much information has appeared about the oxidative stress' roles in the development of number of serious illnesses, such as certain cancers, cardiovascular diseases, and age-related degenerative diseases, and about the possible therapeutic value of antioxidants against these illnesses. The importance of fruits, vegetables, berries and legumes as part of a healthy diet is generally accepted. The possible reason why these foods can promote good health because of the presence of a range of antioxidants in edible plants, for example vitamins D and C, carotenes, selenium, folates, and phenolics compounds including flavonoids **(Barhé and Tchouya, 2016)**.

The aim of this work is to study the effects of Water soluble fractions 'WSFs' of petroleum crude oil as a source of poly aromatic hydrocarbons 'PAHs' on reproduction of hybrids red tilapia and the alleviation impact of zinc and selenium on these effects.

MATERIALS AND METHODS

1. Preparation and determination of water soluble fractions 'WSFs' of petroleum crude oil

The preparation of WSFs of petroleum crude oil was according to **Anderson *et al.* (1974)** method and was determined according to **Parsons *et al.* (1985)** method. LC_{50} was determined by using arithmetic method of karber adapted by **dede and Igbigbi (1997),** and according to results of **Dighiesh *et al.* (2019)**, LC_{25} concentration of 5.25 mg/L was used.

2. Experiment Design

Six treatments including control, triplicate treatment was used for each treatment. Fish was adapted in glass aquaria (100×40×50 cm) filled with 80-liter synthetic sea water for 2 weeks before treatments.

Fingerlings (8.5±1.5g) were divided randomly into 6 groups with stocking rate of 35 fingerlings per aquarium. First group (control) was the untreated, the second group exposed to WSFs of petroleum crude oil only, the third group exposed to zinc only as zinc nitrate, the fourth group exposed to selenium only as selenium dioxide, the fifth group treated with WSFs of petroleum crude oil+ Zn, and sixth group treated with WSFs of petroleum crude oil and selenium as illustrated in Table 1.

Table 1. Experiment design

Material / Treatment	WSFs of crude oil	$Zn(NO_3)_2.6H_2O$	SeO_2	WSFs of petroleum crude oil + $Zn(NO_3)_2.6H_2O$	WSFs of petroleum crude oil + SeO_2
Control (T1)					
T2	■				
T3		■			
T4			■		
T5				■	
T6					■

3. Antioxidants

Zinc and selenium were used as antioxidants with the same concentration of LC_{25} of WSFs of petroleum crude oil.

4. Sex ratio

The sex ratio had been given as males: females (M: F) according to **Vazzoler (1996)** equation total number of males/total number of females.

5. Gonado-somatic Index% (GSI)

GSI (%) had been determined as gonad percentage to whole body weight according to **Das (1997)** equation

GSI = [weight of gonad (g/fish)/weight of fish (g/fish)] *100

6. Hormonal Analysis

Fish gonads "females and males separately" had been homogenized for hormonal analyses according to **Sakaguchi (2004)** method.

7. Histopathological analysis

Specimens of fish gonads were fixed in bouin solution (75% saturated picric acid, 25% formalin, and 5% acetic glacial acid). Tissues was dehydrated in an ethyl alcohol series of ascending concentrations (70, 80, 90, 95, 100%), after that maintained in methyl benzoate overnight and then embedded in paraffin wax, blocked, and sectioned at 5-6 µm. The tissue sections were stained with hematoxylin-eosin (H×E) and examined by "Zeiss" microscope. Three sections of each tissue were examined. Sections of examined organs photographed as requested **(Drury and Wallington, 1980)**.

8. Statistical analysis

Data had been analyzed statistically by using "one-way" analysis of variance (ANOVA); SPSS version 22 had been used, as described method of **Dytham (1999)**. Means had been compared by using **Duncan's test (1955).** All output data had been expressed as means±standard error and the significance level was set at the probability level of $P<0.05$.

RESULTS AND DISCUSSION

Environmental pollution caused by industrial development, technology, and formal settlements threaten many fresh and marine ecosystems. Not only does environmental pollution cause a decrease in water quality, but subsequently affects all living organisms in that system. Fish health may reflect and be a good indicator of specific aquatic ecosystem's health status. Early toxicity of pollution may however be an evident on cellular or tissue level before appearing of significant changes that could be identified in fish behavior or external signs **(Seiyaboh *et al.,* 2013)**.

