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THE COMPARISON OF OXIDATIVE STRESS AND ACETYLCHOLINE STRASE PARAMETERS IN FARMERS' EXPOSED TO THE ORGANOPHOSPHATE PESTICIDE TO THE CONTROL GROUP

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ABSTRACT

The aim of this study was to compare toxicity of organophosphate (OP) pesticides in exposed farmers and control group in terms of oxidative stress, acetylcholinesterase. A causal-comparative cross sectional analysis was done in 40 horticulture farmers who have been exposed OPs in comparison to a control group containing 40 healthy subjects with the same age and sex and education level. Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), DNA damage, total antioxidant capacity (TAC), total thiol molecules (TTM), and acetylcholinesterase (AChE) activity were measured in the blood of subjects. Mental state evaluated by MMSE (Meni Mental state Examination) questionnaire and psychological disorder measured by Scl90-R questionnaire. Electroencephalography was done for assay of cortex activity. Clinical examination was done in order to record any abnormal sign or symptoms. Comparing with controls, the farmers showed higher blood levels of SOD and LPO increased while TAC decreased. The present findings indicate that oxidative stress and inhibition of AChE can be seen in chronically OP-exposed people. Use of supplementary antioxidants would be useful in the treatment of farmers.

Keywords: *Electroencephalogram, Organophosphate Pesticides, Antioxidant, Oxidative Stress, Acetyl Cholinesterase*

INTRODUCTION

Pesticides and Toxic

Toxin or Poison is a substance that has an internal or external origin and reason that cause imbalance of life in the person. A toxin and poison with internal origins means poisons that are produced as a result of natural biochemical reactions occur in body, such as creatinine, urea and ammonia. These toxins are also called endogenous toxin (Haji, 2006).

Exogenous toxins are poisons that enter the body from outside and generally fall into three categories:

1. Natural toxins that maybe of the plant, animal or microbial type. These toxins are analyzing in the science which is called of toxicology.
2. Chemical toxins which may be inorganic or organic.
3. Drug toxins, including pharmaceuticals and cosmetics materials (Haji, 2006).

Intoxication or poisoning is as the result of the, physiological and biochemical imbalance is person caused by a toxin. Intoxication is classified in different points of view. Their classification according to the symptoms time includes:

1. Supra Acute poisoning - the symptoms of poisoning would appear a few seconds to a few minutes after the toxin enters into body and can cause death within a few minutes to one hour.
2. Acute poisoning - poisoning symptoms appears within minutes to four hours and can cause death within hours or a days. Some poison and toxin can cause acute poisoning in a benign type, but it has a delayed effects. For example, if paracetamol orally taken into the body in small amounts, can result in a transient gastrointestinal disturbances, but a few weeks later, the patient will die with pulmonary fibrosis symptoms (Faiz, 2011).
3. Sub-acute poisoning - in this case, the toxin and poison will enter the body with lower amount and can cause to death after a few days to a few months.

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4. Chronic poisoning. In this case, the poison will enter the body several times and during a long time and the poisoning symptom may not appear until many years and then cause to the poisonous death. This type of poisoning may occur as a result of accumulation of toxins in the body. But sometimes, toxin accumulation in the body is not a problem but frequent toxic effects on the body would not let the waste be repaired and causes poisoning like bronchitis due to the stimulatory effect of SO₂.

On the other hand, poisonings can be classified into two categories, based on the origin of toxin: internal and external poisoning. In external type of poisoning, dysfunctioning in excretion organs of the body such as liver and kidney, leads to the accumulation of toxic substances such as uremia, while the toxin entering into the body causes the external poisoning (Ranjbar *et al.*, 2002).

Pests are live organisms which have economic and health losses for the human. The origin of pests may be Animal or vegetable. Pest with animal origin, including insects, snails and rodents and pests of vegetable origin are included fungi and weeds.

There are different ways to control pests such as: modification of the environment, mechanical control, mechanical, biological control and chemical control. Real and successful pest control occurs when a combination of different methods is applied. Pesticides are chemical substances that are used for pest control. These combinations are divided into insecticides, molluscicides, rodenticide, larvicide and fungicide and herbicides based on type of the pests. It should be noted that some of the material may be placed in several categories. For example, arsenic compounds are used as insecticides, rodenticides and herbicides (Atherton *et al.*, 2009).

