Original Research

Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Histopathological changes in the gill and kidney tissues of *Carassius auratus* exposed to acrolein

Authors Info

S. Arman* and S. İ. Üçüncü

Department of Biology,
Faculty of Science, Ege University,
Bornova-Izmir-35100, Turkey

*Corresponding Author Email :
sezgiarman@gmail.com

Key words

Acrolein,
Carassius auratus,
Gill tissues,
Histopathological changes,
Kidney tissues

Publication Info

Paper received : 16.01.2016
Revised received : 11.05.2016
Re-revised received : 21.06.2016
Accepted : 09.11.2016

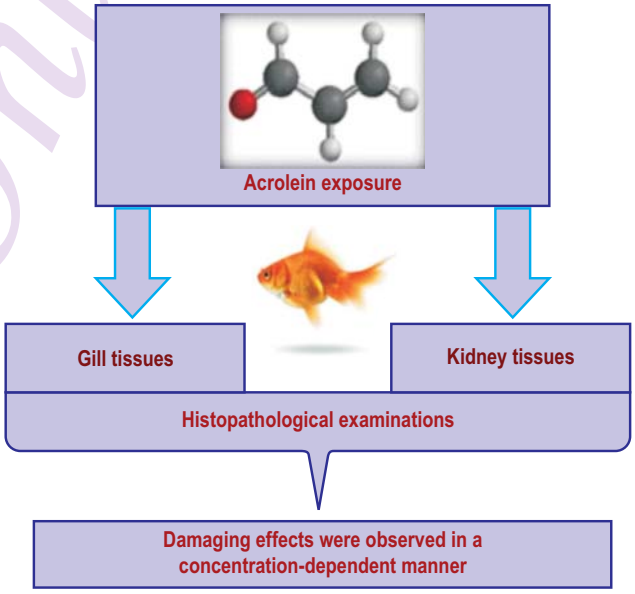
Abstract

Aim: The aim of the present study was to examine the histopathological alterations caused by acrolein, an aquatic herbicide, in the gill and kidney tissues of *Carassius auratus*.

Methodology: The fish were exposed to sublethal concentrations of acrolein (1, 5 and 25 µg l^{-1}) for 96 hr. The gill and kidney tissues were removed, fixed and embedded in paraffin. Histological sections of the treated tissues were investigated by light microscopy and compared with the control group.

Results: Acrolein exposure caused hyperplasia, aneurism, edema, curling and shortened secondary lamellae, epithelial lifting, desquamation, hypertrophied mitochondria-rich cells, dilated central vein, separation of primary lamella epithelium from the cartilaginous tissue and necrosis in the gills. The kidney tissues showed vasodilatation, congestion, enlarged melanomacrophage centers, vacuolization in the renal parenchyma, separation of tubules and collecting ducts from the parenchyma, hyperplasia, vacuolization and deformation in the tubule epithelium, fibrous tissue configuration in the parenchyma and around the collecting duct, hemorrhage, infiltration and necrosis.

Interpretation: Acrolein exposure caused damaging effects on the gill and kidney of goldfish. Alterations in the vital organs probably would affect the metabolic activities and even the survival rate. From the environmental and health protection point, acrolein has to be taken into consideration.



Introduction

Acrolein is a reactive α, β-unsaturated aldehyde and wide spread in the environment due to natural and anthropogenic processes. Acrolein enters the environment from herbicides, industrial discharges, organic combustion, plastic, chemical control agents for fouling organisms and as an endogenous metabolite in biological systems resulting from oxidative processes (Eisler, 2007; Stevens and Maier, 2008; Bein and Leikauf, 2011; Randall *et al.,* 2013). It has been detected in smog, food and water (Eisler, 2007).

Herbicides are used to avoid reduced water delivery capacity caused by submerged weeds in irrigation systems (US EPA, 2007; Montagna *et al*., 2011). MAGNACIDE H®, the trade name of acrolein, is an aquatic herbicide applied directly to agricultural irrigation canals. It is also applied directly to marine environment and cooling water systems of power plants to control fouling organisms (Nebot *et al.,* 2010). Recommended concentrations of acrolein range from 1 to 15 ppm (Nordone *et al.,* 1997). However, the acute toxicity (LC_{50} or EC_{50}) of this herbicide ranges between 14-320 µg l^{-1} for freshwater fish (US EPA, 2009). According to the report of US EPA (1980), acrolein is one of the most toxic herbicides. Acrolein has been used in the United States, Canada, Egypt, Argentina, Mexico and Turkey for channel maintenance (Bowmer and Smith, 1984; Eisler, 2000).

Electrophilic character of acrolein promotes covalent reactions with cellular nucleophiles in the biological systems. Toxic effects of acrolein is thought to be mediated by GSH depletion, disruption of cellular redox systems, loss of protein function (Spiess *et al.,* 2011), protein miss-folding (Haberzettl *et al.,* 2009; Vladykovskaya *et al.,* 2012), cross-linking or aggregation (Burcham and Pyke, 2006; Randall *et al.,* 2013).

