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## **Effect of Polytrin C (Combination Pesticide) on the Ach Ease Inhibition in Plasma and Brain of Wistar Rats**

<sup>1</sup>Sekar Babu Hari Ram, <sup>1</sup>Ch.Uma Devi, <sup>1</sup>Ch. Susma, <sup>2</sup>Venkateswara Rao Jasti, <sup>2</sup>T.M. Vijaya Kumar and <sup>2</sup>G. Thirumurugan

<sup>1</sup>Godavari Institute of Engineering and Technology, NH-5, Chaitanya Nagar, Rajahmundry-533294, India

<sup>2</sup>Research Laboratory, GIET School of Pharmacy, NH-5, Chaitanya Nagar, Rajahmundry-533294, India

*Corresponding Author: Sekar Babu Hari Ram, Godavari Institute of Engineering and Technology, NH-5, Chaitanya Nagar, Rajahmundry-533294, India*

### **ABSTRACT**

The present study has been aimed to screen the sub acute toxicity of a combination pesticide polytrin C which contains an organophosphate and a pyrethroid. First combination pesticide acute studies were carried out in Wistar rats to fix up to LD<sub>50</sub> dosage. Polytrin-C show a lesser toxicity when compare to the earlier literature available regarding the toxicity of its constituents profenofos and cypermethrin. Plasma AChE showed a prominent decrease in the both male and female rats of group 4 (34 mg kg<sup>-1</sup> b.wt.) while a dose dependent decrease of blood AChE was observed only in female rats, the males showing a decrease only in the highest dose group (34 mg kg<sup>-1</sup> b.wt.). In brain, a dose dependent decrease of AChE activity was observed in both male and female rats treated with the pesticide at the doses of 17 and 34 mg kg<sup>-1</sup> b.wt. (G3, G4) while the lowest dose group of 8.5 mg kg<sup>-1</sup> b.wt. remained on par with the untreated control. The study also revealed that the combination pesticides behave differently and exhibit a different toxicological profile when compared with the toxicity of the individual pesticides in the combination.

**Key words:** AchEase activity, organophosphate, pyrethroid, polytrin C, pesticides

### **INTRODUCTION**

Pesticides contaminate air, water, soil and food (Mohan *et al.*, 1998) and thus the toxic effects of these substances may have consequences even for consumers of food. According to World Health Organization (WHO), more than one serious accidental and 2 million suicidal poisonings with insecticides occur worldwide every year, and of these approximately 200,000 die, mostly in developing countries (Jevaratnam, 1990). Also among lethal Chemical Weapon (CW) agents, the organophosphorus nerve agents have had an entirely dominant role since World War II (Schrader, 1963). In the present agricultural practice to control pest animals invading the agricultural commodities farmers use combination pesticide containing one organophosphate and a pyrethroids to have a best pesticidal action and at the same time to reduce environmental toxicity in human and domestic animals. Many works have been attributed regarding screening the toxicity of the constituent pesticide (organophosphate or pyrethroids) but less exploration is done on the effect of mixed formulation regarding the sub acute toxicity. The mechanism of toxic effect of organophosphates is based on Acetylcholinesterase (AChE) inhibition in the nerve system. The Organo phosphates after entering the body of an organism reaches the cholinergic sites of the

nervous system and inhibit the activity of the AChE by binding at its active sites. The organophosphates are efficiently absorbed by inhalation, ingestion and skin penetration and this exposure by the multiple routes can lead to serious additive toxicity (Reigart and Roberts, 1999). As most organophosphates, diazinon attaches to acetyl cholinesterase and prevent it from destroying acetylcholine causing over stimulation of the nerves (Ware, 2000). AChE, a sensitive marker of neurotoxicity is widely distributed within the Central Nervous System (CNS). The AChE inhibition, thus leads to the accumulation of Ach at nerve endings which in turn cause the disruption of the nervous activity resulting in excitation, paralysis and finally the death of the organism (Satoskar *et al.*, 1999; Gaines and Linder, 1986). Fryer *et al.* (2004) demonstrated that the organophosphate insecticides could cause airway hyperactivity in the absence of AChE inhibition by decreasing neuronal receptor function. Alavanja *et al.* (2004) reported that different pesticides including diazinon have been found to be significantly associated with the lung cancer. Hence, recently new combination of pesticides have been introduced to reduce environmental pollution and at the same time to have a maximum action in killing the pest animals (Dinham, 2005; Dede and Dogara, 2004). The recent trend is to mix organophosphate along with pyrethroids in bringing out combination pesticide. Pyrethrins are natural insecticides produced from inter alia pyrethrum of the composite group and are esters of pyrethric or chrysanthemic acids (Ram, 2004). The synthetic pyrethroids are structurally similar compound rendered photo stable by addition of various substituent groups such as chlorine, bromine or cyanide on the basic structure. Some of the newer ones bear a more distance structural relationship to be pyrethrins. Because of their low mammalian toxicity, high insecticidal potency and lack of persistence in the environment, they have achieved widespread usage in agricultural as a household insecticide and in wood preservation. However, they are very toxic to aquatic organisms (Zitco *et al.*, 1979).

