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Studies on Biochemical Changes of Acetyl and Butryl Cholinesterases in the Sprayers Exposed to Organophoshorous Pesticides in Nuziveedu Krishna District A.P., India.

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Abstract- The neurotransmitter enzymes such as acetyl and butryl cholinesterase's effects as changes are studied in human beings exposed to organophosphorous pesticides during agriculture practices of spraying in the areas of Nuziveedu Krishna district, A.P, INDIA. These changes are significant as it was found to be more than 45% in Acetyl cholinesterases (AChE) and 36% in butryl cholinesterase (BChE) decrement in the exposed group when compared to non-exposed group as controls. First absorption, by people resulting toxicity as chronic by the production of oxygen free radicals, being heterotrophic metabolically, results in the alteration of homeostasis leading to oxidative stress that culminates the non maintenance of the antioxidants continuously due to imbalance.

The organophosphorous (OP) residues as contaminants in the body inhibit the two enzymes which are cholinergic rendering the hydrolysis of the acetylcholine not to take place there by disturbance in neuronal transmission. The results in different age groups and smoking habit do not show much variation but the duration of exposure resulted due to ethylated and methylated variation. Results varied both in nicotinic group as well as in the muscarinic group of workers. Significant levels of changes are observed in methylated OP insecticide sprayed individuals only rather than non methylated OP compounds rather than the both as mixtures. It results in tiredness, weakness, nausea, dizziness, headache, sweating, tearing, vomiting, limited vision, diarrhea, polyuria, muscle trembling, hyper tension and breathing disorders.

Keywords: organophosphorous pesticides, acetyl cholinesterase, butryl cholinesterase, methylated, non-methylated, bio-indicator.

I. Introduction

Food, fibre, forage are the three essential necessities for human beings. Concomitant to the demands, of the above pesticide usage is inevitable. This indiscriminate usage leads to contamination. Pesticides, as a diverse group of chemicals destined to kill and control the pests categorized differently into insecticides, bactericides, algaecides, weedicides, etc. To the concomitant increase of world population there is a demand for the basic necessities, which require pest control and storage. This resulted in the usage of different chemical substances like organochlorines, organophosphates, carbamates and synthetic pyrethroid groups during different periods of time.

According to Watson et al., (2002), the most commonly used organophosphates and carbamates accounted for human poisoning and death other than pesticide class organo chlorines. But not only the consumption of residued food and water even the fondeling of such chemicals effect the health, of those who spray them during agricultural practices where in usage is inevitable and that is why the agri sprayers as farmers and labour engaged in such tasks, resulting what it does do. The earlier reports with a variety of neurological dysfunction were by Karalliede eta al., 1999; Vega 1994, Brown et al., 1989.

Not only the environment as ecotoxicology aspects studied but also individual organisms are affected. No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects though disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (Jeyaratnam, 1985).

India, one of the agricultural countries, use of pesticides due to 56.7% of farm sector ranked 12th in the world in its consumption. The first report of poisoning due to pesticides was from Kerala in 1958 where over 100 people died after consuming wheat flour contaminated with parathion (WHO, 1990). This prompted the special committee on Harmful effects of pesticides constituted by Indian Council of agricultural research to focus attention on the problem (Karunakaran, 1958).

Locally, the place popularly known as "Mangoes Paradise" as Nuziveedu, situated in Krishna district of Andhra Pradesh, India and other agricultural crops use lot of pesticides to combat pests and among

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them OP takes the lions share. As they inhibit the enzymes AChE and BChE, the agri sprayers which include farmers and labours as sprayers, an attempt is made to know how ignorance illiteracy and illusion could give a sociological problem regarding the proper care to be taken while spraying, of course this study is an attempt at local area because monitoring plays a major role in abatement.

At present, India is the second largest producers of pesticides in Asia and also ranks twelfth in the consumption of the world pesticides (Eds, et al., 1972). Majority of the population in India (56.7%) are engaged in agriculture and are inevitable to exposure to the pesticides used in agriculture (Gupta, 2004). Organophosphate pesticides can generate reactive oxygen species and alter cellular antioxidant systems (Delescluse, et al., 2001; Bagchi, et al., 1995; Flessel, et al., 1993). ChE enzymes include acetyl and pseudo cholinesterase as butryl cholinesterase. Monitoring plays a major role in protecting the pesticide application workers from sub acute poisoning with organophosphate (OP) insecticides (Ames, 1989). Absorption of Organophosphorus compounds is commonly assessed by monitoring or measuring the decrease in acetylcholinesterase (AChE) or buytyrlcholinesterase (BChE) activities in human blood.

