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INDUCED EFFECT OF PERMAKIL (PYRETHROID) AND SANDAPHOS (ORGANOPHOSPHATE) ON LIVER AND KIDNEY CELLS OF *EUPHLYCTIS CYANOPHLYCTIS*

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ABSTRACT

The present study was done to investigate the effects of pyrethroid and organophosphate on liver and kidney cells of adult *Euphlyctis cyanophlyctis*. During the study, the frogs were treated with two concentrations of both the pesticides. Under the effect of Permakil (pyrethroid) the liver cells of treated frog showed necrosis. The cells were clustered and they were collectively formed. The hepatic parenchyma with focal disruption of lobular architecture and focal necrosis was observed. No further significant calcification was observed. Under the effect of Permakil the histological section of kidney of treated frog showed foci of necrosis. Renal parenchyma with necrosis and tubular atrophy was observed. No significant change in the glomerulus was identified. In the case of Sandaphos (organophosphate) the multinucleated, melanomacrophages were observed in histological section of liver of treated frogs. Malignant neoplasmic cells were also found. Hepatic tissue showed focal disruption of lobular architecture. Focal necrosis was identified. Under the effect of Sandaphos hypertrophy and the ruptured cells were identified in the kidney sections of treated frogs. Atrophy, necrosis and inflammation were observed in nephrons.

Keywords: *Euphlyctis cyanophlyctis*, effects, permakil, sandaphos, liver, kidney.

INTRODUCTION

Amphibians are an important part of aquatic ecosystems, and decline of amphibian population is a major environmental issue (Blaustein and Wake, 1990; Rabb, 1999; Kiesecker *et al.,* 2001; Boone and Bridges, 2003). Since 1980s herpetologists have been researching, and documenting the overall decline in the health and abundance of amphibian population (Rabb, 1999). The most obvious factors contributing to amphibian population declines are habitat destruction and alteration (Alford and Richards, 1999; Khan *et al.,* 2010). A diversity of pesticides and their residues are present in a wide variety of aquatic habitats (Harris *et al.,* 1998; McConnell *et al.,* 1998; Le Noir *et al.,* 1999; Kolpin *et al.,* 2002). However, the effects of these toxic materials remain to be studied on non-target biodiversity in many regions of the world (Khan *et al.,* 2008). A wide array of contaminants may affect amphibian populations which include pesticides, herbicides, fungicides, fertilizers and numerous pollutants (Sparling *et al.,* 2000; Khan and Yasmeen, 2008). The pesticides have the potential to affect many aquatic taxa (Vertucci and Corn, 1996; Houlahan *et al.,* 2000) and the amphibians living in these habitats exhibit physiological signatures of pesticides (Sparling *et al*., 2001) and declining populations are correlated with greater amounts of upwind agriculture where pesticide use is common (Davidson *et al.,* 2001, 2002).

Pesticides can severely affect amphibians in a variety of ways, as destroy the natural biotic balance in agricultural soils and reduce the diversity and abundance of biodiversity with cascading effects at higher trophic levels (Larson *et al.*, 1997). They can kill amphibians directly, effect their behaviour, reduce their growth rates, act as endocrine disrupters or induce immunosuppression (Bishop, 1992; Carey and Bryant, 1995; Alford and Richards, 1999).

The causes of these declines include human involvement in an effort to increase agricultural products and indiscriminate applications of pesticides. The pesticides, organophosphate and carbamate are widely used and have a variety of lethal and sublethal effects on non-target wildlife species (Parsons *et al*., 2000; Khan *et al*., 2003a). The organophosphorus toxicity is believed to be mediated through inhibition of acetylcholinesterase (AChE). Given their widespread distribution in aquatic systems and their ability to undergo chemical transformation, their environmental impacts at sublethal concentrations in non-target organisms have become an important question (Ukpebor *et al*., 2011).