Teleosts are useful models for ecotoxicological experiments and various species are used to investigate the effects of waterborne exposure to environmental pollutants (Koskinen *et al.,* 2004; Palermo *et al.*, 2008; Seok *et al.,* 2008; Xing *et al.,* 2012). In this study, goldfish was selected as a test organism as it is easy to maintain under laboratory conditions and has commercial value.

In light of the above, the present study aimed to investigate the histopathological effects of acrolein in gills and kidney of goldfish.

Materials and Methods

C. auratus (3.7-4.2 cm in length and 3.87-5.8 g in weight) were obtained from a commercial fish dealer in Izmir. The fish were acclimated under laboratory conditions for two weeks before the experiment. They were maintained in a 100 l glass aquarium consisting of dechlorinated tap water at 26±2 ºC and natural photoperiod. Fish were fed with commercial fish food (Sera Goldy) twice a day.

Sublethal treatment concentrations of acrolein were determined as 1, 5 and 25 µg l^{-1} based on the previously reported 96 hr LC_{50} values of the chemical for freshwater fish [ranges between 14-320 µg l^{-1} (US EPA, 2009)]. The fish were divided into four groups randomly in separate aquaria, and five fish were used for each group. Control fish were maintained in chemical-free water during the 96 hr experiment. After treatment, all the fish were anaesthetized with MS222 (tricaine methanesulfonate); gill and kidney tissues were removed and fixed in Bouin's fluid for 24 hr at room temperature. Specimens were dehydrated in ethanol; treated with xylol and embedded in paraffin wax. 5 µm serial sections were stained with Mayer's hematoxylin eosin (H-E) and histopathological changes were examined by light microscopy. Microphotographs were taken with Zeiss Axio Scope. A1 equipped with Zeiss AxioCam ERc5s.

