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Histopathological Alterations in Major Organs of Freshwater Ornamental Goldfish, *Carassius auratus* (L.), Variety Shubunkin Reared in Inland Saline Water

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***Abstract*:** The present study was carried out in glass aquaria (50 liter) for 120 days to assess histopathological alterations in major organs of freshwater ornamental goldfish, *Carassius auratus* (L.), variety Shubunkin reared in inland saline water in different salinity levels (2 to 10 ppt/ ‰) prepared from stock inland saline water (12 ‰). After proper conditioning, fishes were acclimatized (gradual increase in salinity @ 1 ‰ at 1-hr interval) to different salinity levels. After 4 months of salinity exposure in terms of histopathological alterations revealed deviation from normal responses/alterations beyond 4 ‰. The study presented alterations in gills, kidney and liver, which increased gradually from 2‰ up to 10‰. Although fish was capable of adapting and growing up to 6 ‰ in inland saline water, however, <4 ‰ can be considered safe with respect to overall performance of fish especially histopathological alterations. The results of present study suggest <4 ‰ as safe level to rear gold fish in inland saline water.

***Keywords*:** Gold fish, Histopathological alterations, Inland saline water, Salinity stress

Among various abiotic factors, salinity is one of the critical parameters for overall well-being of freshwater fishes as it determines the level of osmoregulatory stress. Preliminary effect of salinity on fish physiology impacts the osmoregulation process where the ionic concentration of the body is maintained through intake or loss of ions by major organs such as gills, kidney and intestine (Al-Hilali and Al-Khshali 2016). The saline water tolerance in freshwater fishes vary between and within the species (Islam et al 2014). As freshwater fishes are stenohaline, growth decreases with the increase in salinity leading to homeostasis imbalance (Enayati et al 2013). Several reports are available on the rearing of freshwater ornamental fishes like gold fish, crucian carp and molly in natural or artificial saline water (Vasagam et al 2005, Schofield et al 2006, Küçük 2013). Schofield et al (2006) reported that goldfish can thrive in low saline environments (<10 ‰) for longer duration, and at higher salinities for short period. Their salinity tolerance is similar to that of *Cyprinus carpio*, but is higher and lower than that of *Hypophthalmicthys molitrix* and *Tilapia zillii*, respectively (Wang et al 1997). Due to its hardy nature, its culture in brackish water and marine environment is being taken up.

Hitherto, no report on the effects of Inland Saline Water (ISW) on histopathological studies of freshwater ornamental fish is available. Hence, optimizing the rearing technology of freshwater ornamental fish, goldfish *C. auratus* L. in North-Western states of India (inland saline or water-logged areas) is imperative. Further, it is also vital to study the stress response at different salinity levels in order to determine the optimal salinity tolerance for sustained culture practices which shall in turn, enhance the socio-economic status of the farmers as well. Moreover, the adaptability and tolerance of this species to physico-chemical changes in water vary greatly due to the differences in ionic composition of ISW to sea water (Dhawan et al 2010). With these limitations and opportunities, the present research was designed to study the adaptability of goldfish in ISW with special reference to histopathological responses.

MATERIAL AND METHODS

Experimental designs: A four-month experiment was carried out in glass aquaria (50 L capacity) at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana to assess the histopathological alterations in gills, liver and kidney of *C. auratus* (L.) at different salinity levels of ISW (12‰) collected from village Shajrana, district Fazilka (Punjab). The experimental fish (Shubunkin) were procured from local market, and then conditioned for 15 days in indoor conditions. After proper conditioning, fishes (average length and weight - 8.04-8.25 cm and 6.30-6.80 g) were acclimatized gradually by increasing the salinity @ 1‰ per hour to 5 salinity levels (0, 2, 4, 6, 8 and 10‰) and were

distributed (10 fishes per replicate) randomly in control and experimental salinity treatments (triplicates). All the experimental aquaria were provided with continuous oxygen supply and fishes were fed ad-libitum twice a day throughout the experimental period. Water quality parameters were analyzed with respect to temperature, pH, salinity, dissolved oxygen (DO), electrical conductivity (EC), total alkalinity (TA), total hardness (TH) and ammoniacal nitrogen (NH_3-N).

