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Impact of malathion stress on lipid metabolism in *Limnonectus limnocharis*

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Abstract

Despite mounting concerns about amphibian population declines, information on impact of pesticides on physiological changes is meager. The present study deals the influence of an organophosphate pesticide—malathion on the lipid metabolism of *Limnonectus limnocharis* under laboratory conditions. Changes in the lipid metabolism were analyzed in the liver, muscle, ovary, and testis of frogs exposed to lethal (10.67 mg L^{-1} for 1, 2, 3, and 4 days) and sub-lethal (2.13 mg L^{-1} for 1, 5, 10, 15, 20, and 25 days) concentrations of malathion. Upon lethal concentration treatment, against the increase of fatty acids, glycerol, and lipase activities in all tested tissues, there was decrease in the total lipids content over different durations. On the other hand, exposure to sub-lethal concentration, the amount of total lipids content, free fatty acids, glycerol and lipase activity increased. Changes in the lipid metabolism due to lethal concentration of malathion exposure could depict the negative impact on the reproductive success, which would result in decline of amphibian population.

Keywords: *Limnonectus limnocharis*; Lipid metabolism; Malathion; Pesticide

1. Introduction

A number of hypotheses have been proposed as underlying causes of the worldwide decline of amphibian populations, including habitat alteration and destruction, predation, competition from exotic non-indigenous species, parasites, disease, ultraviolet radiation, climate change, and environmental contamination [1–6]. Generally, each of these factors are harmful to amphibians and some of these stressors may be solely responsible for injury, while, it is more likely that several factors act simultaneously in the amphibian habitats and induces injury through synergistic interactions [7]. Many amphibians breed and develop through their early life-stages in shallow water. Most of amphibian habitats are vulnerable to chemical contamination, pesticide application, surface runoff from industrial and agricultural and other non-point sources. Amphibian habitats adjacent to agricultural areas would be subjected to agricultural chemicals through runoff, chemical drift and direct application. Such contamination can result in immediate acute responses in amphibians and other organisms, but more likely, injury will occur slowly upon the sub-lethal effects manifested over time. The sub-lethal effects of pesticide residues on adult anuran may be catastrophic to a population given the limited active season in which anurans must emerge, successfully breed, and consume sufficient food to withstand the months of hibernation [8]. Similarly, delayed development and metamorphosis could prove fatal for anuran larvae [9], which must transform into juveniles before the end of the summer season, or before ephemeral pools dry up [10].

Even though, pesticides are important in controlling domestic, agriculture, and industrial pests, including vector organisms, these adversely affect the non-target organisms and are widely used in agriculture eco-system, wherein the amphibians, the non-target species, are major components

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of the wetland biota. Thus the frogs living in agriculture eco-system and in wetlands are exposed to these pesticides [11]. An organophosphate pesticide malathion (diethyl[dimethoxy phosphino thioyl]butanediotae) is one of the five most commonly used pesticides that accounts for 65% of all organophosphate pesticide application in the field [12]. Residue of malathion was frequently detected in the range from 2.62 to 129 $\mu g\,Kg^{-1}$ soil and in water 0.698–298 $\mu g\,L^{-1}$ in the study region [13–15].

Lipids are the integral component of biological systems, which are generally insoluble in aqueous media but soluble in organic solvents. They are synthesized de novo and also supplied from the diet and they are the actual or potential derivatives of fatty acids. Lipids have varied functions; provide high levels of energy as compared to carbohydrates and proteins. They are essential for maintaining good health and facilitate the absorption of fat-soluble vitamins. Lipids are also used for vitellogenic growth of oocyte by the ovary of frog [16]. Fatty acids play a vital role in membrane architecture by maintaining appropriate fluidity of biological membrane [17]. The frog uses the liver, muscle, ovary, and testis for lipid storage. The utilization of the energy released by breakdown of lipids in liver and muscle is most evident in hibernation and that of ovary and testis in reproductive process [18–21].

