African Journal of Biotechnology Vol. 9(49), pp. 8369-8377, 6 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.784
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Biochemical alterations of certain health parameters in cotton growing farmers exposed to organophosphorous and pyrethroid insecticides

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Accepted 15 October, 2010

A study was carried out on cotton growing farmers to assess the impact of organophosphorous pesticides on the biochemical parameters. Three hundred occupationally exposed farmers to pesticide application and equal number of unexposed subjects were selected from 24 randomly selected villages using stratified proportionate random sampling procedure. Multivariate logistic regression analysis was performed to assess the effect of pesticides on acetyl cholinesterase enzyme activity (AchE), in exposed and unexposed subjects. About 80% of exposed subjects used mixture of organophosphates and were exposed to pesticides for a period of 16.3 years at an average of 4 months / year. The severity of symptoms in the exposed subjects was higher in the second tertile (between 12 and 20 years) than in the third tertile (> 20 years). However, inhibition of AchE activity was significantly high ($P < 0.05$) in third tertile than the first (< 12 years) and second tertiles. Overall, there was a significant decrease in AchE activity and antioxidants with increased levels of lipid peroxidation ($P < 0.01$) in exposed subjects than unexposed subjects. The elevated levels of aspartate aminotransferase (AST) and creatinine in the exposed subjects were very marginal and just above the normal values.

Key words: Chronic exposure, pesticides, AchE activity, lipid peroxidation.

INTRODUCTION

Exposure to pesticides among the cotton farmers in India and Southeast Asian countries is common due to their occupation in pesticide spraying. Although certain pesticides are banned for certain crops, excessive use of such pesticides by the farmers who were occupationally engaged in spraying activities without following safety measures has caused repeated exposure. Majority of the rural population in India (56.7%) is engaged in agriculture and exposed to most commonly used pesticides such as organophosphorus, organochlorine, carbamates, and synthetic pyrethroids and 45% of the total pesticides are used on cotton crops to control pests (Chitra et al., 2006, Shadnia et al., 2005; Sharma, 2006). According to W.H.O., approximately 25 million pesticide poisoning cases occur annually among agricultural workers in developing countries. However, pesticide poisoning in agricultural farmers due to occupational exposure is poorly documented in developing countries (Smit et al., 2003).

Studies on agricultural workers from developing countries who were exposed to organophosphate pesticides indicated the commonest clinical symptoms such as headache, giddiness, ocular symptoms and paresthesia (Misra et al., 1988). Exposure to organophosphate pesticides interferes with synaptic transmission peripherally at muscarinic and nicotinic receptors. The most frequent signs are reported to be meiosis and diarrhoea. Muscarinic manifestations include hyper salivation, vomiting, respiratory distress, abdominal pain, depressed level of consciousness and smooth muscle fasciculation. Nicotinic manifestations include increased or decreased

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Abbreviations: AchE, Acetyl choline esterase Enzyme; **TBARS,** Thiobarbituric Acid Reactive Substances; **AST,** Aspartate amionotransferase; **ALT,** Alanine amionotransferase **.**

muscle power and skeletal muscle fasciculations (Sungur and Guven, 2001). Systemic effects mediated through cholinesterase inhibition further leads to an over stimulation and then depression of the nervous system in the exposed humans to organophosphorous and N-methyl carbamate insecticides (Smit et al., 2003).

One of the consequences of acute exposure to pesticides is generation of free radicals leading to oxidative stress, lipid peroxidation and alterations in antioxidant status in animals and humans (Rodriguez et al., 1991; Sungur and Guven, 2001; Ranjbar et al., 2002; Sivapriya et al., 2006). Several studies have demonstrated organophosphate pesticide induced lipid peroxidation in rat brains and human erythrocytes and acute renal tubular necrosis due to reactive oxygen species (Prakasam et al., 2001). They also have been associated with delayed neuropathy (Shadnia et al., 2005), chromosomal aberration, central nervous system alterations and non-Hodgkin's lymphoma (Misra et al., 1988). Studies also reported the adverse effects of pesticides on the male reproductive system such as decrease in the antioxidant enzymes in the epididymis of the testis of the male goats (Abdollahi et al., 2004). Inhibition of AChE activity was another biochemical parameter to detect the severity of chronic exposure of humans and animals to the pesticides.