Exposure of fish to petroleum crude oils induce ethoxyresorufin-deethylase (EROD) activities in fish **(Lee *et al.,* 2011)**. EROD alter fishes' embryonic development and the production of transformation enzymes specially at the highest concentrations play a role in its elimination **(Pauka *et al.,*2011)**.

The Nile tilapia (*O. niloti cus*) can mature sexually within 6 months and spawn when still very small (i.e.below 40 g). This diversion of energy from growth into reproduction is a natural phenomenon shown by wild tilapias under certain conditions **(Iles, 1973)**; but it becomes acute within cultured populations **(Mair and Little, 1991)**. Once mature, tilapia females produce multiple batches of eggs, the oral incubation of each egg batch being followed by only a short period of recovery before they are ready to spawn, approximately 1.0 month on average, is required for a female tilapia to complete each reproductive cycle. Spawning may not occur every month, but under favorable environmental conditions a female of Oreochromis will normally produce several batches of fry in a year.

Fig. 1 shows that the control (T1) group consisted of 78 % females and 22% males, WSFs of petroleum crude oil (T2) group consisted of 73% females and 27% males, zinc (T3) group consisted of 71% females and 29% males, selenium (T4) group consisted of 77% females and 23% males, zinc+ WSFs of petroleum crude oil (T5) group, consisted of 78% females and 22% males, and selenium+ WSFs of petroleum crude oil (T6) group consisted of 75% females and 25% males; the sex ratio (males to females) was around the natural ratio within all treatments.

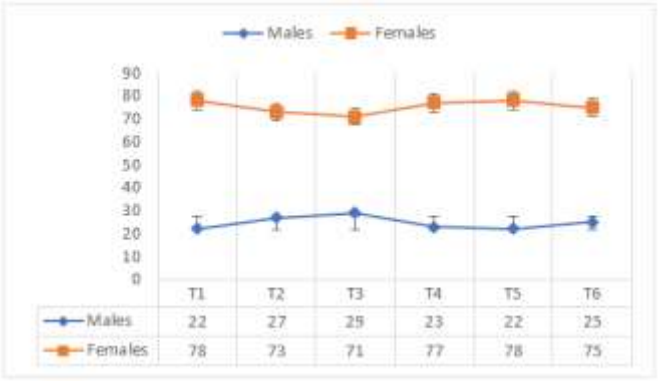

Fig. 1. "Males and females" sex ratio

Figs 2,3 show males and females maturation ratio percentage, where columns refer to immature status and curves refer to mature status.

