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Determination of IC_{50} of Acephate for Acetylcholinesterase in Various Tissues of Chick

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Abstract: Pesticides are widely used to control unwanted pests such as insects, weeds, fungi and rodents. Increasing use of pesticides to protect crops causes harm to the living being in various ways. Many agricultural pesticide and industrial contents are capable of adversely affecting birds directly and indirectly. Birds are at high risk due to direct or indirect consumption of variety of pesticides. Acephate belongs to a large assembly of organophosphorus pesticides, well-known as inhibitors of acetylcholinesterase activity. The measurement of acetylcholinesterase activity is a better indicator to evaluate the toxicity of organophosphate pesticides. The *in vitro* effect of OP acephate was studied in liver, heart, kidney, intestine and muscle of chick. Acetylcholinesterase activity was measured by Ellman's method (1961) and 50% inhibitory concentration (IC_{50}) was determined *in vitro*. The result showed significant variation in IC_{50} of acephate to AChE in chick organs, which indicates different sensitivity of AChE to acepahte in these organs of chicks.

Keywords: Acephate, Acetylcholinesterase, $IC_{50,}$ Chick, liver, heart, kidney, muscle, intestine.

Introduction

Organophosphate (OP) pesticides are a preferred and major class of pesticides in agriculture because of their relatively low persistence in the environment. The primary target for OPs is acetylcholinesterase (AChE, EC 3.1.1.7), which plays a critical role in the vertebrate nervous system by terminating acetylcholine-mediated neuro transmission (Davies, 1963). OP insecticides elicit acute toxicity by inhibiting the enzyme AChE and causes accumulation of acetylcholine at cholinergic synapses, with overstimulation of cholinergic receptors of the muscarinic and nicotinic type. As a result various CNS effects viz. dizziness, inhibition of central respiratory centers, convulsions and coma occurs (Lotti, 2010). Majority of OPs used as insecticides are phosphorothioates (i.e., they have a P=S bond Bartlettn Terry (2017) and need to be bioactivated in vivo to their oxygen analogs to exert their toxic action however, some (eg. dichlorvos, methamidophos, or the nerve agents sarin or soman) have a P=O bond and do not need any bioactivation (Chambers, 2010). A recent *in vitro* study of Gao *et al.* (2017) reveals that chlorpyrifos and its oxon could inhibit axonal transport at concentrations below those required for inhibiting AChE activity.

Acephate (*O , S*-dimethyl acetylphosphoramidothioate) is a racemic organophosphorus insecticide widely used for foliar treatment of vegetable, fruit and field crops, cotton, commercial ornamentals, and in around poultry houses and dairies (WHO Report, 2002). Acephate itself considered toxic but after the decomposition to methamidophos become more toxic. Acephate itself acts as a weak cholinesterase but inhibited brain cholinesterase nine times more it did than erythrocyte cholinesterase, methamidaphos and acephate both insecticides have similar insecticidal potency, but different mammalian toxicity i.e. methamidaphos > acephate (Maul *et al.,* 2004; Md. Mahajna, *et al.,* 1997; White *et al.,* 1998).

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular inhibitor is required to inhibit a given biological process i.e. an enzyme or so by half. In other words, it is the half maximal (50%) *in vitro* inhibitory concentration (IC) of a substance (50% IC, or IC50). It is very useful as a measure of antagonist drug potency in pharmacological research (Ramsay and Tipton, 2017). The present investigation is aimed

at the determination of IC 50 of acephate for in vitro AChE inhibition in various organs viz. the liver, heart, kidney, muscle and intestine of chick.

Materials and method

Chemicals:

Acetylthiocholine iodide (ATCI) (Himedia), 5, 5 Dithio-bis-2-nitrobenzoic acid (DTNB) (sisco research laboratories PVT LTD), and other chemicals used were of analytical grade and Acephate (O-S Dimethyl acetylphosphor-dithiote 75% purity) was of technical grad.