II. Material and Methods

Blood Acetyl cholinesterase (AChE) and Buytyrlcholinesterase (BChE) activity was determined by the method of Ellman (Ellman et al, 1961). Venous blood is collected into heparinized tubes and subjected to centrifugation for plasma without removing any erythrocytes. Then the erythrocytes are suspended into water to makeup to the same volume of the whole blood and to this 0.1M phosphate buffer is added and then frozen in order to haemolyse the erythrocytes. After thawing, the suspension is further diluted with buffer and thiol reagent DTNB added. Ten minutes after DTNB, acetyl thiocholine (ATCh) is added. The absorbance is read at 412 nm against a blank containing hemolysed erythrocytes suspended in buffer.

The BChE activity in plasma is also with 1.0Mm ATCh using the same buffer and DTNB reagent. The enzyme activities are measured at 25or 37oC. The concentration of haemglobin in the erythrocyte suspension is determined spectrophotometrically at 546 nm at room temperature. The activities of AChE and BChE were expressed as micromoles of hydrolysed ATCh per minute and per milliliter of whole blood and plasma (Worek, 1999).

III. Results and Discussion

In the present study both acetyl cholinesterase (AChE) and butyrlcholinesterase (BChE) as per the effects of OPI values are given in figures1, 2, 3, 4, 5, 6. As per the illustration in figure I the RBC, AChE and the plasma BChE levels are measured in the controls and the exposed workers. All the OP pesticides are suppressors of AChE activities and both the parameters serve as an important as bio-markers of toxicity. The activities of both the enzymes (AChE and BChE) were decreased significantly in the OP pesticide exposed group as compared to the values recorded in the control group. The decrease in the activity of AChE was found to be more than 45% and 36% in case of BChE. The decreased levels of cholinesterase in the exposed group may be resulting from dephosphorylation of the enzymes due to direct action of OP pesticides.

The AChE and BChE levels in relation to age in controls and exposed group are provided in figure 2. The effect of age on AChE and BChE activities in controls and exposed workers was not discernable in this study as the two age groups ($<$30 years and $>$30 years) did not differ significantly in the controls as well as OP pesticide exposed workers.

The effect of smoking on the AChE and BChE levels in the controls and exposed workers is displayed in figure 3. The results indicated no significant differences in the mean values of AChE and BChE in the controls as well as in the exposed group. The study failed to demonstrate any significant effect of smoking in the decrease of the activity of AChE and plasma BChE in both the groups.

The levels of AChE and BChE as per the duration of exposure of OPs insecticides are summarized in figure 4. The workers in the chronic exposure group ($>$10 years) showed significant decrease in the mean values of both enzymes as compared to the mean values observed in the acute exposure group ($<$1 year). The significant decrease of activities following prolonged exposure to OP insecticides may be resulting from ageing of both, is which the cholinesterase is active. In fact the continuous exposure does not allow the recovery of inactive AChE and BChE to their active form as the process any more remains as reversible.

The AChE and plasma BChE levels in normal asymptomatic and symptomatic exposed workers are given in figure 5. The levels were significantly reduced both in the nicotinic group as well as in the muscarinic group of workers as compared to asymptomatic workers. The significant reduction in RBC AChE and plasma BChE in both nicotinic and muscarinic groups is indicative of OPI intoxication and in such cases the symptoms are the outcome of the tremendous decrease is true and pseudocholinesterases levels (BChE).

In RBC AChE and plasma BChE activity measured in all the three subgroups of workers is shown in figure 6. The maximum decrease of RBC AChE was recorded in the exposed workers-group I who sprayed only methylated OP insecticides 407.6$\pm$23.9 while group III showed the highest reduction in plasma BChE

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419±23.6 as compared to other groups. Both group I and group III workers showed significant decrease in the mean values of AChE while this was not observed in the case of plasma BChE which did not show any significant variation when compared within the three sub groups of pesticide sprayers.

