Sains Malaysiana 50(10)(2021): 2869-2876
http://doi.org/10.17576/jsm-2021-5010-02

Ultrastructural Changes of the Digestive Tract of *Pomacea canaliculata* Exposed to Copper at Lethal Concentration

(Perubahan Ultrastruktur Saluran Pencernaan *Pomacea canaliculata* Terdedah kepada Tembaga pada Kepekatan Maut)

VIPAWEE DUMMEE, MALEEYA KRUATRACHUE, SOMBAT SINGHAKAEW & PHANWIMOL TANHAN*

ABSTRACT

The present study was undertaken to elucidate the basis of cellular reactions and to verify the suitability of Pomacea canaliculata *digestive tract ultrastructure as a biomarker for assessing the Cu pollution in freshwater environments. Two-month-old* P. canaliculata *were exposed to 96-h lethal concentration of Cu (0.15 mg L^{-1}) for 96 h. Electron microscope investigations showed different alterations of organelles in the epithelial cells lining the esophagus and intestine. The most striking changes were damages to the mitochondria, RER, and nucleus typified by loss of cristae and degeneration of mitochondria; degranulation and fragmentation of RER. In nucleus, karyolysis and rupture of nuclear envelope were observed. These changes were attributed to membrane destabilization and increased membrane permeability to ions under the influence of toxicants or heavy metals. These findings indicate the possibility of using the* P. canaliculata *as biomonitor for Cu contamination in the freshwater environment.*

Keywords: Cu; digestive tract; Pomacea canaliculata; *ultrastructure*

ABSTRAK

Kajian ini dijalankan untuk menjelaskan asas reaksi sel dan untuk mengesahkan kesesuaian ultrastruktur saluran pencernaan Pomacea canaliculata *sebagai penanda biologi untuk menilai pencemaran Cu di persekitaran air tawar.* P. canaliculata *yang berusia dua bulan terdedah kepada kepekatan maut Cu 96 jam (0.15 mg L^{-1}) selama 96 jam. Penyelidikan mikroskop elektron menunjukkan perubahan organel yang berlainan pada sel epitelium yang melapisi esofagus dan usus. Perubahan yang paling ketara adalah kerosakan pada mitokondria, RER dan nukleus yang ditandai dengan kehilangan krista dan degenerasi mitokondria; degranulasi dan pemecahan RER. Dalam nukleus, kariolisis dan sampul nuklear pecah diperhatikan. Perubahan ini disebabkan oleh ketidakstabilan membran dan peningkatan kebolehtelapan membran pada ion di bawah pengaruh racun atau logam berat. Penemuan ini menunjukkan kemungkinan menggunakan* P. canaliculata *sebagai biomonitor untuk pencemaran Cu di persekitaran air tawar.*

Kata kunci: Cu; Pomacea canaliculata*; saluran pencernaan; ultrastruktur*

INTRODUCTION

The widespread use of Cu-based compounds as fertilizers and fungicides in agriculture has resulted in Cu contamination in waters, sediments, and soils (de Oliveira-Filho et al. 2004). High levels of Cu (>200 mg kg^{-1}) were reported in the sediments of tributaries flowing into Beung Boraphet reservoir (1300 ha), Nakorn Sawan province, central Thailand (Dummee et al. 2012). The lake is surrounded by agricultural areas (mostly rice paddies) treated with fertilizers and pesticides, including Cu. These rice paddies are flooded intermittently, converting dry aerobic environments to relatively undisturbed anaerobic sediments which promote the release of Cu. Sediment is therefore a natural significant source of Cu (Eisler 1998). Copper has been demonstrated to cause dysfunction of biochemical and physiological processes in invertebrates. The major lethal effects of Cu in snails were disruption of the transporting surface epithelium, and osmoregulation resulting in a possible increase in water accumulation in tissues (Atli & Grosell 2016; Hoang et al. 2008; Kulac et al. 2013). Copper causes toxicities in aquatic invertebrates including DNA damage and histopathological alterations in various organs which were observed in various species of mollusk such as *Mytilus edulis* and *Marisa cornuarietis* exposed to Cu (Al-Subiai et al. 2011; Sawasdee et al. 2011). The

histopathological alterations were changed in the tissue structure of epidermis, swelling of hepatopancreatic digestive cells, alterations in the number of basophilic cells, abnormal apices of digestive cells, irregularly shaped cilia as well as changes in the amount of mucus in the gills (Sawasdee et al. 2011).