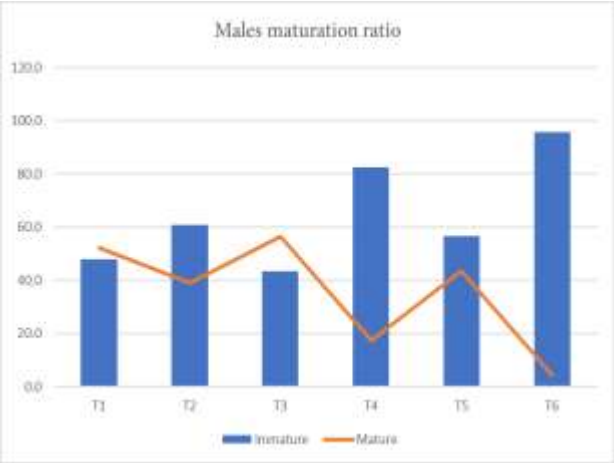

Fig. 2. Males' maturation percentage

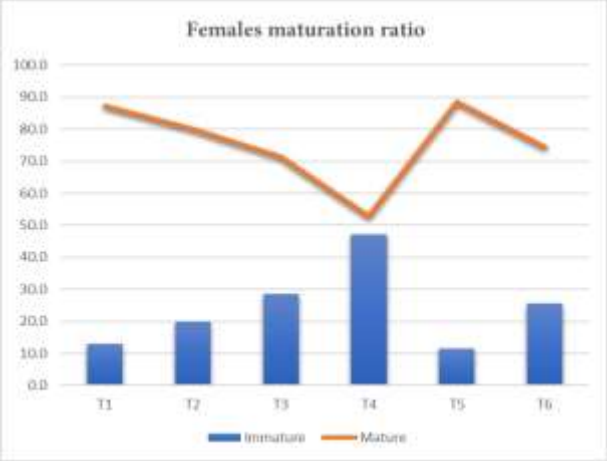

Fig. 3. Females' maturation percentage

Trewaves (1983) indicated that *Sarotherodon melanotheron* can actively achieve sexual maturity at a very small body size, a trait which is an adaptive advantage for an opportunistic invasive species. Females produce a clutch of respectively 50 orange eggs that vary from 1.5-4 mm in diameter depending on the size of the female. Incubation within the buccal cavity "mouth brooding" by the male ranges from 6-22. Black chin tilapia inhabits estuarine habitat such as mangrove marshes, and travel freely between fresh and saltwater environments.

Results in Table 2 show that males' gonado-somatic index was slightly significant differences (P≤0.05) and the results was 0.48± 0.57, 0.83±0.22, 1.36±0.28, 1.13±0.38, 0.40±0.07 and 0.43±0.11 for control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil treatments, respectively, while females' gonado-somatic index results showed that there is no significant differences between treatments and the results was 2.97± 0.21, 2.86 ± 0.23, 2.80±0.26, 2.74±0.34, 3.08±0.19, and 3.25±0.21 for control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil respectively.

Additional complication is due to that the reproduction is the ultimate step in a long-term process, beginning from germ-cells' migration in embryos to gametes' maturation in reproducing adults, all these steps can be disrupted throughout life stages and lastly results in reproduction fail. Complication is also may due to the environment xenobiotics are present as complex mixtures of compounds which taken separately can have pro/ or anti-estrogenic properties **(Kloas *et al.,* 2009)**. PAHs showed an interference with reproduction in fish and other animals, including invertebrates **(Seruto *et al.,* 2005; Tetreault *et al.,* 2003)**.

Long-term or chronic exposure to pollutants almost leads to a decrease in gonado-somatic index GSI (as agreed with our males GSI), smaller, less-developed oocytes, and fewer mature oocytes and on the other hand an increase in the numbers of atretic follicles **(Sukumar and Karpagaganapathy, 1992)**.

Table 2. The effect of sublethal level of water soluble fractions of crude oil, zinc, and selenium on gonado- somatic index of hybrid red tilapia (males, females)

Treatments	*Gonado- somatic index %*	
	Males	Females
Control (T1)	0.83^{abc} ±0.22	2.97^{a} ± 0.21
WSFs of petroleum crude oil (T2)	0.48^{bc} ±0.57	2.80^{a} ± 0.23
Zinc(T3)	1.36^{a} ±0.28	2.86^{a} ± 0.26
Selenium (T4)	1.13^{ab} ±0.38	2.74^{a} ± 0.34
Zinc+ WSFs of petroleum crude oil (T5)	0.40^{c} ±0.07	3.08^{a} ± 0.19
Selenium+ WSFs of petroleum crude oil (T6)	0.43^{c} ±0.11	3.25^{a} ± 0.21

Means followed by different letters in the same column differ significantly (p ≤0.05).

Reproduction is considered a key function to all species survival, but also it is the target of some xenobiotics at the same time. Disruption of reproduction may be the results of direct passives such as reproduction hormones modification in the case of endocrine disruptor compounds or may be through the indirect consequence of many general physiological degradation that includes growth or behavioral defects **(León -Olea *et al.,* 2014)**.

In normal reproductive development, the relationship between steroid hormones and their receptors plays a major role. Maintenance of appropriate levels of hormone ligands in both circulation and at target tissues depends on coordinated biosynthesis and degradation. Much experimental evidence shows that WSFs of petroleum crude oil may interfere with specific receptor activation, steroid biosynthesis, and degradation, but the possible biological consequences are less well understood. Induced CYP3A (and other) activities may result in increased clearance of endogenous steroid hormones. However, this may be compensated by regulatory mechanisms for steroid homeostasis **(You, 2004)**. In fish, increased releasing of estrogens in the liver may represent a particular problem because the liver is the main production site for pivotal components of the oocytes, Vtg, and Zrp **(Arukwe and Goksøyr, 2003)**.

