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Bioaccumulation of Heavy Metals and Physiological/Histological Changes in Gonads of Catfish (*Clarias gariepinus*) inhabiting Lake Maryout, Alexandria, Egypt

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ABSTRACT

The current study investigated the bioaccumulation of heavy metals, as well as, physiological and histological alterations, in gonads of Catfish (*Clarias gariepinus*) inhabiting the polluted location (main basin) and a relatively clean area (southwest basin) at Lake Maryout. A significant (P<0.05) decrease was recorded in muscle total protein and lipid in fish collected from the main basin in comparison to the southwest basin. In contrast, a significant (P<0.05) increase in muscle water content was detected in fish collected from the main basin. A significant (P<0.05) decrease in LH, FSH, estrogen, and progesterone hormones, as well as the activities of the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), were detected in fish collected from the main basin in comparison to the southwest basin. Histological observation of ovary showed lytic ovary with some stages of oocytes include early pre-vitologenic, atretic late pre-vitologenic, wide inter-follicular space and loose tunica albugenia. On the other side, testes pathologies showed completely disorganized lobule structure, accompanied by a reduced number or disappeared of germinal cells, increased interstitial space with reduced interstitial cells. In conclusion, this study confirmed that the main basin of Lake Maryout suffered from great pollution that affected completely on the fish population and the situation needs the rapid intervention of the Egyptian government to stop agricultural, industrial and health drainage in Lake Maryout by establishing treatment units before direct drainage in the Lake

INTRODUCTION

Millions of pounds of toxic chemicals are flushed into Egyptian waterways each year. According to recent records, Egyptian Delta Lakes (Maryout; Manzala; Edku and Borollus) are heavily polluted and unsafe for fishing (Annabi *et al.*, 2013). Saad (2003) recorded that the levels of pollution in these Lakes are in the following order: Lake Maryout> Lake Manzalah> Lake Edku> Nozha Hydrodrome> Lake Brullus. Lake Maryout is now considered a major source of pollution to the Mediterranean Sea through El-Mex pumping station, which receives the surplus water from the Lake and disposes it to El-Mex Bay (EEAA, 2009). Lake Maryout receives most of its water from a heavily polluted drain (El-Qalaa drain). Therefore, it has changed from being the most productive fisheries resource to the least productive in a couple of decades.

Lake Maryout has been divided artificially into five basins: The fishery, the northwest, the southwest, the west, and the main basin, which is the heavily polluted part of the Lake (Adham, 2002).

Heavy metals are what one might call "the unknown killers" since these probably cause and aggravate most health conditions in our bodies. These tiny pieces of metals easily embedded in body tissues as a result of the toxic environment we live in now (Abdullahi, 2013). Bioaccumulation of heavy metals in the aquatic environment poses a serious threat to biodiversity and human health (Annabi *et al*., 2013). Among heavy metals dispersed in the environment, lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are widely dispersed in the environment. The problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to the biomagnifying potential of toxic metals in the human food chain (Mohanty *et al.*, 2013).

Several investigations had concerned with the effect of toxic heavy metals on the biochemical composition in gonads of fishes (Abou EL-Naga *et al*., 2005; Mohamed and Gad, 2008; Arafa and Ali, 2008; Yousafzai and Shakoori 2009; Padmini and Usha Rani, 2009; Carvalho *et al*., 2012); tissue histological structures (Mohamed and Gad, 2008; Ebrahimi and Taherianfard 2011) ; and accumulation of heavy metals in gonads of fishes (Ebrahimi and Taherianfard 2011; Osman, 2012; Vergilio *et al*., 2013). These studies confirmed that such low-level pollution caused an effect on reproduction either directly on the free gametes (sperm or ovum) or indirectly *via* accumulation in the reproductive organs, which are released into the water. Control of reproduction in fish is difficult and controlled by a wide range of factors, and low-level pollution have been affected any part of this pathway (Shafiei *et al*., 2009)

Therefore, the goal of this study was to investigate some biochemical composition, histological alterations, and bioaccumulation of heavy metals in gonads of Catfish, *Clarias gariepinus* caught from polluted location (main basin) and a relatively clean area (southwest basin) at Lake Maryout.