The Pyrethroid appears to effective voltage-dependent neuromuscular sodium channels producing tremors, hyperexcitation and convulsions (Van den Bercken, 1977; Vijverberg *et al*., 1982; Ruigt and Van den Bercken, 1986; Berril *et al*., 1993, 1994; Materna *et al*., 1995). Therefore, pesticides have been reported to have reduced enzyme activity of cholinesterase in frog *Rana tigrina*

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(Khan *et al.*, 2002 a,b; Khan *et al.*, 2003a,b) in skittering frog *Rana cyanophlyctis* (Khan *et al.*, 2003b,c,d ; Khan and Yasmeen, 2005; Khan *et al.,* 2007a; Khan *et al.,* 2008; Ali *et al.,* 2011). Khan *et al. (*2007b) observed the induced effect of chlorpyrifos (organophosphate) on skin of *Euphlyctis cyanophlyctis.* Yasmeen *et al.* (2009) determined the effects of pesticide β-Cypermethrin on skin of adult skittering frog *Euphlyctis cyanophlyctis*. The objective of present study was to investigate the effects of Permakil and Sandaphos on liver and kidney cells of skittering frog *E. cyanophlyctis.*

MATERIALS AND METHODS

The experimental work was carried out on adults skittering frog *E. cyanophlyctis* collected from selected areas of Province of Sindh. Collected frogs were brought in laboratory and kept in glass aquarium in the Wildlife Lab, Department of Zoology, University of Karachi, and frogs were fed with prawns and insects. Two concentrations of both pesticides were applied, i.e. 5 and 10% of Sandaphos whereas 0.05 and 0.1% of Permakil. The pesticide was injected in the sub-cutaneous abdominal region of frog by using insulin syringe. The effects of pesticide were observed after 24 hours of the treatment. To observe the effects of pesticides on treated frogs the liver and kidney tissues were fixed in 10% Formalin. Histological study of liver and kidney cells was carried out by sectioning of fixed tissue by paraffin section technique. The tissue was fixed, dehydrated in graded series of alcohols, cleared in xylene and finally embedded in paraffin wax. The embedding or block making is the orientation of tissue in melted paraffin, which solidifies and provides a firm medium for keeping intact all parts of tissues at the time of the section cutting. A clean dust free slide was inserted into the floated water bath. The sections were put on glass slides. Hematoxylin and Eosin were used for staining the nucleus and the cytoplasm respectively, and mounted in a synthetic resin DPX (Kiernan, 1990).

RESULTS AND DISCUSSION

In this study after 24 hours of the treatment samples from liver and kidney of *E. cyanophlyctis* were examined. It was found that under the effects of Permakil and Sandaphos the necrosis was significantly observed in liver and kidney tissues of treated frogs.

The microscopic studies of liver cells of treated *E. cyanophlyctis* showed complete necrosis under the effect of Permakil low conc. (0.05%). The cells were clustered and collectively formed. No significant calcification was observed (Fig. 1). In the case of Permakil high conc. (0.1%) the focal necrosis was observed in the liver section of treated *E. cyanophlyctis*. No other significant calcification was identified, while the hepatic parenchyma showed focal disruption of the lobular architecture (Fig. 2).

In the case of Permakil low conc. (0.05%) the histological section of kidney of treated *E. cyanophlyctis* show foci of necrosis and calcification at different places (Fig. 3). Under the effect of Permakil high conc. (0.1%) the kidney section of treated *E. cyanophlyctis* showing inflammation in renal parenchyma. Necrosis and tubular atrophy was observed. No significant change in the glomerulus was identified (Fig. 4).

Under the effect of Sandaphos low conc. (5%) the multinucleated, pigment cells were observed in liver section of treated *E. cyanophlyctis.* Malignant neoplasmic cells were also found (Fig. 5). Under the effect of Sandaphos high conc. (10%) the liver section of treated *E. cyanophlyctis* revealed hepatic tissue with necrotic foci and atrophy of lobular architecture (Fig. 6).

In the case of Sandaphos low conc. (5%) the kidney section of treated *E. cyanophlyctis* revealed hypertrophy and atrophic glomeruli are ruptured (Fig.7). Under the effect of Sandaphos high conc. (10%) the kidney section of treated *E. cyanophlyctis* showing atrophy, necrosis and inflammation in renal parenchyma (Fig.8).