Histopathological studies: After completion of the experiment, histopathological studies were carried out from the control and salinity treatments. The targeted tissue (gills, kidney and liver) samples were fixed in 10% neutral buffered formalin (NBF; Cat #501128, Sigma Aldrich). They were washed in running tap water followed by alcohol dehydration, clearing with xylene and then embedding in paraffin wax. Thin sections (4 µm) were cut and mounted on gelatinized slides using a rotary microtome (Leica RM2125 RT, Germany). After this, the sections were mounted on microscope slides (Super Frost, #G10106, Abdos, India) and then dried at 33°C overnight. After observing the sections under a microscope (LEICA DM3000 LED), the good quality sections having no nicks were chosen for hematoxylin and eosin staining (Shanthanagouda et al 2014). The slides were mounted using Dibutylphthalate Polystyrene Xylene (DPX) mount (product code-23140, Molychem., Mumbai) and were examined. These slides were photographed under a light microscope (Nikon 80i) and captured using digital camera.

RESULTS AND DISCUSSION

During the experimental period, range of the water quality parameters *viz*. water temperature pH, DO, EC ,TA ,TH, NH_3-N was 14.2 - 30.5 °C, 7.14 - 8.99, 5.50 - 9.52 mg l^{-1}, 0.64 - 18.91 mS cm^{-1}, 232 - 336 $CaCO_3$ mg l^{-1}, 305 - 2930 $CaCO_3$ mg l^{-1} and 0.010 - 0.298 mgl^{-1}. During the present study, fishes were under chronic salinity stress for 120 days, resulting in prominent histopathological alterations in the major organs (gills, liver and kidney). Such alterations were occuring even at the lowest salinity treatment 2‰, which became severe with the increase in salinity to the extent of physiological breakdown (8‰ and 10‰).

The photomicrographs for the histopathology of gills showed remarkable changes with lamellar bending, thinning of inter lamellar region (Fig. 1 B, C, D), fused secondary lamellae, talengiactasia, hyperplasia, blood congestion and complete degeneration of secondary lamellae in highest salinities (Fig. 1 E, F). The alterations implicate the defense mechanisms (Fernandes and Mazon 2003; Akaishi et al 2004) and these alterations not only result in reduced secretary and excretory functions of the gills (Tilak et al 2006), but possibly lead to destruction of gill structure resulting in asphyxia (only 60 % fish survival in present study at highest salinity of 10‰). Further, the severity of gill alterations also indicated that the fishes were approaching tolerance limit to salinity resulting in osmoregulatory failure (Lawson and Alake 2011) causing mortality (6-10 ‰ in the present study).