Biomonitoring is a key processes in environmental risk assessment (Binelli et al. 2010). Gastropod and bivalve species were used for biomonitoring and bioindication purposes (David et al. 2012; Kim et al. 2008; Rodriguez-Iruretagoiena et al. 2016). They have been broadly used to predict environmental risk since they are at low trophic levels and the entrance doorway of these elements in the trophic web (Burger & Gochfeld 2001; Gupta & Singh 2011). The introduced golden apple snail, *Pomacea canaliculata*, is a widespread agricultural pest in Asia. At Beung Boraphet, apple snails feed on rice, lotus, and other aquatic plants, which considered harmful to these crops. They are an ecologically important component of freshwater food webs and are a primary food source for various species of waterbirds in the reservoir. They are particularly vulnerable to Cu accumulation and toxicity because they feed on the Cu contaminated periphyton and macrophytes (Eisler 1998). Based on their behavioral ecology, they are exposed to Cu through water, sediments, and dietary uptake (Hoang et al. 2011).

Monitoring of metals in the aquatic ecosystems can be evaluated by using a combination of chemical and/or biological parameters. Biomarkers are biological parameters that originate from an organism's response to any toxin and reflect the change in the status of the organisms (Chambers et al. 2002). An increasing amount of research is now incorporating histo-cytological biomarkers in practical ecological risk assessment methodologies (Au 2004; Wester et al. 2002). A wide range of histo-cytological alterations in fish and bivalves have been identified and recommended as biomarkers for monitoring the effects of pollution (Au 2004). Cellular biomarkers including histopathological and ultrastructural responses typically occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters (Triebskorn et al. 1997).

In contrast to histopathological responses at the light microscopic level, ultrastructural studies reflect alterations of metabolic processes resulting in structural changes of organelles and occur earlier than histopathological effects (Gernhöfer et al. 2001). In addition, ultrastructural responses are sensitive enough to indicate early effects on living organisms of low concentrations of chemicals, which makes them potentially useful biomarkers for assessing environmental pollution (Pawert et al. 1998). However, there are few references to the cellular responses of prosobranch mollusks to heavy metals. The digestive gland of the marine prosobranch *Nerita saxtilis* was shown to accumulate high concentrations of Pb and Cd and electron-dense granules in digestive cells were suggested to be the site of storage of detoxified metal (AbdAllah & Moustafa 2002). Histopathological alterations were reported in the digestive tract of another marine prosobranch, *Babylonia areolata* exposed to Pb and Cd (Supanopas et al. 2005; Tanhan et al. 2005); in the digestive gland of *Marisa cornuarietis* exposed to Cu and Li (Sawasdee et al. 2011); and in the digestive tract of *P. canaliculata* exposed to Cu (Dummee et al. 2015, 2012). However, to the best of our knowledge, the ultrastructural alterations in caenogastropoda organs caused by metal exposure have not been studied.

In the present study, ultrastructural alterations of the digestive tract (esophagus, and intestine) from *P. canaliculata* exposed to lethal concentration of Cu were examined in order to investigate the basis of cellular reactions and to verify the suitability of digestive tract ultrastructure as a biomarker to assess the Cu pollution.

Materials and Methods

P. canaliculata eggs were collected from unpolluted natural ponds in Kanchanaburi province, Thailand. They were hatched in the laboratory and juvenile snails were reared under laboratory conditions (25-27 °C, mild aeration) until they were 2 months old. Healthy and uniform juvenile *P. canaliculata* (2-3 cm in shell length) were selected and transferred to a 3 L glass chamber containing acclimatized tap water (pH 6.8-7.5, 25-28 °C) with aeration. They were acclimatized for 10 days before metal exposure.

The static technique was used for acute toxicity exposure. 1000 mL of acclimatized tap water were introduced into each test chamber and Cu solution (as $CuSO_4$, 96-h LC_{50} concentration of 196 µg L^{-1}) was added (Dummee et al. 2015). Ten snails were then placed in each test chamber. Snails which were not exposed to Cu served as controls. There were 3 replicates for each experiment. Snails were not fed 24 h before starting and 96 h during the experiment.

At the end of exposure (96 h), five snails from each tank were randomly collected for cytological observation. Collected snails were cracked to remove the shell. The esophagus and intestine in the digestive tract were removed, dissected into small pieces, and fixed in cold 2.5% glutaraldehyde in 0.05 M sodium cacodylate

buffer, pH 7.3 for 24 h. They were postfixed in 1% osmium tetroxide overnight, rinsed in buffer, dehydrated in a graded series of acetone and embedded in Araldite. Ultrathin sections were cut on an ultramicrotome (GMbH A1170-Wien, Austria) with a diamond knife (Swiss Diamond Knife HC 2530), stained with uranyl acetate and lead citrate, and examined under a JEOL 2100 electron microscope (Japan) operating at 75 kv.

