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Short Communication

A STUDY ON THE INDUCED EFFECT OF β-CYPERMETHRIN ON SKIN OF *EUPHLYCTIS CYANOPHLYCTIS*

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INTRODUCTION

The pollution of lakes and rivers with chemicals of anthropogenic origin may have adverse consequences the waters become unsuitable for drinking, irrigation, fish cultivation, household purposes, and the animal communities living in them may suffer seriously (Clickman and Lech, 1982). The amphibians are important part due to their value as indicators of environmental stress (Blaustein, 1994; Blaustein and Wake, 1995). They are in close contact with water as larvae and mostly have some contact with land as adults. Therefore, they experience both aquatic and terrestrial stressors. They have moist, permeable skin and unshelled eggs that are directly exposed to soil, water and sunlight, more sensitive to environmental toxins or to changes in patterns of temperature or rainfall than are other terrestrial vertebrate groups (Blaustein and Wake, 1990; Vitt, *et al*., 1990). The most obvious factors contributing to amphibian population decline are habitat destruction and alteration (Alford and Richards, 1999). A variety of pathogens affect wild amphibian populations. These include viruses, bacteria, parasites, protozoans, oomycetes, and fungi (Blaustein *et al.,* 1994; Jancovich *et al.,* 1997; Kiesecker and Blaustein, 1997; Longcore *et al*., 1999; Johnson *et al*., 2002). These pathogens can be the proximate causes of mortality or they can cause sublethal damage such as severe developmental and physiological deformities. Pathogens may infect amphibians at various life stages. There are some observations of pathogens causing massive die-offs of amphibians. Of particular concern to a number of investigators is whether the diseases of amphibians are novel or if they are being triggered by an environmental change. A wide array of contaminants may affect amphibian populations which include pesticides, herbicides, fungicides, fertilizers and numerous pollutants (Sparling *et al.,* 2000). 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).

Many pyrethroids have emerged as an important class of agricultural pesticides due to their high bio-efficacy and relatively low toxicity in comparison to several organochlorine (OC) and organophosphorous (OP) pesticides (Casida *et al*., 1983) and are used in households, cereals, vegetable, cotton, tobacco, and other crops (Clickman and Lech, 1982; Smith and Stratton, 1986). During last 50 years, the application of pesticides and chemical fertilizers has increased rapidly in Pakistan, and the volume of pesticides has been used from 665 tons in 1980 to 70,000 tons in year 2002 (Iftikhar, 2003). Several studies already have been reported that some pesticides reduced cholinesterase (ChE) in frog *Rana tigrina* (Khan *et al*., 2002[a,b] and 2003[a]) and in *Rana cyanophlyctis* (Khan *et al*., 2003[b,c,d] ; Khan and Yasmeen, 2005; Khan *et al*., 2006; Khan *et al*., 2007[a]). There is some indication that field application of these pesticides may be deleterious to amphibians (Jolly *et al*., 1978; Thybaud, 1990; Berril *et al*., 1993; Materna *et al*., 1995). Pyrethroids appear to affect voltage-dependent neuromuscular sodium channels producing tremors, hyperexcitation and convulsions (Van den Bercken, 1977; Vijverberg *et al.,* 1982; Ruigt and Van den Bercken, 1986).

The histology of the normal amphibian skin has been described by Patt and Patt (1969) and Green (1999). The present study was aimed to determine the induced effects of pesticide β-Cypermethrin (pyrethroid) on skin of *E. cyanophlyctis*.

MATERIALS AND METHODS

The experimental work was carried out on adults of skittering frog *E. cyanophlyctis,* collected from selected areas of Sindh Province. Collected frogs were brought in laboratory and kept in glass aquarium in Wildlife and Fisheries Lab, Department of Zoology, University of Karachi. Frogs were fed with prawns and insects. For the treatment of β-Cypermethrin, 0.08ml was injected in the sub-cutaneous abdominal region of frog by using insulin syringe. Weight of each frog was measured before and after treatment. The effects of pesticide were observed on

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the skin during 90 days after the treatment. Histological study of skin 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. Hematoxylin & Eosin (HE) and periodic acid-schiff stain (PAS) were used for staining. Mounted in a synthetic resin DPX (Kiernan, 1990).