The combination pesticides are gaining popularity in pest control programmes as they exhibit a broad spectrum of activity coupled with better efficacy and economy. But for the registration of these pesticides, acute toxicity data is sufficient and therefore the long-term toxicity of these compounds remains unexplored. Alternatives are required for long term studies as they are difficult to carryout, time consuming and expensive. From this study it can be understood hat a well designed sub-acute study clubbed with neurotoxicological assessment can provide a major part of the information obtained from a long-term study.

## **MATERIALS AND METHODS**

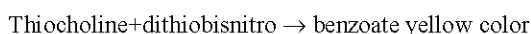
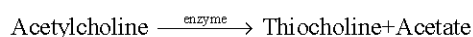
**Collection of sample:** The AChE, a sensitive marker of neurotoxicity is widely distributed within the Central Nervous System (CNS). Some sub cortical areas like nucleus caudatus and globus pallidus are particularly rich in this enzyme. It exists in several molecular forms of which the soluble globular form is present in the brain. Animals of both the sexes were fed ad libitum in the controlled environmental condition. Animals were divided in to groups as G1, G2, G3 and G4 from the four groups G1 is treated with distilled water and they are designated as control group whereas G2, G3 and G4 were treated as experimental groups fed with 1/10, 1/20 and 1/40 th of LD<sub>50</sub> dosage of mixed pesticide polytrinC dissolved in distilled water for 28 days through oral incubation.

The sub acute dosages are as follows:

- G1 only distilled water control group
- G2 8.5 mg kg<sup>-1</sup> b.wt.
- G3 17 mg kg<sup>-1</sup> b.wt.
- G4 34 mg kg<sup>-1</sup> b.wt.

On the 29th day of study Blood collected through retro orbital puncture and the animals were sacrificed to dissect to collect the brain sample which is used to screen the AchEase activity in blood and brain. During the course of sub acute study the in life parameters such as body weight gain and food intake was recorded. The works were carried out in GIET Campus, Rajahmundry, India during Feb.-May 2009.

**Principle:** The AChE was estimated in whole blood and tissues extracts by Ellmans's method (Ellman *et al.*, 1961). This method estimates AChE using acetylcholine iodide (substrate) and dithiobisnitro benzoic acid. The enzymatic activity is measured by the yellow colour produced by thiocholine when it reacts with dithiobis nitrobenzoate ion.



The color intensity can be measured on a spectrophotometer and the enzyme activity expressed as the rate of reaction per minute.

## RESULTS

In the results the activity of AchEase activity in both blood and plasma is separately studied to assess the inhibition caused by the combination pesticide polytrin C. From the results it is evident that in male rats, a significant decrease of AChE activity in blood was observed in the highest dose group (GIV) when compared to untreated control. But in case of female a dose dependent decrease of AChE activity in blood was observed in the pesticide treated groups at all the doses (Table 1). In plasma, a decrease of AChE activity was observed in groups 3 and 4 in female rats but in males a decrease was found only in Group IV (Table 1). In brain, a dose dependent decrease of AChE activity was observed in both male and female rats treated with the pesticide at the doses of 13 and 26 mg kg<sup>-1</sup> b.wt. while the lowest dose group of 6.5 mg kg<sup>-1</sup> b.wt. remained on par with the untreated control (Table 1).

In the results the activity of AchEase activity in both blood and plasma is separately studied to assess the inhibition caused by the combination pesticide polytrin C. From the results it is evident that in male rats, a significant decrease of AChE activity in blood was observed in the highest dose group (GIV) when compared to untreated control. But in case of female a dose dependent decrease of AChE activity in blood was observed in the pesticide treated groups at all the doses (Table 1).