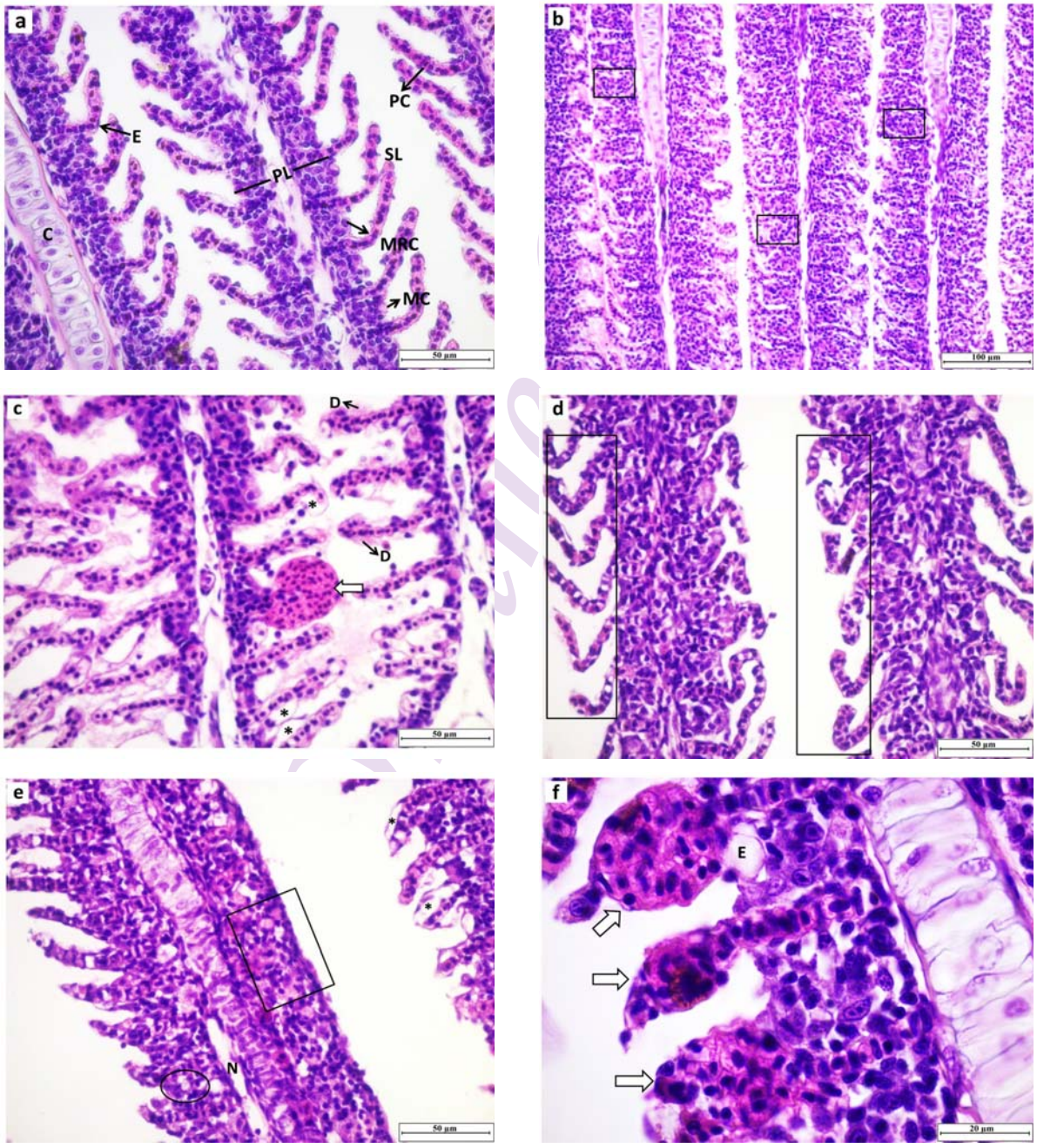
Results and Discussion

Histopathological changes were not observed in the gills of control fish. The structural details of gill are shown in Fig. 1a. Gills are the first organs that interact with the chemicals. Therefore, alterations in gill structure can affect gaseous exchange, osmoregulation and other physiological processes (Brand *et al.,* 2001). Hyperplasia and lifting of secondary lamellae epithelium were noted in all treatment groups (Fig. 1b, 1e, 1i). Additionally, aneurism and desquamation were noticed in 1 µg l^{-1} concentration (Fig. 1c). Curling of secondary lamellae, lamellar fusion, edema, aneurism and hypertrophied mitochondria-rich cells were observed in 5 µg l^{-1} treatment group (Fig. 1d, 1e, 1f, 1g). The most striking changes in 25 µg l^{-1} concentration were dilatation in the central vein, shortened secondary lamellae, separation of primary lamellae epithelium from cartilaginous tissue and necrosis (Fig. 1h, 1i).

Several authors have also reported histopathological changes in the gills caused by various herbicides. Glyphosate exposure resulted in proliferation of filament cell, lamellar cell hyperplasia in lamellar cell, lamellar fusion, epithelial lifting and aneurism in *Oreochromis niloticus* (Jiraungkoorskul *et al.,* 2003) and pillar cell system enlargement, filament epithelium hyperplasia, mucus cell proliferation in filament epithelium, pavement and chloride cell hypertrophy in *Piaractus mesopotamicus* (Shiogiri *et al.,* 2012). Glyphosate caused congestion, cellular infiltration, hemorrhage and necrosis in the gills of *Clarias gariepinus* (Ayoola, 2008). *Labeo rohita* fingerlings exposed to atrazine showed epithelial hyperplasia, curling of secondary lamellae, changes in chloride cells, pyknotic nuclei, vacuolization and degradation of epithelial and pillar cells in their gills (Jayachandran and Pugazhendy, 2009). Alachlor treatment induced necrosis, vacuolar degeneration, fusion and atrophy of primary and secondary gill lamellae in *Channa punctatus* (Butchiram *et al.,* 2009). Clomazone exposure led to hypertrophy

of mucus cells, hyperplasia of the interlayer epithelium and fusion of secondary lamellae in *Rhamdia quelen* (Brum *et al.,* 2014). Swelling of top gill lamellae, hyperplasia of surface epithelial cells and forming a slice of epithelial cell plate were reported in the gills of *C. auratus* exposed to butachlor (Xu *et al.,* 2015). In the present study, 96 hr acrolein exposure caused substantial histopathological changes in the gills of *C. auratus*. Such alterations in the gills should be considered as an important indicator of early response to herbicide poisoning.

Histopathological changes were not observed in the kidney of control fish (Fig. 2a, 2b). In each three treatment groups, melanomacrophage centers, hyperplasia in the tubule epithelium, degenerative tubules and progressive necrosis were