Oxidative Stress and Free Radicals

Free radicals are molecules that are chemically very active, and producing these radicals is a natural reaction in the body's metabolism. Radicals will be removed from the body through a combination with an antioxidant. Free radicals, oxidative stress and antioxidant are the names which are often used when speaking of the mechanisms of disease. It seems that the metabolism of free radicals is a central and clear location in the mechanisms of many human diseases which appear to be unrelated. Oxidation reactions - Resuscitation (redox) are at the center of our metabolic machinery. Redox reactions include electron transfer or hydrogen atoms from one reactant to another.

Research Questions

1. Does the amount of oxidative stress parameters in farmers exposed to organophosphate pesticides is different from the control group?
2. Does the average amount of acetyl cholinesterase in farmers exposed to organophosphate pesticides is different from the control group?

MATERIALS AND METHODS

Research Population and Samples

The samples in this study were: a) farmers in the province of Kerman which has been exposed to the organophosphate pesticide in the first half of the year of 2014.

B) Healthy subjects which were matched with farmers' population in terms of age, sex, level of education, place of residence.

Instruments

Measuring Instrument of Mental Disorders (SCL-90), Symptom Checklist

SCL-90-R Questionnaire is one of the most common used instruments for psychiatric diagnosis. This questionnaire includes 90 questions for evaluating and assessment of mental symptom and disorder and is reported by a respondent. This questionnaire was introduced by Dragotis *et al.*, (1973) and was reviewed based on clinical experience and psychometric analysis, and the final version was prepared in 1976. Deragotis (1976) has reported the internal reliability of the test. The most correlation coefficients value was reported for the depression with 0.95 and the least was for psychosis with 0.77. Reliability was calculated on 94 psychiatric patients after a week of performing and the obtained correlation coefficients was between 0.78 and 0.90. The most correlation coefficient was reported for depression case with 0.73 and the lowest correlation for fear disease with 0.36 in the validity of the test.

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Each of the questions concludes from a 5 option range which shows Discomfort level from zero (none) to four "heavily". The questionnaire consists of 9 dimensions which are: (somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, aggression, phobia, paranoia and psychosis).

Measuring Oxidative Stress

Pars test kits were used to measure blood parameters. Electroencephalogram device was also used to EEG recording.

DTNB (2 di-thio nitro benzoic) was used for Evaluation of plasma thiol groups (colorimetric method HU). DTNB makes a yellow complex with these groups which have a maximum absorption at a wavelength of 412 nm (Hu and Dillard, 1994).

- Ferric Reducing Ability of plasma or FRAP was used to measure serum total antioxidant. This method is based on the plasma ability to restore ions of $Fe + 3$ (ferric) to $Fe + 2$ (Ferro) in the presence of a substance called TPTZ and complex $Fe + 2 - TPTZ$ which is a blue complex with the maximum absorption of 593 nm, which serum restoration power or plasma is measured by a spectrophotometer device through an increase in above complex concentration (Benzie *et al.*, 1999).

TBA method was used to assess lipid peroxidation. Different aldehydes would produce as the result of free radicals attacks to lipid like MDA (malon dialdehyde) which reacts with thio barbituric acid in acid PH and high temperature. The maximum of pink Complex absorption is 532 nm (Esterbeur and Cheeseman, 1990).

ELISA device, Gen5 models, made in America: ELISA Gen 5 model device and special kits for Cayman company was used to measure the activity of catalase enzyme, superoxide dismutase, glutathione peroxidase and Myeloperoxidase.

Procedure

Lipid peroxidation, catalase enzyme activity, superoxide dismutase, glutathione peroxidase, DNA damage, total antioxidant capacity, total thiol molecules and acetyl cholinesterase was measured in the blood of patients.

Brief questionnaire MMSE was used for the assessment of cognitive condition and psychiatric disorders questionnaire SCL-90-R to assess mental disorders and symptoms of the disease. Criteria to start the study includes: lack of a history of any type of drug, alcohol, smoking, and antioxidant and non-affected by specific diseases such as diabetes, cancer, and thyroid, cardiovascular and respiratory disorders.