MATERIALS AND METHODS

Fish sampling

Samples of *Clarias gariepinus* were collected from the main basin and southwest basin (Lake Maryout) (length 25±5cm and weight 118±5g) during May 2017. Fish were directly dissected to carry out physiological and histopathological examinations in gonads.

Determination of Metal pollution index

The metal pollution index (MPI) was calculated according to Usero *et al*., (1997).

$$MPI= (M_1 \text{ x } M_2 \text{ x } M_3 \text{ x } M_4 \text{ x } \ldots\ldots\ldots\ldots\ldots M_n)^{1/n}$$

Where M_n is the concentration of metal (n).

In the present study, Cd, Pb, Hg and As were used to calculate the MPI.

Heavy metals in tissue samples

Concentrations of heavy metals were measured in gonads according to methods described by (Bervoets and Blust, 2003). Tissue samples were dried at 105°C for 48 hours and then grounded to a fine powder. The dried samples were digested in which 0.5g (dry powder) was digested in a solution of nitric "acid (HNO3 - AR grade) (5 ml) on a hot plate at 80-90°C until the sample becomes clear. After cooling, the solution was filtered and the filtrate was made up to a known volume

G5ml with deionized distilled water. The concentrations of Pb, Hg, Cd and As in gonads were measured by Atomic Absorption Spectrophotometer (Perkin Elmer 3110, Waltham, MA, USA, model 1200 A) and the results were expressed in "µg/g" of the dry weight of the tissue

Determination of body composition

Sample of 0.1 g of muscle was homogenized in a glass homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein content and total lipid in tissues according to Doughaday *et al*. (1952); Knight *et al*. (1972), respectively. Water content in tissues was determined according to methods described by Sidwell, (1970). Caloric values were determined by using conversion factors: 4.19 cal/mg for protein and 9.5 cal/mg for lipid according to Prosser and Brown, (1961).

Hormone Analysis

Blood samples were collected from the caudal vein of adult female fish into a clean, dry, sterile container. They was centrifuged at 3000 rpm for 15 minutes and the serum was collected in (Eppendorf) capped sterile tubes which was kept frozen at -20°C till assaying FSH, LH, estradiol, and progesterone (Ebrahimi., 2004). They were quantitatively determined using fish enzyme-linked immunosorbent assay (ELISA) kits- (Sunlong biotech Co, Ltd), used Sandwich ELISA.

Determination of Antioxidant enzymes

The activity of glutathione peroxidase (GPX) (EC 1.11.1.9) was measured by The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (A340) providing a spectrophotometric means for monitoring GPx enzymes activity according to Paglia and Valentine (1967), but superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by the ability of the enzyme to inhibit the phenazine methosulphate –mediated reduction of nitroblue tetrazolium dye according to Nishikimi *et al*. (1972). While, catalase (CAT) (EC 1.11.1.6) activity was determined by the destruction of the H_2O_2 concentration at 240 nm according to Abei, (1984).

Histopathological Examinations

The light microscopic examinations were reported according to Bancroft and Gamble (2002). Tissue samples of gonads were quickly removed, fixed in 10% formalin solution and routinely processed for paraffin embedding. Sections were cut at 5 mm and stained routinely with Haematoxylin and Eosin (H&E).

Statistical analysis

The data were analyzed by one-way ANOVA using the Statistical Processor System Support (SPSS 20, Armonk USA). Data are expressed as means ±SD. Values of $P < 0.05$ were considered statistically significant. Means in each row are significantly different ($P<0.05$) with no common superscripts.

RESULTS AND DISCUSSION

Bioaccumulation of heavy metals in gonads

In this study, the concentrations of heavy metals in gonads of C. *gariepinus* collected from the main basin and the southwest basin are shown in Table (1). Metal pollution index for testes and ovary in the main basin were 1.762 and 1.756 respectively, while in southwest basin were 0.738 and 0.646, respectively. In general, fish ovary compared to testes contains the lowest level of metals. The obtained results are in accordance with Honda (1983) who reported that relatively high concentrations of Cd and Hg were found in the testes in comparison to the ovary of

Pagothenia boechgreninki. In this study, the mean Pb concentrations in gonads showed a highly significant increase (*P*<0.05) of the studied fish in the main basin compared to the southwest basinLead is also known to damage the brain, the central nervous system, kidneys, liver and the reproductive system (Ademoroti, 1996).