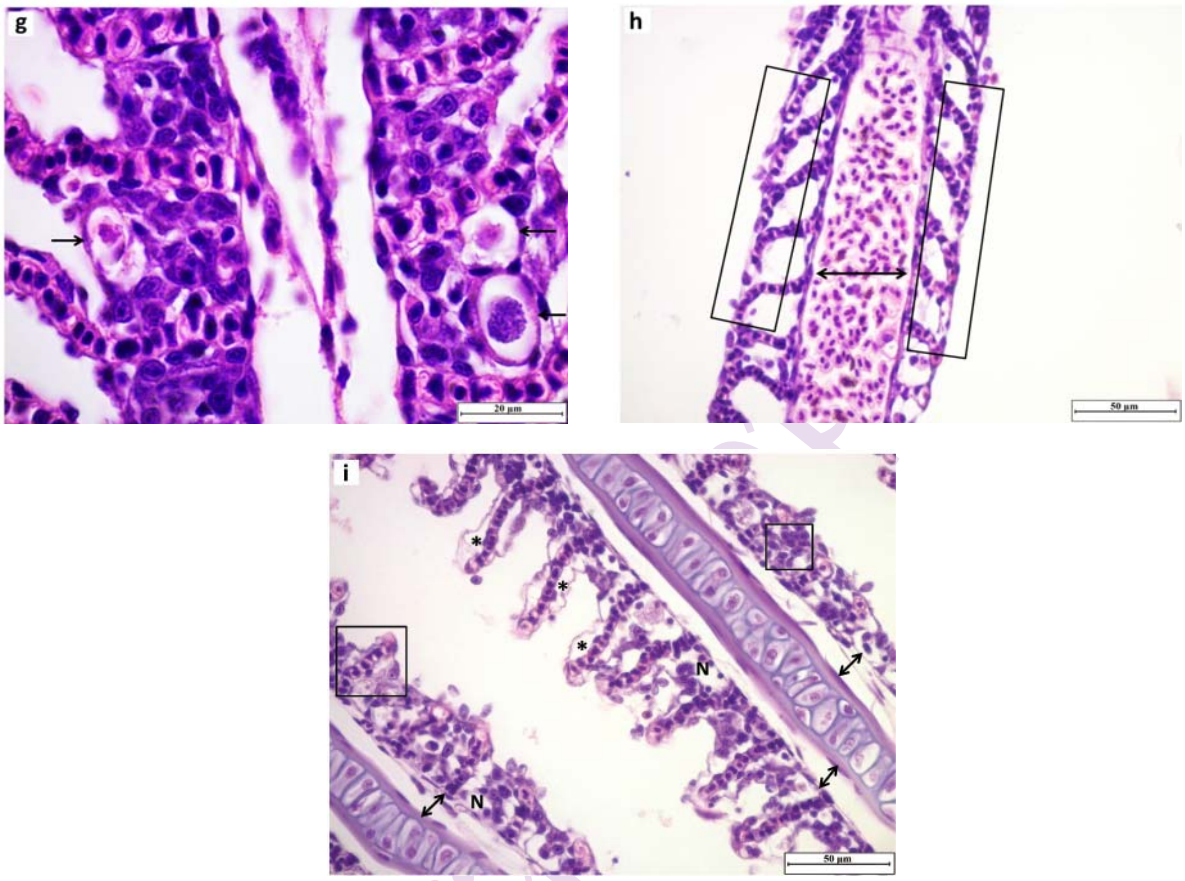


Fig. 1 : Gill tissues of *C. auratus*. (a) Control - PL: Primary lamella, SL: Secondary lamellae, E: Epithelial cell, MRC: Mitochondria-rich cell, MC: Mucus cell, PC: Pillar cell, C: Cartilaginous (original magnification x40, H-E); (b) Exposed to 1 µg l^{-1} acrolein – Epithelial hyperplasia (in rectangles) (original magnification x20, H-E); (c) Exposed to 1 µg l^{-1} acrolein – Epithelial lifting (*), aneurism (white arrow), desquamation (D) (original magnification x40, H-E); (d) Exposed to 5 µg l^{-1} acrolein – Curling of secondary lamellae (in rectangles) (original magnification x40, H-E); (e) Exposed to 5 µg l^{-1} acrolein – Epithelial hyperplasia (encircled), lamellar fusion (in rectangle), epithelial lifting (*), necrosis (N) (original magnification x40, H-E); (f) Exposed to 5 µg l^{-1} acrolein – Edema (E), aneurism (white arrows) (original magnification x100, H-E); (g) Exposed to 5 µg l^{-1} acrolein - hypertrophied mitochondria-rich cells (arrows) (original magnification x100, H-E); (h) Exposed to 25 µg l^{-1} acrolein – Early fusion of secondary lamellae (in rectangles), dilated central vein (double arrow) (original magnification x40, H-E); (i) Exposed to 25 µg l^{-1} acrolein – epithelial lifting (*), shortened secondary lamellae (larger square), epithelial hyperplasia (smaller square), separation of primary lamella epithelium from the cartilaginous tissue (double arrows), necrosis (N) (original magnification x40, H-E)

noted in a concentration-dependent manner (Fig. 2c, 2e, 2f, 2g, 2i, 2l). Probably, kidneys of *C. aurata* were damaged during the elimination and excretion processes of acrolein.

Vasodilatation, congestion, hyperplasia in the epithelium of collecting duct and vacuolization in renal parenchyma were noticed in 1 µg l^{-1} acrolein treated group (Fig. 2c, 2d, 2e). At 5 µg l^{-1} concentration, hyperplasia in collecting duct epithelium, separated tubules from the parenchyma with narrowed lumens and prominent necrosis were observed (Fig. 2f, 2g). In 25 µg l^{-1} treated group, vasodilatation, hemorrhage, infiltration, vacuolization and hyperplasia in the epithelium of collecting duct surrounding fibrous tissue, degenerative tubules separated from parenchyma and fibrillation in parenchyma were noticed (Fig. 2h, 2i, 2j, 2k, 2l).

