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Research Article

NUCLEIC ACID CHANGES IN *HOPLOBATRACHUS TIGERINUS* FROGS INDUCED BY SUBLETHAL IMIDACLOPRID EXPOSURE DURING THE LARVAL STAGE

Padmaja B[1]*., Ch Vivek[2]., K Usha Rani[1] and K Veeraiah[2]

[1]Department of Zoology, D.N.R College, Bhimavaram, West Godavari, A.P, India
[2]Department of Zoology, Acharya Nagarjuna University, Guntur, A.P, India

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ABSTRACT

The widespread use of neonicotinoids has led to harmful effects on the environment and human health. Wide ranging application of manufactured pesticides in agriculture has resulted in contamination of soil ecosystems. Frogs are universal in aquatic habitats such as marshes, boundaries of water bodies and paddy fields. High occurrence of deformed frogs as well as decrease in body length, head length, limb size, growth rate and increase in liver weight are known from different frog species occurring in pesticide contaminated sites. The current study exemplifies that these sub-lethal yet environmentally considerable concentrations of imidacloprid has toxicological implications on *Hoplobatrachus tigerinus*. The validation of the study was to assess nucleic acids analysis in vital organs of *Hoplobatrachus tigerinus* exposed to Imidacloprid, after 24, 48, 96 h, 8, 15 and 30 days of exposure. The results obviously illustrate that exposure to sub-lethal concentration of imidacloprid $1/10^{th}$ of 96 h LD_{50} was returned in tissue concentrations of pesticide with significant alterations in the nucleic acids. In the present study, the DNA and RNA contents decreased in all the tissues in response to Imidacloprid sub-lethal exposure at time periods of 24, 48, 96 h, 8, 15 and 30 days. Decrease in nucleic acids suggests the decrease in protein synthesis and damage to the liver is the major metabolic organ of drug detoxification. Hence, it is the accumulation of alteration in the DNA due to pesticides in the environment that seems to be a causative agent. We put forward detailed studies on food availability and consumption by frog in the paddy fields and urban wetlands to understand factors influencing the metabolic and growth patterns.

INTRODUCTION

In order to increase food production, India has adopted new agriculture practices and achieved remarkable success. Pesticide exploit in agriculture can cause undesirable effects on the natural environment and humans (Moza *et al.,* 1998). One of the objectives of integrated agriculture is the removal or diminution of possible sources of environmental pollution such as pesticides. Amphibians use wetlands in urban and agricultural landscapes for breeding, growth and development (Malvin *et al.,* 2018). To achieve this objective, farmers need a method to support them in estimating the environmental impact of pesticide use. The present study predictable to help fill the gap on the shortage of information concerning the Imidacloprid health hazards to frogs. Imidacloprid, 1-(6-chloronicotinyl)-2-nitroiminoimidazolidine belonging to the class of chloronicotinoids is a new insecticide with high activity against sucking pests, including ricehoppers, aphids, thrips and white flies. Imidacloprid is chemically related to the tobacco toxin "nicotine". Like nicotine, it acts on the nervous system (Caroline Cox, loc. cit.). The chemical works by interfering with the transmission of stimulus in the insect nervous system causing blockage in a type of neuronal pathway. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventually death. It is effective on contact and via stomach action.

The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in the industry, urbanization or in agriculture enters in to the bodies of marine and other aquatic organism (Islam and Tanaka, 2004). These pollutants build up in the food chain and are responsible for adverse effects and death in aquatic organism. Pesticides and related chemicals destroy the delicate balance between species that characterizes a functioning ecosystem. Pesticides produce many physiological and biochemical changes in fresh water organisms by

Corresponding author:* **Padmaja B
Department of Zoology, D.N.R College, Bhimavaram, West Godavari, A.P, India

influencing the activities of several enzymes. These tests measure the direct effects of chemicals or effluents on aquatic organisms and are useful in determining the relative toxicities of chemicals and in discerning the relative sensitivities of species (Finger *et al.,* 2018). Alterations in the chemical composition of the natural aquatic environment usually affect behavior and physiological system of the inhabitants, particularly those of the amphibians (Khan and Law, 2005). Hormone disrupting effects in biota as a result of chemicals are caused by a wide variety of mechanisms. Amphibians are important organisms in aquatic and agricultural ecosystems; and play vital roles in both aquatic and terrestrial ecosystems. They are among the most important natural enemies of many agricultural pests. Because their sensitivity to changes of their habitat and their larvae in the aquatic environment. Concern over the decline of amphibians globally has highlighted the importance of using this group as a bio indicator of environmental contamination and climate change. Although this approach gives significantly further coverage of the effects of $1/10^{th}$ of 96 h LD_{50} sub-lethal concentration of Imidacloprid on the different tissues of *Hoplobatrachus tigerinus*.