Preparation of Stock Solution of Acephate:

Fresh stock solution of 0.1 g/ml acephate was prepared in double distilled water for *in vitro* study.

Experimental Animals:

Healthy chicks of 2 weeks of both sexes procured from local Govt. poultry farm. 10 chicks of similar size were selected for experiment; weighing 125±150g was used for the investigation. They were acclimatized for 10 days according to standard laboratory conditions at room temperature of 32-35°C and 12 hours, light/dark in separate cages, floor litter consisted of wood shavings. They were provided free access of water and commercial feed *ad libitum.*

In vitro enzyme inhibition

Chicks were euthanized to obtain the liver, heart, kidney, muscle and intestine for determination of AChE activity and in vitro 50% enzyme inhibition by acephate was calculated using Ellman's method (1961). For determining IC_{50} values, homogenates were incubated without and with varying concentrations of acephate (0.27- 69.94µm). Added to 2.6 ml, 0.1 M phosphate buffer (pH 7.4) contained 100 mM DTNB as a chromogenic reagent and kept for half hour before the addition of the substrate ATCI (75 mM) and rate of enzyme activity was immediately noted. Absorbance was noted at 412 nm in spectrophotometer. IC_{50} values were calculated by plotting graphs with concentration of acephate against AChE % inhibition with regression analysis and compared for toxicity in various tissues**.** The percentage of AChE inhibitory activity was calculated by using the following equation:

$$\text{Inhibition } \% = [(Cc\text{-}Cp)/Cc] \text{ X } 100$$

Where: Cc is the control activity and Cp is the experimental (with pesticide) activity.

Statistical analysis

The results in the figures are average values of 5 replicates ± standard deviation and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. All statistical analyses were performed using SPSS statistical software.

Results

In vitro IC_{50} of acephate was determined and the sensitivity of acetylcholinesterase in liver, heart, kidney, muscle and intestine of chick to acephate was observed. Tissues were incubated with different concentrations of acephate and residual acetylcholinesterase activity was measured as shown in fig.1 and table 1. The IC50 values of acephate was recorded in the range of 49.1- 78.9 µM in the tissues studied. The lowest IC_{50} was found in muscle (46.561±233) and highest in the intestine (78.923±112). As evident in table -1 the 50% inhibitory concentration of acephate was in increasing order as intestine**>** kidney> heart> liver > muscle**.** Therefore our results suggest the sensitivity of AChE to acephate in chick organs in as Muscle > Liver > Heart > Kidney > Intestine.

Table–1: IC_{50} values of Acephate for Acetylcholinesterase in various organs of Chick.

Organs	Liver	Heart	Kidney	Muscle	Intestine
$IC_{50} X\ 10^{-6}$ M of Acephate for AChE in Chick tissues	49.1 ± 120	55.2 ±189	73.684 ±204	46.561 ± 233	78.923 ± 112

Each value is mean ± S.D. of 5 individual observations.

