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Preliminary Histological Investigation of East Asian Bullfrog (*Hoplobatrachus rugulosus*) Tadpoles Exposed to Potassium Chlorate

Wararut Buncharoen[1], Monruedee Chaiyapo[1]*, Supap Saenphet[1], Athibeth Buajai[1], Phornphan Phrompanya[1] and Kanokporn Saenphet[1]

[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*
*Corresponding author's e-mail address: chaiyapo@gmail.com

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ABSTRACT

Potassium chlorate has been used widely in northern Thailand to induce off-season flowering of longan trees. Therefore, it is highly possible that potassium chlorate could contaminate the surrounding waters. Although adverse effects of agrochemicals on the aquatic vertebrates are well documented, the exploration of histopathological changes under potassium chlorate toxic effects on amphibians is limited. The present study aimed to histopathologically investigate the effect of potassium chlorate on the kidney, liver, and gonad of east Asian bullfrog *Hoplobatrachus rugulosus* larvae. The 3-day-old tadpoles were divided into 0.1, 0.5, 1.0, and 1.5 ppm exposure groups to potassium chlorate for 24 weeks. Larval samplings were conducted for histopathological examination in pro- and complete- metamorphic stages. The histopathological changes of all investigated organs were observed in potassium chlorate treated groups, especially at the high concentrations (1.0 and 1.5 ppm). Kidney tissues showed glomerular shrinkage together with congestion, while vacuolization and degeneration of hepatocytes, expanded sinusoidal spaces, and leukocyte infiltration were found in the liver. Moreover, atretic oocytes and karyorrhexis were recognized in the ovary. Our results suggested that the use of potassium chlorate at high concentrations adversely affected vital organs of *H. rugulosus* tadpoles and might lead to the decline of the amphibian population.

INTRODUCTION

Longan is one of the major economic fruit crops in Thailand, especially for exportation [1]. To induce off-season flowering of longan trees and increase productivity, potassium chlorate has been used widely in the upper northern provinces of Thailand [2-4]. Besides the benefits of agrochemical usage, potassium chlorate could be washed into water bodies after raining and contaminates the water resources to be affected for aquatic organisms including amphibians [5-7].

The results of prior studies indicated that chlorates could cause a broad spectrum of adverse effects on non-target animals. Sodium perchlorate ($NaClO_4$) can lead to harmful effects, such as thyroid follicular cell hypertrophy, metamorphic inhibition, and disruption of metamorphosis- and growth-related gene expression in wood frog (*Lithobates sylvaticus*), African clawed frog (*Xenopus laevis*), Asiatic toad (*Bufo gargarizans*), zebrafishes, and sticklebacks [8-12]. Potassium perchlorate ($KClO_4$) had toxicity to overstimulate the thyroid gland and thyroid follicular cells in the common toad (*Bufo arenarum*) leading to interruption of mechanisms involving a decrease in thyroxine (T4) level, and an increase in hypothalamo-hypophyseal endocrine secretory stimulation [13]. In addition, potassium perchlorate could increase number but reduce in size of thyroid follicular cells, cause severe hyperplasia, and lower production of thyroxine in zebrafish [14].

Typically, potassium chlorate ($KClO_3$) has been applied to improve on-season flowering and induce off-season flowering in popular economic plants, e.g., longan, lychee, mango, rambutan, and orchid [3, 15-22]. Up-to-date, there has been only one report that mentioned the impact of potassium chlorate on the survival rates of earthworms during 4 months of the treatments [23].

Hoplobatrachus rugulosus (the east Asian bullfrog), a common species of the eastern and southeastern regions of Asia, generally inhabits freshwater marshes, ponds, rice fields, swamps, and flooded agricultural land [24]. Due to the unique biology and life cycle of amphibians, water resources are required for their developmental and reproductive stages. Hence, *H. rugulosus* is suitable to be a bioindicator of environmental pollution due to their susceptibility to chemicals during their freshwater cycles [25]. Especially, in the larval stage, anurans are quite sensitive and necessary to be submerged in the water consistently. If the increased agricultural chemicals run off and contaminate the surrounding water resources, it may be harmful to metamorphosis and population decline of amphibians directly [26-28].

Although adverse effects of several agrochemicals on the aquatic vertebrates are well documented [8-14], the histopathological information about the toxic effects of potassium chlorate on amphibian larvae has not been well explored. Therefore, the present study aimed to histopathologically investigate the effect of potassium chlorate on the kidney, liver, and gonad of *Hoplobatrachus rugulosus* larvae.