Most of the agricultural farmers In Guntur district of Andhra Pradesh have taken up cotton farming as it is considered to be the most important commercial crop. During the cultivation of cotton, the farmers use pesticides re-peatedly for the control of cotton pests to obtain better yield. Therefore, the present study was undertaken to assess the association between the biochemical para-meters viz., acetylcholinesterace (Ache), lipid peroxiation, catalse activity, reduced glutathione (GSH), aspertate aminotransferase (AST), Alanine aminotransferase (ALT), Creatinine and Urea among the cotton growing farmers of Guntur district of Andhra Pradesh, South India who were occupationally exposed to pesticide spraying. This study focused on various signs and symptoms among these agricultural farmers on exposure to organophosphate pesticides.

MATERIALS AND METHODS

Study area

A cross sectional study was carried out in Guntur district, Andhra Pradesh, India, targeting mainly the agricultural community where the cultivation of cotton crop is predominant and the use of pesticides is very high as compared to the other districts of Andhra Pradesh. The district was divided into three administrative revenue divisions, out of which two divisions were selected randomly. There were about 571 villages in both the divisions and around 1000 inhabitants were residing in each village. Since large numbers of farmers are exposed to pesticides, 5% (24 villages) of the villages were randomly selected from each of the division. Exposed (n=300) and unexposed (n=300) subjects were selected out of the randomly selected 1000 households. Age, gender and socio economically matched subjects who were not exposed to any form of pesticides but were engaged in other professional activities such as tailoring, carpentry, hotel vending, etc., were selected as control subjects (Unexposed) from the same village. Ethical clearance was obtained from the Institutional Ethical Committee to include human subjects with prior informed consent and also for drawing of blood samples (Agricultural farmers and non-agricultural subjects) in the study. A structured questionnaire was developed and pre-tested in consultation with an expert medical epidemiologist from the National Nutrition Monitoring Bureau (NNMB) of the National Institute of Nutrition to assess the exposure and the details about muscarinic, nicotinic, central nervous system and general signs and symptoms as self reported by the farmers during the spraying activities. This questionnaire was administered by trained research assistants to elicit the socio-demographic information on land ownership, types of pesticides used and duration of exposure.

Determination of erythrocyte AchE, vitamin E and TBARS levels

Blood samples were drawn from each of the exposed (n=300) and unexposed subjects (n=300). About 10ml (each) of blood sample from both exposed and unexposed subjects was collected in EDTA (ethylenediamine tetraacetic acid) vials (used for reduced glutathione and AchE tests) and heparin (for the remaining tests) vials, which were transported to the laboratory within 24 hours in frozen condition using gel packs. Erythrocyte AchE levels were measured immediately by Ellman et al. (1961).

The samples were centrifuged at 2000 rpm for 15 min and the plasma and leukocyte layers were separated and used for other assays. The erythrocyte sediment in the tubes was washed thrice with 0.9% saline for carrying out the catalase and glutathione (GSH) assays. The levels of vitamin E (α-tocopherol) and thiobarbituric acid reactive substances (TBARS) were quantified in plasma. All the samples were stored at -80℃ until assayed. Lipid peroxidation was quantified by plasma Thiobarbituric Acid Reactive Substances by spectrophotometric method (Bhat, 1991). Vitamin E was determined using high performance liquid chromatography (HPLC) method (Allard et al. 1998).

Determination of catalase, ALT, AST activities and GSH level

Catalase activity was determined spectrophotometrically and reduced glutathione was determined fluorimetrically by O-phthalaldehyde (OPT) method (Aebi et al. 1974). Liver function tests (serum ALT and AST) and kidney function tests (levels of creatinine and urea) were assayed using the automated kits (supplied by M/s. Biosystems India Ltd).

Statistical analysis

Descriptive statistics, and cross tabulations were computed. Two tailed student's 't' test was used to compare the mean difference between exposed and unexposed subjects for biochemical parameters such as AchE, antioxidant levels, lipid peroxidation parameters, and liver and kidney function tests. The data were log transformed to stabilize the variation. Based on the response to the questionnaire the exposed subjects were categorized into three tertiles depending on the number of years of exposure (<12 years, between 12-20 years and >20 years) and the groups were related to AchE levels using one-way analysis of variance. A post hoc analysis of Least Significant Difference (LSD) was used to assess the significance between the groups.

Table 1. Profile of the study subjects.

