

SCREENING OF ACH EASE INHIBITION IN BLOOD PLASMA AND BRAIN OF WISTAR RATS BY NEURELLA D [COMBINATION PESTICIDE]**Dr.H.SEKAR BABU, Dr.P.JAYARAMAN AND P.AARTHY**

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Corresponding Author* drhsekarbabu@yahoo.comABSTRACT**

Ach Ease is a very important biomarker to assess the lesions in the nervous system caused by pesticides organophosphates and pyrethroids. The present trend in agriculture is 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 of the combination] but less exploration is done on the sub acute toxicity of a combination pesticide. Hence the present work has been designed to study the sub acute toxicity for 28 days on wistar strain rats to screen the biomarker AchEase enzyme inhibition in the plasma and in brain. Results are quite interesting to note that the toxicity exhibited by the mixed formulation differ from the toxicity exhibited by its constituents. In lower doses the combination pesticide did not show significant inhibition in AchEase activity due to synergistic effect whereas in the earlier literature inhibition is seen in all the experimental groups with relation to its constituent pesticide in the selected combination pesticide. Therefore it is very much 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 subacute studies on AChEase inhibition will help us to identify a combination pesticide that have better efficacy in pest control. With lesser mammalian toxicity compared to its constituents in the combination.

INTRODUCTION

In recent days the combination pesticides are gaining popularity in pest control programmes as they exhibit a broad spectrum of activity coupled with better efficacy in pest control. But for the registration of these pesticides, acute toxicity data is sufficient and therefore the long-term toxicity of these compounds remains unexplored. It is presumed that the combination pesticides behave differently and exhibit a different toxicological profile when compared with the toxicity of the individual pesticides in the combination. As we are aware that the residues of the constituent pesticides in the

combination contribute significantly to contamination of air, water, soil and food (Koeman, 1979) and thus the toxic effects of these substances may have consequences even for consumers of food. AChE, a sensitive marker of neurotoxicity which is widely distributed within the entire central nervous system (CNS). Some subcortical areas like nucleus caudatus and Globus pallidus are particularly rich in this enzyme (Foldes et al., 1962). It exists in several molecular forms (Taylor et al., 1987). Of which the soluble globular form is present in the brain

MATERIALS AND METHODS

Study is carried out to evaluate the short-term sub acute (28 days) toxicity of the combination pesticide Nurelle-D (chlorpyrifos and cypermethrin) in Wistar rats. The acute toxicity of these combination pesticides was evaluated to arrive LD 50 (Finney, 1971) dosage. From the LD50 dosage the acute doses were fixed as 1/10, 1/20 and 1/40th of LD50 dosage of pesticide. The selected combination Neurella D is dissolved in distilled water and administered orally through oral gavage for 28 days for the animals were grouped as G1, G2, G3 and G4.

Out of four groups G1 treated as controls whereas G2, G3 and G4 animals were fed with 1/10, 1/20 and 1/40th of LD50 dosage of pesticide. During 28 days sub acute studies in life parameters such as body weight gain and food intake were also recorded. On 29th day animals were sacrificed to collect blood sample and brain tissue extract of both control and experimental animals to be screened for AChE enzyme studies. AChE was estimated in whole blood and tissues extracts by Ellman'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 due to the activity of AChE. The colour intensity can be measured on a spectrophotometer and the enzyme activity expressed as the rate of reaction per minute.

RESULTS

Blood and Plasma

In male rats, a significant decrease of AChE activity in blood was observed in the highest dose group (G IV) when compared to untreated control. But in case of female a dose dependent decrease of AChE activity was observed (Table 1 Figure 1 and 2). In plasma a dose dependent decrease of AChE activity was observed in both male and female rats treated with the pesticide (Table 1; Figure 1 and 2).

Brain

In brain, a dose dependent decrease of AChE activity was observed in both male and female rats treated with the pesticide when compared with the untreated control (Table 1; Figure 1 and 2).

Table 1.
Levels of Acetyl cholinesterase in wistar rats treated with different concentrations of Nurelle-D

Groups	AChE (μ moles of substrate hydrolyzed/minute/l (or) g)					
	Plasma		Blood		Brain	
	Male	Female	Male	Female	Male	Female
I Control	1.858 ^a \pm 0.07	1.714 ^a \pm 0.03	2.00 ^a \pm 0.03	2.013 ^a \pm 0.06	4.00 ^a \pm 0.37	3.932 ^a \pm 1.28
II 6.5 mg/kg b.w.	1.796 ^a \pm 0.07	1.431 ^b \pm 0.08	2.020 ^b \pm 0.04	2.01 ^b \pm 0.02	3.462 ^b \pm 0.17	3.035 ^b \pm 0.19
III 13.0 mg/kg b.w.	1.806 ^a \pm 0.05	1.343 ^c \pm 0.02	1.930 ^c \pm 0.06	1.714 ^c \pm 0.05	2.479 ^c \pm 0.14	1.518 ^c \pm 0.14
IV 26.0 mg/kg b.w.	1.615 ^c \pm 0.07	1.176 ^d \pm 0.04	1.698 ^d \pm 0.04	1.642 ^d \pm 0.06	1.931 ^d \pm 0.22	1.047 ^d \pm 0.13

Values are presented as mean \pm standard Error

Values having similar superscripts are not statistically significant ($p > 0.05$).