It is also observed that the fall in the activity of plasma BChE was not that pronounced as in the case of RBC, AChE. It is therefore RBC AChE is considered as more reliable bio-indicator of OP toxicity in contrast to plasma BChE.

Cholinesterase is one of the enzymes of neurological system and OPs have interfered in Cholinesterase activity by way of inhibition. The effects of this compound can result from breathing, eating, or contact with skin and by mucous secretion during the different stages of working, preparation of the solutions and spraying etc.

The prolonged exposure resulted adverse effects and infact showed different signs and symptoms due to degree of toxicity of the concentration. In low exposure, symptoms like tiredness, weakness, vertigo, nausea and dimness were seen, in median exposure headache, sweating, tearing, saliva increasing, vomiting and limited vision and in high exposure abdominal AChE, polyuria, diarrhea, muscle trembling tittering pupil stricture, hypotension, bradycardia, breathing disorders and probably dead according to (Parron, et al., 1996) who considered, them as the three separate target groups.

In the organophosphorus compounds, insecticides like malathion, diazinon, acephate etc., are mostly used as insecticides in agriculture and hygiene pest control. It should be mentioned that some of these workers working in some seasons and there is no control on their cholinesterase activities. However, this association is independent of the utilization of Ops pesticides other than OPs can also decrease AChE (Hernandez, et al., 2006; Banerjee, et al., 1999). McCauley et al, (2006) reported the role of acetylcholinesterase (AChE) in rural workers due to OPs exposure. Such effects noticed, observed and repoted to have profound influence on cellular respiration culminating the metabolism, in working people which can be considered as occupational hazard.

Erythrocyte AChE was significantly decreased in pesticide–exposed farmers compared to the control. Organophosphates inhibit AChE in RBC. The depletion of AChE found in the exposed workers occupationally to OP compounds correlated well the with period of exposure. This observation was clearly seen when acute exposure group was compared with the chronic with regard to impairment of erythrocyte acetyl cholinesterase. The latter group showed significantly reduced mean value, as compared to that found in acute exposure group, which showed a decreasing trend.

The levels are significantly reduced both in the nicotinic group as well as in the muscarinic group of workers as compared to asymptomatic workers. The significant reduction in RBC AChE and plasma BChE in both nicotinic and muscarinic groups is indicative of OPI toxicity, in such cases the symptoms are the outcome of the tremendous decrease in true and pseudo cholinesterase levels.

A maximum decrease of RBC AChE was recorded in the exposed workers, sprayed only methylated OP pesticides such as methyl parathion, diazinon, malathion, dichlorovos, dimethoate, when compared to ethylated OP pesticides such as chlorpyrifos, diazinon, methidathion, phorate and in mixed op pesticide applicators and further it showed the highest reduction in plasma BChE.

The fluctuations in AChE levels with an increasing severity of pesticide exposure may reflect the amount of pesticide inhaled or absorbed. The significant reduction in RBC AChE levels in the chronic exposure group could also be attributed to irreversible dephosphorylation of AChE i.e. “Aged AChE” or inactive. Due to prolonged and consistent environmental exposure to OP pesticides as found in this study, and the absence of time lag is the key factor in chronic exposure while acute exposure is intermittent giving time allowance to recover to its active form. It is synchronizing with the early reports of some. Such reports are earlier by Amar Santosh Dhalla and Mohammd Fareed et al, 2013; Suman Sarma 2013; Quazi et al, 2012; Tilak, et al, 2011, 2010; Fernanda Simoniella et al, 2010; Manel Araoud et al 2010; Rohlman et al, 2010; Rastogi et al, 2009; 2008; Zhou et al 2007; Lopez et al 2007, Boiko et al, 2005; Vidya Sagar, 2004; Patil, 2003; Verma et al, 2003 and Pay Mino et al, 2002.

Hence as per the earlier reports and present study it may be concluded that both AChE and BChE serve as an indices of pesticide toxicity and if applied as a biomarker for giving any pesticide representation in usage can be a part of monitoring the pollution abatement and control.

With the increase of the scientific knowledge a mechano revolution finding sprayers using machines needs to be revolutionized and agri engineering must take care of a minimum exposure by the human beings so that contamination can be reduced to a maximum extent.