Results and Discussion

The esophagus and intestine were lined by ciliated columnar epithelium (Figures 1 & 2). The apical surface of epithelium contained both long microvilli and cilia (Figures 1(a), 1(b), 2(b)). There were 3 cell types: columnar storage cells, columnar secretory cells, and mucous-secreting goblet cells (Figures 1(c), 1(d), 2(a)). There were fewer mucous cells in the intestine. The columnar cells had richer cytoplasm filled with numerous mitochondria, RER, Golgi body and ribosome, than the mucous cells. The columnar storage cells contained numerous mitochondria of varying sizes and shapes in the apical region (Figures 1(b) & 2(b)). In addition, there were abundant ribosomes, RERs, and a few Golgi bodies in the central region (Figure 1(b) & 1(c)). A long nucleus was located in the basal region (Figure 1(c)). It usually contained euchromatin with a single nucleolus. The columnar secretory cells were characterized by numerous secretory granules, RER, SER, ribosomes, and Golgi bodies (Figures 1(c) & 2(c)). The nuclei were basally situated, irregular in shape, possessed a huge

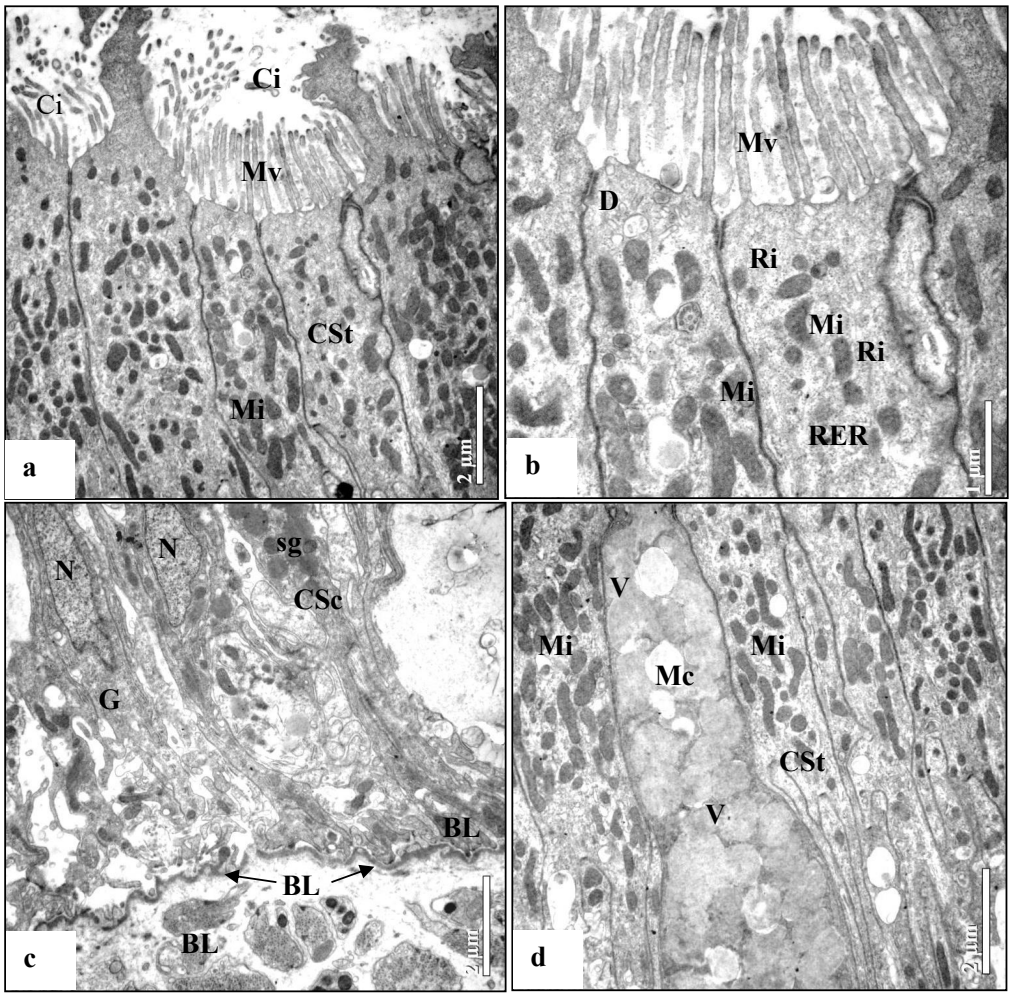

FIGURE 1. Epithelium of esophagus in control snail. a) Apical area of columnar storage cells (CSt) with microvilli (Mv) and cilia (Ci). Notice numerous mitochondria (Mi); b) Cytoplasm of CSt with mitochondria (Mi), ribosomes (Ri), RER. Cells are connected by desmosome (D); c) Basal area (BL) of CSt showing Golgi body (G), nucleus (N) and columnar secretory cell (CSc) containing secretory granules (sg); d) Mucous cell (Mc) with abundant vacuoles (V) and CSt with mitochondria (Mi)