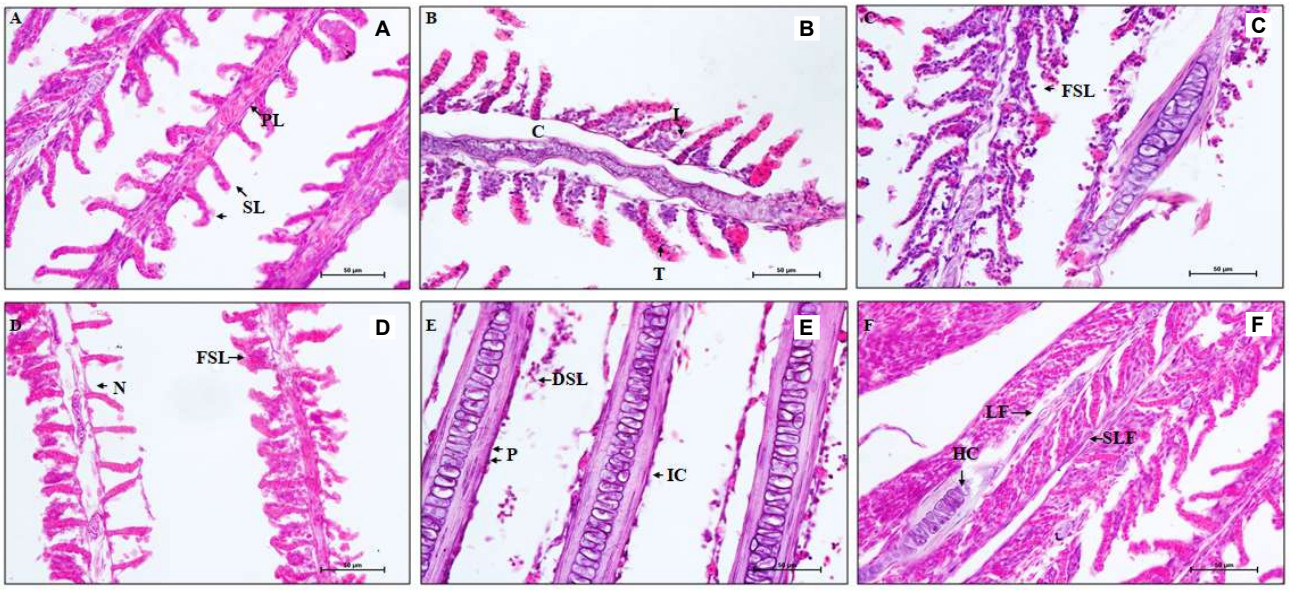

Fig. 1. Representative pictures of histomorphology of the gills of gold fish exposed to different salinity conditions for 120 days. A) 0‰ (freshwater) Primary lamellae (PL), Secondary lamellae (SL); B) 2 ‰ Infilteration (I), Telangiectasia (T), Chondrocytes (C); C) 4 ‰ Fused secondary lamellae (FSL); D) 6 ‰ Fused secondary lamellae (FSL), Necrosis (N); E) 8 ‰ Degenerated secondary lamellae (DSL), Increased Chondrocytes (IC), Perichondrium (P) and F) 10 ‰ Lamellar fusion (LF), Hyaline cartilage (HC), Secondary lamellar fusion (SLF). H & E stain. Scale bar= 50 µm

The photomicrographs for the kidney structures in control (Fig. 2 A) showed normal proximal and distal convoluted tubule structures, while the major histological alterations in kidney during increasing salinity conditions were observed in terms of degeneration of glomerular and distal convoluted tubules (Fig. 2 B, C). These alterations became severe