Table 1: Metal concentration in gonads of *Clarias gariepinus* from the main basin and the southwest basin at Lake Maryout.

Metals (μg/g dry weight)	Location	Testes	Ovary
Lead	Main Basin	3.44± 0.54[b]	3.42± 1.10[b]
	Southwest Basin	1.40± 0.30[a]	1.25± 0.20[a]
Mercury	Main Basin	1.53± 0.50[b]	1.46 ± 0.30[b]
	Southwest Basin	0.45± 0.60[a]	0.40± 0.10[a]
Cadmium	Main Basin	2.06 ± 0.40[b]	2.05 ± 0.20[b]
	Southwest Basin	1.07± 0.10[a]	0.83 ± 0.20[a]
Arsenic	Main Basin	0.84 ± 0.20[b]	0.84 ± 0. 14[b]
	Southwest Basin	0.44 ± 0.09[a]	0.42 ± 0.14[a]

Values are expressed as mean ± SD, (n=5). Means in a row with no common superscripts are significantly different (*P*<0.05).

On the other hand, the mean Hg concentrations in tests and ovary showed also a highly significant increase (*P*<0.05) of the studied fish in the main basin compared to the southwest basin. Mercury is one of the most toxic elements among the studied heavy metals and exposure to a high level of this element could permanently damage the brain, kidneys and developing fetus (Castro-González and Méndez-Armenta, 2008). Also, Cd concentrations in tests and ovary showed a highly significant increase (*P*<0.05) of the studied fish in the main basin compared to the southwest basin. Cd toxicity in human may affect some organs such as kidney, lung, bones, as well as the central nervous system (Castro-González and Méndez-Armenta, 2008). Finally, the mean (As) concentrations in tests and ovary showed a highly significant increase (*P*<0.05) of the studied fish in the main basin compared to the southwest basin. Arsenic is the most toxic element and considered as a Group (A) human carcinogenic followed by damage of lung, kidney, and skin (ATSDR, 2003). The concentrations of the studied heavy metals in gonads of *Clarias gariepinus* were found to be within unsafe limits as suggested by various authorities (FAO/WHO, 2004; EC, 2006) and indicated of pollution. From the above, we can see that, although these data may be not fully representative for the most recent situation, they still should provide a strong warning to the Egyptian population and authorities regarding the quality of fish. Our results were similar to the results of Ebrahimi and Taherianfard (2011) who studied the accumulation of heavy metals (Pb), (Cd), (Hg), and (As) in cyprinid fish from the Kor River and reported that no significant differences (p > 0.05) were detected between the two sexes and species, which may be due to a similar degree of accumulation in both sexes and species, as their feeding habits and habitats are similar (Singh *et al*., 2006). Osman, (2012) studied the River Nile Pollution using Nile Tilapia fish and showed that the Nile tilapia sampled from the downstream river of the Nile accumulated higher levels of all the detected heavy metals than those collected from upstream sites.

Biochemical composition

Proximate body composition is a good indicator of fish physiological condition and health (Saliu *et al*., 2007). Variations of protein, lipid, energetic values and water content of *Clarias gariepinus* collected from the main basin and the southwest basin at Lake Maryout are shown in Table (2). Overall, a significant difference (*P*< 0.05) was observed in the chemical composition of *Clarias gariepinus*. Analysis of fish muscles showed that the highest protein and lipid contents value was recorded in fish caught from the southwest basin, respectively. In contrast, the lowest concentrations of protein and lipid were observed in fish caught from the main basin, respectively.

Table 2: Body compositions of *Clarias gariepinus* from the main basin and the southwest basin at Lake Maryout.

Parameter	Main Basin	Southwest Basin
Total protein (mg/g)	56.00 ± 8.30^{b}	72.00 ± 4.90^{a}
Total Lipid (mg/g)	26.00 ± 5.00^{b}	32.00 ± 3.50^{a}
Total calories (cal./g)	483.54 ± 63.58^{b}	609.26 ± 45.63^{a}
Water content (%)	75.00 ± 4.40^{b}	53.00 ±7.70^{a}

Values are expressed as mean ± SD, (n=5). Means in a row with no common superscripts are significantly different ($P<0.05$).

