Egyptian Journal of Pure and Applied Science

Neurotransmitters and Antioxidants in Organophosphorous Insecticide Poisoned Patients

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A R T I C L E I N F O

Article history:
Received 19 September 2017
Accepted 15 March 2018

Keywords:
Organophosphates;
Neurotransmitters;
Antioxidants.

A B S T R A C T

Organophosphates (OPs) have been widely used in agriculture and domestic applications. They are lipophilic compounds which has caused significant human health effect. The objective of this study is to identify the correlation between antioxidants and neurotransmitters which can lead to oxidative stress in OPs poisoning patients. This study was conducted on 60 adult patients of both sexes who were admitted with acute OPs poisoning to the poison control center, Ain Shams University Hospitals. In addition to 10 healthy volunteers, all patients and control were subjected to the following Parameters: Pseudocholinesterase (PChE), Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Zink (Zn), Vitamin E (Vit E), Vitamin C (Vit C), Malonalaldehyde (MDA), Nitric Oxide (NO), Norepinephrine (NE), Dopamine (DA), Serotonin (5-HT), Aminotransferase (ALT, AST), Albumin (Alb.), Urea, Creatinine (Creat.). Results showed that OPs poisoning induces oxidative stress leading to generation of free radicals, as evidenced by the elevated MDA, DA, 5-HT levels, and alterations in antioxidants and scavengers of oxygen free radicals, as evidenced by the reduced levels of GPx and CAT activity. In addition to impairment of kidney and liver, as evidenced by the abnormal levels of Urea, Creat, ALT, AST, Alb.

Introduction

Organophosphates poisoning is a major health problem all over the world. Insecticide poisonings remain a serious public health problem worldwide [1]. Organophosphates are used extensively throughout the world. The main sources of contamination for humans are dietary ingestion and occupational exposures. The major concerns related to OP exposure are delayed effects following high level exposures as well as the impact of low-level exposures during the lifespan which are suggested to be a risk factor for nervous system chronic diseases. Use of OPs came as an alternative to chlorinated hydrocarbons due to their easy degradability.

Although these xenobiotics degrade under natural condition, their residues have been detected in soil, sediments, and water due to their non-regulated usage practice. The over-reliance on pesticides has not only threatened our environment but contaminations of OP residues have been also detected in certain agricultural products like tea, sugars, vegetables, and fruits [2]. However, their extensive use has polluted the environment and increased human vulnerability to various chronic diseases. Organophosphates exposure causes genetic and epigenetic modifications, endocrine disruption, mitochondrial dysfunction and oxidative stress [3]. Both high- and low-level exposures may have a particularly high impact in population subgroups such as aged or genetically vulnerable populations. Apart from the principle action of OPs which involves inhibition of the AChE enzyme, several molecular targets, such as hormones; neurotransmitters; neurotrophic factors; enzymes related to the metabolism of beta amyloid protein as well as inflammatory changes have been identified for OP compounds [4]. Organophosphates inhibit both ChE & PChE enzymatic activity and leads to cholinergic signs and symptoms [5]. Patients die mostly from respiratory failure and lung injury, although there is variability in the clinical symptoms and signs depending on nature of compounds, amount consumed, severity, time gap between exposure, and presentation in the hospital [6]. Uses of antioxidants

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have been recommended as adjunct treatment to OP poisoning. The biochemical estimations of oxidative stress parameters revealed oxidative stress in many OP induced subjects [7].

This study aims to identify the correlation between antioxidants status and neurotransmitters in OPs poisoning patients.

Subjects and methods

The study was conducted on a total number of 70 individuals divided into two groups. Control group, and Patient group (60 adult acute patients); (27 males, 33 females) aged 17-50 years. Patients group were divided according to the level of PChE into mild, moderate and severe groups (20 patients per each group). All patients were selected from the poison control center, Ain Shams University Hospitals during the period of 6 months from (Feb. 2014) to (July 2014), in addition to control group; 10 healthy volunteers without any complications (5 males, 5 females) aged 18-52 years. The initial screening of OP poisoning cases for the study was selected with low level of PChE as a reference biomarker for clinical score described by Peradenya Organophosphorous Poisoning (POP) scale .