The results of estradiol indicated that there were s significant differences between treatments (P≤0.05) and the results was 15.83±6.83, 56.16±22.57, 9.00±0.00, 9.00±0.00, 147.43±43.94 and 9.00±0.00 for control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil, respectively.

Regarding to the results of progesterone**,** there was significant differences (P **≤** 0.05) between treatments 2.58±0.22, 4.47^{b}±0.46, 3.25±0.38, 5.87±0.32, 4.57±0.82 and 10.18±1.13 for control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil, respectively.

The results of testosterone indicated that there were slight significant differences (P≤0.05) between treatments 1.01±0.09, 3.26±0.82, 1.19± 0.18, 0.68±0.19, 3.06±0.54 and 0.52±0.06 for control, WSFs of petroleum crude oil, zinc, selenium, zinc+ WSFs of petroleum crude oil and selenium+ WSFs of petroleum crude oil, respectively.

Table 3. The effect of sublethal level of water soluble fractions of crude oil, zinc and selenium on estradiol, progesterone, and testosterone levels of hybrid red tilapia

Treatments	Estradiol, E2 pg/ml	Progesterone ng/ml	Testosterone ng/ml
Control (T1)	15.83^{b} ± 6.83	2.58^{c} ± 0.22	1.01^{b} ± 0.09
WSFs of petroleum crude oil (T2)	56.16^{b} ± 22.57	4.47^{bc} ± 0.46	3.26^{a} ± 0.82
Zinc(T3)	9.00^{b} ± 0.00	3.25^{c} ± 0.38	1.19^{b} ± 0.18
Selenium (T4)	9.00^{b} ± 0.00	5.87^{b} ± 0.32	0.68^{b} ± 0.19
Zinc+ WSFs of petroleum crude oil (T5)	147.43^{a} ±43.94	4.57^{bc} ± 0.82	3.06^{a} ± 0.54
Selenium +WSFs of petroleum crude oil (T6)	9.00^{b} ± 0.00	10.18^{a} ± 1.13	0.52^{b} ± 0.06

Means followed by different letters in the same column differ significantly (p ≤0.05).

Role of zinc in modulating oxidative stress has recently been recognized. Oxidative stress is an important contributory factor in many chronic diseases, like atherosclerosis and related vascular diseases, mutagenesis and cancer, immunologic disorders, neurodegeneration, and the aging process **(Castro and Freeman, 2001; Lachance *et al.,* 2001)**.

The study of reproductive physiology provides a complete measure of the effects of pollutants on whole organisms and important insights into the potential risks to populations. It is therefore very serious to study the long-term/ chronic effects of petroleum compounds at ecologically relevant doses, which may affect physiological processes in organisms, further propagating to changes at the population and ecosystem levels **(Bender *et al.,* 2016)**. 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 **(Hatekeyama and Yasuno, 1987; Wang *et al.,* 2014)**.

Chronic/ long-term pollution may lead to a decrease in quality of gametes, thereby deteriorating reproductive success and resulting a significant threat to the sustainability of fish population **(Doherty *et al.,* 2013)**.

In the present study, testes from the control group fish showed normal seminiferous tubules with the different developmental stages of spermatogonia, spermatids, and mature spermatozoa in their lumenae Fig. 4-a. The testes treated with WSFs of petroleum crude oil revealed focal degeneration and necrosis of some seminiferous tubules that had either degenerated spermatogonial cells or complete depletion of all spermatogeneic stages Fig. 4-b. The testes treated with selenium revealed normal structure of testicular tissue Fig. 4-c. The testes treated with zinc revealed normal histological picture as well Fig. 4-d. The testes treated with WSFs of petroleum crude oil and protected with selenium showed pronounced improvement of the testes with occasional degenerated seminiferous tubules Fig. 4-e. The testes treated with WSFs of petroleum crude oil and protected with zinc showed fairly normal spermatogonial cells, spermatids and spermatozoa Fig. 4-f.