On the other side, the highest water content was recorded in the main basin, while the lowest was in the southwest basin. Similar to the above results, the energetic values were recorded in fish caught from the southwest basin (609.26 cal./g), while in the main basin, it was (483.54 cal./g). Also, Authman *et al.* (2013) reported that the muscle total protein contents of fish collected from El-Rahawy drain showed a highly significant decrease, while muscles water content was increased as compared with the values of the river Nile fish. The elevation in the lipid content observed in the muscles of *Clarias gariepinus* from the southwest basin could be the result of enhanced lipid synthesis and/or reduced utilization as like as the results observed by Mohamed and Gad (2008). While, the current results were disagreement with Palaniappan *et al*. (2008) that recorded depletion in lipid and protein contents in musculature of *Catla catla* exposed to lead may be due to tissue organization and their utilization in cell repair with the formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in cytoplasm. (Filipovic and Raspor, 2003). Moreover, the significant changes in fish body composition in the main basin and the southwest basin may be due to differences in water quality, feeding conditions, sex, maturity state, environmental conditions and the period during which the organism was captured (Saeed, 2013; Younis *et al.,* 2014).

Hormone Analysis

The concentration of FSH, LH, estrogen and progesterone hormones of female *Clarias gariepinus* collected from the main basin and the southwest basin at Lake Maryout are shown in Table (3). A significantly (P < 0.05) increased in these hormones were recorded in fish caught from the southwest basin. The trends of these hormones were similar to the findings by Zhang *et al*. (1995) studied the effect of lead on reproductive endocrine function in pregnant rats. Samarawickrema et al. (2008) explained that the decrease in the concentration of LH and FSH may be due to direct action of cypermethrin on anterior pituitary gonadotrophs, responsible for the secretion of LH and FSH; or hypothalamic neurons, responsible for the secretion of gonadotropin-releasing hormone (GnRH) that exercises tropic action on anterior pituitary gonadotrophs. Our results were an agreement with Thomas (1990) who recorded a significant decrease in plasma estrogen of Atlantic croaker after exposing fish to lead. The same results were recorded by Saxena *et al*. (1989) after exposing Asian swamp eel (*Monopterus albus*) to cadmium.

Ebrahimi and Taherianfard (2011) recorded that the concentration of estradiol in female fish in Band-e-Amir and Korbal village in both sexes of cyprinid fish from the Kor River were significantly decreased, while decreased of progesterone in

Doroudzan-Dam from the Kor River fish showing the direct effect of heavy metal contamination on steroidogenesis in female fish, either due to the harmful effects of metals on either the hypothalamus–pituitary axis (Song *et al*., 2002) or on the germinal cells capacity of estradiol production (Hinck *et al*., 2007).

Also, Mohamed and Gad (2008) showed the heavy metals may have direct effects on fish gonads (testes and ovaries), resulting in a disturbing development of germ cells. Moreover, Heckers *et al., (*2002) found that exposure of fish in the Elbe River Germany exposed to heavy metals having endocrine disruptor effects leading to a decrease in the levels of sex steroid hormones. Agrawal (2012) and Salim (2015) suggested also that heavy metals can modify hormone production and activity through the blocking the synthesis of hormones, mimicking the natural hormones, and providing receptors that inhibit cell synthesis of hormones. Heavy metal toxicity in natural water is the major source of contamination, which have adverse effects of on the hypothalamic–pituitary–gonadal relationship of fish and disturbs the aquatic biodiversity that is responsible for maintaining and supporting overall environmental health (Drevnick and Sandheinrich (2003).

Table 3: Hormone analysis of *Clarias gariepinus* from the main basin and the southwest basin at Lake Maryout.

Hormone	Main Basin	Southwest Basin
FSH (IU/ml)	0.89 ± 0.17^{b}	1.75 ± 0.30^{a}
LH (IU/ml)	0.48 ± 0.17^{b}	1.10 ± 0.20^{a}
Estrogen (ng/ml)	103.40 ± 24.2^{b}	182.80 ± 57.76^{a}
Progesterone (ng/ml)	0.61 ± 0.20^{b}	1.49 ± 0.80^{a}

Values are expressed as mean ± SD, (n=5). Means in a row with no common superscripts are significantly different (P<0.05).