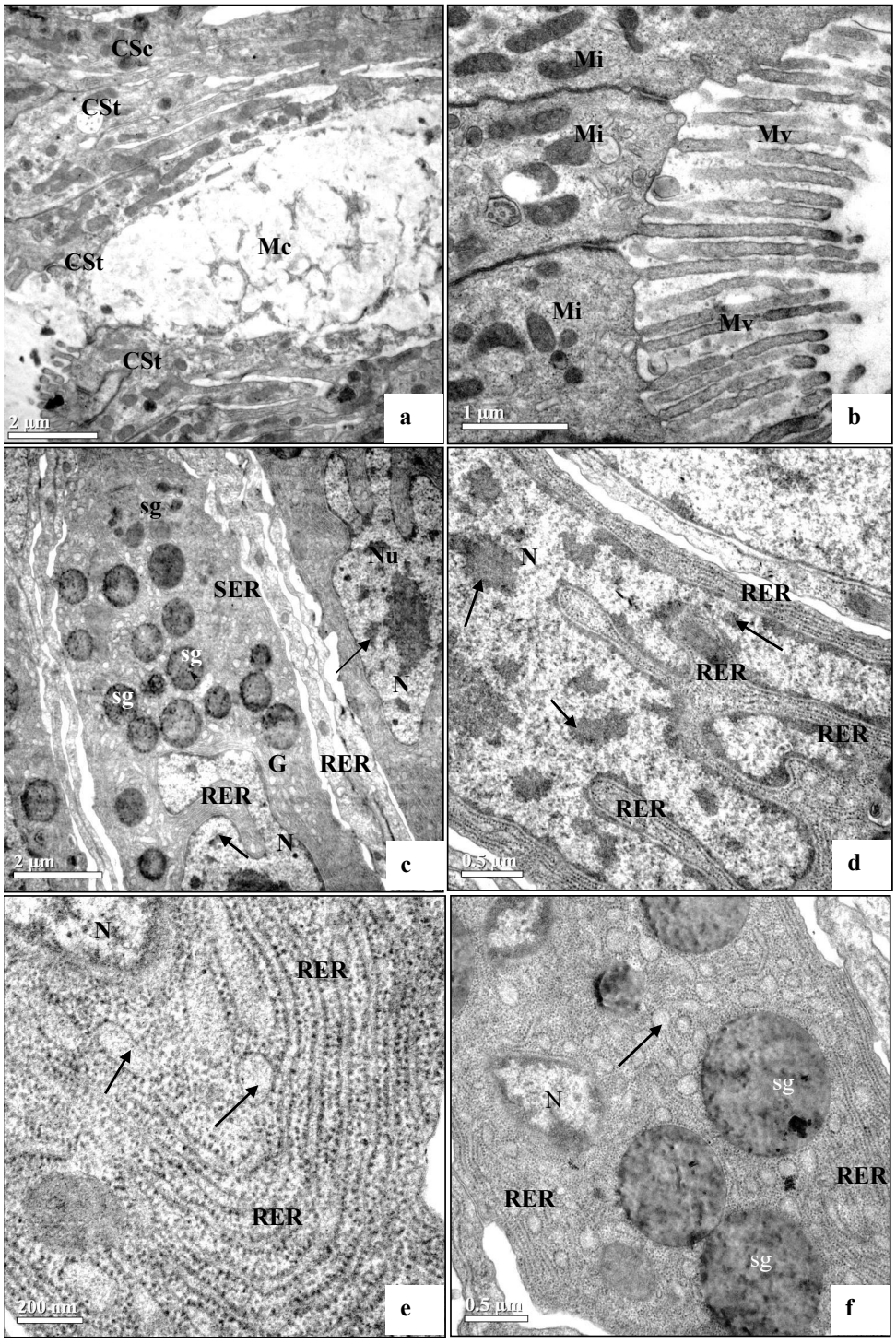

FIGURE 2. Epithelium of intestine in control snail. a) Three cell types: columnar storage cell (CSt), columnar secretory cell (CSc), and mucous cell (Mc); b) Apical area of CSt with microvilli (Mv) and abundant mitochondria (Mi); c) CSc with numerous secretory granules (sg), SER and RER. Notice irregular shaped nucleus (N) with nucleolus (Nu) and patches of heterochromatin (arrows), and Golgi body (G); d) Nucleus (N) of CSt with heterochromatin (arrows). Notice nuclear infoldings filled with RER; e & f) CSt with stacks of tubular RER, vesicular RER (arrows), secretory granules (sg) enclosed in membrane. N, nucleus