Career of all the subjects will be more than two years. The subjects would attend in this study after being informed of the aim and the method and way of this research, orally and in a written form. All subjects in the study filled the questionnaire of of quality of life, psychological disorders and cognitive neuropsychology.

- Add 2.5 ml tri chloro acetic acid To 5.0 ml of plasma, and 20% more chloroethyl and placed it for 10 minutes at room temperature and then it will centrifuged in 3000 g for ten minutes.

- Upper liquid will be discarded and the remaining sediment will be washed with sulfuric acid 0.05

- 2.5 ml of sulfuric acid 0.05 M and 3 ml of TBA 0.2% is added to the sediment.

- It will place in iling water (100) for 30 minutes.

- 4 ml of n- butyl alcohol will be added and mixed well and then the sample is centrifuged for 10 min at g 3000, after cooling tubes.

- Separate the organic phase and the optical attraction and absorption will be read at 532 nm of wavelength.

- Assessment the result: standard curve was plotted for different concentrations of the standard solution (tetra Methoxypropane) based on the obtained absorption values.

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And the considered samples the TPARS values are calculated on the curve calculated according to this curve and are rated on microliter (Esterabeur and Cheeseman, 1990).

RESULTS AND DISCUSSION

Independent t-test was used to analyze the given questions.(Table 1). The results of this test are shown in Table 1.

Table 1: oxidative stress markers level and acetyl cholinesterase in the farmers and controls groups

acetyl cholinesterase (KU/L)	Pg /mL), glutathione peroxidase	8-hydroxy (Pg/mL)	Total thiol molecule (nmol/mg protein)	catalase (mol/mg protein)	superoxide dismutase (U/mg protein)	Total anti-oxidant (μ M)	(μ M)	Subject group
35/73 ± 4/89	30/33 ± 11/91	526/29± 113/24	11/35 ± 4/78	2/85 ± 1/4	0/99 ± 0/32	824/92± 198/72	135/01± 16/55	Control group
31/24 ± 7/11	30/91 ± 8/82	550/15± 71/5	13/43 ± 6/84	3/4 ± 1/84	1/12 ± 0/29	717/44± 198/1	145/28 ± 18/44	case
0/001	0/806	5800/	0/119	0/138	0/018	0/001	0/011	p Value

Data and information are based on the average and standard deviation. So, table 1 shows oxidative stress bio markers and acetyl cholinesterase. the average levels of superoxide dismutase enzyme activity ($p = 0 / 018$) and lipid peroxidation($p = / 011$) has showed a significant increase in farmer's to the control group and the concentration of total anti-oxidant $p = / 001$ has showed a significant decrease in the farmer's to the control group which is statistically significant and this indicates that there is a significant different in terms of oxidative stress in the farmer's and control group and since there is a more oxidative stress in the farmer's, so the response to question 1, is positive.

Total thiol groups, DNA damage and catalase level and values didn't show a significant difference in the farmer's to the control group.

Acetylcholine strase concentration was lower in farmers to the control group and this significance showed that there is a difference between two groups and since the concentration is lower in the farmers, so the answer to the second question is positive too.

Athreton *et al.*, (2009) through the study on Spanish farmers and comparison with the control group, has shown that, in July, which are more exposure to pesticides, Acetylcholine strase activity level is 30 % less than control group, while the level of DNA damage and oxidative stress is higher than the control group. And there is a positive relation between pesticides metabolites in blood and DNA damage, (Atherton, 2009).

Another study has shown that Acetylcholine strase activity in the spray has been controlled in comparison with control group, significantly (Singh *et al.*, 2006; Rastvegi *et al.*, 2009). While Salovii *et al.*, (2003) study has shown that Acetylcholine strase activity was normal in all group and there was no difference between the spraying and non-spraying period and time (Salovi *et al.*, 2003).

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Conclusion

The results in this study showed that there is a significant difference in terms of the oxidative between exposed farmers and control group and since, the level of oxidative stress is more in farmers, So the answer to research question is one yes or positive. the amount and level of total thiol, DNA damage and catalase group didn't show a meaningful difference in exposed group compared to the control group.

Acetyl cholinesterase concentration ($p = 0.001$), was lower in farmers to the control group. This suggests that there was a significant difference in terms of Acetyl cholinesterase between farmers and control groups. So the answer to this question was also positive.

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