METHODOLOGY

Animal, chemical, and experimental design

The present study was performed with *H. rugulosus* larvae. Three-day-old tadpoles from Thunpisit Farm, Chiang Mai were purchased and acclimatized for a week before starting the experiment. The tadpoles were reared in plastic containers equipped with a continuous supply of well-aerated and dechlorinated tap water (1L) under a natural photoperiod condition (12 h light/dark cycle) and constant temperature (25+ 1˚C). Bullfrog tadpoles were fed with frozen blood worms twice daily. Rearing water was changed every 3 days and dead tadpoles were removed immediately if present. All same-size of 450 tadpoles were chosen randomly and divided into 5 groups: control group, 0.1 ppm, 0.5 ppm, 1 ppm, 1.5 ppm of potassium chlorate exposure groups, respectively, for 24 weeks. A commercial grade of purchased potassium chlorate ($KCLO_3$) from Sigma-Aldrich was chosen for this experiment.

Histopathological examinations

Larval samplings were conducted for histopathologic examination at 2 developmental stages, pro-metamorphosis, and complete metamorphosis. The tadpoles were overnight fixation with Bouin's solution. Then the tissues were processed and embedded in paraffin. Six µm sections were made and stained with hematoxylin and eosin (H&E). The samples were analyzed by assessing the histopathological alteration under a light microscope and photograph taken by Olympus BX41. Two individuals of tadpoles in each group and each stage (pro- and complete metamorphosis) were randomly collected to examine the histopathological alterations of kidney, liver, and gonad tissues. The present experiment was approved by Institutional Animal Ethics Committee of Chiang Mai University, Thailand (Approval number RE002/14).

RESULTS

The histopathological alterations observed in the kidney, liver and ovary of *H. rugulosus* exposure to potassium chlorate were represented in terms of the descriptive report as follows:

1. Histopathology of kidney tissues

In control group of pro-metamorphosis (Figure 1A), renal tissues were distinguished by characteristics of the renal corpuscle, which consisted of an outer envelope of Bowman's capsule surrounding a fluid-filled Bowman's space and a glomerulus, which was a tuft of endothelial cells. In groups of pro-metamorphic tadpoles treated with 0.1, 0.5, and 1.0 ppm potassium chlorate, there were no histopathological changes in kidney tissues. After treatment with 1.5 ppm of potassium chlorate in pro-metamorphosis, the glomerular shrinkage was evidenced that led to wider Bowman's space and congestion (Figure 1B).

The renal tissues of 1.0 ppm treated bullfrog tadpoles in a complete metamorphosis (Figure 2C) represented vacuolization and degeneration of renal tubules. Whereas, there was marked glomerular shrinkage increasing the Bowman's space and epithelial lifting of renal tubules in a 1.5 ppm treated group (Figures 2D, 2E) compared to kidney tissues of a control group (Figures 2A, 2B) and groups of tadpoles exposed to 0.1 and 0.5 potassium chlorate in a complete metamorphosis with no observed pathological alterations.

2. Histopathology of liver tissues

Hepatic tissues of a control group in pro-metamorphosis (Figure 3A) consisted of hepatocytes arranged in hepatic cords intervening with hepatic sinusoids. Hepatocytes were typically hexagonal in shape with large, round central nuclei, and the cytoplasm was regularly distributed with very fine granules of glycogen and lipid droplets. In pro-metamorphic tadpoles induced by 0.1 and 0.5 potassium chlorate, hepatopathological changes were not recognized. In contrast, peribiliary leukocyte infiltration and congestion (Figures 3B, 3C) were recognized in hepatic tissues of tadpoles exposed to 1.0 and 1.5 ppm of potassium chlorate in pro-metamorphosis.

To compare with a control group (Figure 4A) in complete metamorphosis, necrosis of hepatocytes, vacuolization, and sinusoidal dilation were observed from hepatic tissues in tadpoles exposure to 1.0 and 1.5 ppm of potassium chlorate during complete metamorphosis (Figures 4B, 4C). Whereas, no alterations were evidenced in liver tissues of complete metamorphic tadpoles treated with 0.1 and 0.5 ppm of potassium chlorate.

3. Histopathology of ovarian tissues

Ovarian tissues of a 1.5 ppm treated group in pro-metamorphosis (Figure 5B) had no differences compared to those of the control group, which comprised a number of various stages of oocytes inside the ovary (Figure 5A), and other treatment groups.

In complete metamorphosis, karyorrhexis and atretic oocytes were recognized in ovarian tissues of a 1.5 ppm treated group of *H. rugulosus* tadpoles (Figure 6B) compared to a control group (Figure 6A) in this stage. In groups of a complete metamorphic tadpoles exposed to 0.1, 0.5, and 1.0 ppm of potassium chlorate, no histopathological changes in ovarian tissues were observed.