Ten ml of Venous blood samples were collected from study participants in a sterile heparin containing vials. Samples were immediately centrifuged at 3000 rpm for 15 minutes to separate the plasma. The cells were washed with normal saline and RBC's were subjected to lysis. Pseudocholinesterase was estimated according to Young [8], Using a kit purchased from BEN-Biochemical Enterprise, Italy. Total Antioxidant Capacity (TAC) activity was determined in plasma according to Koracevic et al. [9], using a kit purchased from Biodiagnostic, Egypt.

Catalase (CAT) activity was determined according to Aebi [10], Superoxide dismutase (SOD) activity was determined according to Nishikim et al. [11], Gultathione peroxidase (GPX) activity was determined according to Paglia & Valentine [12], using kits purchased from Biodiagnostic, Egypt

Ascorbic acid (Vit C) activity was determined according to Harris & Ray [13], using a kit purchased from Biodiagnostic, Egypt. Vitamin E was performed with a HPLC method according to Gliszczyńska-Świgło & Sikorska [14], using a Symmetry C18 column (150 mm×3.9 mm, 5 µm) fitted with a µBondapak C18 cartridge guard column (all from Waters, Millford, Ma, USA). Zinc activity was determined according to Hayakawa & Jap [15], using a kit purchased from Biodiagnostic, Egypt.

Malondialdehyde (MDA) was estimated according to Kei [16], Nitric Oxide (NO) activity was determined according to Ingram et al. [17], using kits purchased from Biodiagnostic, Egypt. Catecholamines (NE, DA and 5-HT) were determined by HPLC method according to Pagel et al. [18], The HPLC system consisted of quaternary pump; a column oven, Rheodine injector and 20µl loop, UV variable wavelength detector. The report and chromatogram taken from data acquisition program purchased from chemstation. 4.6 mm 5µ C18, purchased from Phenomenex, USA.

Liver functions (ALT & AST) were determined according to Young [19], Albumin concentration was determined according to Tietz [20], Using kits purchased from Spinreact, Spain.

Kidney functions (Creatinine & Urea) were determined according to Young [8], using kits purchased by BioSystem, UK.

In the present study, all data were statistically analyzed by SPSS software version (21.0). Results were expressed as mean ± standard deviation of the mean. Significant values were $P < 0.05$ and highly significant at $P < 0.01$.

Results

Results presented in the following tables.

Table 1: Effect of OP poisoning on PChE, and antioxidants activity for mild, moderate and severe groups compared to control.

Group	Mean ±S.D.			
	Control	**Mild**	**Moderate**	**Severe**
PChE (U/L)	8519 ±11	2254 ±16*	1090 ±51*	408 ±17*
TAC (mM/l)	0.9 ±0.3	1.7 ±0.1*	1.6 ± 0.1*	1.6 ±0.1*
SOD (U/ml)	249 ±41	251 ±35	252 ±35	264±17
CAT (U/L)	488 ±36.7	586 ±38*	429 ±63**	375 ±75*
GPx (mU/ml)	409 ±27	292 ± 44*	314 ±59*	292±43*
Zn (µg/dl)	13 ±4.8	32 ±6.6*	29 ±3.9*	29 ±4*
Vit. E (mg/dl)	1.0 ±0.3	1.2 ±0.2	1.1 ±0.4	0.9 ±0.4
Vit. C (mg/l)	31 ±5.5	68 ±118	110 ±114	39 ±34

* Highly significant against control

** Significant against control

Table 2: Effect of OP poisoning on Lipid peroxidation, and neurotransmitters activity for mild, moderate, and severe group compared to control.