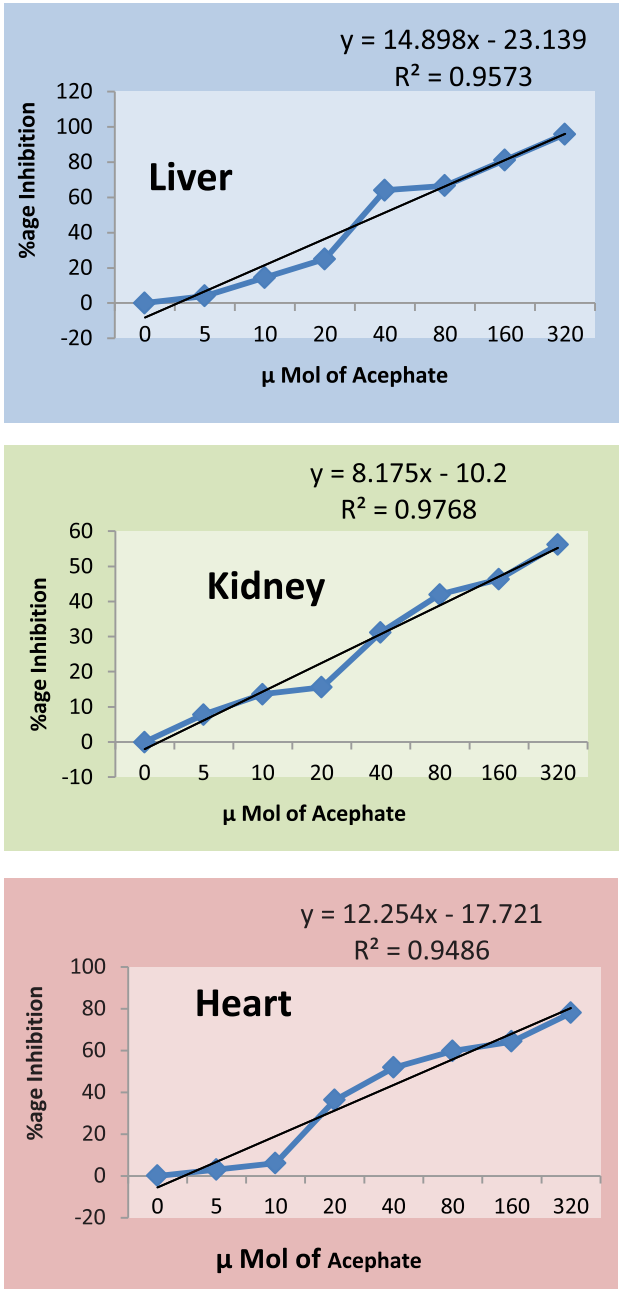

Fig- 1: IC_{50} ($x10^{-6}$) values of acephate for acetylcholinesterase in different organs of Chick.

Discussion

In the current investigation IC_{50} values of acephate for AChE in chick organs was determined to assess the difference in the sensitivity of these tissues to acephate.

Based on IC_{50}, values Bannet and Morimotto (1982) have reported that acephate was a weak inhibitor of cholinesterase in erythrocyte, plasma and brain cholinesterase of rat, monkey and human. Acephate was more toxic to rat brain and erythrocyte cholinesterase (1.6×10^{-3} and 1.3×10^{-3} mol/lit.) than monkey (3.4×10^{-3} and 2.7×10^{-3} mol/lit.) and human (5.4×10^{-3} and 2.7×10^{-3} mol/lit.) respectively. Similar findings of Gupta and Moretto, (2005) also revealed IC_{50} of acephate on brain and erythrocyte AChE and plasma ChE in vitro for rat, monkey and humans. Acephate was found slight more effected inhibitory of brain and erythrocyte activity in rat (IC_{50} = 1.6 and 1.3nM, respectively) than in monkey (IC_{50} = 3.4 and 2.7mM, respectivity) or human (IC_{50} = 5.4 and 2.7nM, respectively). Chattopadhyay *et al.,* (1986) has been reported species difference in the in vitro inhibition of brain AChE by mepafox, paraoxon and soman. They found IC_{50} value of mepafox for brain AChE of chicken (261×10^{-3}), rat (470×10^{-8}) and frog (1.60×10^{-8}), While, IC_{50} of paraoxon for chicken was (245×10^{-12}), for rat (310×10^{-3}) and for frog it was 229×10^{-12}. Their result revealed that chicken brain AChE showed more sensitivity to all OPs while frog brain AChE was least sensitive.

The result of present study also supports the findings of Chattopadhyay *et al.* (1986). We have also found

different IC_{50} of acephate for AChE of heart, liver, muscle, kidney and intestine of chick has differential sensitivity in these organs. Rahman *et al.* (2004) determined the IC_{50} in liver AChE and observed 7.69, 8.63 and 0.18mM IC_{50} for RPR-II, RPR-V and MCP, respectively. These investigators suggested that in vitro AChE assay is a sensitive assay and can be used as biochemical marker for the exposure of organophosphorus compound.

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