Fig 1

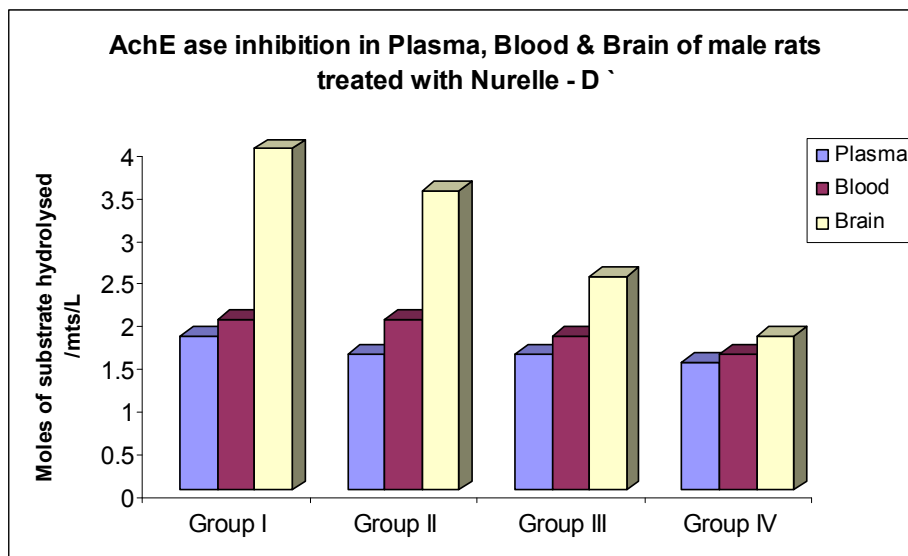
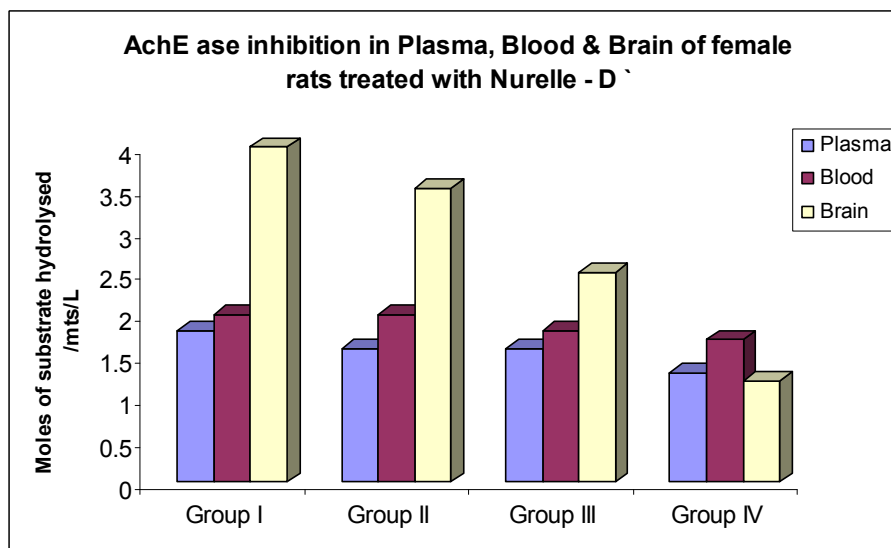


Fig 2



DISCUSSION

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 AChEase inhibition. Acute OP poisoning causes various neurological signs in human and experimental animals (Wadia et al., 1947). From the present

investigation it could be observed that the combination pesticide Neurella D did exhibit the characteristic toxicity signs of its constituent insecticides like suppression of AChE (induced by chlorpyrifos an organophosphates in the combination)lead to convulsions and tremors induced by its constituent cypermethrin Although, body

weight gain and behavioral changes observed in the study were similar to the findings reported by earlier studies in cypermethrin the time of incidence and severity of these parameters were found to be different. So it could be understood that the toxicity of neurella D was not the same as that of the constitute pesticides namely chlorpyrifos[organophosphate] and cypermethrin pyretheroids.

Plasma AChE showed a dose-dependent decrease in the pesticide treated in female rats but the males showing a decrease only in the highest dose group hence a sex wise difference was observed in the case of biochemical parameters in plasma. Blood AChE showed a dose-dependent decrease in the pesticide treated rats Brain AChE showed a dose dependant decrease in both males and females of the treated groups. The reduction in the body weight and feed composition in the present study is supported by the findings of Crown and co-workers but is not in agreement with the observations reported by Couston and co-workers.

The decrease in plasma AChE observed by both Couston and co-workers and Crown and co-workers was similar to the decrease noticed in the present study. But the decrease of brain AChE observed in the Nurelle-D treated groups in the present study was not similar to the observations made by Couston and co-workers [1971]. Regarding the results in lower doses it has not showed significant inhibition in AchEase activity. Whereas the earlier literature on its constituents showed inhibition in all experimental groups which may be because of the synergetic effect of its constituents.hence this type of Neurotoxic short term sub acute studies will be an

alternative for the long term subacute studies which should be made mandatory before releasing a new combination pesticide in the agricultural practice instead of purely depending on the acute studies (Sekar Babu 2010).

Summary:

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 [. Murali Mohan and co-workers 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 (Murali Mohan et al., 1998). The synergetic effect of constituent pestide unexplored in the form of long term sub acute studies.Hence long-term toxicity studies are very essential for evaluations of combination pesticides having their constituents as organophosphates and pyrethroids to asses the synergetic effect of its constituents. From this study it can be understood that a well designed short time sub-acute study clubbed with neurotoxicological assessment AchEase can provide a major part of the information as 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 (Sekar Babu Hariram et al., 2010).

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