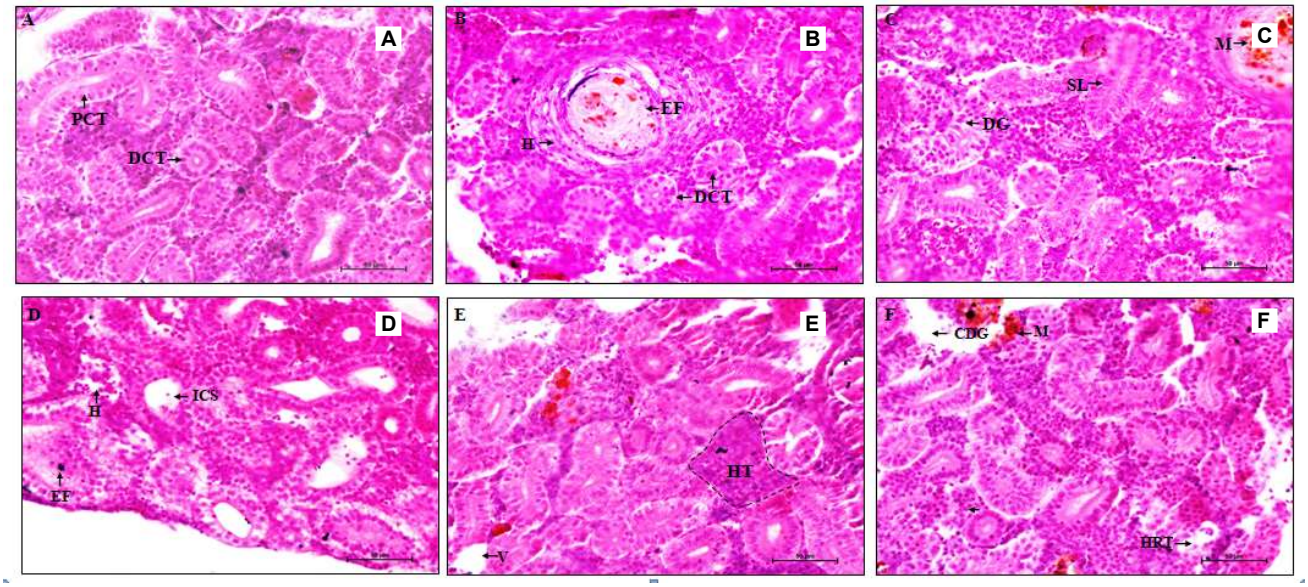

Fig. 2. Representative pictures of histomorphology of the kidney of gold fish exposed to different salinity conditions for 120 days. A) 0‰ (freshwater), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT); B) 2 ‰ Edmatous fluid (EF), Hyperplasia of tubules (H), Distal convoluted tubule (DCT); C) 4 ‰ Degeneration of glomerulus (DG), Shrunken lumen (SL), Melanomacrophage (M); D) 6 ‰, Edmatous fluid (EF), Haemorrhages (H), Increased capsular space filled with fluid (ICS); E) 8 ‰ Hematopoetic tissue (HT), Vacuolization (V) and F) 10 ‰ Complete degeneration of glomerulus and emptying of Bowman's capsule (CDG), Hypertrophy in renal tubules (HRT), Melanomacrophage (M). H & E stain. Scale bar= 50 µm