Chemicals are primarily eliminated and excreted from the body through kidneys. In teleosts, beside renal activity, kidneys have also hematopoietic and endocrine functions. Accordingly, alterations in kidneys induced by the environmental pollutants have to be examined intently. In common carp, atrazine exposure caused cloudy swelling of epithelial cells of renal tubules, contraction of glomerulus, expansion of Bowman's space and necrosis in tubular epithelium (Xing *et al.,* 2012). *Oreochromis niloticus* kidneys displayed dilation of Bowman's space,

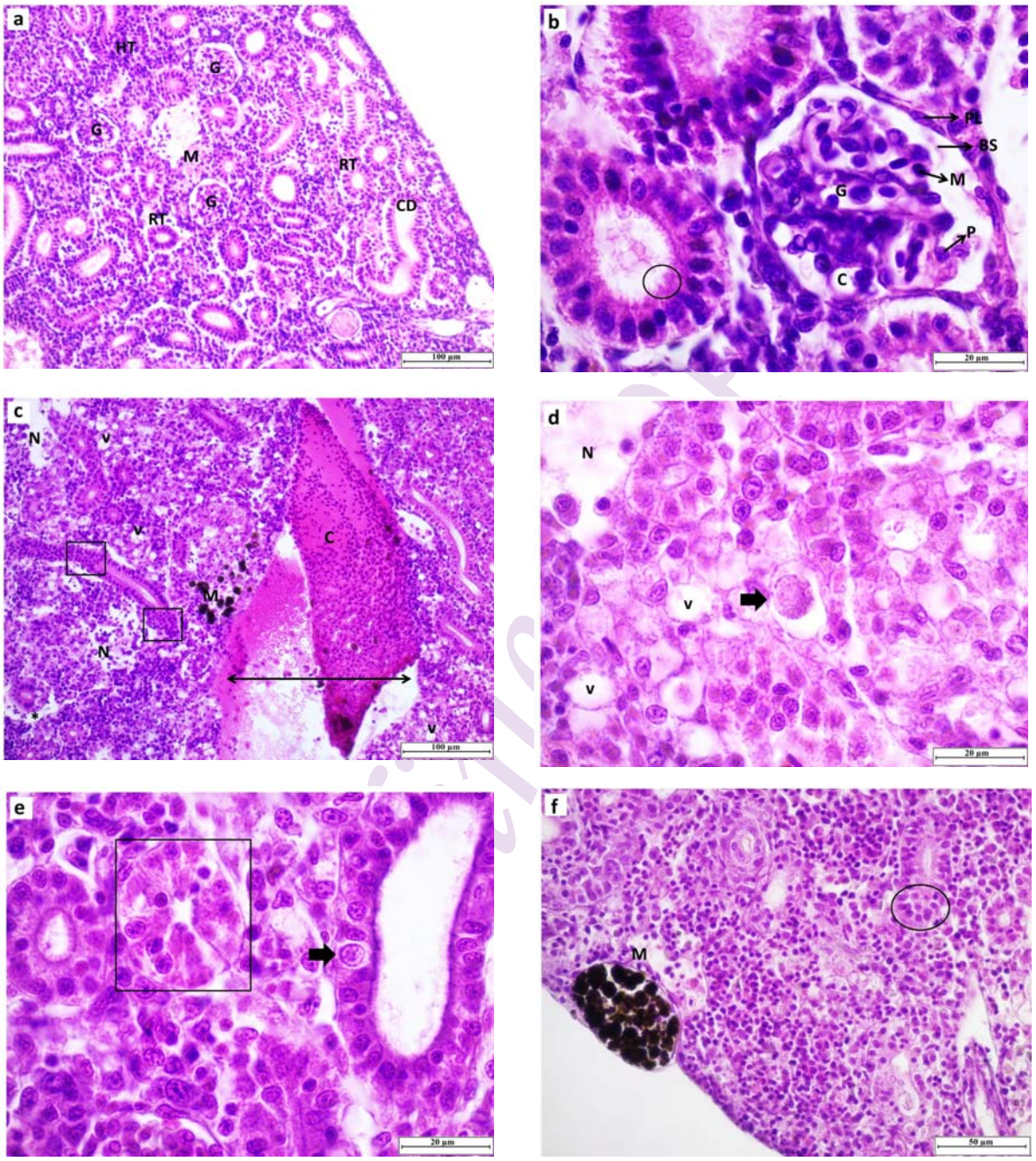


Fig. 2 : (a) Control – Glomeruli (G), Hematopoietic tissue (HT), Collecting ducts (CD), Renal tubules (RT), Melanomacrophage aggregates (M) (original magnification x20, H-E); (b) Control - Brush border (encircled), Bowman's space (BS), Glomerulus (G), capillaries (C) of a renal corpuscle surrounded by outer parietal layer (PL), and the main cells of visceral layer; podocytes (P) and mesangial cells (M) (original magnification x100, H-E); (c) Exposed to 1 µg l^{-1} acrolein – congestion (C) in a dilated vein (double arrow), melanomacrophages (M), hyperplasia in the collecting duct epithelial cell (in square), vacuoles in renal tubule epithelial cells (*) separated from the parenchyma and necrosis (N) (original magnification x20, H-E); (d) Exposed to 1 µg l^{-1} acrolein – substance accumulation (arrow) in large vacuoles (v) in the parenchyma and necrosis (N) (original magnification x100, H-E); (e) Exposed to 1 µg l^{-1} acrolein – a degenerative renal tubule (in square) and hyperplasia in the epithelium (arrow) (original magnification x100, H-E); (f) Exposed to 5 µg l^{-1} acrolein – An enlarged melanomacrophage center (M) and surrounded by a capsule, and hyperplasia in tubule epithelium (encircled) (original magnification x40, H-E)