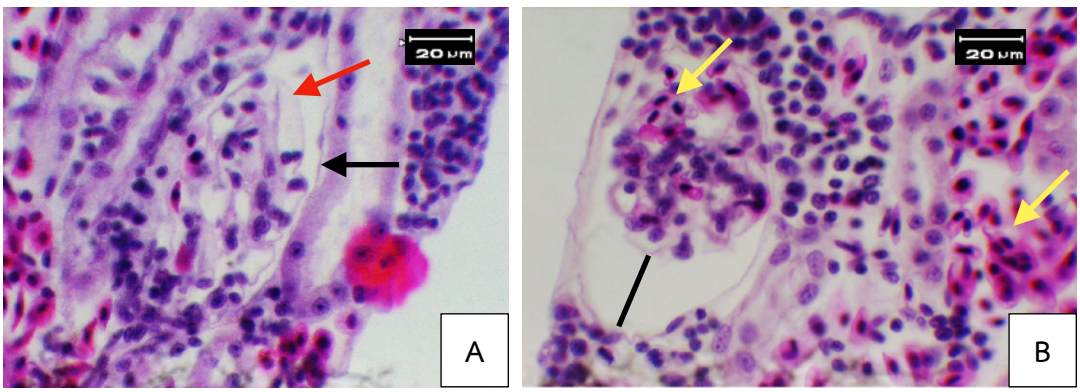

Figure 1. Histomorphology of bullfrog tadpole renal tissues in pro-metamorphosis of a control group and a potassium chlorate treatment group. (A) A control group showed glomerulus (red arrow) and Bowman's capsule (black arrow), and of 1.5 ppm. (B) A 1.5 ppm potassium chlorate treatment group showed glomerular shrinkage (black line) and congestion (yellow arrows). H&E staining. Scale bar = 20 µm, 400x magnification.

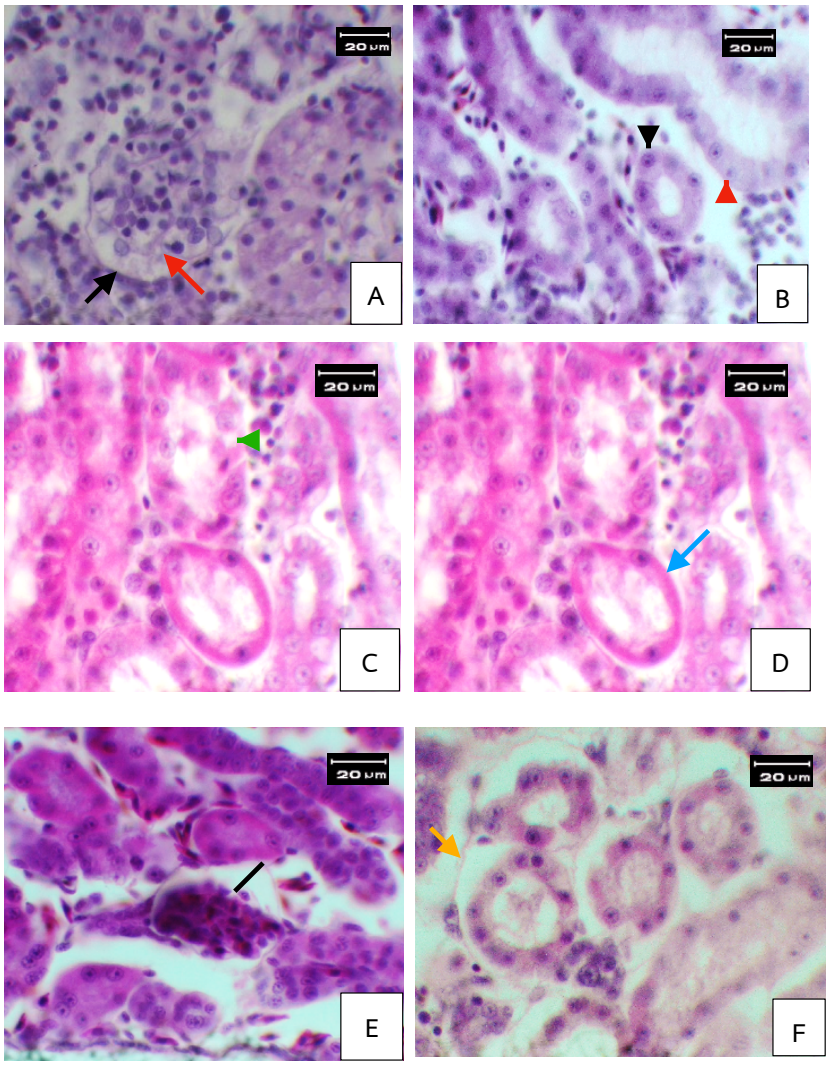

Figure 2. Histomorphology of bullfrog renal tissues in a complete metamorphosis of a control group and potassium chlorate treatment groups. (A) Glomerulus (red arrow) and Bowman's capsule (black arrow) and (B) proximal tubule (black arrow head) and distal tubule (red arrow head) of a control group. (C) Cytoplasmic vacuolization (green arrow head) and (D) renal tubular degeneration (black line) of a 1.0 ppm potassium chlorate treatment group. (E) Glomerular shrinkage (black line) and (F) epithelial lifting (orange arrow) of renal tublues of a 1.5 ppm potassium chlorate treatment group. H&E staining. Scale bar = 20 µm, 400x magnification.