Parameter	Exposed (n = 300)	Un- Exposed (n = 300)
Age (yrs)	37.8 (10.8)	37.3 (12.3)
Average (yrs) Smoking habit (any time)	42% (n = 100)	44% (n = 110)
Daily Smokers	60% (n = 100)	74%
Average smoking (per day)	7.0 (10.64)	9.1 (8.9)
Average tobacco chewing	6.9 (7.3)	4.7 (4.5)
Alcohol consumption (ml/ day)	195.8 (221.2)	196 (163.6)

Values are expressed as mean (standard deviation); P > 0.05.

RESULTS

Majority (70%) of the exposed subjects were engaged in agriculture on leased land for the cultivation of cotton crops, 12.1% were agricultural labourers and 17.9% were other labourers. The mean extent of land holding of the farmers was 5.9 acres. The mean age of the exposed subjects was 37.8 ± 10.8, while that of the unexposed subjects was 37.3 ± 12.2 years. The prevalence of smoking in exposed and unexposed subjects was 42% and 44%, respectively. The difference in the habit of smoking between the exposed and unexposed subjects was not statistically significant (P>0.05) (Table 1).

The survey indicated that majority (80%) of the farmers (exposed) used mixture of organophosphate pesticides. The most widely used pesticides were acephate (O, S-dimethyl acetylphosphoramidothioate, $C_4H_{10}NO_3PS$), chlorpyriphos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate, $C_9H_{11}C_{l3}NO_3PS$), monocrotophos (36% E.C.) (dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate,$C_7H_{14}NO_5P$) (supplied by M/s. Bayer India Pvt. Ltd., M/s. Dhanuka Pvt. Ltd.) and synthetic pyrethroids such as ((RS) -α-cyano-3-phenoxybenzyl (1RS,3RS; 1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate, $C_{22}H_{19}C_{12}NO_3$), alphamethrin (racemate comprising (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, $C_{22}H_{19}C_{12}NO_3$) (supplied by M/s. Rallis India Pvt. Ltd.,) for their pest control operations. About 20% of the farmers used combination of commercially available pesticides. The farmers were occupationally involved in pesticide spraying activities on an average of 6 hours per day for a period of about 4 months in a year ranging from 11-20 years or more. About 78% of the farmers did not use any protective devices while handling the pesticides.

The exposed subjects reported several symptoms immediately after pesticide spraying. The major symptoms were of general nature like chest pain, red eyes, skin itching and tingling sensation while nicotinic symptoms viz., weakness, muscle twitching and paralysis. Similarly, some of the exposed subjects reported muscarinic symptoms like, salivation, nausea, vomiting, abdominal pain, diarrhoea, running nose, fainting, burning sensation, lacrimation in eyes, blurred vision and central nervous system symptoms such as headache, dizziness, confusion, convulsions, coma, difficulty in breathing and sleepiness. Most of the symptoms were observed in the second tertile as compared to the first and third tertiles (Figs 1-4). The symptoms related to muscarnic and nicotinic exhibited by the exposed subjects were not observed in the unexposed subjects, as they were not exposed to pesticide spraying activities at any given point of time which shows the severity and consequent health effects. However, general symptoms such as giddiness, nausea, headache, abdominal pain observed in the exposed population were also recorded among un-exposed population.

The inhibition of AchE activity in RBC was significantly high (P<0.05) in the exposed subjects (1674.2 ± 113.8) as compared to the unexposed subjects (2088.6 ± 106.2). However, the results of the AchE levels in the exposed subjects when distributed in three tertiles indicated that there was significant alterations (P<0.05) in the AchE levels in the third tertile exposed subjects (>20 years) when compared to the first tertile [subjects exposed to <12 years] (Table 2). The oxidative stress parameters such as catalase was significantly high (P<0.01) by 0.023 units per mg protein in the exposed subjects (0.058 ±0.001) when compared to unexposed subjects (0.035 ± 0.001) (Fig 5). There were significant (P<0.01) alterations in antioxidant levels viz., reduced glutathione (0.0405 ± 0.0015), and α-tocopherol (7.6 ±0.22) (P<0.01) in exposed subjects (Fig 6) when compared to unexposed subjects. Similarly, lipid peroxidation in terms of thiobar-bituric acid reactive substance (2.68 ± 0.056) was also significantly altered (P<0.01) (Fig 2).