In this study, after 24 hours of the treatment of Sandaphos and Permakil the necrosis was significantly observed in liver and kidney tissues of treated frogs as compared to Lab standard skittering frog *E. cyanophlyctis*.

Global declines in amphibian populations have been attributed to a number of anthropogenic activities, including habitat destruction, habitat alteration and introduction of exotic species, exposure to environmental contaminants, climate change, increased acid precipitation and UV flux associated with ozone depletion. Many studies have illustrated that declines in amphibian population have also taken place in relatively pristine habitats such as national parks and reserves, where specific environmental stressors are not readily apparent (DAPTF, 2001; Khan *et al*., 2010).

The enzyme inhibition occurs in a number of species and reduction can result in sub-lethal toxicity and death (Cooper, 1991). Internationally the use of pesticides and fertilizers has been increased. Over two billion pounds of pesticides are sold in USA each year for agriculture, commercial and home (Khan *et al.,* 2007a). Amphibians are known to be vulnerable to pesticides that are cholinesterase inhibitors (Wang and Murphy, 1982). Amphibian declines have been occurring for decades, and much of this decline is attributed to environmental contaminants. In Pakistan pesticides have been reported to have reduced enzyme activity of cholinesterase in frog *Rana tigrina* (Khan *et al.*, 2002 a,b) and in *Rana*

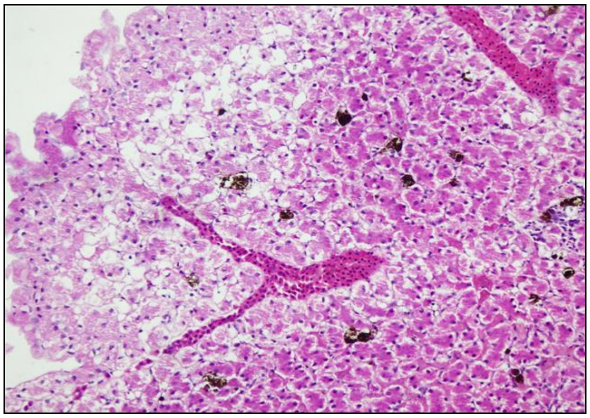

Fig. 1. Liver section of treated *E. cyanophlyctis* showing necrosis. The cells are clustered.

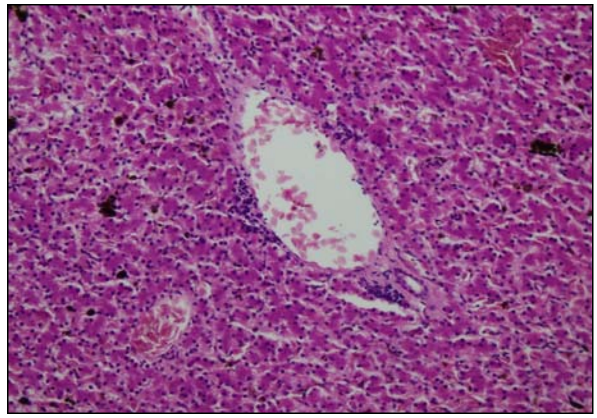

Fig. 2. Liver section of treated *E. cyanophlyctis* showing hepatic parenchyma with focal disruption of lobular architecture and focal necrosis.

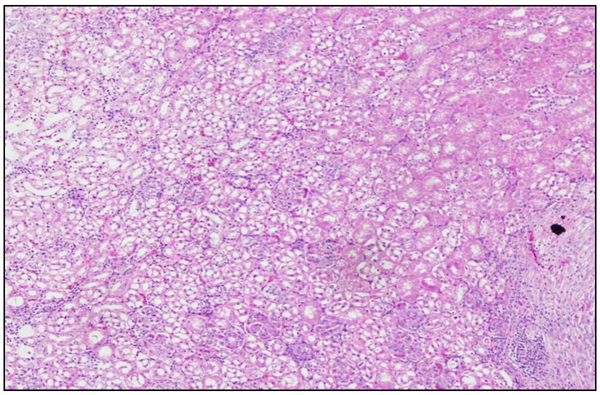

Fig. 3. Kidney section of treated *E. cyanophlyctis* showing foci of necrosis.