DISCUSSION

Histopathological alterations of renal tissues

According to this examination, potassium chlorate may cause histopathological changes on kidney of *H. rugulosus* as follows: glomerular shrinkage leading to the increase of Bowman's space, congestion, vacuolization, and necrosis in renal tubular epithelium. Although the available documents about the nephrotoxicity of xenobiotics in amphibians were limited, the results of the present study on this organ were consistent with the previous reports either induced laboratory or natural conditions.

The glomerular shrinkage leading to the increase of Bowman's space was frequently observed in renal tissues of amphibians exposure to pesticides and metals. For example, *H. rugulosus* larvae induced by chitosan, cypermethrin, and methomyl [29, 30], green toad (*Bufotes variabilis*) tadpoles exposed to carbaryl [31], marsh frog (*Pelophylax ridibundus*) induced by Talstar 10EC insecticide [32], *Xenopus laevis* frogs treated with 500 µg/L atrazine [34], juvenile Italian tree frog (*Hyla intermedia*) after chronic exposure to pyrimethanil [35], and Indian green frogs (*Euphlyctis hexadactylus*) induced by a mixture of heavy metals from polluted sites [40].

Congestion was particularly evidenced in kidney tissues of *H. rugulosus* tadpoles exposed to methomyl [30], juvenile Italian tree frog (*Hyla intermedia*) after chronic exposure to pyrimethanil [35], *Microhyla fissipes* induced by cadmium [36], *Rana esculenta* sub-lethal exposure to environmental pollutants (heavy metals and insecticides) [39], and Indian green frogs (*Euphlyctis hexadactylus*) induced by a mixture of heavy metals [40].

Vacuolization of tubule epithelial cells was previously mentioned in green toad (*Bufotes variabilis*) tadpoles exposed to carbaryl [31], marsh frog (*Pelophylax ridibundus*) induced by Talstar 10EC insecticide [32], unidentified species of male frogs caused by alpha-cypermethrin [33], *Xenopus laevis* frogs induced by 500 µg/L atrazine [34], and *Rana esculenta* sub-lethal exposure to environmental heavy metals and insecticides [39].

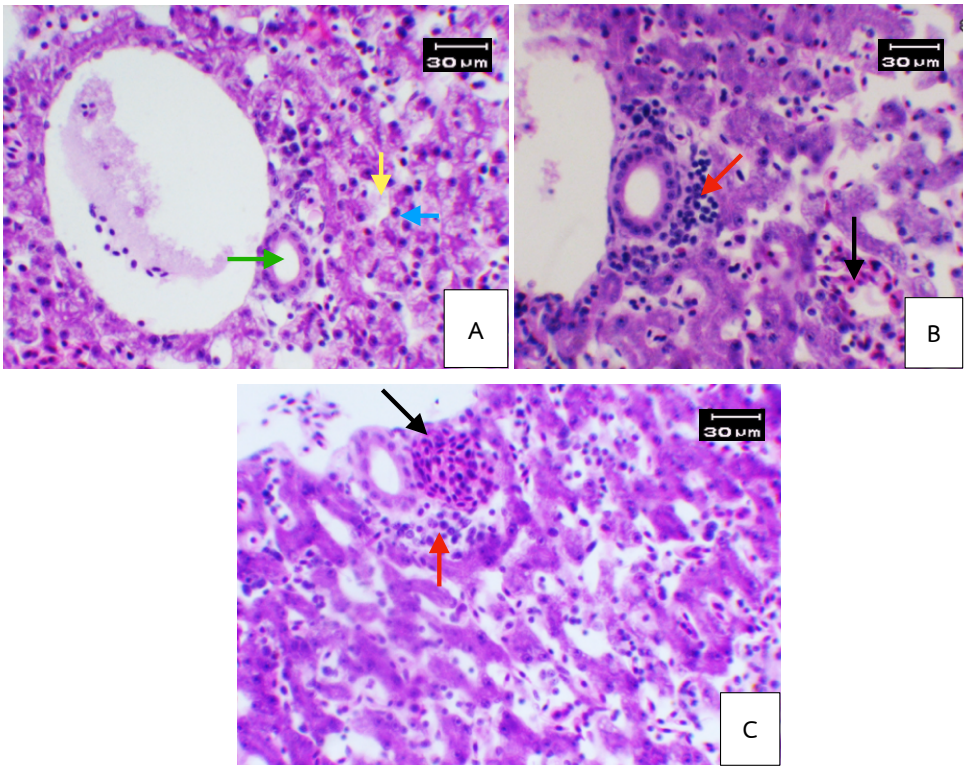

Figure 3. Histomorphology of bullfrog hepatic tissues in pro-metamorphosis of a control group and potassium chlorate treatment groups. (A) portal triads showed bile duct (green arrow), hepatocyte (blue arrow), and hepatic sinusoid (yellow arrow) of a control group. Peribiliary leukocyte infiltration (red arrow) and congestion (black arrow) of (B) a 1.0 ppm and (C) a 1.5 ppm potassium chlorate treated groups. H&E staining. Scale bar = 30 µm, 200x magnification.