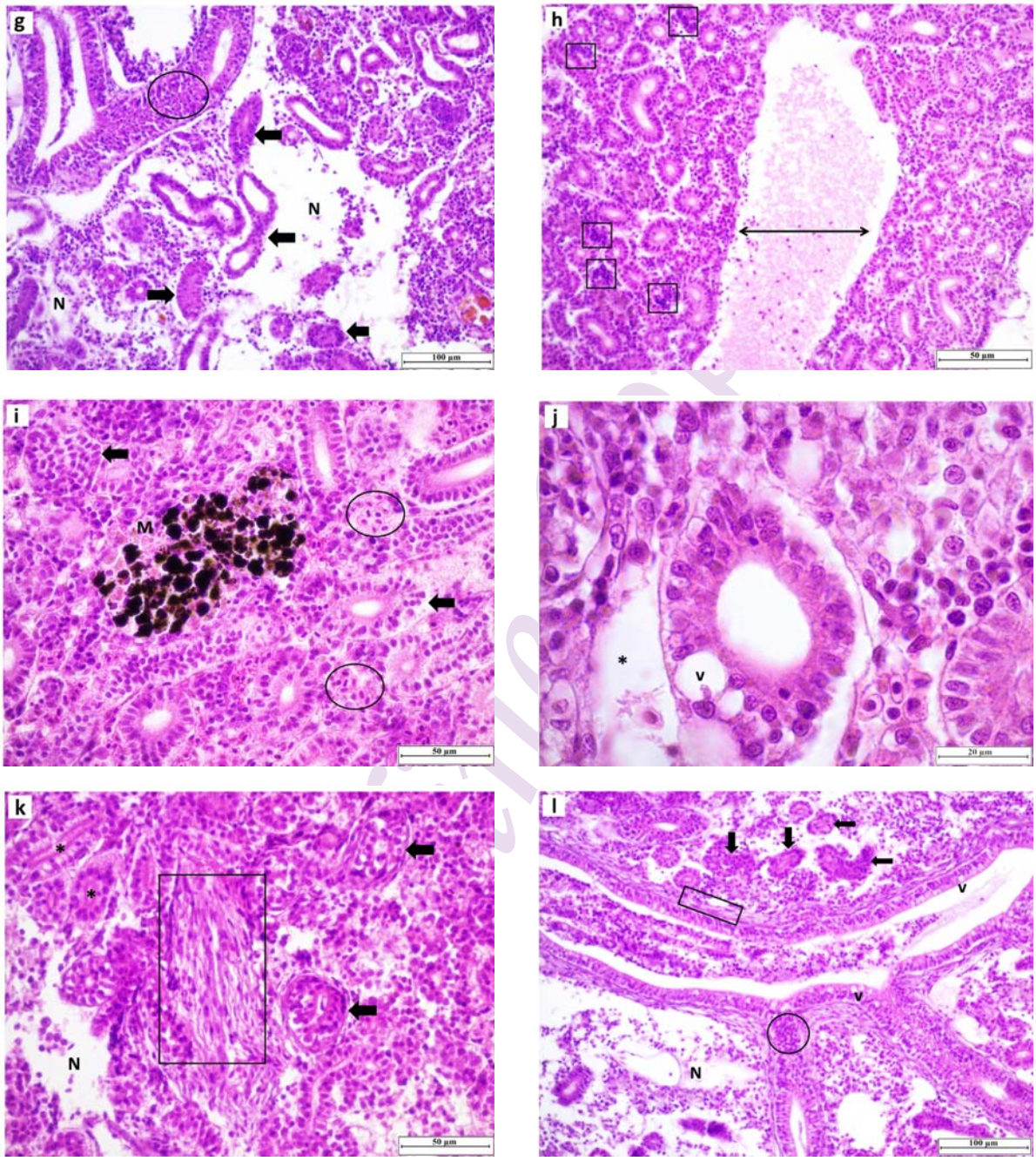


Fig. 2 : (g) Exposed to 5 µg l^{-1} acrolein – hyperplasia (encircled) in the collecting duct epithelium, deformation in the tubules (arrows) and enlarged necrotic areas (N) (original magnification x20, H-E); (h) Exposed to 25 µg l^{-1} acrolein – Distinct vasodilatation (double arrow) and infiltration (in squares) (original magnification x40, H-E); (i) Exposed to 25 µg l^{-1} acrolein – an enlarged melanomacrophage center (M), degenerative tubules (arrows) and hemorrhage (encircled) (original magnification x40, H-E); (j) Exposed to 25 µg l^{-1} acrolein – vacuolization (V) in the epithelium of a tubule separated from the parenchyma (*) (original magnification x100, H-E); (k) Exposed to 25 µg l^{-1} acrolein- fibrillation in the parenchyma (in rectangle), substance accumulation in degenerative tubules(arrows) with narrowed lumen (*) and necrosis (N) (original magnification x40, H-E); (l) Exposed to 25 µg l^{-1} acrolein- epithelial vacuolization (v) and hyperplasia (encircled) in a collecting duct with peripheric fibrous tissue compositon (in rectangle), degenerative tubules (arrows) and prominent necrosis (N) (original magnification x20, H-E)

accumulation of hyaline droplets in tubular epithelial cells and necrosis after glyphosate treatment (Jiraungkoorskul *et al.,* 2003). *O. niloticus* kidney showed hydropic swelling of tubular cells, accumulation of lipid vacuole in many tubules, and nuclear pyknosis after acute exposure to alachlor (Peebua *et al.,* 2008). Metribuzin treatment caused peritubular congestion of blood (teleangiectasia) and hyaline degeneration of epithelial cells of renal tubules in the kidney of *Cyprinus carpio* (Velisek *et al.,* 2009). Simazine exposure induced globular eosinophilic droplets within the cytoplasm of proximal tubular epithelial cells, melanomacrophage aggregates and necrosis in hematopoietic tissue in the kidney of *C. carpio* (Oropesa *et al.,* 2009). Butachlor treatment gave rise to increased size of renal epithelial cells and increased transparency of cell base in *C. auratus* (Xu *et al.,* 2015).