Antioxidant enzymes

Antioxidant enzymes believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are harmful products generated during normal aerobic respiration. As shown in Table (4) marked significant (P < 0.05) decreases in catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities were observed in *Clarias gariepinus* collected from the main basin comparing with the southwest basin. The obtained results are in agreement with Saliu and Bawa-Allah (2012) that showed a reduction in the activities of SOD and CAT in fishes *Clarias gariepinus* exposed to lead. Also, Vutukuru *et al*. (2006) showed decreased antioxidant activities of superoxide dismutase, catalase and lipid peroxidation in copper treated the freshwater teleost fish, *Esomus danricus*. Moreover, Sujatha et al (2011) showed decreased activities of CAT, GPX, and SOD in rats exposed to lead acetate.

CAT is responsible for the reduction of hydrogen peroxide, while GPx catalyzes the reduction of both hydrogen peroxide and lipid peroxides. However, our results indicated that the decreased of the GPx and SOD activities in tissues of the *C. gariepinus*, from the main basin, could be indicated that the abilities to protect against hydrogen peroxide were reduced and are not scavenged by these antioxidant enzymes. In addition, the marked decrease in SOD activity may result from direct binding of the metal to the enzyme leading to oxidative stress and lipid peroxidation (Bainy, 1993; Hamed *et al.*, 2003). Low levels of GPx in fish may result in a significant accumulation of the high levels of H_2O_2. This could be associated to the O_2^- production or to the action of metals on enzyme synthesis (Padmini and Usha Rani, 2009), and causing a number of cellular damage for the reason that the impairment in the radical formation.

Table 4: The activities of the antioxidant enzyme (U/ml) of *Clarias gariepinus* from the main basin and the southwest basin at Lake Maryout.

Antioxidant enzyme	Main Basin	Southwest Basin
Catalase	17.80± 5.50[b]	45.60±7.70[a]
Glutathione peroxidase	18.60±6.50[b]	52.40±7.70[a]
Superoxide dismutase	31.20±5.80[b]	72.40±11.50[a]

Values are expressed as mean ± SD, (n=5). Means in a row with no common superscripts are significantly different (*P*<0.05).

Histopathological alterations

Ovary

In the present study, the histology of ovaries of C. *gariepinus* collected from the main basin of Lake Maryout showed lytic ovary with some stages of oocytes including early pre-vitologenic, atretic late pre-vitologenic, wide inter-follicular space and loose tunica albugenia (Fig. 1). Groups of deformed late pre-vitologenic oocytes with a gradual degeneration, as a centrically aggregated nucleoli in the disintegrating nucleus (Fig. 2). The atretic follicles were present throughout the year, but they were increasing during autumn (spent ovaries) and winter (resting ovaries); and these atretic follicles determined the spawned individuals (Emam and Badia, (2014). The pre-vitellogenic follicles found all over the year, but in autumn were abundant as the spent ovaries and in winter as the resting ovary and less common during spring and summer (spawning seasons) (Emam and Badia., 2014). In this study, atresia may be due to environmental stress (Mehta *et al.,* 2015). Heavy metals pollution cause greater loss to advance stages of oogenesis (Raksha and Sharma. 2012). These effects may disturb the oogenesis and may reduce the ability of the fish to reproduce (Mehanna, 2005). Wahbi and El-Greisy (2007) recorded that in females S*iganus rivulatus*, effluents (industrial, mixed and domestic) resulted in extensive necrosis of oolema, hypertrophy, and hyperplasia of the follicular cells of oocytes. Also, ovaries showed atresia in the large vacuolated mature follicles where atresia characterized by broken zona radiata, the proliferation of follicular cells and break down of yolk granules. Abou Shabana *et al*. (2008) observed that oogenesis is disrupted on applying concentrations (3.12%, 6.25% &12.5%) for, 7, 14 and 28 days to the wastewater effluent. Atretic oocytes and degenerated follicles are observed lead to the absence of ripe oocytes. Nuclear disintegration is detected in perinucleolus oocytes, infiltration of blood tissue which disturbs the development of chromatin nucleolus and perinucleolus oocytes. While, exposure to low concentration doses for short duration resulted in nuclei disintegraion, pycnosis in perinucleolus oocytes and hyperplasia of granulosa layer and zona radiata. Inhibition of reproduction of catfish is caused by trace element accumulation, which inhibits gonadal development (Yamaguchi *et al*., 2007). While, ovaries of C. *gariepinus* collected from the southwest basin showed group-synchronous oocytes; early pre-vitellogenic with small cytoplasmic volume, many late pre-vitellogenic, abundant cortical follicles with cortical alveoli, vitellogenic oocytes and post- vitellogenic oocytes (Fig. 3). Moreover, Yolk globules are observed at vitellogenic oocytes frequently fill the entire center of the oocyte, cortical alveoli coated by demarcated theca folliculi consist of follicular epithelium, granulosa and zona radiata well attached to the basement membrane of the oocyte, late pre-vitellogenic with many pleiomorphic nucleoli bordering the nuclear envelope inside the ooplasm (Fig. 4). These results conform to those reported by Abou Shabana *et al*. (2008); Cek and Yelmaz (2005). The mature or post-vitellogenic follicles were common during spring and summer as they were in the spawning and ready to spawn and ovulate (Raksha and Sharma, 2012).