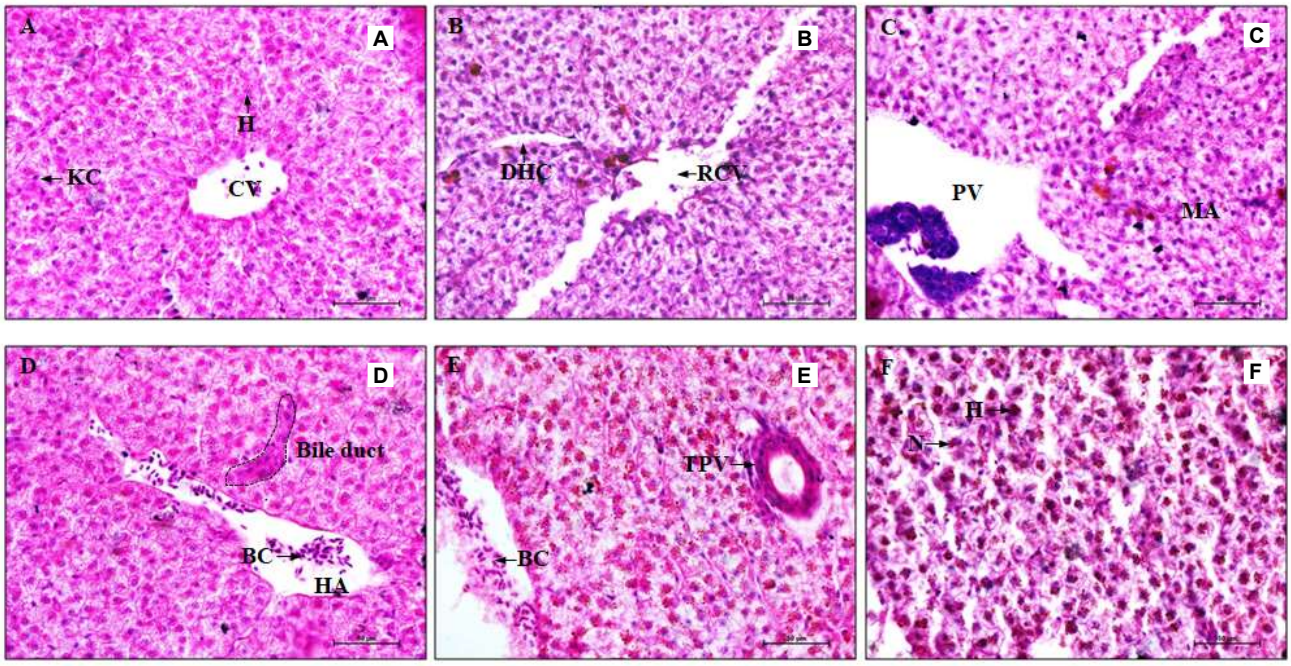

Fig. 3. Representative pictures of histomorphology of the liver in gold fish exposed to different salinity conditions for 120 days. A) 0‰ (freshwater) Hepatocytes (H), Central vein (CV), Kupffer cells (KC); B) 2 ‰ Rupturing of central vein (RCV), Disorganization of hepatic chords (DHC); C) 4 ‰ Melanomacrophage aggregates (MA), portal vein (PV); D) 6 ‰ Blood congestion (BC), Hepatic artery (HA); E) 8 ‰ Thinning of portal vein (TPV), Blood congestion (BC); F) 10 ‰ Haemorrhages (H), Necrosis (N). H & E Stain. Scale bar= 50 µm

leading to complete degeneration of glomerulus, emptying of Bowman's capsule (Fig. 2D, E), hypertrophy of renal tubules, haemorrhages and mononuclear cellular infiltration in higher salinity treatments (Fig. 2 F). Similar alterations were observed by Raskovic et al (2013) in common carp exposed to water having high pH and low DO (stream water), but were comparatively milder (Silva and Martinez 2007). Additionally, results in terms of shrinking and degeneration of glomerulus along with increased amount of edematous fluid were in accordance with the observations of Abdelhamid and El-Ayouty (1991) w.r.t. pollutant exposure/water quality alterations. In liver, the photomicrographs of control fish showed normal Kupffer cells, hepatocytes containing homogenous cytoplasm with a centrally placed nucleus and central vein (Fig. 3 A). Ruptured central vein and disorganization of hepatic chords were observed in 2‰ (Fig. 3 B). Further, there was an increase in the density of melanomacrophage aggregates in the parenchymal tissue of liver (Fig. 3 C) which indicated degenerative and necrotic processes (Pacheco and Santos 2002). In 6 and 8‰ hepatic artery, blood congestion and thinning of portal vein were observed (Fig. 3D, E). Hence, haemorrhages and necrosis at 10‰ can be explained as inhibition of DNA synthesis required for the growth and maturation of liver under abnormal conditions (Sanad et al 1997).

The most of the previous studies w.r.t. histological alterations in different organs of fishes were carried out to observe the effect of chemical, xenobiotics and pollutants or variations in water quality (especially temperature) (Velmurugan et al 2009, Hadi and Alwan 2012, Banaee et al 2013, Sharma and Tamot 2013, Drishya et al 2016, Sultana et al 2016). There is a lack of information regarding the effect of salinity on freshwater fish with special reference to histological alterations. Hence, the present study can be considered as the baseline for conduction of elaborative studies in near future.

CONCLUSION

The freshwater ornamental Shubunkin gold fish, *Carassius auratus* (L.) reared under different salinity regimes (0-10‰) depicted histopathological alterations w.r.t. gills, kidney and liver. The alterations started gradually from 2 ‰ onwards which became more severe at 10‰. Although fish was capable of adapting and growing under salinity conditions up to 6 ‰ in inland saline water, however, <4 ‰ salinity can be considered safe w.r.t. overall performance of the fish. Hence, freshwater ornamental Shubunkin gold fish, *Carassius auratus* (L.) can be reared for longer periods in inland saline water and it is further recommended to conduct field trials for better understanding of the effect of salinity under dynamic environmental conditions.

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