The liver function test, alanine aminotransferase (ALT) and kidney function tests (creatinine and urea levels) were not significantly different between the unexposed and exposed subjects, respectively. However, a significant increase in the levels of aspartate aminotransferase

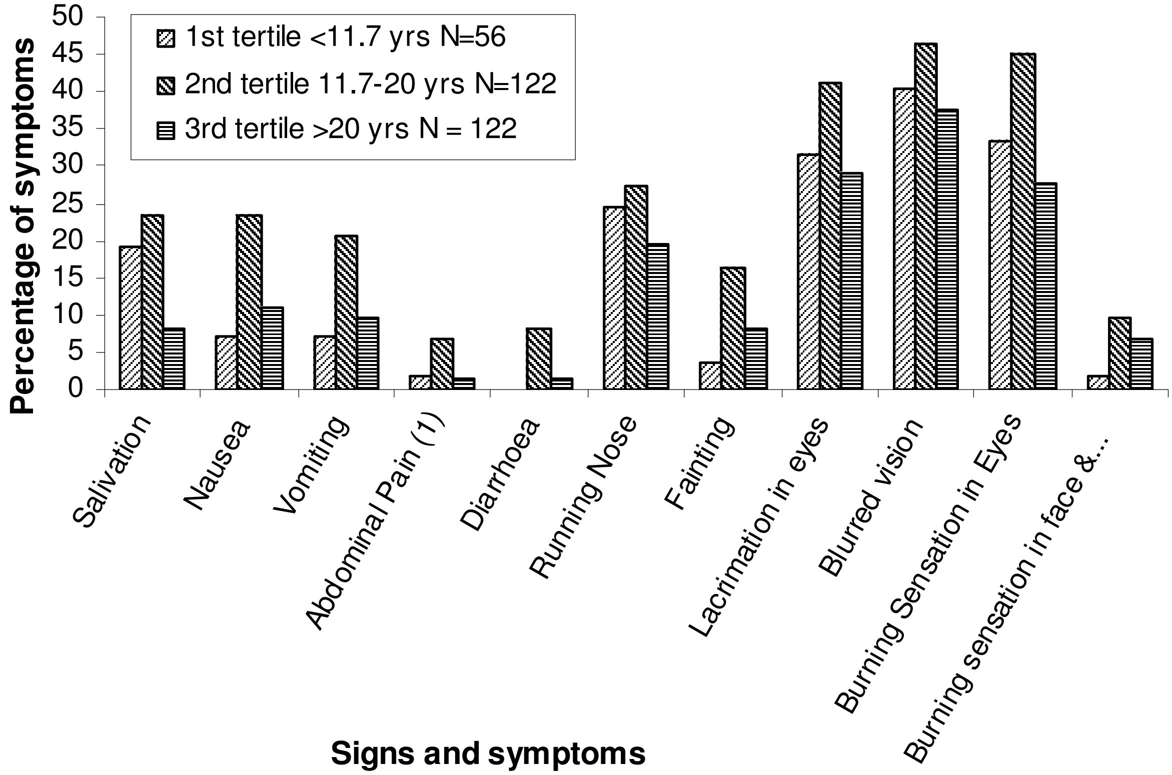

Figure 1. Muscarinic signs and symptoms (%) of the exposed subjects.