Testes

The present results showed testes of C. *gariepinus* collected from the main basin of Lake Maryout with completely disorganized lobule structure, accompanied by a reduced number of, or the absence of, germinal cells, increased interstitial space with reduced interstitial cells (Fig. 5). Also, seminiferous tubules showed highly vacuolation and all developmental stages; spermatogonia, spermatocytes cyst, spermatid cyst showed spongy vacuolation (Fig. 6). Severe testicular atrophy with arrested spermatogenesis, necrotic spermatogenic cells, and vacuolization in the interstitial tissue Permanent testicular damage resulted from increasing degree levels of heavy metal accumulation (Hanna *et al.*,2008). Shan *et al*. (2009) observed slight degeneration of germ cells lining seminiferous tubules with desquamation of germ cells and the tubular lumen were filled with degenerated germ cells. Also, interstitial spaces were abnormally widening with degeneration of Leydig cells after the rat testes treated with lead. Also, El-Sayed *et al*. (2015) showed that lead acetate caused necrosis of spermatogenic cells in the rat's seminiferous tubules, congestion of interstitial blood vessels and severe interstitial edema. The testes of C. *gariepinus* collected from the southwest basin showed seminiferous tubules supported by thin connective tissue (Fig. 7), spermatogonia stage, spermatocytes cyst, spermatid and the lumen filled with more amounts of spermatozoa (Fig. 8). The annual reproductive cycle of testes in teleost fish is dependent on environmental conditions such as temperature that determines the time and duration of the spawning period (Krol *et al*., 2006). Annual reproductive cycles of testes can be divided into spring, spring summer or autumn ones (Abou Shabana *et al.*, 2008). The tunica albuginea of ovary and testes with no uniform thickness throughout the year but, it reached a high thickness during winter and became thin during spring and summer because of pressure exerted on it by the distended testicular lobules or enlarged mature follicles, and began to increase again during autumn (El-Morshedi *et al*. 2014). The spermatids increased during spring to produce spermatozoa and became few during summer as most of them were changed into spermatozoa. The spermatozoa began to appear within the lumen of testicular lobules during spring.

CONCLUSION

It is concluded that this study confirmed that the main basin of Lake Maryout suffered from great pollution that affected completely on the fish population. So, the situation needs rapid recovery from the Egyptian government to stop that agricultural, industrial and health drainage disposes of in Lake Maryout by establishing treatment units before direct drainage in the lake