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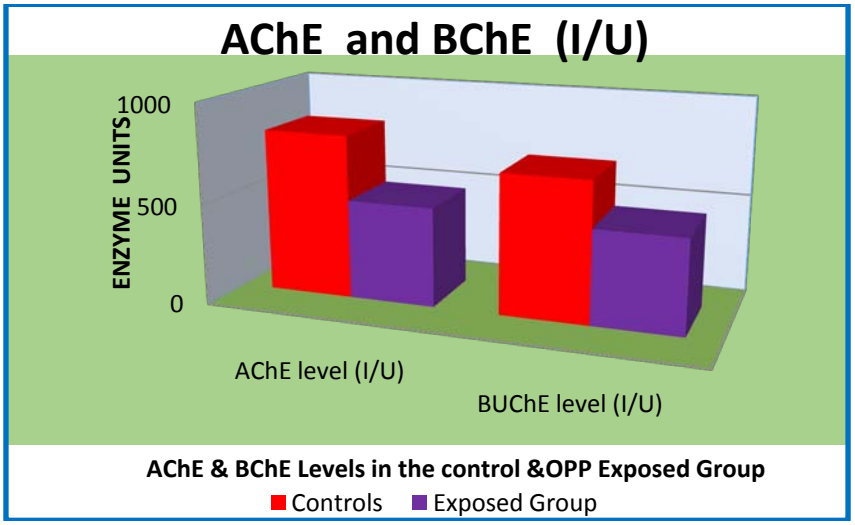

Figure 1 : The Effect of AChE and BChE in the Controls and in the OPP Exposed Group

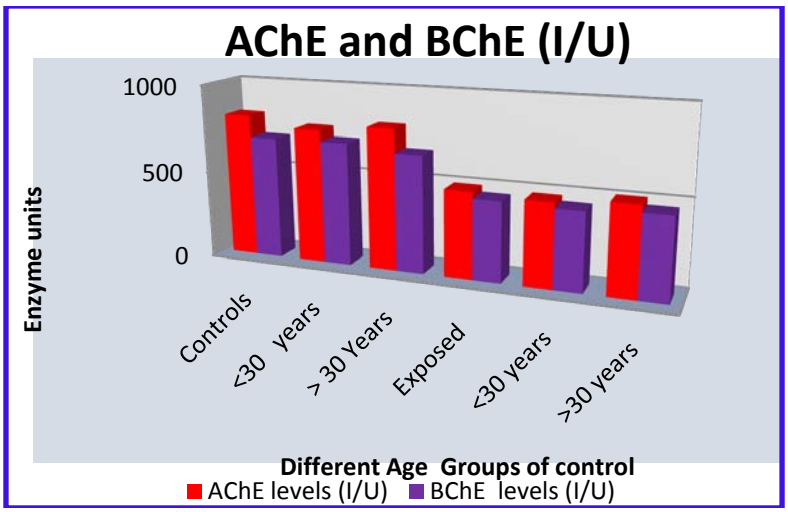

Figure 2 : The effect of AChE and BChE in different age groups on the controls and in the OPP Exposed Group

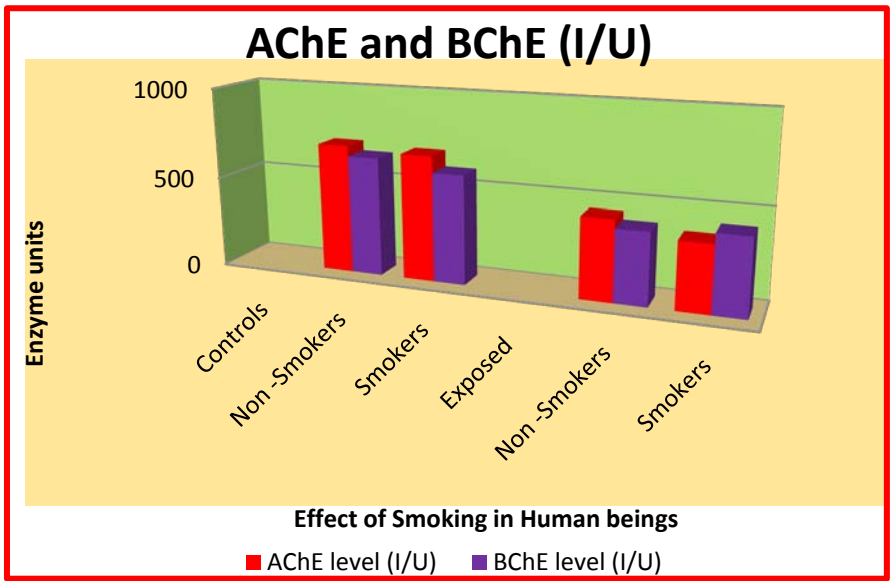

Figure 3 : The effect of smoking on AChE and BChE levels in the controls and OPP Exposed Group