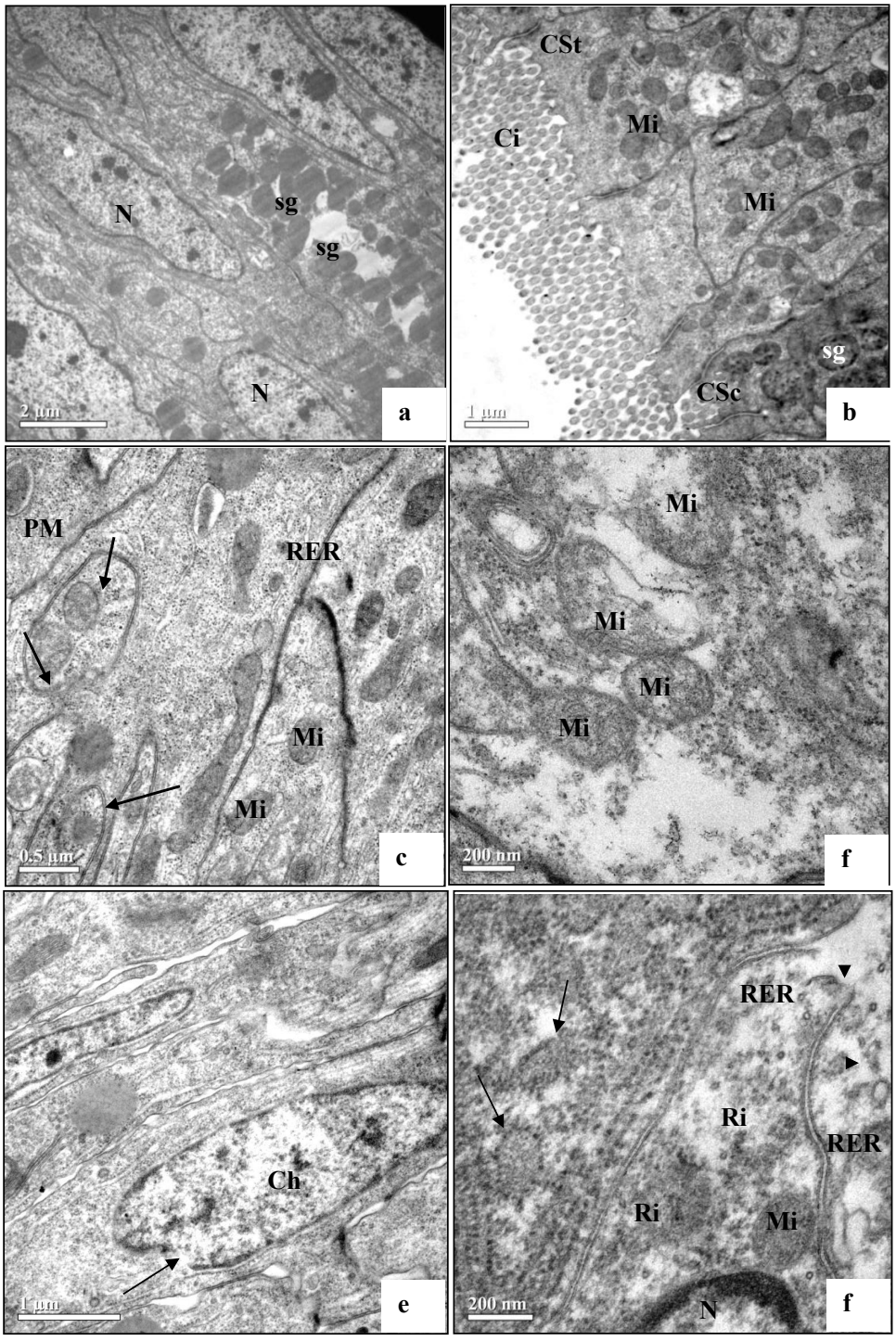

FIGURE 3. Esophagus and intestine of Cu-treated snails. a) Esophagus: columnar storage cells with nucleus (N) and columnar secretory cell with secretory granules (sg). Notice loss of heterochromatin in nucleus; b) Intestine: CSt with mitochondria (Mi) and CSc with secretory granules (sg). Notice loss of density and well-defined membrane in sg. Ci, cilia; c) Intestine: some alterations were observed in CSt such as loss of cristae in mitochondria (Mi), invagination of plasma membrane (PM) around mitochondria (arrows); d) Esophagus: mitochondria (Mi) showing various stages of degeneration; e) Intestine: nucleus with decreased heterochromatin (Ch) and ruptured nuclear envelope (arrow); f) Esophagus: high magnification showing stack of degranulated RER, dilated RER (arrows) and fragmented RER (arrowheads). Ri, ribosome; Mi, mitochondria