RESULTS AND DISCUSSION

The amphibian's skin plays important role in survival and their ability to exploit a wide range of habitats and ecological conditions (Clarke, 1997). Frog does not drink like higher animals but absorbs the required water through its skin. In the present study, after 90 days of post treatment of β-Cypermethrin, the induced effects were observed on the skin of *E. cyanophlyctis*, samples were examined, and it was found that the skin was covered with fibrocollagenous tissue and skeletal muscle tissue. Mild chronic inflammatory cells were also identified in the fibrocollagenous tissue with focal areas of necrosis.

The microscopic studies of skin cells of treated *E. cyanophlyctis* showed complete necrosis of the skin, with loss of the glands and even necrosis of the underlying cutis or possibly musculature. The subcutaneous lymph sac has also disappeared (see Fig.1). The skin section shows distinctly accumulation of inflammatory material in the subcutaneous lymph sac (see Fig. 2), this most probably is a bacterial infection. The Hematoxylin and eosin (HE) stained slide (see Fig. 3) elucidates the same elements as the periodic acid-schiff (PAS) stained slide (see Fig. 4) which shows the details of figure 3 (near the upper right corner). This again explains the presence of cellular exudates in the subcutaneous lymph sac. The PAS stained slide (see Fig. 4) exhibits the normal epidermis, normal cutis and glands. The open space lined with a very delicate endothelium (slender nucleus at the epidermal side) is a lymph space. The dark violet irregular strands are normal constituent of the frog skin, and this layer is related to water management. In the right lower corner, the dense connective tissue of normal sub-cutis is visible, and no any alterations were recognized.

Little is known about the diseases of wild amphibians. Many disease agents are present in healthy animals and disease occurs when immune system is compromised (Crawshaw, 1992; Alford and Richards, 1999). The observations that amphibians are dying in large numbers due to infectious disease raise the critical question concerning why amphibian immune systems are not successful in combating these pathogens? 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) some information exists on the influence of environmental factors on the effectiveness of these defenses (Carey *et al.,* 1999**)**, this phenomenon could be due to 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.

The 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). 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, besides few neutrophils, lymphocytes and macrophages were observed 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 macrophages and neutrophils in response to a foreign invader (Janeway and Travers, 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.*, 2002[a,b]; Khan and Yasmeen, 2005 and 2008; Khan *et al.*, 2006, 2007[a] and 2008), thus it is conceivable, though it has not been proved that pesticide could affect on blood vessels permeability.

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. However, the 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 in mycotic epidermal infections suggests that formation of memory T and B cells and antibodies would be limited. Few amphibians appear to survive chytrid infections (Berger *et al.,* 1999), but if some do, they would likely face a repeat attack with little improvement in their resistance. Another study Khan *et al.* (2007[b]) observed the induced effect of pesticide Chlorpyrifos (organophosphate) on skin of *E. cyanophlyctis*, and reported that skin covered with fibrocollagenous tissue exhibiting areas of necrosis and aggregates of mild chronic inflammation.

In the present study, some sections examined revealed skin is covered with fibrocollagenous tissue and skeletal muscle tissue. Mild chronic inflammatory cells were identified in the fibrocollagenous tissue with focal area of necrosis. In a case complete necrosis of the skin, with loss of the glands and even necrosis of the underlying cutis or possibly musculature was recorded, where it seems subcutaneous lymph sac has disappeared. However, it could be that the mass of cellular exudates in the subcutaneous lymph sac. Though, in this respect inflammatory material might have appeared as a result of other factors, therefore, it could not be a sole consequence of pesticide effect, hence, it remained for confirmations. The purpose of the study was to investigate the effect of β-Cypermethrin (pyrethroid) on skittering frog *E. cyanophlyctis* though histopathology conclusion indicated no strong treatment-related findings. Further study is necessary to evaluate the relative susceptibility of adult frog to key pesticides in order to utilize these species as sentinels of pesticide toxicity.

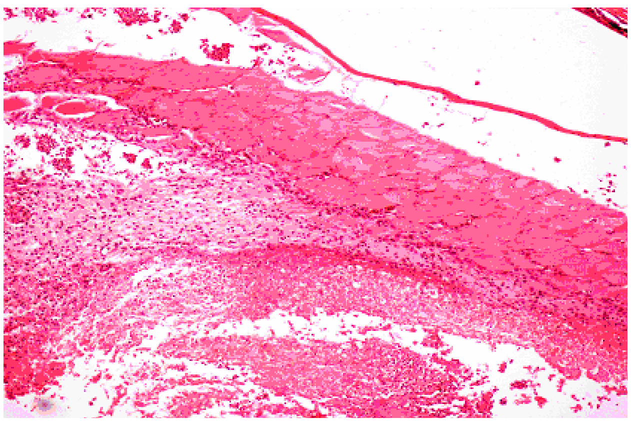

Fig. 1. Skin section of treated *E. cyanophlyctis* showing complete necrosis of the skin.