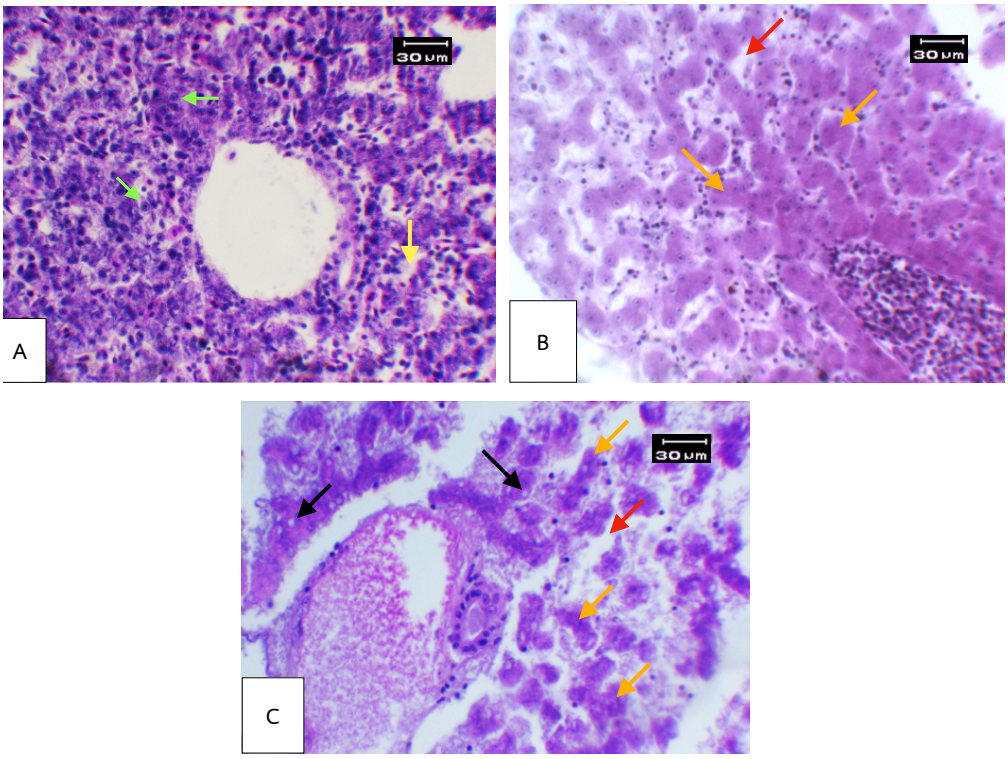

Figure 4. Histomorphology of bullfrog hepatic tissues in a complete metamorphosis of a control group and potassium chlorate treatment groups. (A) Hepatocyte (green arrow) and hepatic sinusoid (yellow arrow) of a control group. Hepatocyte necrosis (orange arrows), hepatocyte vacuoles (black arrows), and expanded sinusoidal spaces (red arrows) of (B) a 1.0 ppm and (C) a 1.5 ppm potassium chlorate treatment

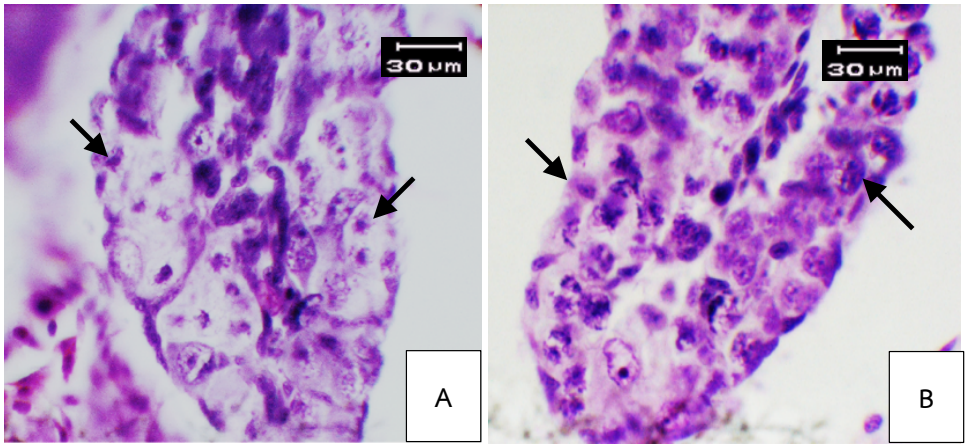

Figure 5. Histomorphology of bulfrog ovaries in pro-metamorphosis of (A) a control group and (B) a 1.5 ppm potassium chlorate treatment group showed primordial follicle cell (black arrows). H&E staining. Scale bar = 30 µm, 200x magnification.