Özlem and Sema (2015) said that WSFs of petroleum crude oil caused tubular degeneration in both (*Poecilia sphenops* and *Xiphophorus helleri*). The incidences of disorganized tubules were

Mohamed (2003) investigated the effects of the polluted water from El-Salam Canal in *Oreochromis niloticus*, *Tilapia zillii*, and *Synodoniis schall*, and determination of 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.

In the present study, ovaries from the control group fish showed normal ovarian histological structure with presence of different developmental stages in ovarian tissue and mainly, chromatin nucleolar oocyte, perinuclear oocytes, cortical alveolar oocyte, and mature ripped oocyte Fig. 5-a. On the other hand, ovaries treated with WSFs of petroleum crude oil showed degeneration, edema, and necrosis of most of the stages Fig. 5-b. The ovaries treated with selenium revealed normal ovarian structure Fig. 5-c.

The ovaries treated with zinc revealed normal histological picture of the ovaries Fig. 5-d The ovaries treated with WSFs of petroleum crude oil and protected with selenium showed pronounced protection of ovarian tissue with mild edema of interstitial tissue and mild degeneration of some mature ripped oocytes Fig. 5-e The ovaries treated with WSFs of petroleum crude oil and protected with zinc showed mild and occasional degeneration of ripped oocytes Fig. 5-f.

Oocyte atresia is a degenerative and resorptive process, most often of vitellogenic eggs is a normal physiological event. A number of investigators have described atresia in teleost ovaries **(Mytilineou 2000; Schulz and Blüm, 2002)**. Atresia is characterized by the disintegration of the nucleus, vitelline envelope breakdown and increase in number and size of follicular (granulosa) cells; liquefaction of yolk globules with follicular cells entering the oocyte to phagocytize degenerating material; degeneration of the follicular cells once yolk resorption is complete and eventually fibroblast like cells around yellowish-brown material (lipofuscin/ceroid) remain.

Histological defects similar to those found in the present study have already been described before by other authors **(Dutta and Dalal, 2008; Pieterse *et al.,* 2010)**.

No histopathologic changes were observed in the gonads of cunners, *Tautogolabrus adspersus* Walbaum),