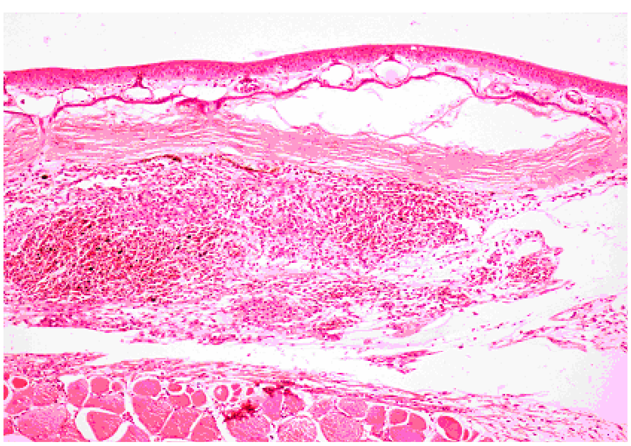

Fig. 2. Skin section of treated *E. cyanophlyctis* showing accumulation of inflammatory material in the subcutaneous lymph sac.

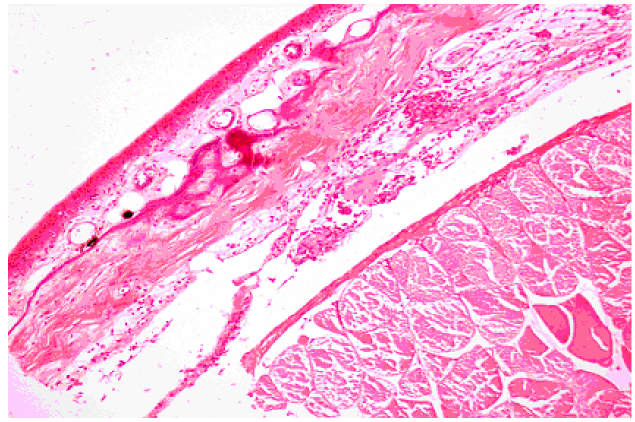

Fig. 3. Skin section of treated *E. cyanophlyctis* showing the presence of cellular exudates in the subcutaneous lymph sac.

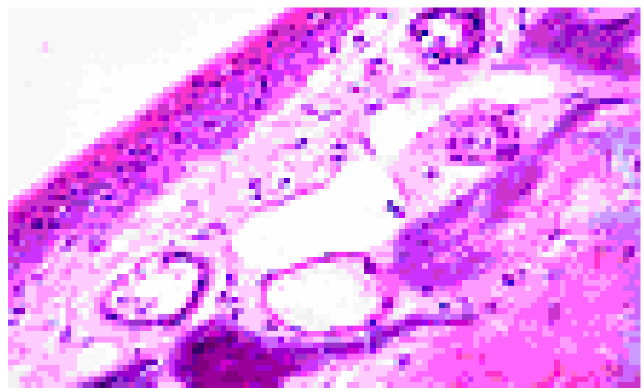

Fig. 4. Skin section of treated *E. cyanophlyctis* showing the normal epidermis, glands and dense connective tissue of normal sub-cutis.

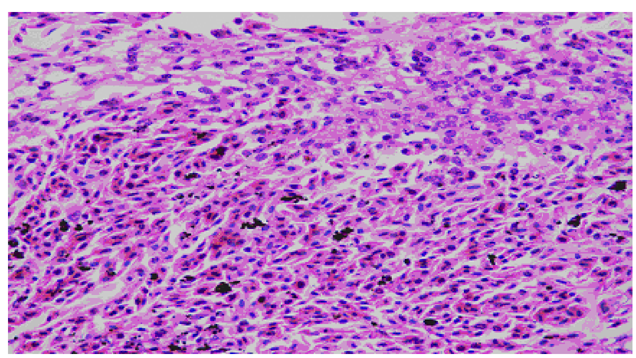

Fig. 5. Skin section of lab standard of *E. cyanophlyctis* showing normal structure of skin cells.

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