nucleolus and scattered patches of heterochromatin (Figure 2(c) & 2(d)). They contained many infoldings filled with well-stacked RER (Figure 2(d)). There were 2 types of RER: tubular RER and vesicular RER (Figure 2(e)). The secretory granules were enclosed within a single membrane (Figure 2(f)). The mucous cells were characterized by the presence of mucous vacuoles filled with mucus (Figures 1(d) & 2(a)), peripheral cytoplasm with RERs and ribosomes. The nucleus was located in the basal region of the cell.

In the Cu-treated *P. canaliculata*, most of the cytological alterations were observed in the mitochondria, RER, and nucleus (Figure 3(a) & 3(b)). The mitochondria exhibited various changes which indicated the severity of Cu toxicity (Figure 3(c) & 3(d)). Damage to the mitochondria displayed varying degree of severity from swelling and reduction of cristae and presence of various inclusions in the matrix to severe vacuolization and engulfment by the plasma membrane (Rez 1986; Triebskorn 1989). Goyer and Rhyne (1975) proposed that the swelling of the organelles resulted from inhibition of ion transport and protein synthesis. The toxin most likely interacted with the mitochondria membrane so as to change its permeability to ions (Triebskorn 1989). In this study, some mitochondria were still intact with double membranes but a reduction in cristae (Figure 3(d)). Others had undergone severe damages such as the breakdown of cristae and membrane (Figure 3(c) & 3(d)). The most severe case observed was mitophagy (Figure 3(c)) or the removal of damaged mitochondria by autophagy (Lin et al. 2012; Priault et al. 2005). Autophagy lysosome pathway (ALS) is one of the major intracellular degradation systems in eukaryotic cells. ALS degrades soluble proteins, large aggregates, and organelles in the cytoplasm. However, these alterations are often considered unspecific symptoms (Rez 1986; Triebskorn 1989). They were also observed in digestive tract of other mollusks such as *Deroceras reticulatum* (Triebskorn 1989), in collembolan midgut cells (Pawert et al. 1998), and neuronal cells of vertebrates (Lin et al. 2012) exposed to various toxicants.

In the columnar cells of the digestive tract of Cu-treated *P. canaliculata*, the RER also underwent different changes such as degranulation or the loss of ribosomes, fragmentation of tubular RER (Figure 3(f)). Some detachment of ribosomes was probably due to disruption of the energy-dependent interactions between ER membrane and its ribosome (Cotran et al. 1994). Dilation or swelling of ER cisternae can be caused by an impairment of cell volume regulation by plasma membrane. The toxicant interfered with oxidative phosphorylation resulting in a decrease in ATP and ATPase, which led to the accumulation of Na ions in the cell. The cell therefore acquired more water resulting in membrane dilation and swelling of organelles (Cotran et al. 1994).

Another cellular structure that exhibited apparent alterations was the nucleus. The nucleus of the columnar cell showed a condensation of chromatin into small clumps (Figure 3(a)). Ruptured nuclear envelope was observed (Figure 3(e)) and some even became fragmented (Figure 3(a)). Ghadially (1988) also described a condensation of the chromatin in different organisms exposed to various toxicants. As a result, it leads to a reduction of the transcription rate and hence, a decrease of the metabolic activity.

Reactions such as cytoplasm condensation, mitochondrial swelling (Goyer & Rhyne 1975; Triebskorn 1989) and dilation of endomembranes (Triebskorn 1989) as well as ER-membrane proliferation or destruction (Moore 1985, 1979; Nott & Moore 1987) resembled those already described in bivalves, gastropods, and vertebrates as cellular stress symptoms after intoxication with different xenobiotics (Triebskorn 1989). Most of these reactions could be attributed to membrane destabilization and increased membrane permeability to ions under the influence of toxicants followed by osmotic effects and finally cell death (Sparks 1972).

Conclusion

In conclusion, it is apparent the use of transmission electron microscope is highly effective in elucidating the cellular responses of *P. canaliculata* to Cu. The present study provides additional evidence for the usefulness of a set of digestive ultrastructural biomarkers in assessing the health of freshwater systems. Therefore, it is important to note that the responses of ultrastructural biomarker depend on the Cu concentration and the frequency of exposure. In addition, the ultrastructural alterations observed in the esophagus and intestine indicated that the freshwater gastropod *P. canaliculata* could serve as a biomonitor for assessing Cu concentrations in the freshwater environment.