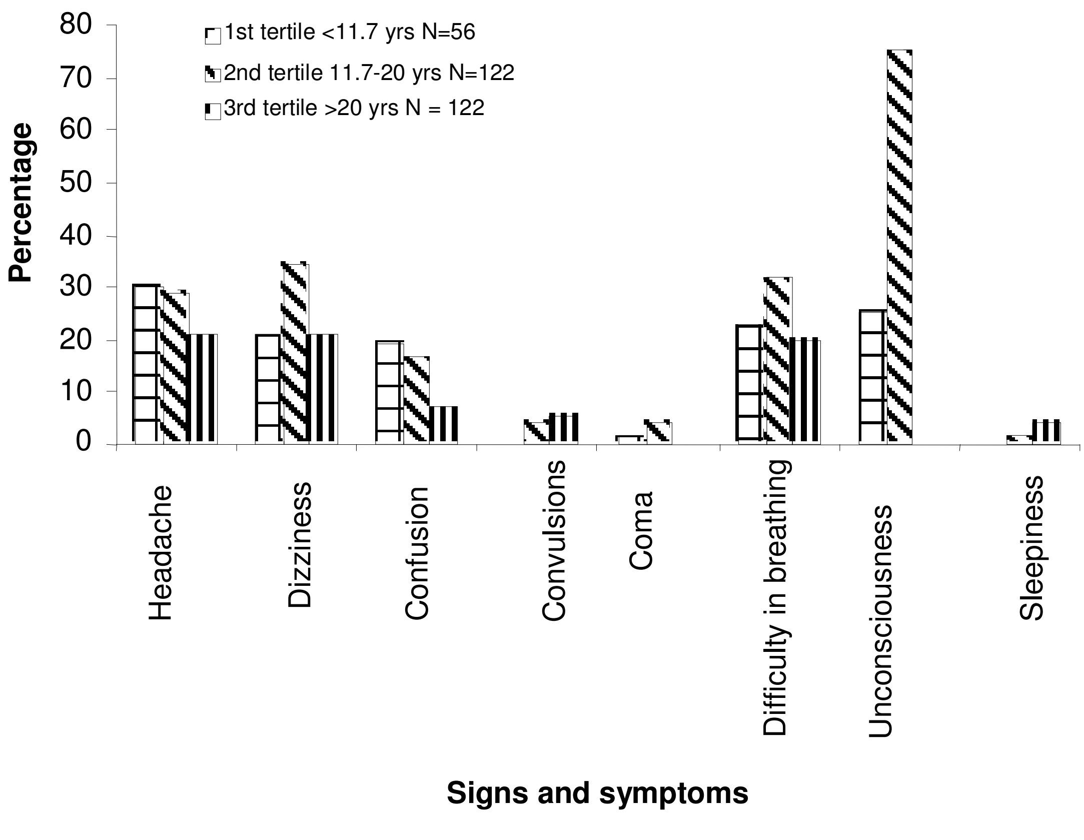

Figure 2. Signs and symptoms (%) of the central nervous system.

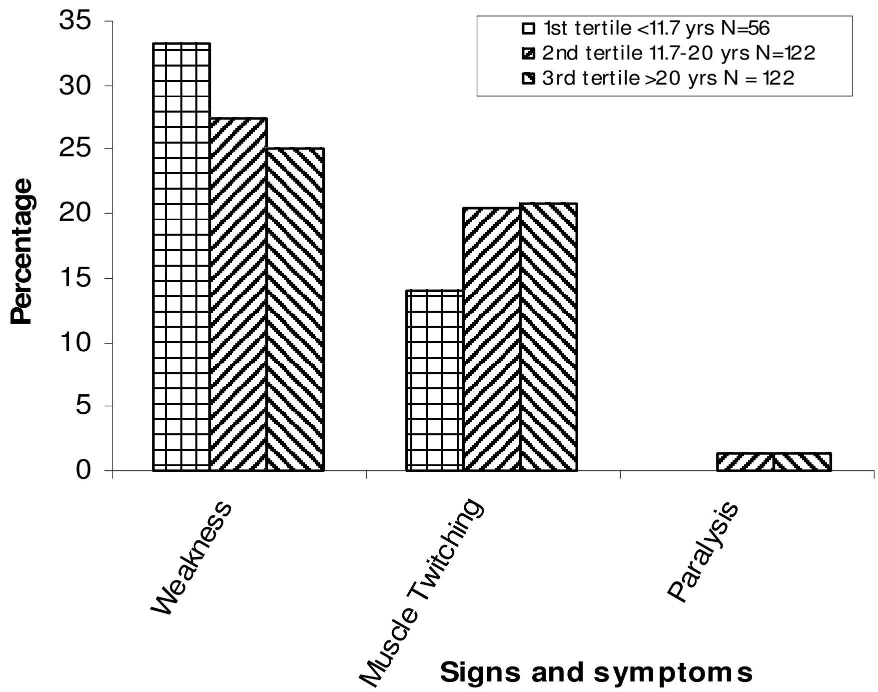

Figure 3. Nicotinic signs and symptoms (%) of the exposed subjects.