Experimental Section

Collection of Test Organism

Indian bull frog *Hoplobatrachus tigerinus* of both sex were collected by hand net from their spawning ponds in unpolluted and non-agricultural sites of Bhimavaram, West Godavari district Andhra Pradesh, India. The frogs were transported to the laboratory in covered baskets and acclimatized to the laboratory conditions for a period of 7 days. Adult frogs of the same size and almost same weight (35.87± 0.04 g) were acclimatized in glass tanks ($51\times32\times33cm^3$ **)** containing two liters of dechlorinated tap water for seven days prior to the experiment (Vogiatzis and Loumbourdis, 1997). Tanks were placed on a slant to provide the option of both aqueous and dry environment. Water was changed for every two days and the tank was cleaned thoroughly. Frogs were fed with earth worms twice in a week. Uneaten earth worms and faecal wastes were removed and water replenished regularly**.** In any batch during acclimatization, if 5% mortality observed, the total batch was discarded.

Preparation of imidacloprid (17.8% SL) stock

Imidacloprid, a soluble pesticide was dissolved in acetone without any agitation immediately prior to use. Doses of Imidacloprid were prepared and incubated into the experimental animals according to the design of the experiment.

Selection of sub-lethal concentrations

In the current study, $1/10^{th}$ of 96 h LD_{50} value was taken as sub-lethal concentration to study the behavioral alterations and physiological alterations (Anon, 1975). The data on the mortality rate of frogs were recorded. The dead frogs were removed immediately. The toxicity tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h. Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LD_{50} values. The respective probit values were taken from Table IX of Fisher and Yates. For the determination of the 95% confidence limits, LD_{50} values and a normal variant of 1.96 were taken into consideration.

Method of Administration

Imidacloprid was given orally to all the experimental animals. At sub-lethal doses after every test period of 24 h, the pesticide was administered orally with the help of a syringe fitted with a 16 gauze oral blunt feeding needle. The oral feeding needle was placed into the mouth and passed back into the stomach; this is called oral intubation. Control animals of were treated with distilled water without giving pesticide.

Estimation of Nucleic acids

The nucleic acids, deoxyribo nucleic acid (DNA) and ribo nucleic acid (RNA) were estimated by the method of Searchy and Maclinnis (1970). Five per cent homogenates of gill, brain, muscle, liver and kidney were prepared in 5 ml of 0.5 N perchloricacid and heated at 90^0C for 20 minutes. After cooling, the tissue homogenates were centrifused at 3000 rpm for 10 minutes. The supernatant was separated into two volumes and used for DNA and RNA analysis.

DNA: The first half or one half of the homogenate was mixed with diphenylamine reagent and kept aside for 20 h. Then the colour developed was read at 595 nm. The standard graph was plotted with standard DNA (calf thymus) supplied by the Sigma Chemical Company with the aforesaid method.

RNA: The other part of the homogenate was mixed with Dischi-orcinol and heated at 90^0C for 15 minutes. After cooling at room temperature, the colour developed was read at 655 nm for RNA. The standard graph was plotted with standard RNA (Bakers yeast) supplied by Sigma chemical company. Analysis of variance (ANOVA) with repeated measures and Scheffe and Dunnetts comparison test was used to compare the means. Differences were deemed statistically significant at $p<0.05$. Statistical analysis was carried out with SPSS v.17.0 for windows.

RESULT AND DISCUSSION

Nucleic acids play an important role in maintaining the physiological configuration of the frog. Nucleic acid and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating different activities of cells. Their ratios also provide significant information about the way in which the mechanism of these contents regulates the multifaceted activities of cells.