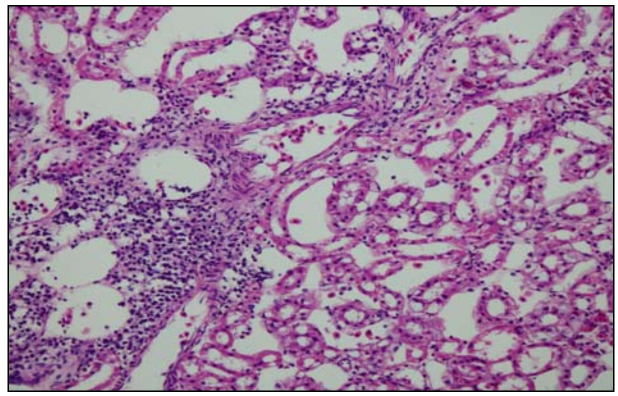

Fig. 4. Kidney section of treated *E. cyanophlyctis* showing inflammation in renal parenchyma. Necrosis and tubular atrophy was observed.

cyanophlyctis (Khan *et al.*, 2003b,c,d; Khan and Yasmeen, 2005; Khan *et al.*, 2006; Khan *et al*., 2007 a; Khan *et al*., 2008; Khan and Yasmeen, 2008).

The liver plays important role in frog by the regulation of blood glucose level, metabolism, bile secretion, breakdown of old red blood cells and detoxification. Whereas the kidneys perform the essential function of removing the waste products from the blood and regulating the water fluid levels. In the present work, the liver and kidney of *E. cyanophlyctis* under the effects of two selected pesticides Permakil and Sandaphos were studied and compared with the lab standards. The necrosis was observed in liver and kidney cells of treated frogs under the effect of both the pesticide groups (Figs.1-8) as compared to lab standard *E. cyanophlyctis* which shows normal structure of liver and kidney cells.

Yasmeen *et al*. (2009) investigated the effects of pesticide β-Cypermethrin on skin of *E. cyanophlyctis*, and effects were observed after 90 days of the treatment. Under the effects of β-Cypermethrin sections revealed that skin covered with fibrocollagenous tissue and skeletal muscle tissue. Mild chronic inflammatory cells were identified in the fibrocollagenous tissue with focal area of necrosis. Histopathological conclusion indicated no strong treatment-related findings. In present work the effect of Permakil on the liver and kidney cells of treated *E. cyanophlyctis* were observed after 24 hours of the treatment showing the focal necrosis in liver and kidney cells. Therefore, present results are in agreement with the earlier reports.

The anticholinesterase pesticides function by binding with this enzyme in animals and disrupting nervous system activity, usually causing death by respiratory failure. Decreased cholinesterase activity can indicate exposure to some commonly used pesticides and can be harmful to wild animals (Catherine and Gloria, 2000).

In the present work, under the effect of Sandaphos, the multinucleated, pigment cells were observed in histological section of liver of treated *E. cyanophlyctis.* Malignant neoplasmic cells were found. Hepatic tissue showed necrotic foci and atrophy of lobular architecture, while the histological sections of kidney of treated *E.*

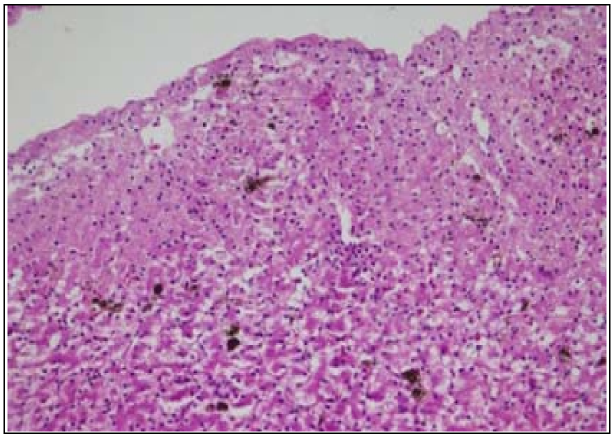

Fig. 5. Liver section of treated *E. cyanophlyctis* showing pigment cells. Multinucleated cells are also present.