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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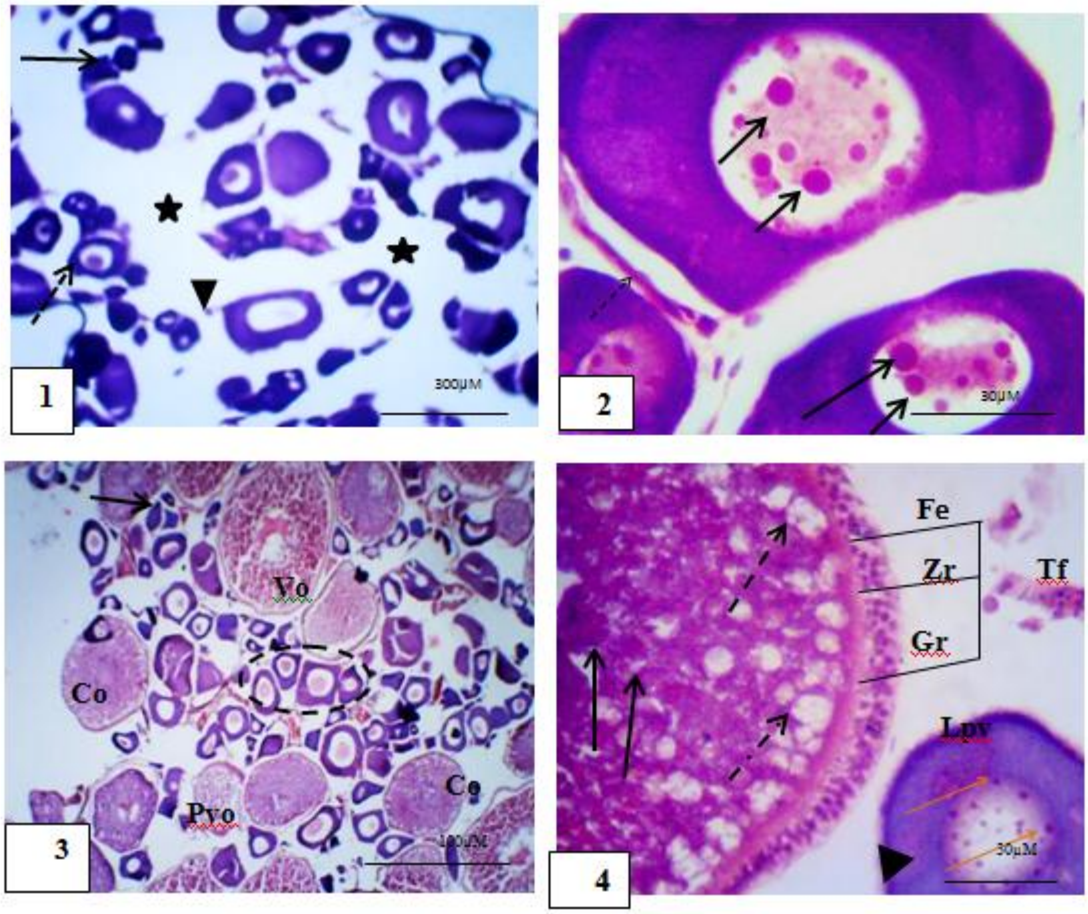

Fig. 1: Ovary tissues of C. *gariepinus* collected from the main basin showed lytic ovary with some stages of oocytes includes early pre-vitologenic (arrow), atretic late pre-vitologenic (dashed arrow), wide inter-follicular space (stars) and loose tunica albugenia (arrowhead) (5X. H&E stain).

Fig..2: Ovary tissues of C. *gariepinus* collected from the main basin showed groups of deformed late pre-vitologenic oocytes with a gradual degeneration, as a centrically aggregated nucleoli in the disintegrating nucleus (arrows) (40X. H&E stain).

Fig. 3: Ovary tissues of C. *gariepinus* collected from the southwest basin showed group-synchronous oocytes, early pre-vitellogenic with small cytoplasmic volume (circle), many late pre-vitellogenic (dashed circle), abundant cortical follicles (Co) with cortical alveoli, vitellogenic oocytes (Vo), post- vitellogenic oocytes (Pvo) (10X. H&E stain).

Fig. 4: Ovary tissues of C. *gariepinus* collected from the southwest basin showed part of vitellogenic oocytes includes yolk globules (arrows) frequently fill the entire center of the oocyte, cortical alveoli (dashed arrows) coated by demarcated theca folliculi (Tf) consist of follicular epithelium (Fe), granulosa (Gr) and zona radiata (Zr) well attached to the basement membrane of the oocyte, late pre-vitellogenic (Lpv) with many pleiomorphic nucleoli (orange arrows) bordering the nuclear envelope inside the ooplasm (arrowhead) (40X. H&E stain).