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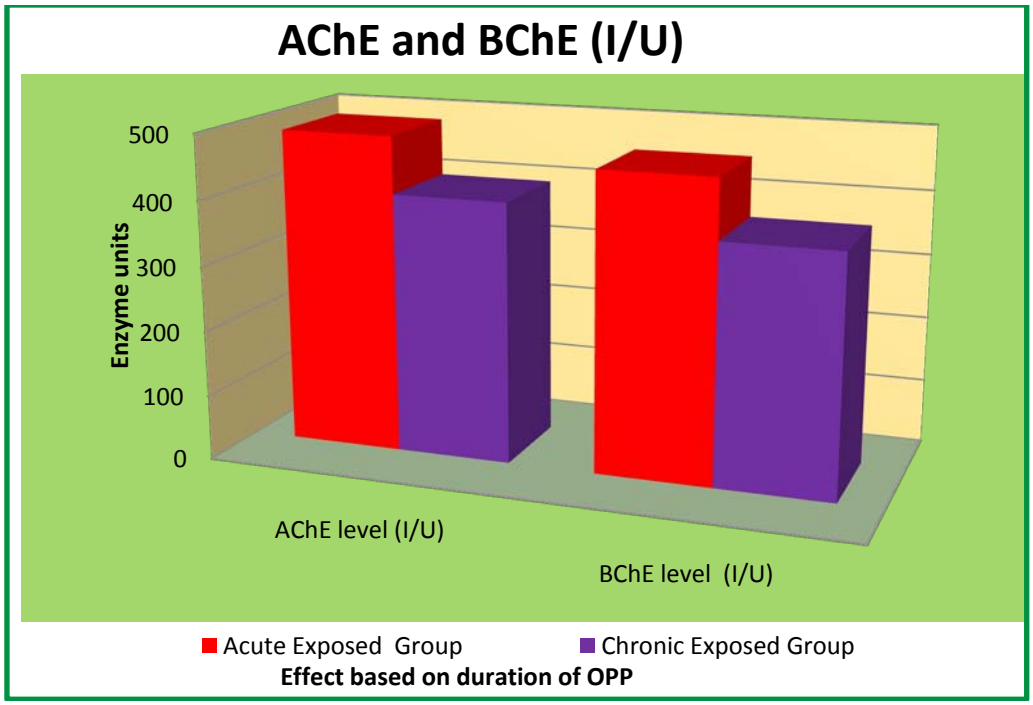