The histopathological changes induced by the treatment of sublethal concentrations of acrolein in gills and kidneys of *C. auratus* was concentration dependent. Such alterations in the vital tissues, that have important physiological functions, may cause serious malfunctions in metabolic processes and even death in long term.

References

Ayoola, S.O.: Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus)* juvenile. *Afr. J. Agric. Res*., **3**, 825-834 (2008).

Bein, K. and G.D. Leikauf: Acrolein – a pulmonary hazard. *Mol. Nutr. Food Res*.**55**, 1342–1360 (2011).

Bowmer, K.H. and G.H. Smith: Herbicides for injection into flowing water: Acrolein and endothal-amine. *Weed Res.*, **24**, 201-211 (1984).

Brand, D.G., R. Fink, W. Bengeyfield, I.K. Birtwell and C.D. Mcallister: Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stress-related visceral lesions after 10-day exposure to sublethal concentrations of water-soluble fraction of North Slope crude oil. *Toxicol. Pathol.,* **29**, 574–584 (2001).

Brum, A., G. Dotta, K. Roumbedakis, E.L.T. Gonçalves, L.P. Garcia, P. Garcia, V.M. Scussel and M.L. Martins: Hematological and histopathological changes in silver catfish *Rhamdia quelen* (Siluriformes) exposed to clomazone herbicide in the Madre River, Santa Catarina State, Southern Brazil. *J. Environ. Sci. Heal. B.,* **49**, 169-175 (2014).

Burcham, P.C. and S.M. Pyke: Hydralazine inhibits rapid acrolein-induced protein oligomerization: Role of aldehyde scavenging and adduct trapping in cross-link blocking and cytoprotection. *Mol. Pharmacol.*, **69**, 1056–1065 (2006).

Butchiram, M.S., K.S. Tilak and P.W. Raju: Studies on histopathological changes in the gill, liver and kidney of *Channa punctatus* (Bloch) exposed to Alachlor. *J. Environ. Biol.,* **30**, 303-306 (2009).

Eisler, R.: Handbook of Chemical Risk Assessment: Health Hazards to humans, plants and animals. Three Volume Set (Vol. 1). CRC Press (2000).

Eisler, R.: Eisler's encyclopedia of environmentally hazardous priority chemicals. *Elsevier,* 2007.

Haberzettl, P., E. Vladykovskaya, S. Srivastava and A. Bhatnagar: Role of endoplasmic reticulum stress in acrolein-induced endothelial activation. *Toxicol. Appl. Pharmacol.*, **234**, 14–24 (2009).

Jayachandran, K. and K. Pugazhendy: Histopathological changes in the gill of *Labeo rohita* (Hamilton) fingerlings exposed to atrazine. *Am. Eurasian. J. Sci. Res.,* **4**, 219-221 (2009).