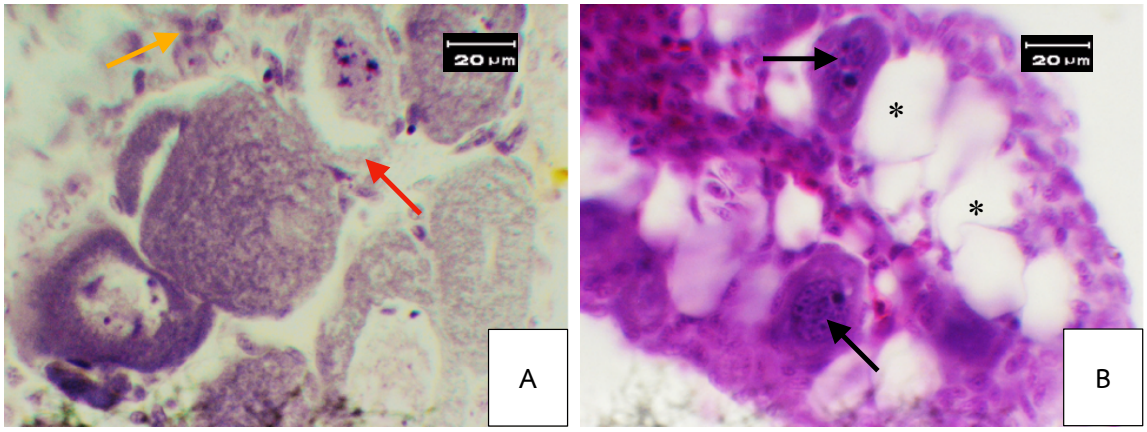

Figure 6. Histomorphology of bullfrog ovaries in a complete metamorphosis of a control group and potassium chlorate treatment group. (A) Primordial follicles (orange arrow), and follicle cell (red arrow) of a control group. (B) A 1.5 ppm potassium chlorate treatment group showed karyorrhexis (black arrows) and atretic oocytes (black asterisks). H&E staining. Scale bar = 20 µm, 400x magnification.

Necrosis of the renal tubules was recognized in green toad (*Bufotes variabilis*) tadpoles exposed to carbaryl [31], marsh frog (*Pelophylax ridibundus*) induced by Talstar 10EC [32], unidentified species of male frogs contaminated with alpha-cypermethrin [33], *Microhyla fissipes* induced by cadmium [36], Italian crested newt (*Triturus carnifex*) toxicated by cadmium [37], *Rhinella arenarum* treated with cadmium [38], and Indian green frogs (*Euphlyctis hexadactylus*) induced by a mixture of heavy metals [40].

The kidney of amphibians is one of the vital organs that plays a major role in the homeostasis of the body's extracellular fluid. It has been considered as an important organ to evaluate the toxicity of the environmental contaminants due to its primary functions and high sensitivity [31, 41, 42]. Nevertheless, the histopathological changes of renal tissues to toxicants in amphibians were diversified and non-specific to types of chemicals (insecticide, heavy metals, fungicide) or purity (single or mixture) of chemicals. However, the degree of histological damage might depend on concentration, exposure period, and mechanism of each xenobiotic in amphibians.

Alterations of kidney tissues observed in this study revealed the distinct toxicity of potassium chlorate on *H. rugulosus* larvae. Glomerular shrinkage and enlargement of Bowman's space indicated the accumulation of fluid inside the kidney and fluid retention of treated frogs lead to kidney damage and acute renal failure. Congestion indicated the rupture of small vessels in the hepatic tissues and discharge red blood cells into the surrounding tissue. While vacuolization and necrosis in renal tubular epithelium including the epithelial lifting of renal tubules were correlated to the collapse of renal tubular structures and the severe injury entering the point of no return [31, 43].

Histopathological changes in hepatic tissues

In the current study, the obvious histopathological changes in the livers of *H. rugulosus* tadpoles exposure to potassium chlorate were evidenced by leukocyte infiltration, necrosis of hepatocytes, vacuoles in hepatocytes, enlarged sinusoids and congestion.