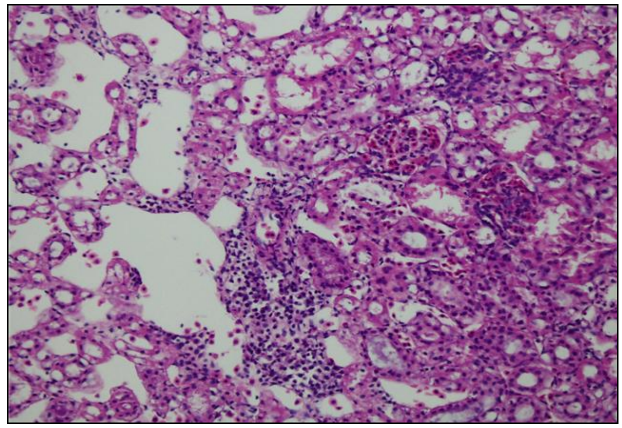

Fig. 6. Liver section of treated *E. cyanophlyctis* showing necrotic foci and atrophy of lobular architecture.

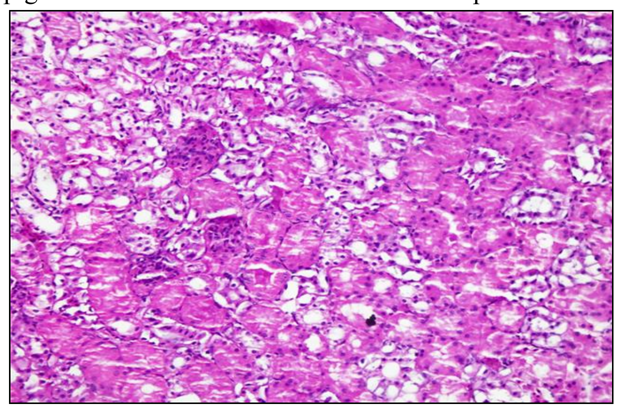

Fig. 7. Kidney section of treated *E. cyanophlyctis* showing hypertrophy and atrophic glomeruli are ruptured.

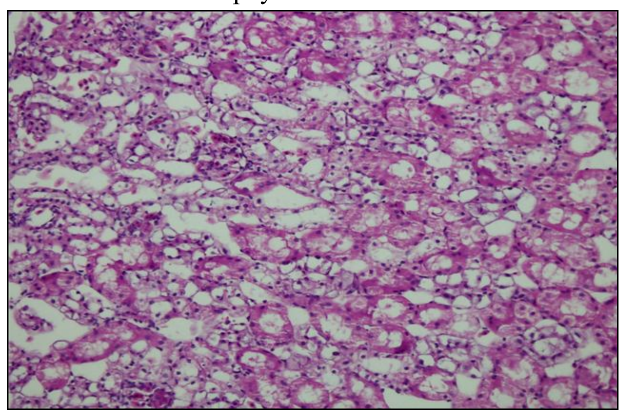

Fig. 8. Kidney section of treated *E. cyanophlyctis* showir atrophy, necrosis and inflammation in renal parenchyma.

cyanophlyctis revealed hypertrophy and atrophic glomeruli were ruptured. However, atrophy, necrosis and inflammation were noted in nephrons.

The reports regarding amphibians' mortality in a large numbers due to infectious disease raise the critical question concerning why are amphibian immune systems not successful in combating these pathogens? While amphibian immune defenses involve both innate and adaptive components that together lack only a few elements of mammalian immune systems (Carey *et al*., 1999; Du Pasquier *et al*., 1989) as some information exists on the influence of environmental factors on the effectiveness of these defenses (Carey *et al*., 1999**),** this phenomenon could be interpreted as a result of abiotic factors that could be a pesticide as well. However, the interaction of amphibian immune systems with viruses and the defenses against fungal skin infections have not been examined in detail (Khan *et al*., 2007b).