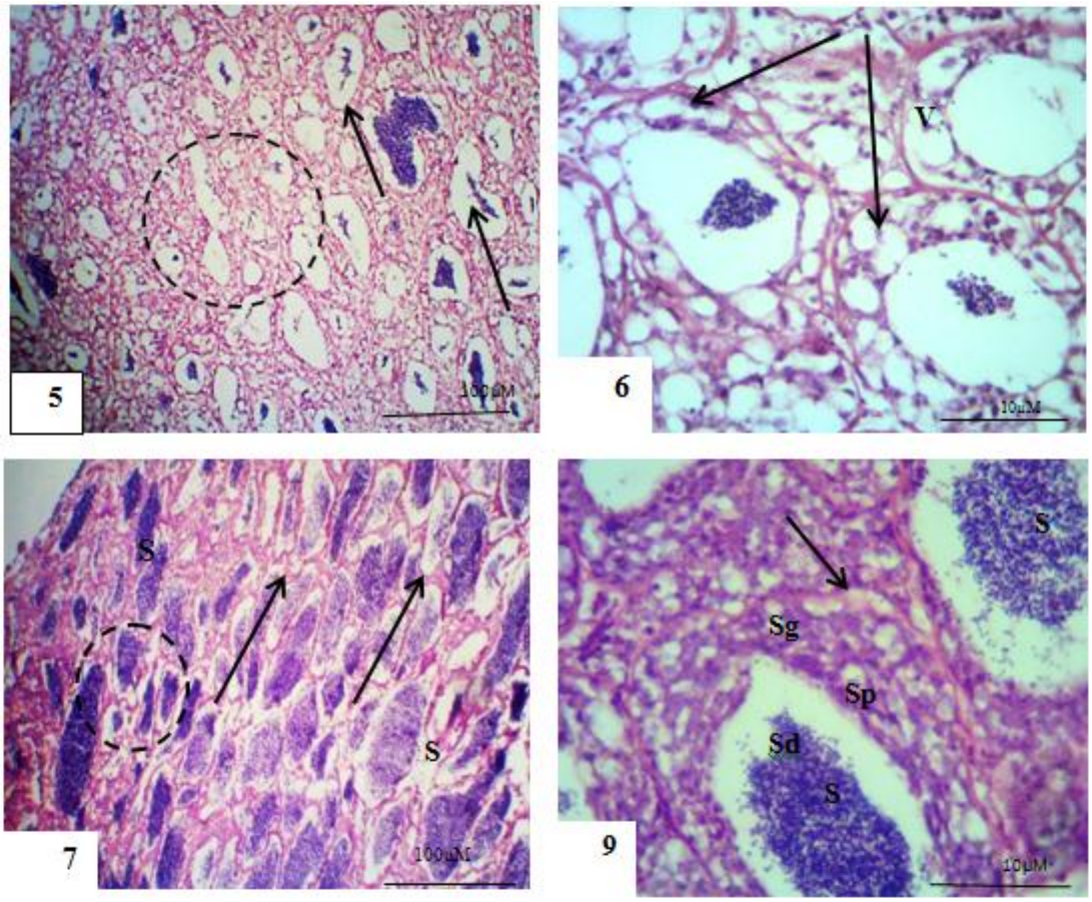

Fig. 5: Testes tissues of C. *gariepinus* collected from the main basin showed completely disorganized lobule structure accompanied by a reduced number (circle) of, or the absence of, germinal cells, increased interstitial space with reduced interstitial cells (asterisk) (10X. HE stains).

Fig. 6: Testes tissues of C. *gariepinus* collected from the main basin showed seminiferous tubule much affected with highly vacuolation. All developmental stages (spermatogonia, spermatocytes cyst, spermatid cyst) showed spongy vacuolation (40X. H&E stain).

Fig. 7: Testes tissues of C. *gariepinus* collected from the southwest basin seminiferous tubules (St) supported by normal thin connective tissue accompanied with more amounts of spermatozoa (S) (10X. H&E stain).

Fig. 8: Testes tissues of C. *gariepinus* collected from the southwest basin seminiferous tubules (St) supported by thin connective tissue (arrow), spermatogonia stage (Sg), spermatocytes cyst (Sp), spermatid (dashed-arrow), the lumen filled with spermatozoa (S) (40X. H&E stain).