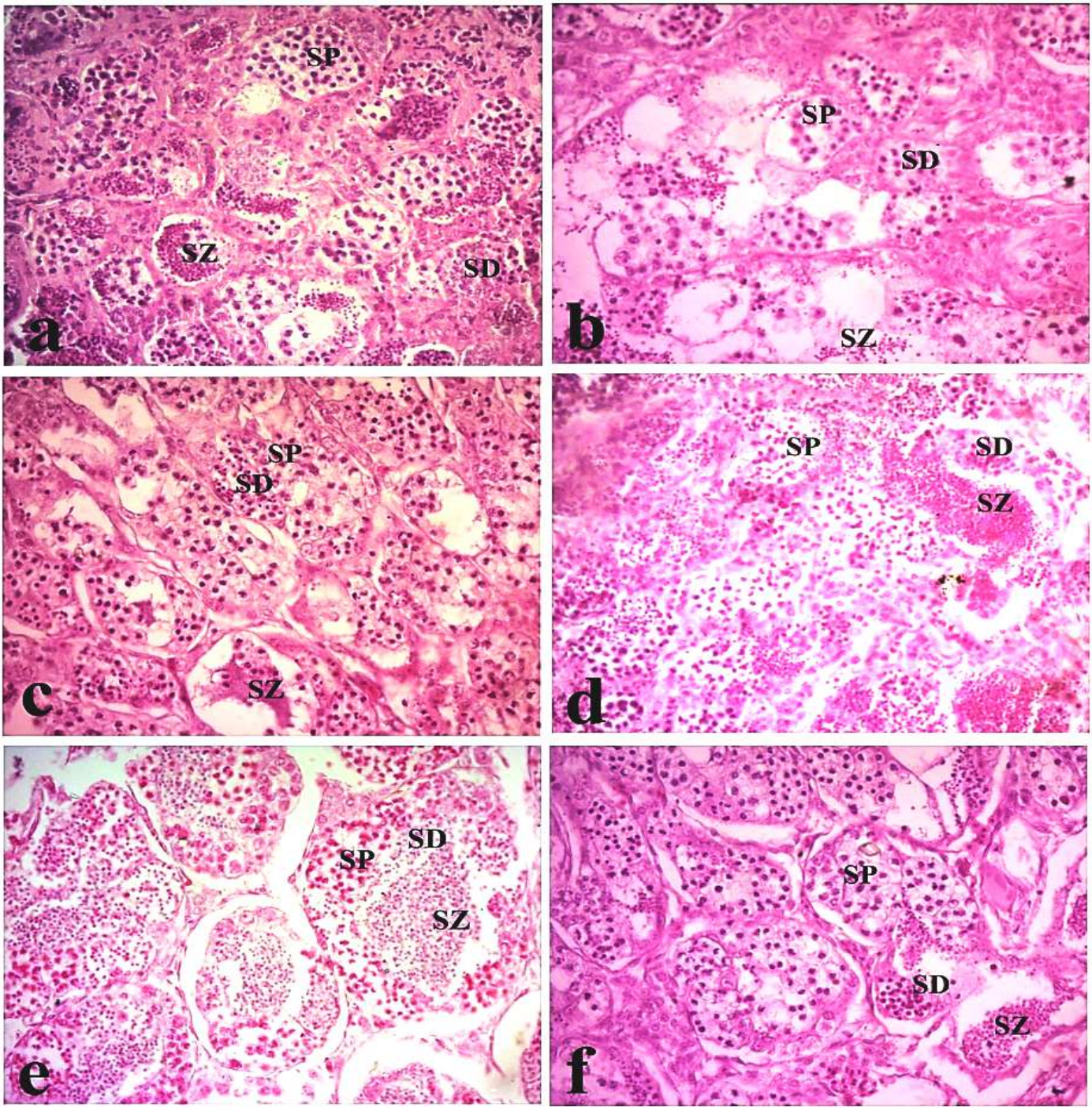

Fig. 4. Testes of hybrid red tilapia showing intensive number of spermatocytes (SP), spermatids (SD) and presence of spermatozoa in the lumen of seminiferous tubules (SZ) in control fish (a), severe reduction in all spermatogenic stages in WSFs of petroleum crude oil treated fish (b), normal spermatogenesis in zinc treated fish (c) and in selenium treated fish (d), mild degeneration of some seminiferous tubules in fish treated with selenium+ WSFs of petroleum crude oil (e), fairly normal seminiferous tubules with normal spermatogenic stages in fish treated with Zinc+ WSFs of petroleum crude oil (f). H and E, X 400.