Acknowledgements

This study was funded by the Center for Environmental Health, Toxicology and Management of Chemicals under Science and Technology Postgraduate Education and Research Office (PERDO) of the Ministry of Education, Thailand; Faculty of Science, Mahidol University; and Mahidol University. We also thank Associate Professor Philip D. Round for editing the manuscript.

REFERENCES

AbdAllah, A.T. & Moustafa, M.A. 2002. Accumulation of lead and cadmium in the marine prosobranch *Nerita saxtilis*, chemical analysis, light and electron microscopy. *Environmental Pollution* 116(2): 185-191.

Al-Subiai, S.N., Moody, A.J., Mustafa, S.A. & Jha, A.N. 2011. A multiple biomarker approach to investigate the effects of copper on the marine bivalve mollusc, *Mytilus edulis*. *Ecotoxicology and Environmental Safety* 74(7): 1913-1920.

Atli, G. & Grosell, M. 2016. Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (*Lymnaea stagnalis*) during acute copper exposure. *Aquatic Toxicology* 176: 38-44.

Au, D.W.T. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: A review. *Marine Pollution Bulletin* 48(9-10): 817-834.

Binelli, A., Cogni, D., Parolini, M. & Provini, A. 2010. Multi-biomarker approach to investigate the state of contamination of the R. Lambro/R. Po confluence (Italy) by zebra mussel (*Dreissena polymorpha*). *Chemosphere* 79(5): 518-528.

Burger, J. & Gochfeld, M. 2001. On developing bioindicators for human and ecological health. *Environmental Monitoring and Assessment* 66(1): 23-46.

Chambers, J.E., Boone, J.S., Carr, R.L., Chambers, H.W. & Straus, D.L. 2002. Biomarkers as predictors in health and ecological risk assessment. *Human and Ecological Risk Assessment: An International Journal* 8(1): 165-176.

Cotran, R.S., Kumar, V. & Robbin, S.L. 1994. *Pathologic Basic of Disease*. Philadelphia: W.B. Saunders Company.

David, E., Tanguy, A., Riso, R., Quiniou, L., Laroche, J. & Moraga, D. 2012. Responses of Pacific oyster *Crassostrea gigas* populations to abiotic stress in environmentally contrasted estuaries along the Atlantic coast of France. *Aquatic Toxicology* 109: 70-79.

de Oliveira-Filho, E.C., Lopes, R.M. & Paumgartten, F.J.R. 2004. Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere* 56(4): 369-374.

Dummee, V., Tanhan, P., Kruatrachue, M., Damrongphol, P. & Pokethitiyook, P. 2015. Histopathological changes in snail, *Pomacea canaliculata*, exposed to sub-lethal copper sulfate concentrations. *Ecotoxicology and Environmental Safety* 122: 290-295.

Dummee, V., Kruatrachue, M., Trinachartvanit, W., Tanhan, P., Pokethitiyook, P. & Damrongphol, P. 2012. Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet reservoir, Thailand. *Ecotoxicology and Environmental Safety* 86: 204-212.

Eisler, R. 1998. Copper hazards to fish, wildlife, and invertebrates: A synotic review. US Geological Survey, Biological Resources Division, Biological Science Report USGS/BDR/BSR 1997-0002.

Gernhöfer, M., Pawert, M., Schramm, M., Müller, E. & Triebskorn, R. 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *Journal of Aquatic Ecosystem Stress and Recovery* 8(3-4): 241-260.

Ghadially, F.N. 1988. *Ultrastructural Pathology of the Cell and Matrix*. 3rd ed. Guildford: Butterworth-Heinemann.

Goyer, R.A. & Rhyne, B.C. 1975. Pathological effects of lead. *International Review of Experimental Pathology* 12: 1-77.

Gupta, S.K. & Singh, J. 2011. Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: A review. *The IIOAB Journal* 2(1): 49-57.

Hoang, T.C., Pryor, R.L., Rand, G.M. & Frakes, R.A. 2011. Bioaccumulation and toxicity of copper in outdoor freshwater microcosms. *Ecotoxicology and Environmental Safety* 74(4): 1011-1020.

Hoang, T.C., Rogevich, E.C., Rand, G.M., Gardinali, P.R., Frakes, R.A. & Bargar, T.A. 2008. Copper desorption in flooded agricultural soils and toxicity to the Florida apple snail (*Pomacea paludosa*): Implications in Everglades restoration. *Environmental Pollution* 154(2): 338-347.

Kim, Y., Powell, E.N., Wade, T.L. & Presley, B.J. 2008. Relationship of parasites and pathologies to contaminant body burden in sentinel bivalves: NOAA status and trends 'Mussel Watch' program. *Marine Environmental Research* 65(2): 101-127.