Table 1: Levels of Acetyl cholinesterase in wistar rats treated with different concentrations of compound polytrin C

Groups	AChE (μ moles of substrate hydrolysed/minute/l (or) g)					
	Plasma		Blood		Brain	
	Male	Female	Male	Female	Male	Female
I Control	1.98±0.13 <sup>a</sup>	2.01±0.12 <sup>a</sup>	2.01±0.11 <sup>a</sup>	1.91±0.05 <sup>a</sup>	4.215±0.42 <sup>a</sup>	3.986±0.43 <sup>a</sup>
II 6.5 mg kg <sup>-1</sup> b.wt.	1.93±0.10 <sup>a</sup>	1.90±0.07 <sup>a</sup>	2.02±0.06 <sup>a</sup>	1.80±0.05 <sup>a</sup>	4.01±0.06 <sup>a</sup>	4.212±1.17 <sup>a</sup>
III 13.0 mg kg <sup>-1</sup> b.wt.	1.84±0.08 <sup>a</sup>	1.76±0.14 <sup>b</sup>	1.94±0.07 <sup>a</sup>	1.79±0.06 <sup>b</sup>	3.641±0.27 <sup>b</sup>	3.501±0.16 <sup>b</sup>
IV 26.0 mg kg <sup>-1</sup> b.wt.	1.67±0.06 <sup>b</sup>	1.50±0.08 <sup>c</sup>	1.72±0.07 <sup>b</sup>	1.72±0.07 <sup>b</sup>	2.316±0.25 <sup>c</sup>	2.023±0.06 <sup>c</sup>

Values are presented as Mean±SE. Values having similar superscripts are not statistically significant (p>0.05)

In plasma, a decrease of AChE activity was observed in groups 3 and 4 in female rats but in males a decrease was found only in Group IV (Table 1). In brain, a dose dependent decrease of AChE activity was observed in both male and female rats treated with the pesticide at the doses of 13 and 26 mg kg<sup>-1</sup> b.wt. while the lowest dose group of 6.5 mg kg<sup>-1</sup> b.wt. remained on par with the untreated control (Table 1).

## DISCUSSION

Acute OP poisoning causes various neurological signs in human and experimental animals (Wadia *et al.*, 1974). This includes behavioral changes; sleep disturbances, tremors, convulsions, coma and respiratory/circulatory failures. Early signs and symptoms of OP poisoning like depression, emotional lability, insomnia and tremors, exhibit as a result of the disturbances to the Central Nervous System can be correlated with substantial of blood AChE. Acetyl cholinesterase, a biomarker of organophosphate poisoning, did not show any specific pattern in the blood and plasma of the pesticide treated groups, in order to arrive at a conclusion on which combination pesticide was the least toxic. Plasma AChE showed a prominent decrease in the both male and female rats of group 4 (26 mg kg<sup>-1</sup> b.wt.) while a dose dependent decrease of blood AChE was observed only in female rats, the males showing a decrease only in the highest dose group (26 mg kg<sup>-1</sup> b.wt.) brain AChE showed a dose-dependent decrease in both males and females in groups treated with Polytrin-C at the doses of 13 and 26 mg kg<sup>-1</sup> b.wt. The decrease in plasma AChE observed by Vijerberg co-workers was similar to the decrease noticed in the present study.

From the study it could be seen that the combination pesticides exhibit toxicity in a different fashion compared to the toxicity manifested by the constitute pesticides. Mohan *et al.* (1998) have also reported previously that long term toxicity of the combination pesticide in most of the cases vary compared to that of the individual pesticide in the combination. A decrease in the toxicity of Polytrin-C when compared to their respective constituent pesticides pointed towards the unpredictability in the toxicity of the combination pesticides. Therefore, long-term toxicity studies are very essential for combination pesticides. Short-term sub acute studies combined with neurotoxicological evaluations can be used an alternative in predicting the long-term toxicity that could be manifested by the combination pesticides having their constituents as organophosphates and pyrethroids.

## CONCLUSIONS

The combination pesticides are gaining popularity in pest control programmes as they exhibit a broad spectrum of activity coupled with better efficacy and economy. But for the registration of these pesticides, acute toxicity data is sufficient and therefore the long-term toxicity of these compounds remains unexplored. Alternatives are required for long term studies as they are difficult to carryout, time consuming and expensive. From this study it can be understood hat a well designed sub-acute study clubbed with neurotoxicological assessment can provide a major part of the information obtained from a long-term study. The study also revealed that the combination pesticides behave differently and exhibit a different toxicological profile when compared with the toxicity of the individual pesticides in the combination. Therefore it is very necessary that all the toxicological studies have to be carried out (as for a new pesticide) for a combination pesticide before it can be brought to the field. Similar studies can also help to identify combination pesticides that have lesser mammalian toxicity compared to the individual pesticides used in the combination while retaining or obtaining better efficacy.

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