The information available on the effects of pesticides to amphibians, at biochemical level, particularly on lipid metabolism, is scanty [22,23]. Hence, the present investigation is taken up to analyze the effect of lethal and sub-lethal concentrations of malathion on the fat reserves of the liver, muscle, ovary, and testis of the anuran *Limnonectus limnocharis*, which inhabits the paddy fields of Western Ghats.

2. Materials and methods

2.1. Animals

Frogs of *L. limnocharis* collected from the paddy fields of the Western Ghats were the experimental animals. They were fed with insects every day and were acclimatized to the laboratory conditions for 10 days prior to experimentation. Water was changed daily. After complete acclimation only healthy and active frogs weighing 1.5 ± 0.1 g were selected for exposure to pesticide.

2.2. Chemical

The pesticide used in this experiment was 50% active ingredient malathion—diethyl[dimethoxy phosphino thioyl]butanediotae (Registration No. VI, 711 (7) malathion 601, Batch No. 41 Agro-chemical Industries, Bangalore).

2.3. Treatment

The frogs were exposed to nine different concentrations of malathion (active ingredient), starting from 0 (control) to 40 $mg\,L^{-1}$ with an interval of 5 $mg\,L^{-1}$ for a period of 96 h. Mortality was recorded for every 24 h, and numbers of dead were converted into percent mortality. Later, the 50% of lethal concentration (LC_{50}) was determined using the Spearman Karbar Method Version 5.0 [24] statistical computer program provided by the Ecological Exposure Research Division (EERD) of US Environmental Protection Agency. The 96 h LC_{50} of malathion is 10.67 $mg\,L^{-1}$ [25]. One-fifth of the LC_{50} value (2.13 $mg\,L^{-1}$) of malathion was used for sub-lethal treatment in the present study. The frogs were categorized into three groups. Group-I served as control, group-II was exposed to malathion 10.67 $mg\,L^{-1}$ lethal concentration for four days, and the group-III was exposed to 2.13 $mg\,L^{-1}$ sub-lethal concentration for 25 days. Both treated and untreated frogs were sacrificed at an interval of 1, 2, 3, and 4th day in lethal and 1, 5, 10, 15, 20, and 25th day in sub-lethal concentration. Liver, muscle, ovary, and testis of both treated and control frogs were isolated transferred to cold amphibian ringer solution for the estimation of the lipid metabolism.

2.4. Estimation of lipid metabolites

Total lipids were estimated by the gravimetric method as described by Folch et al. [26]. Free fatty acids were extracted from lipids by using Dolls extraction mixture and estimated by employing the method of Dumcabe [27]. Using 5% of tissue homogenate prepared in tri-carboxylic acid, the glycerol in the tissues was estimated using the method of Fletcher [28]. Whereas, lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) activity was estimated using the procedure of Colowick and Kaplan [29]. The enzyme activity was expressed as units/g wet wt of tissue.

2.5. Statistical analysis

The data between treatment and control were analyzed by S tudent '*t*' test using SPSS programme (ver. 10.5) to derive the significance. The values were expressed as means ± SE. The values obtained were expressed as percent change (+ or −) over the control.

3. Results

3.1. Total lipids

The results of the effect of lethal and sub-lethal concentrations of malathion on total lipids in *L. limnocharis* are summarized in Table 1. Even though there was reduction in total lipids content from 1st day of exposure, the highest reduction was observed in testis (39.22%) on the exposure to 4 days lethal concentration. The reduction in all the tissues analyzed for all the days of exposure was significant compared to control ($p < 0.05$). Contrary to this, there was gradual increase of total lipids content from 1st day of exposure to 25th in the 4 tissues studied. However, significant increase was observed on 15th, 20th, and 25th day of exposure in liver (including 10th day), muscle and ovary and only on 25th day of exposure in case of testis ($p < 0.05$).