In the present study, the DNA content was found to be decreased in all the tissues in response to Imidacloprid sub-lethal exposure at time periods of 24, 48, 96 h, 8, 15 and 30 days. Decrement in the DNA level might be due to activation of some dormant regulating factors or increase in activity of the essential factors controlling DNA synthesis or may be degeneration of hepatic cells due to the effect of Imidacloprid. Decrease in nucleic acid suggests the decrease in protein synthesis and damage to the liver is the major metabolic organ of drug detoxification. The calculated values of nucleic acid, DNA content along with standard deviation, error bars with standard error and the per cent change over the control for 24, 48, 96 h, 8. 15 and 30 days were presented (Figures.1). In control frog, the total DNA content of brain, liver, kidney and muscle, were more or less stable during the 30 days cycle of

the experiment. The maximum level of total DNA content was found in brain and minimum in liver. Under exposure to sub-lethal dose of Imidacloprid for 24, 48, 96 h, 8, 15 and 30 days the amount of DNA content was found to decrease in all the tissues of the frog, *Hoplobatrachus tigerinus.* Under exposure to sub-lethal dose for 24, 48, 96 h, 8, 15 and 30 days maximum decrease was noticed in liver, brain and minimum in kidney.

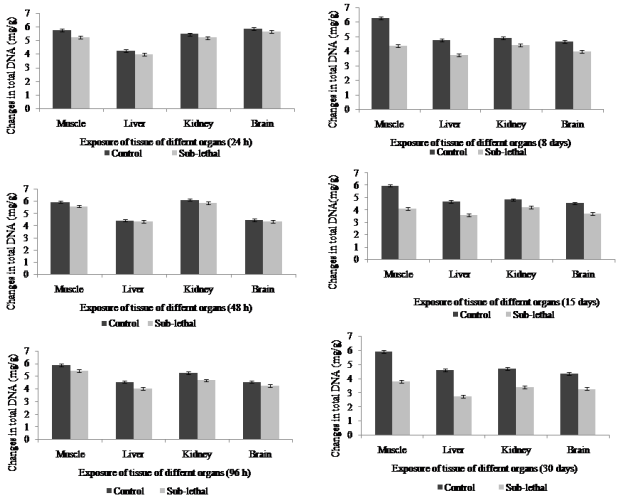

Fig I Changes in Deoxy ribonuclic acid (DNA) content (mg/gram wet weight of the tissue) in different tissue of the frog *Hoplobatrachus tigerinus* on exposure to sub-lethal dose of Imidacloprid for 24, 48, 96 h, 8, 15 and 30 days.

Randhir Kumar and Banerjee (2012) reported that the DNA content was progressively decreased in the gills and was statistically significant, after *Clarias batrachus* exposed to sub-lethal dose (1 mg/L; 5% of 96h LC_{50} value) of sodium arsenite (NaAsO2). The heterogeneous levels of DNA and RNA in the tissue of brain, liver, muscle gill and kidney were observed after exposure to sub-lethal and lethal concentrations of cadmium chloride in freshwater fish *Cirrhinus mrigala* (Veeraiah, *et al.,* 2013). The change noticed in DNA and RNA for low dose of imidacloprid was insignificant (Ajay Kumar, 2014). The DNA, RNA and protein contents were significantly reduced, whereas the amino acid content was significantly enhanced (Ansari and kumar., 1988; Plummer *et al*., 2018).

The calculated values of nucleic acid RNA content, along with standard deviation and the per cent change over the control frog after exposure to 24, 48, 96 h, 8,15 and 30 days were presented (Figure.2). In control frog, the total RNA content of brain, liver, kidney and muscle of control fish were more or less stable during the 30 days cycle of the experiment. Under exposure to sub-lethal doses of Imidacloprid, the total RNA content was decreased in most of the tissue of the test frog, *Hoplobatrachus tigrinus.* Maximum decrease was noticed in muscle and minimum in brain at 24, 48, 96 h and 8, 15 and 30 days.

In the present study, a cumulative decreasing trend in total RNA content was observed under the sub-lethal dose exposure of Imidacloprid. Durairaj and Selvarajan (1992) stated the decrease of RNA may be suggested that the daily addition of pesticides results in the swelling and chromatolysis of nissle bodies which are rich in RNA, Interference in the nucleic acid synthesis or inhibiting the function of RNA polymerase, non–coding for the process of protein synthesis. Nucleic acid content is considered as an index of the capacity of an organism for its protein synthesis.