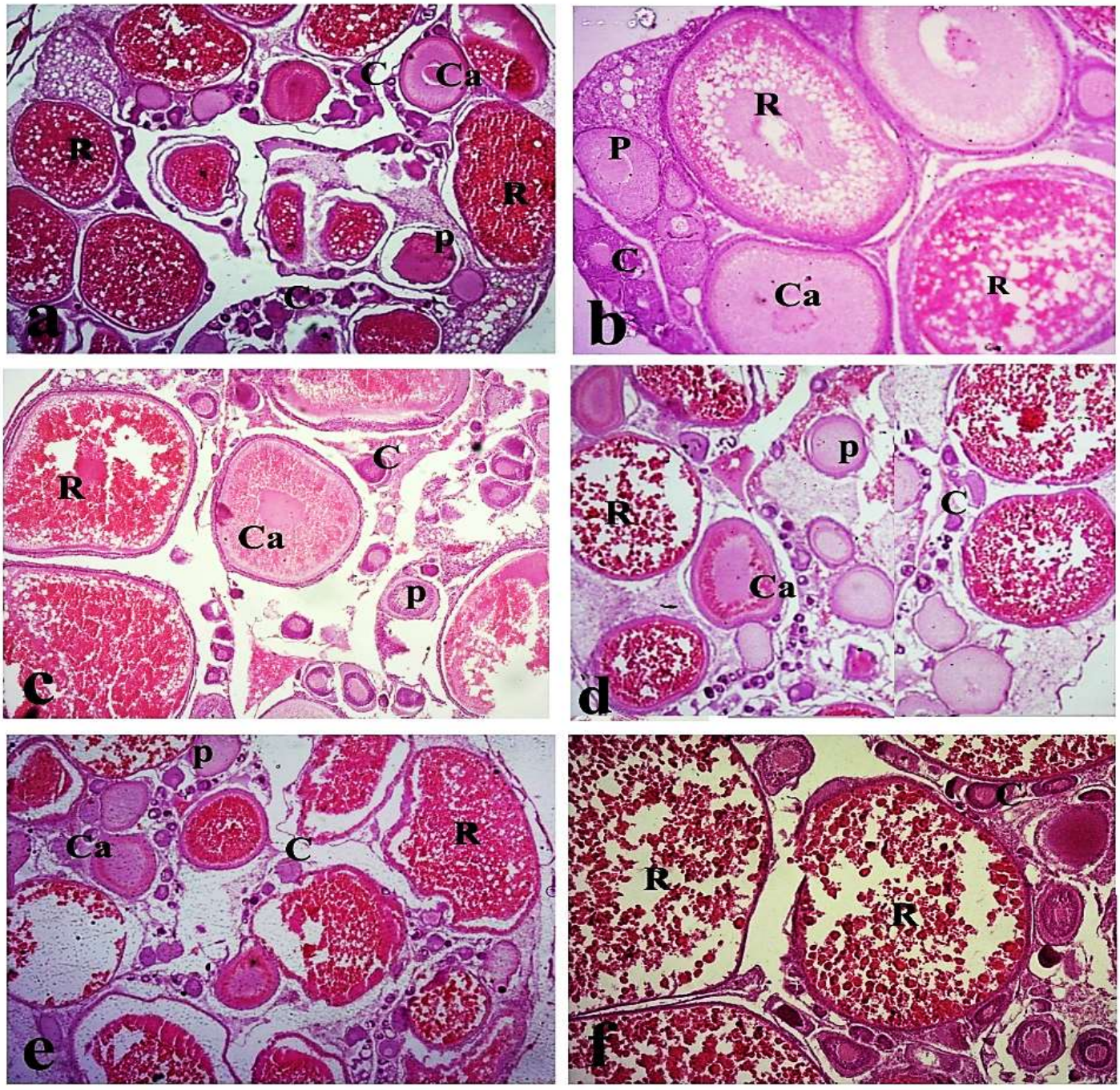

Fig. 5. Ovary of hybrid red tilapia showing normal chromatin nucleolar oocyte (C), perinucleolar oocytes (P) cortical alveolar oocyte (Ca) and mature ripped oocyte (R) in control fish (a), edema and degeneration of mature ripped oocytes in WSFs of petroleum crude oil treated fish (b), normal oocytes stages in selenium treated fish (c) & in zinc treated fish (d), mild degeneration of mature ripped stage with mild edema in interstitial tissue in fish treated with selenium+ WSFs of petroleum crude oil (e), mild degeneration of some ripped mature oocytes in fish treated with Zinc+ WSFs of petroleum crude oil (f). H and E, X 400. A, d & e at X 200.

(exposed to Venezuelan crude oil **(Payne *et al.,* 1978)**.

In the study of **Louiz *et al.,* (2009)**, The main histopathological findings in female ovaries from Bizert lagoon -contaminated with oil-was retraction of the cytoplasm from the follicular cells, karyoplasmic clumping which generate a space between cytoplasm and karyoplasms and follicular atresia where follicles had changes within their supportive cells (e.g., breakdown of yolk granules) characterized by broken zona radiata and proliferation of follicular cells.

CONCLUSION

In the current study, it appeared that the water soluble fractions of petroleum crude oil had negative and bad effects on reproduction organs of hybrid red tilapia, this was clear in case of “red tilapia” which was the subject of the present study, at any concentration more than the allowed levels or by bioaccumulation, and it is clear that using of resistant or antioxidant materials may have positive effect on eliminating these effects if it is always in its safe limits.

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