Kulac, B., Atli, G. & Canli, M. 2013. Response of ATPases in the osmoregulatory tissues of freshwater fish *Oreochromis niloticus* exposed to copper in increased salinity. *Fish Physiology and Biochemistry* 39(2): 391-401.

Lin, M., Chandramani-Shivalingappa, P., Jin, H., Ghosh, A., Anantharam, V., Ali, S., Kanthasamy, A.G. & Kanthasamy, A. 2012. Methamphetamine-induced neurotoxicity linked to ubiquitin-proteosome system dysfunction and autophagy-related changes that can be modulated by protein kinase C delta in dopaminergic neuronal cells. *Neuroscience* 210: 308-332.

Moore, M.N. 1985. Cellular responses to pollutants. *Marine Pollution Bulletin* 16(4): 134-139.

Moore, M.N. 1979. Cellular responses to polycyclic aromatic hydrocarbons and phenobarbital in *Mytilus edulis*. *Marine Environmental Research* 2(4): 255-263.

Nott, J.A. & Moore, M.N. 1987. Effects of polycyclic aromatic hydrocarbons on molluscan lysosomes and endoplasmic reticulum. *Histochemistry Journal* 19: 357-368.

Pawert, M., Müller, E. & Triebskorn, R. 1998. Ultrastructural changes in fish gills as biomarker to assess small stream pollution. *Tissue and Cell* 30(6): 617-626.

Priault, M., Salin, B., Schaeffer, J., Vallette, F.M., di Rago, J.P. & Martinou, J.C. 2005. Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death & Differentiation* 12(12): 1613-1621.

Rez, G. 1986. Electron microscopic approaches to environmental toxicity. *Acta Biologica Hungarica* 37: 31-45.

Rodriguez-Iruretagoiena, A., Rementeria, A., Zaldibar, B., de Vallejuelo, S.F.-O., Gredilla, A., Arana, G. & de Diego, A. 2016. Is there a direct relationship between stress biomarkers in oysters and the amount of metals in the

sediments where they inhabit? *Marine Pollution Bulletin* 111(1): 95-105.

Sawasdee, B., Köhler, H.R. & Triebskorn, R. 2011. Histopathological effects of copper and lithium in the ramshorn snail, *Marisa cornuarietis* (Gastropoda, Prosobranchia). *Chemosphere* 85(6): 1033-1039.

Sparks, A.K. 1972. *Invertebrate Pathology. Noncommunicable Diseases*. New York: Academic Press.

Supanopas, P., Sretarugsa, P., Kruatrachue, M., Pokethitiyook, P. & Upatham, E.S. 2005. Acute and subchronic toxicity of lead to the spotted babylon, *Babylonia areolata* (Neogastropoda, Buccinidae). *Journal of Shellfish Research* 24(1): 91-98.

Tanhan, P., Sretarugsa, P., Pokethitiyook, P., Kruatrachue, M. & Upatham, E.S. 2005. Histopathological alterations in the edible snail, *Babylonia areolata* (spotted babylon), in acute and subchronic cadmium poisoning. *Environmental Toxicology* 20(2): 142-149.

Triebskorn, R. 1989. Ultrastructural changes in the digestive system of *Deroceras reticulatum* (Mollusca: Gastropoda) induced by lethal and sublethal concentrations of the carbamate molluscicide cloethocarb. *Malacologia* 32: 89-106.

Triebskorn, R., Köhler, H.R., Honnen, W., Schramm, M., Adams, S.M. & Müller, E. 1997. Induction of heat shock proteins, changes in liver ultrastructure, and alterations of fish behavior: Are these biomarkers related and are they useful to reflect the state of pollution in the field? *Journal of Aquatic Ecosystem Stress and Recovery* 6(1): 57-73.

Wester, P.W., van der Ven, L.T.M., Vethaak, A.D., Grinwis, G.C.M. & Vos, J.G. 2002. Aquatic toxicology: opportunities for enhancement through histopathology. *Environmental Toxicology and Pharmacology* 11(3-4): 289-295.

Vipawee Dummee, Maleeya Kruatrachue & Sombat Singhakaew
Department of Biology
Faculty of Science
Mahidol University
Bangkok 10400
Thailand

Vipawee Dummee
Faculty of Technology and Environment
Prince of Songkla University
Phuket Campus, Kathu, Phuket 83120
Thailand

Phanwimol Tanhan*
Department of Pharmacology
Faculty of Veterinary Medicine
Kasetsart Univesity
Bangkok 10900
Thailand

*Corresponding author; email: fvetpmt@ku.ac.th

Received: 2 July 2020
Accepted: 19 February 2021