The inflammatory condition characterized by mononuclear cell infiltration was observed in the liver tissue of *H. rugulosus* in this study. This alteration corresponded with those reported *H. rugulosus* tadpoles induced by methomyl [30], in *Bufotes variabilis* tadpoles exposed to carbaryl [31], *Pelophylax ridibundus* induced by Talstar 10EC insecticide [32], *Xenopus laevis* frogs treated with 500 µg/L atrazine [34], *Hyla intermedia* after chronic exposure to fungicide pyrimethanil [35], *Microhyla fissipes* contaminated by cadmium [36], and *Euphlyctis*

hexadactylus toxicated by a mixture of heavy metals [40], In addition, an inflammatory response typically accompanied necrosis.

Necrosis of hepatocytes was previously mentioned in studies of green toad (*Bufotes variabilis*) exposed to carbaryl [31], *Xenopus laevis* frogs induced by 500 µg/L atrazine, herbicide [34], *Hyla intermedia* after chronic exposure to pyrimethanil [35], *Microhyla fissipes* contaminated by cadmium [36], Nile tilapia (*Oreochromis niloticus*) treated with paraquat [46], and the African catfish *Heterobranchus bidorsalis* exposure to cypermethrin [47], and *Bufo gargarizans* tadpoles exposed to triclosan [48].

Vacuolization in hepatocytes was apparently stated in histopathological examinations of livers in *H. rugulosus* treated with methomyl [30], green toad (*Bufotes variabilis*) exposed to carbaryl [31], *Microhyla fissipes* contaminated by cadmium [36], *Rhinella arenarum* contaminated by cadmium [38], chronic pesticide exposure *Lysapsus limellum* and *Rhinella bergi* sampling from rice field crops [45], Nile tilapia (*Oreochromis niloticus*) treated with paraquat [46], and the African catfish (*Heterobranchus bidorsalis*) exposure to cypermethrin [47].

Hepatic sinusoids dilatation was recognized in marsh frog (*Pelophylax ridibundus*) response to Talstar 10EC insecticide [32], *Hyla intermedia* after chronic exposure to pyrimethanil [35], Italian crested newt (*Triturus carnifex*) exposed to cadmium [37], *Lysapsus limellum,* and *Rhinella bergi* with chronic exposure to pesticides [45].

The sinusoidal congestion was mentioned in histopathological changes of the east Asian bullfrog in this study similar to other aquatic living organisms, amphibians, and fishes. For example, hepatic tissues of *H. rugulosus* tadpoles are induced by methomyl [30], green toad (*Bufotes variabilis*) exposed to carbaryl [31], Italian tree frog (*Hyla intermediao*) juveniles after chronic exposure to pyrimethanil [35], Italian crested newt (*Triturus carnifex*) is induced by cadmium [37], and the African catfish (*Heterobranchus bidorsalis*) treated with cypermethrin [47].

Necrosis is the irreversible cell injury and eventual cell death due to pathological processes. It is an uncontrolled cell death that results in swelling of the cell organelles, plasma membrane rupture, eventual lysis of the cell, and spillage of intracellular contents into the surrounding tissue leading to tissue damage [44]. Some primary histopathological alterations (vacuolization in hepatocytic tissues, hepatic sinusoidal dilatation, and congestion) leading to necrosis of hepatocytes were described in tadpoles of *H. rugulosus* exposure to potassium chlorate in this study and also acknowledged in other previous reports about hepatotoxicity of chemicals to aquatic animals.

Due to the crucial role of the liver in the detoxification of most xenobiotic compounds, it is considered as the main target organ for the assessment of histopathological changes [35]. Hence, we demonstrated that east Asian bullfrog exposure to potassium chlorate resulted in a histopathological alteration of hepatic tissues related to concentrations.

Histopathological evaluations of ovarian tissues

According to results of this study, potassium chloride could induce the ultrastructural alterations of *H. rugulosus* ovarian tissues as characterized by karyorrhexis of oocytes and atretic oocytes in complete metamorphosis. In contrast, no histopathological changes were observed in ovary of pro-metamorphic tadpoles exposure to any concentrations of potassium chlorate.

Gonad has primary functions in sexual development and reproduction of vertebrates [49]. Due to its vulnerability to chemical exposure, gonad is the suitable organ to evaluate consequences of early life-stage of amphibians exposure to endocrine disrupting chemicals [50]. Therefore, a number of studies concentrating in the adverse effects of toxicants on this organ in amphibians were reported. For instance, low concentrations (1, 10, 100 nM) of tetrabromoethylcyclohexane (TBECH) caused the intersexuality in *Pelophylax nigromaculatus* tadpoles with 12%, 13% and 17%, respectively and induced gonadal masculinization of this frog species [51]. Besides, development of gonadal intersex (ovotestes) was noted to increase in all atrazine exposure groups of *H. rugulosu*s [52]. Reduced proportions of oocytes at immature, vitellogenic and mature stages, and the increased proportions of previtellogenic oocytes indicating the inhibition of oocyte development were recognized in adult frogs of *Xenopus tropicalis* exposure to environmental progestin concentrations since the lowest concentration (1.3 ng/L). However, no atretic oocytes were observed [53]. Paraquat exposed Nile tilapia (*Oreochromis niloticus*) tended to have a greater increase of late-vitellogenic and mature percentage of oocytes (mainly at 27˚C), but a lower percentage of primary oocytes, which is partly correlated to an increase in hepatic metabolic activity [46]. However, most of previous documents mainly emphasized in toxic assessment of contaminants to amphibian gonad in terms of morphological and developmental abnormalities.