Jiraungkoorskul, W., E.S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams and P. Pokethitiyook: Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environ. Toxicol.,* **18**, 260-267 (2003).

Koskinen, H., P. Pehkonen, E. Vehniäinen, A. Krasnov, C. Rexroad, S. Afanasyev and A. Oikari: Response of rainbow trout transcriptome to model chemical contaminants. *Biochem. Biophys. Res. Commun.*, **320**, 745-753 (2004).

Montagna, C.M., A.M.P. De D and A. Venturino: Toxicology of the herbicide acrolein: Risk assessment in aquatic environments. INTECH Open Access Publisher (2011).

Nebot, E., J.F. Casanueva, R. Solera, C. Pendon, L.J. Taracido, T. Casanueva-Robles and C. Lopez-Galindo: Marine biofouling in heat exchangers. Biofouling: types, impact and anti-fouling (Eds.: J. Chan and S. Wong). NY: Nova Science Publishers, New York, pp. 65-104 (2010).

Nordone, A.J., M.F. Kovancs and R. Doane: [14] C Acrolein accumulation and metabolism in leaf lettuce. *B. Environ. Contam. Tox.,* **58**, 787-792 (1997).

Oropesa, A.L., J.P. Garcia-Cambero, L. Gomez, V. Roncero and F. Soler: Effect of long-term exposure to Simazine on histopathology, hematological and biochemical parameters in *Cyprinus carpio. Environ. Toxicol.,* **24***,* 187-199 (2009).

Palermo, F.A., G. Mosconi, M. Angeletti and A.M. Polzonetti-Magni: assessment of water pollution in the Tronto River (Italy) by applying useful biomarkers in the fish model *Carassius auratus*. *Arch. Environ. Contam. Toxicol*., **55**, 295-304 (2008).

Peebua, P., M. Kruatrachue, P. Pokethitiyook and S. Singhakaew: Histopathological alterations of Nile tilapia, *Oreochromis niloticus* in acute and subchronic alachlor exposure. *J. Environ. Biol.*, **29**, 325 (2008).

Randall, M.J., M. Hristova and A. van der Vliet: Protein alkylation by the α, β-unsaturated aldehyde acrolein. A reversible mechanism of electrophile signaling? *FEBS Letters*, **587**, 3808-3814 (2013).

Seok, S.H., M.W. Baek, H.Y. Lee, D.J. Kim, Y.R. Na, K.J. Noh and J.H. Park: *In vivo* alternative testing with zebrafish in ecotoxicology. *J. Vet. Sci.*, **9**, 351-357 (2008).

Shiogiri, N.S., M.G. Paulino, S.P. Carraschi, F.G. Baraldi, C. da Cruz and M.N. Fernandes: Acute exposure of a glyphosate-based herbicide affects the gills and liver of the neotropical fish, *Piaractus mesopotamicus*. *Environ. Toxicol. Phar*., **34**, 388-396 (2012).

Spiess, P.C., B. Deng, R.J. Hondal, D.E. Matthews and A. van der Vliet: Proteomic profiling of acrolein adducts in human lung epithelial cells. *J. Proteomics*, **74**, 2380–2394 (2011).

Stevens, J.F. and C.S. Maier: Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.,* **52**, 7-25 (2008).

US Environmental Protection Agency (US EPA): Ambient Water Quality Criteria Document for Acrolein, EPA-440/5-80-016, NTIS PB81-117277 US EPA, Washington, DC, (1980).

US Environmental Protection Agency (S EPA): Environmental fate and ecological risk assessment for the registration of acrolein, US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic substances, Washington DC (2007).

US Environmental Protection Agency (US EPA): Ambient aquatic life water quality criteria for acrolein, Protection Agency, Office of Water Office of Science and Technology, Health and Ecological

Criteria Division, Washington DC (2009).
Velisek, J., Z. Svobodova, V. Piackova and E. Sudova: Effects of acute exposure to metribuzin on some hematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). *B. Environ. Contam. Tox.,* **82**, 492-495 (2009).
Vladykovskaya, E., S.D. Sithu, P. Haberzettl, N.S. Wickramasinghe, M.L. Merchant, B.G. Hill and D.A. Schuschke: Lipid peroxidation product 4-hydroxy-trans-2-nonenal causes endothelial activation by inducing endoplasmic reticulum stress. *J. Biol. Chem.,* **287**, 11398–11409 (2012).
Xing, H., S. Li, Z. Wang, X. Gao, S. Xu and X. Wang: Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. *Chemosphere,* **88**, 377-383 (2012).
Xu, H.D., J.S. Wang, M.H. Li, Y. Liu, T. Chen and A.Q. Jia: 1 H NMR based metabolomics approach to study the toxic effects of herbicide butachlor on goldfish (*Carassius auratus*). *Aquat. Toxicol.,* **159**, 69-80 (2015).