Figure 4 : The effect of exposure on AChE and BChE levels

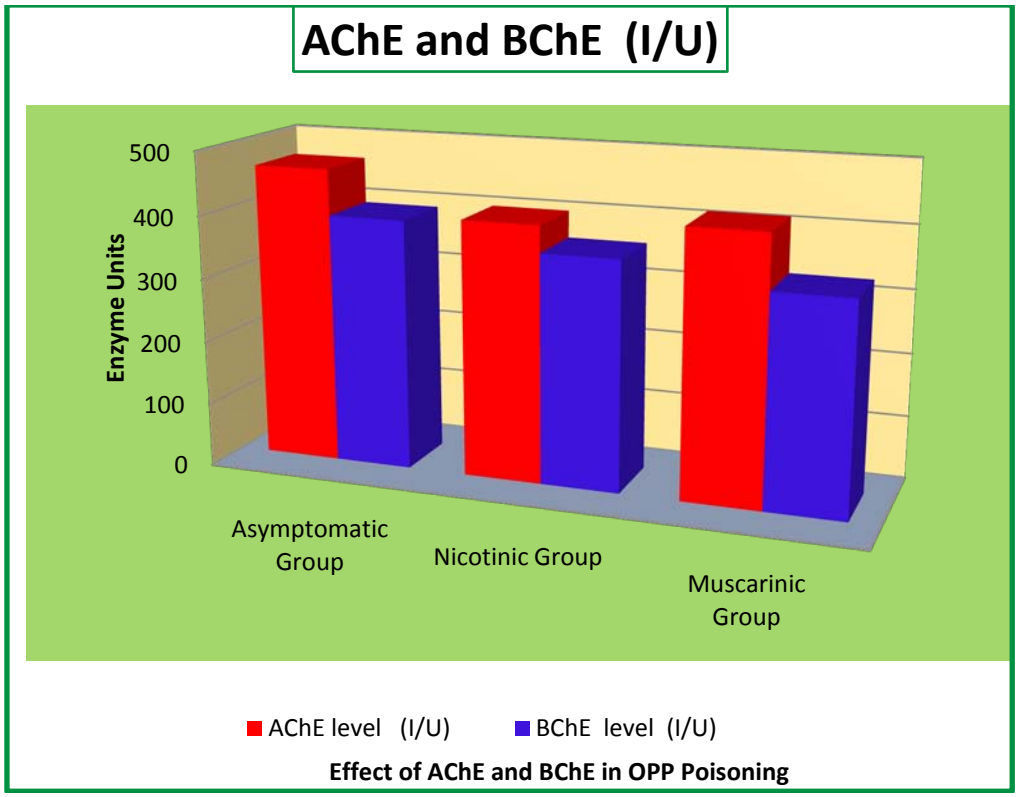

Figure 5 : The activity of AChE and BChE in OP Pesticide Poisoning

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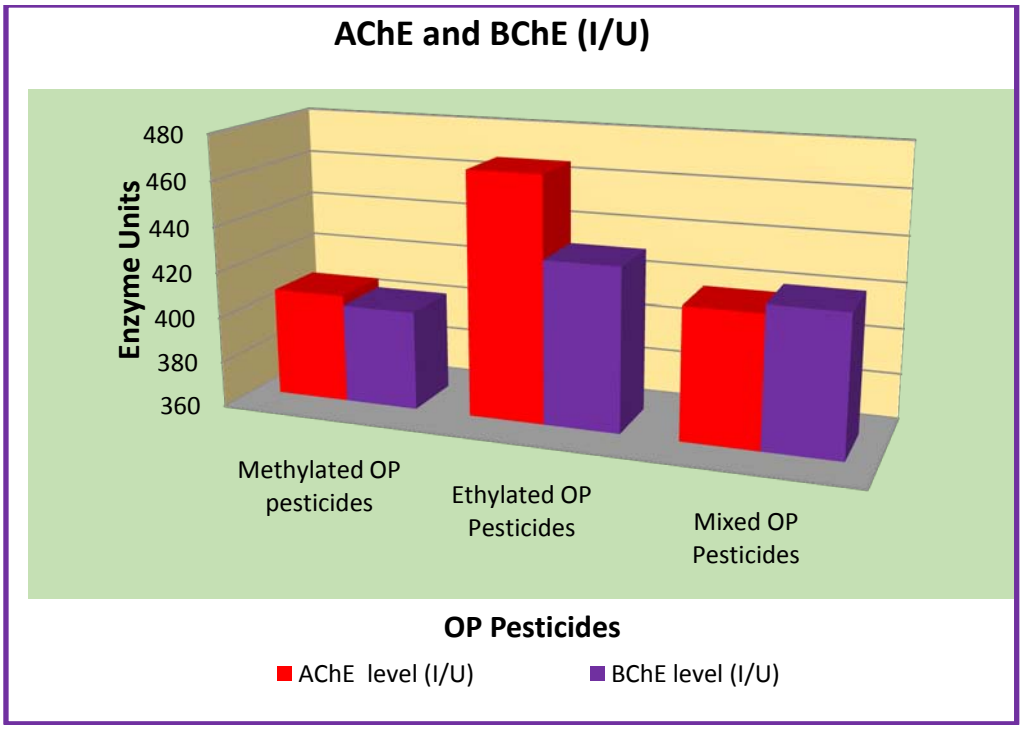

Figure 6 : Blood and Plasma levels of AChE and BChE in the different OP Pesticide Exposed Group

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