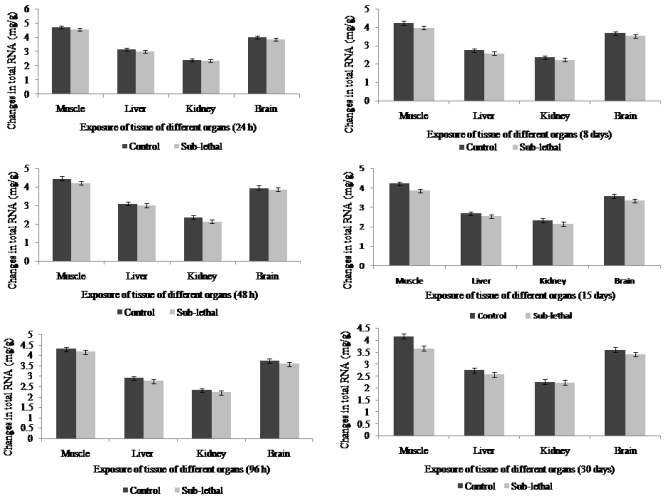

Fig II Changes in Ribonucleic acid (RNA) content (mg/gram wet weight of the tissue) in different tissue of the frog *Hoplobatrachus tigerinus* on exposure to sub- lethal dose of Imidacloprid for 24, 48, 96 h, 8,15 and 30 days.

Various studies on the effects of toxicants on the nucleic acid content in fish have been reported. Quinolphos induced decrease in RNA content of liver, muscle and gill and DNA content of brain of fish, *Oreochromis mossambicus* was reported by Durairaj and Selvarajan (1992). Decreased level of RNA in liver and brain of fish was also observed by Holbrook (1980). Holbrook (1980) observed the maximum inhibition of uridine incorporation occured after 6-48 h of toxicant administration in the rat. He also stated that the increasing trend of RNA in liver, muscle and gill at the end of 72 and 96 h might be due to increase of polymerase activity.

The results of the present study were in agreement with earlier authors (Thenmozhi *et al.,* 2011; Sunitha, 2012; Anisudden Siddiqui, 2013 and Veeraiah *et al.* 2013). Thenmozhi *et al.* (2011) stated that the effects of pesticide on nucleic acid contents in different tissues showed a remarkable observation. The tissues of muscle, liver, kidney, and brain showed significant decrease in the nucleic acid content of RNA and DNA during exposure period. This decrease in nucleic acid suggests the decrease in protein synthesis and further damage to the liver, which is the major metabolic organ of drug detoxification. Inhibition of DNA synthesis might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesizing machinery. The results of the present study suggest that Imidacloprid exposed to freshwater frog, *Hoplobatrachus tigerinus* is a moderate inhibitor of DNA synthesis, which in turn results in the reduction of RNA level. The above results indicate that the sub-lethal dose of Imidacloprid have shown moderate affect on the DNA and RNA contents of the tissue in the test frog, *Hoplobatrachus tigerinus*. The effects of sub-lethal dose of Imidacloprid on DNA and RNA contents show moderate toxicity on the main biochemical machinery of the freshwater frog, *Hoplobatrachus tigerinus*.

CONCLUSIONS

In the present study, the DNA content was found to be decreased in all the tissues in response to Imidacloprid sub-lethal exposure at time periods of 24, 48, 96 h, 8, 15 and 30 days. Decrement in the DNA level might be due to activation of some dormant regulating factors or increase in activity of the

essential factors controlling DNA synthesis or may be degeneration of hepatic cells due to the effect of Imidacloprid. Decrease in nucleic acid suggests the decrease in protein synthesis and damage to the liver is the major metabolic organ of drug detoxification. Under exposure to sub-lethal doses of Imidacloprid, the total RNA content was decreased in most of the tissue of the test frog, *Hoplobatrachus tigrinus.* Maximum decrease was noticed in muscle and minimum in brain at 24, 48, 96 h and 8, 15 and 30 days. The alterations in DNA levels in the present study could be due to the disturbances in the normal synthesis and turnover rate of DNA besides degenerative changes. The RNA levels reflect the intensity of protein synthesis and metabolic activity of the tissue. The depletion of RNA level suggests increased proteolysis and possible utilization of the products of their degradation for metabolic purposes. The changes in the biochemical markers like DNA and RNA which may be due to the increased activity of the enzyme DNAase and the inhibition of RNA polymerase function.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

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