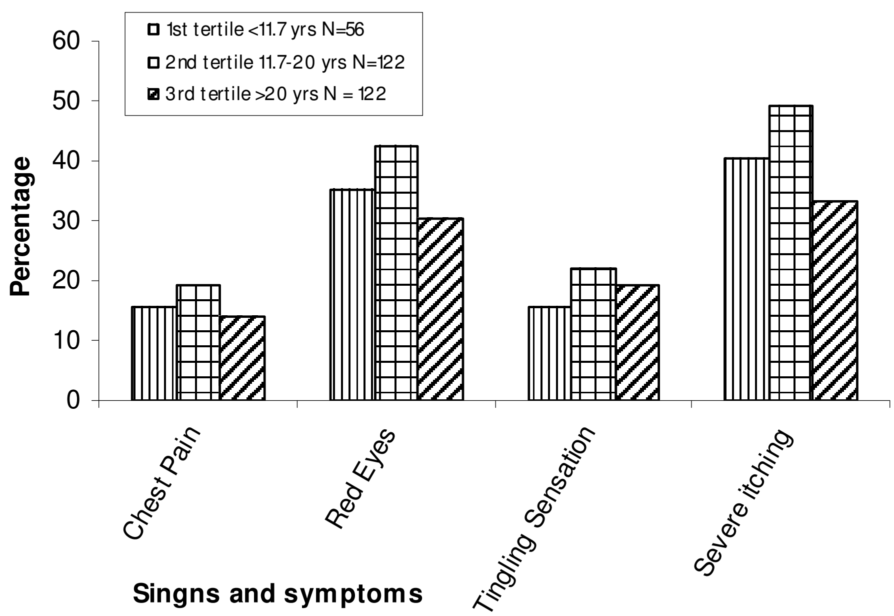

Figure 4. General signs and symptoms (%) of the exposed subjects.

(AST) and creatinine were observed in exposed subjects when compared to unexposed subjects (Table 3).

DISCUSSION

The frequency of applications of pesticides was found to be high in cotton crop in Guntur district of Andhra Pradesh and the cotton growing farmers were repeatedly exposed to different types of pesticides among which organophosphate pesticides (Ops) was predominant. The study results indicated that most of the symptoms were observed n the second tertile (11.7 to 20 years). Studies reported elsewhere showed a wide variety of

Table 2. AchE levels in various degree of exposure to pesticides (U/L).

Number of years of exposure	Erythrocyte AchE	95% CI
< 12 (N = 102)	1916.2 (282.95)[a]	1351.1 – 2481.2
12 - 20 (N = 130)	1786.0 (173.86)[ab]	1439.3 – 2132.8
> 20 (N = 68)	1317. 4 (81.34)[b]	1155.0 – 1479.8

Values of erythrocyte AchE are expressed as mean (standard error). Different superscripts are statistically significant at P < 0.05 by Least Significant Difference Method (LSD)

Table 3. Liver and kidney function parameters in exposed and unexposed subjects.

Parameter	Group	N	Mean ± SE	95% CI
AST (IU/ml)	Exposed	224	22.79 ± 1.04*	20.75 - 24.83
	Unexposed	195	20.37 ± 0.57	19.3 - 21.5
ALT (IU/ml)	Exposed	224	16.59 ± 1.80	13.1 - 20.1
	Unexposed	195	13.29 ± 0.46	12.4 - 14.2
Creatinine (mg/dl)	Exposed	223	0.84 ± 0.029*	0.78 - 0.90
	Unexposed	220	0.75 ± 0.026	0.69 - 0.81
Urea (mg/dl)	Exposed	226	22.71 ± 0.91	20.9 - 24.5
	Unexposed	193	20.66 ± 0.95	- 24.6

*Significant at P < 0.05; SE = standard error; CI = class interval; N = total number of samples.