Not only a few studies of histopathological effects to gonads of aquatic livings exposure to chemicals were examined, but these works also primarily focused in male reproductive structure. Histological alterations in gonads but only testes of *Lysapsus limellum* and *Rhinella bergi* contaminated by sublethal effects of agrochemicals in rice field crops were recorded. The results mentioned the higher proportion of cysts with early spermatognenesis cell stages and different proportions of tubules with free spermatozoa in *L. limellum*, Bidder organ with vacuoles, atretic follicles, and pigmentary cells in *R. bergi* [45]. Abnormalities of sperm in number, morphology, motility, and histology correlated with exposure time and concentration were revealed. In histological changes, vacuoles in spermatogenetic cells, cell dispersion, incomplete cell structures, and deformed nuclei were observed in *Rana nigomaculata in vivo* contaminated by 1 µg/L microcystin-LR (toxin of cyanobacteria) [54].

This study provided evidence based on histopathological responses of *H. rugulosus* ovarian tissues exposed to 1.5 ppm potassium chlorate in complete metamorphosis. Karyorrhexis and atretic oocytes were observed in a complete metamorphosis of the present study correlated to results of the prior experiments following some pollutants induction. The gonadal toxicity of 1.44 ppm methomyl (sublethal concentration) to *H. rugulosus* larvae characterized by vacuolization in various stages of oocytes affected its oocyte development and fertilization [30]. Similarly, cytoplasmic vacuolization of the oocytes at the early stages of development with the highest dose and the increase of atretic oocytes were also mentioned in gonadal tissues of *Rhinella arenarum* in the adult stage with sublethal (0.5 and 5 mg/kg) exposure to cadmium [55]. Abnormal follicles and cytoplasm regression were stated in oocytes of ranid frog (*Rana clamitans*) inhabiting metals polluted water sites [56]. Exposure to pyrimethanil had no effect on sex reversal or abnormal gonadal intersex in *Hyla intermedia* [35]. Despite well-developed ovaries morphologically, severe abnormalities in both oogenesis progression and histological features were observed in pyrimethanil exposed groups as follows: underdevelopment of the meiotic oocytes nests, none or few newly formed diplotene oocytes, delayed gonad differentiation, conspicuous degeneration phenomena involving all germ cell types, large presence of macrophages and apoptotic bodies, enlargement of intercellular spaces and detachment of diplotene oocytes from the enveloping follicular cells, and diffused mononuclear cell infiltration [35].

Mechanism and effects of potassium chlorate to reproductive systems of amphibians have not been documented. Nevertheless, exposure to perchlorate could induce feminization male offspring in the early development of amphibians [57]. Perchlorate (CLO_4^-) and sodium chlorate ($NaCLO_3$) were deeply researched in several experimental animals and disclosed their actions in disrupting thyroid synthesis due

to their competition with iodide (I^-) to be uptaken by the sodium-dependent iodide symporter (NIS) into thyroid follicular cells [58, 59]. Therefore, it could be implied that potassium chlorate might have the similar actions and potential outcomes of perchlorate and sodium chlorate in interrupting thyroid production of amphibians reflecting their metamorphosis and reproduction [60].

In the current study, histopathological changes of *H. rugulosus* were not observed in pro-metamorphosis in any exposure doses, which was correlated to results of atrazine (0.001, 0.01, 0.1, 10, 25, 100 and 1,000 ppb) to gonad development in *H. rugulosus* under laboratory condition [61]. It might be explained with insensitivity of gonadal tissues in this stage of amphibians or underdose of potassium chlorate for histopathological changes in this species.

CONCLUSION

According to the descriptive reports of histopathological changes in the kidney, liver, and ovary of the current study, it may concluded that there was a potential of potassium chlorate to be toxic and had an adverse effect on the east Asian bullfrog (*H. rugulosus*), which could be an important bioindicator of aquatic ecosystems in terms of consumer and biocontrols to other livings. Contamination of potassium chlorate in water resources not only directly affected to amphibian tadpoles but also impacted other aquatic livings and humans. Therefore, the consequences of its contamination should be regarded as urgent issues for environmental safety. Our results also suggested that the use of potassium chlorate at high concentrations may adversely affect the vital organs of *Hoplobatrachus rugulosus* tadpoles and might lead to the decline of amphibian populations environmentally.

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