Before consideration of a provision of pesticide's contribution among abiotic factor for the failure of immune system, it is noteworthy that components of the innate immune system, such as macrophages, neutrophils, and antimicrobial peptides, provide the primary protection against fungal skin infections (Carey *et al*., 1999). The histological examinations of the epidermis of amphibians suffering from fungal skin infections reveal relatively few immune responses. Fungal penetrations into the outer layer of epidermis caused little or no inflammation of the epidermis. A few neutrophils, lymphocytes, and macrophages have been reported in infected skin (Pessier *et al*., 1999; Taylor *et al*., 1999). This sort of inflammation has been described to be caused by cytokines produced by the macrophages and neutrophils in response to a foreign invader (Janeway, 1996). Therefore, inflammation is an important component of immune defense because it results in increased permeability of blood vessels. Since the pesticide could exert effects on blood properties membrane permeability and other physiological parameters (Khan *et al.*, 2006), thus it is consiveable, though it has not been proved that pesticide could affect on blood vessels permeability. Under such circumstances however, the role of pesticide still has not been established with the change in permeability fosters, the release of soluble mediators, such as immunoglobulins and complement, and also assists in recruitment of circulating leucocytes to the site of infection, while the lack of an inflammatory response could result from a number of factors. Nevertheless, some fungi could produce compounds that inhibit the inflammatory response, whereas macrophages and neutrophils may not recognize these fungi as pathogens, and consequently these fungi cause insufficient tissue necrosis to stimulate the inflammatory response or other factors. This type of relatively low involvement of macrophages suggests that formation of memory T and B cells and antibodies would be limited (Khan *et al*., 2007b).

The skin plays key roles in the survival of amphibians and their ability to exploit a wide range of habitats and ecological conditions (Clarke, 1997). During a study Khan *et al*. (2007b) observed the effects of pesticide Chlorpyrifos (Organophosphate) on skin of adult skittering frogs after 90 days of the treatment. The sections examined revealed skin covered with fibrocollagenous tissue exhibit areas of necrosis and aggregate of mild chronic inflammation. The pigment containing basal keratinocytes were identified in epidermis. Variable size measured fibers with degenerated and regenerative changes were also noted. Chronic granulomatous inflammation was not identified. Inflammatory process in the subcutaneous lymph sac was observed. Some necrosis or mineralization along with multinucleated giant cell in superficial dermis was observed. However, in this respect inflammatory processes in the lymph sacs might be appeared as a result of other factors, therefore, it could not be a sole consequence of the pesticide affect, hence, it remained for confirmations. In present study the effect of Sandaphos (organophosphate) on the liver and kidney cells of treated *E. cyanophlyctis* were observed after 24 hours of the treatment showing the necrosis in liver and kidney cells. So, the present results are in agreement with the earlier reports.

Fenoglio *et al*. (2005) examined the liver of *Rana esculenta* adult frogs collected from two rice fields, one was heavily polluted and the other was relatively unpolluted. The possible changes observed were induced by contamination in the metabolic processes which depend on the function of the liver. The production of reactive oxygen species (ROS) were also evaluated through histochemical techniques. In the polluted samples, hepatocytes showed variations in the activity of G_6PDH, AlkPase, and SDH and a moderate to intense ROS expression. Prominent changes were observed in Kupffer cells (KCs) and melanomacrophages, both showed intense reactivity for AcPase and catalase and variations in melanin content and distribution. Results thus indicated a general adaptive response of liver parenchyma to environmental pollution. In present study under the effects of organophosphate and pyrethroid the histological sections of liver of treated *E. cyanophlyctis* shows the inflammation and necrosis in the liver parenchyma. In general the present results are in agreement with the earlier reports.

CONCLUSION

The selected pesticides Permakil and Sandaphos may cause the premature death of cells and living tissue in the liver and kidney of *E. cyanophlyctis.* The cells that die due to necrosis do not usually send the chemical signals to the immune system. The sudden failure of one part of the cell triggers a cascade of events. In addition to the lack of chemical signals to the immune system, cells undergoing necrosis can release harmful chemicals into the surrounding tissue. On the basis of present findings it is concluded that Permakil and Sandaphos both are harmful for amphibians.

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