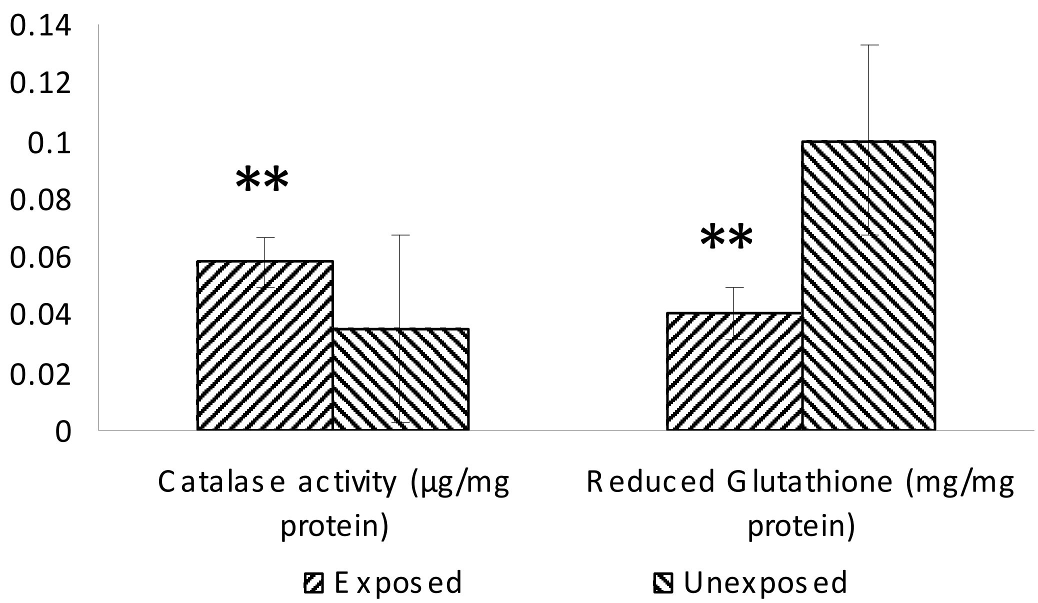

Figure 5. Catalase and reduced glutathione in the exposed and unexposed subjects. ** Significant at P<0.01.

signs and symptoms in the farmers exposed to organophosphate pesticides (Misra et al., 1985; Thamaz et al., 2003). These observations are in conformity with the present study. However, firm conclusions on neurophysiological effects of exposure to pesticides are difficult to draw, as information is meager.

Most of the symptoms expressed by the exposed subjects were self reported based on their experiences during or after spraying the pesticides.Therefore, it was difficult to discriminate and confirm the occurrence of a specific sign or symptom was only due to pesticide exposure or to other environmental factors like hot

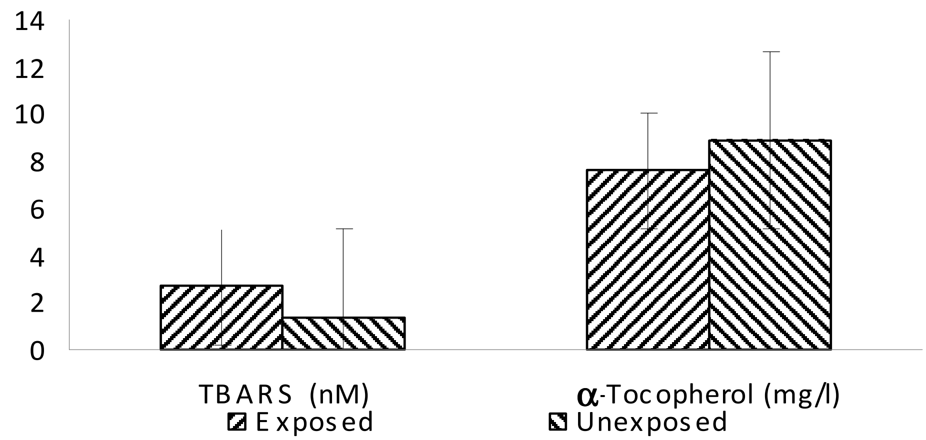

Figure 6. Plasma TBARS and α-tocopherol in the subjects.

weather or excessive humidity (Grace et al., 2000).

