TOXICITY STUDIES OF AGROCHEMICALS TO ADULTS OF FROG RANA TIGRINA USING PHARMACOLOGICAL BIOASSAYS

A. R. CHOPADE, N. S. NAIKWADE, A. Y. NALAWADE, V. B. SHINDE AND K. B. BURADE*

Appasaheb Birnale College of Pharmacy, Sangli 416 416, M.S., India
* Govt. College of Pharmacy, Karad, Dist. Satara 415 124, M.S., India

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ABSTRACT

The wide scale use of agrochemicals and their hazardous impact on biotic components of aquatic and terrestrial ecosystems has been the subject of debate since last two years. Recently many workers have reported toxicity of metals and agrochemicals to frog. Present study was aimed to determine the acute toxicity of different groups of pesticides to the adults of Indian bullfrog Rana tigrina. Isolated rectus abdominus preparation was used for the evaluation of toxicity to the skeletal tissues. While cardiac toxicity was determined by using isolated heart perfusion technique. Acute toxicity of three agrochemicals, one herbicide Paraquat and two fungicides Indofil and Fytolan was estimated to the adults of frog Rana tigrina. Among these bipyridium herbicide was found to be highly toxic followed by carbamate fungicide indofil and copper based fungicide fytolan.

INTRODUCTION

The wide scale use of agrochemicals and their hazardous impact on biotic components of aquatic and terrestrial ecosystems has been the subject of debate since last two years. Broad spectrum of agrochemicals is applied to the farmland to increase crop production. These include insecticides, herbicides,
fungicides, rodenticides and fertilizers. The surface runoff from these agricultural fields contains pesticide residues. An amphibian larval stage qualifies the criteria for being used as an indicator of harmful levels of pollutants in bioassay tests (Cook 1981). Recently many workers have reported toxicity of metals and agrochemicals using frog tadpoles as test organism in bioassay tests. Studies have been conducted with heavy metals (Khangarot et al 1978) pesticides (Alfredo 1992) (Alvarez 1995) and herbicides (Dial & Baur, 1984) (Norman et al. 1987).

Present study was aimed to determine the acute toxicity of different groups of pesticides to the adults of Indian bullfrog *Rana tigrina*.

**MATERIALS AND METHODS**

Adults of frog’s, bred in the frogery at the G. C. O. P. Karad, were used as the animal models for the evaluation of the pharmacological effect of the pesticides.

The toxicants used in the current study were, Paraquat dichloride (Bipyridinium herbicide) 24% SL United phosphorus ltd. Vapi, Gujrat. Indofel (Carbamate fungicide) mancozeb 75% w/p of Indofel chemical company, Mumbai and Fytolan (Copper containing fungicide) of Standard pesticide Pvt. Ltd. was procured from the market. Acetylcholine chloride (100 µ/mL) was used as a standard. Frog ringer solution [NaCl-6.5g, KCl -0.14g, CaCl₂-0.12G, NaHCO₃-0.4g, Glucose - 1.5g and Distilled water up to 1000mL].

**Fig. 1** Log dose response curves of Agrochemicals on rectus abdominus muscle of *Rana tigrina*.

**Fig. 2** Log dose response curve of Agrochemicals on isolated heart of *Rana tigrina*.

**Table 1** Statistical evaluation of responses of agrochemicals on isolated rectus muscle and heart of *Rana tigrina*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Responses on Rectus abdominus muscle</th>
<th>Responses on Isolated heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>34.667 ± 0.8819</td>
<td>34.667 ± 0.8819</td>
</tr>
<tr>
<td>Indofel</td>
<td>25.667 ± 0.8819</td>
<td>25.667 ± 0.8819</td>
</tr>
<tr>
<td>Paraquat</td>
<td>19.667 ± 0.8819</td>
<td>19.667 ± 0.8819</td>
</tr>
<tr>
<td>Fytolan</td>
<td>13.667 ± 0.8819</td>
<td>13.667 ± 0.8819</td>
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</tbody>
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**Part A. Isolated rectus abdominus muscle preparation technique.**

Rectus abdominus muscle of frog was dissected out and suspended in 5ml bath containing frog ringer solution. The bath was continuously bubbled with air. After proper relaxation and confirmation of the stability of tissue, the responses were recorded under the tension of 0.5g. Each response was recorded for 90 seconds, maintaining the cycle of 5 minutes. The effects of various pesticides were studied in different set of experiments. (Kela et al. 1995)

**Part B. Isolated heart perfusion technique**

The frog was pithed and pined to the frog board and was given a midline incision on the abdomen and pectoral girdle was removed and heart was exposed. Pericardium was removed and the heart was canulated with the help of a venous canula. The tip of the ventricle was hooked to a pin with the help of fine thread and tied it to the free limb of the universal lever, which was fixed to a stand. Proper tension and magnification was adjusted by altering the height of lever and the contraction of the heart was recorded. (Kulkarni 2004)

The effects of skeletal and cardiac muscle were studied at various dose levels increasing in a geometric fashion and observations were taken before and after administration of the test pesticides.

**Statistical analysis**

Means ± SEM (standard error of mean) of values obtained in the assays were compared for the statistically significant differences using Students Neumans – Keuls test with standard parametric method for Guassian distribution method.
RESULTS AND DISCUSSION

The comparative profile of acute toxicity study of all three agrochemicals is summarized in Table 1. The log toxicity dose response curves are depicted in Figure 1 and 2. The bioassays revealed there was an increased toxicity with increasing concentrations of the test chemicals. Among the three pesticides used, paraquat was found to be the most toxic to the adults of frog Rana tigrina followed by Indofil and Fytolan. The pesticides showed negative chronotropic and negative ionotropic effects.

Paraquat (1, 1’-dimethyl-4, 4’- bipyridinium dichloride) is widely used in agriculture to control weeds and has been reported to affect the development of amphibians (Dial & Baur 1984) (Tomar & Pandey 1988) and is highly toxic to aquatic species (Kam & Furtado 1977) (Tortorelli et al. 1990).

The acute toxicity of organophosphate carbamate fungicide, Indofil has also been reported in the current study cohort. The toxic contractile response showed by these agrochemicals supports the results of (Alvarez et al. 1995) which focus on the skeletal malformations induced by the insecticides.

The study demonstrates that acute susceptibility of adults of frog R. tigrina to these agrochemicals, which were previously reported to affect the embryonic stage (Dutta et al. 1978) and the growth of tadpoles (Khangarot 1985 & Alfredo 1992).

REFERENCES


Cook, A.S. 1981. Tadpoles as indicators of harmful levels of pollution in the field. Environmental Pollution. 25 : 123-133.