The organophosphate induced acute toxicity by inhibiting AchE in synapses and neuromuscular junctions which lead to the accumulation of acetylcholine at synapses, causing overstimulation of both central and peripheral nervous systems (Curwin et al., 2007; Sungur and Guven, 2001). The results of the present study indicates that the RBC AchE activity was reduced in farmers who were exposed to pesticides for more than 20 years suggesting that the duration of exposure is very important in altering AchE activity. It is well known that the AchE is a membrane bound enzyme and its determination in the blood is of great importance in diagnosis of poisoning cases caused by reversible/ irreversible inhibitors such as pesticides or certain drugs (Bukowska and Katarzya, 2006). The restoration of activity is dependent on the half-life of RBC's. Under normal conditions, the half life of RBC is around 120 days and the symptoms are expected to be within this period, provided the population is not occupationally exposed to the pesticide application (Storm et al. 2000). Decrease in the AchE activity in the exposed cotton farming community may be due to a delay in the restoration of normal RBCs which was obviously due to sub chronic/chronic occupational exposure to pesticides, since no other exposure to environmental contaminants are known to lower erythrocyte cholinesterase activity (Smit et al. 2003). AchE alterations are the indicators of the potential adverse neurotoxic effects of organophosphates which were observed both in human and animal studies. Exposure to pesticides not only decreased the AchE activity but also altered antioxidant and lipid peroxidation parameters in the farmers occupationally exposed to pesticides. (Prakasam et al., 2001; Sulak et al., 2005; Gokalp et al., 2005; Ogutcu et al, 2006). The increase in plasma TBARS in the exposed subjects was also accompanied by stimulation of catalase activity as a compensatory mechanism. The antioxidant levels such as reduced glutathione and Vitamin E were also decreased in the farmers exposed to pesticides in the present study and are suggestive of increased utilization of antioxidants to scavenge the reactive oxygen species in these subjects which is in agreement with earlier reports (Abdollahi et al., 2004; Dillard et al., 1978; Calzada and Bruckdorfer, 1997; López et al., 2007). When these results were analysed in terms of duration of exposure to pesticides there was a consistency observed in the long-term/ chronic mild exposure to pesticides, particularly organophosphate insecticides, suspected to cause adverse effects on the nervous system. Other studies reported wide variety of signs and symptoms in the farmers exposed to organophosphorus pesticides (Misra et al., 1985; Thamaz et al., 2003). These observations are in conformity in the present study. However, firm conclusions on neuropsychological effects of chronic exposure to pesticides are difficult to draw, as information in developing countries is meager.

The decrease in the AchE activity was maximal in the third tertile group, but these alterations in AchE levels did not increase the signs and symptoms among the exposed group as compared to first and second tertile groups. This could be attributed to the development of tolerance to the pesticide exposure in the study subjects as a result of long-term exposure to pesticides due to the repeated applications (Ciesielski, 1994). Studies elsewhere indicated, the decrease in the AchE activity in workers occupationally exposed to cholinesterase-inhibiting pesticides was proportional to the period of exposure (Misra et al., 1985). Further, these results indicated that the signs and symptoms reported in the exposed subjects did not have any significant difference among the first and second tertile groups suggesting that the exposure period had no influence. The symptoms such as fainting and giddiness reported by the subjects exposed to pesticides were all self reported and expressed as their experiences during or after exposure to pesticide spraying activities. Therefore, it was difficult to discriminate and confirm the occurrence of a specific sign or symptom to pesticide exposure or to other environmental factors like hot weather, excessive humidity (Grace et al., 2000). Most of the symptoms were based on their experiences during spraying or after the spraying and the classification was done accordingly (Poulsen et al. 2008). It was further observed that plasma ALT and Urea levels were not significantly different between the exposed and unexposed group. The significant difference in the AST and creatinine levels between the exposed and un-exposed subjects was well within the normal range and is just above the normal values. This difference in these groups could be due to the variation in the creatinine levels in the individuals which can be accepted in human subjects. Similar observations were also made by the earlier workers (Hernández et al., 2006, Azmi et al., 2006). It is well known that the decrease in antioxidants and increase in lipid peroxidation suggests oxidative stress has been a causative factor for many of the non-communicable degenerative diseases (Abdollahi et al., 2004).

It was evident from the present study that the mean age of the subjects was 37.8 ± 10.8 years, as many of the degenerative diseases will initiate at the later stage of life and the exposed subjects may become susceptible to non-communicable diseases at a later stage of life. Following up of the same subjects at later stages may provide some clinching evidence. Adequate steps should be initiated through Regional Farmers' Schools to impart pesticide safety education to the farming community involved in spraying activities. Usage of protective devices such as masks, gloves, aprons, shoes etc. should be encourage among farmers as it prevents from exposure to pesticides to a great extent.

ACKNOWLEDGEMENTS

The first author thanks the Department of Science and Technology, (DST–Grant No SR/WOS- A/LS-683/2003.), New Delhi, India, for providing the financial support to the study. The co-operation provided by Department of Agriculture, Govt. of Andhra Pradesh, Guntur and Dr. K. R. Sambasivarao, Prof. & Head, Dept. of Biotechnology, Nagarjuna University, Guntur A.P., India, is gratefully acknowledged.

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