Group	Mean ±S.D.			
	Control	**Mild**	**Moderate**	**Severe**
MDA (nmol/ml)	3.2 ± 2	38 ±14*	12 ±6*	5 ±3
NO (µmol/L)	1166 ±28	821 ±88*	730 ±67*	109 ±38*
NE (µg/ml)	0.74 ± 0.1	0.75 ±0.2	0.72 ±0.09	0.7 ±0.07
DA (µg/ml)	0.34 ±0.1	0.5 ± 0.1*	0.5 ± 0.1*	0.4 ±0.1
5-HT (µg/ml)	0.27 ±0.02	0.32 ±0.06*	0.28 ±0.02	0.3 ±0.06

* Highly significant against control

Table 3: Effect of OP poisoning on Liver &Kidney Function for mild, moderate, and severe group compared to control.

Group	Mean ±S.D.			
	Control	**Mild**	**Moderate**	**Severe**
ALT (U/L)	11 ±5	35 ±18*	37 ±6*	41 ±6*
AST (U/L)	13 ±7	35±7*	37 ±7*	40 ±5*
Alb (g/dl)	4.3 ±0.5	3.4 ±0.4*	3.2 ±0.5*	3 ±0.4*
Urea (mg/dl)	27 ±9	33 ±8	31±8	42 ±14*
Creatinine (mg/dl)	0.9 ±0.1	1.2 ±0.6	1.15±0.1*	1.2 ±0.3**

* Highly significant against control
** Significant against control

Discussion

In our study, PChE activity was inhibited with increasing OP poisoning severity, PChE showed significant decrease all through mild, moderate, and severe groups against control. Our study agreed with Ganesan and Moorthy [21]; who stated that PChE in plasma is more sensitive than AChE to inhibition by a number of compounds and is depressed well below the normal range of 60% before any symptoms due to systemic anticholinesterase intoxication is evident, so low PChE activity can be taken as good diagnostic test for OP poisoning. In addition, Mishra et al., [22] reported a significant decrease in both PChE and TChE activities in acute OP poisoning patients resulting in the buildup of AChE at the cholinergic synapses resulting in repeated electrical firing causing the symptoms of OP toxicity, where the extent to which ChE are inhibited depend upon the rate at which the OP pesticide is activated.

Total antioxidant capacity (TAC) in mild moderate, and severe groups was significantly higher than their healthy control. Our results disagreed with Hundekari et al., [30] who reported significantly decreased plasma TAC as compared to control, this indicates that the OPs pesticides altered antioxidants status may be caused by more generation of free radicals.

The antioxidant enzyme SOD in mild moderate, and severe groups was insignificantly higher than their healthy control. The acute poisoning lead to increased activity of SOD. Our results are in accordance with Sidhu et al., Mishra et al., Hundekari et al., Tripathi et al, [27, 28, 30, 31], they reported elevated SOD activity in OPs poisoning patients. The increased activity of SOD seen in the poisoning cases coupled with an increase in MDA level suggests an insufficient antioxidant defense, SOD and AChE lacked any correlation at the low exposure period, very likely because of the absence of any relevant toxic effects due to a lower exposure to pesticides [28]. SOD activity was reduced in platelets and polymorphonuclear leukocytes (PMNs) but increased in the plasma [31]. In the other hand, these results disagreed with López et al., [33] they reported that exposure to pesticides was strongly and inversely associated to the erythrocyte enzymes at both high and low exposure periods of the growing season assessed. Catalase in mild group was significantly higher than their healthy control, while it significantly decreased again in both of moderate and severe group compared to healthy control. It was observed that the acute OP poisoning lead to significantly decrease activity of CAT in moderate and severe states. Our study agreed with Tripathi et al, Umosen et al., and Kumar et al. [31, 32, 34]. The significant increase in SOD but decrease in CAT activity in the plasma could be due to an increase in the level of hydrogen peroxide owing to too much conversion from superoxide. Low activity of CAT in the plasma could not scavenge hydrogen peroxide, thereby inducing oxidative stress [34]. CAT activity was increased in platelets; however, it was reduced in the plasma [31].

On the other hand, these results disagreed with Sidhu et al., Mishra et al., and Hundekari et al., [27, 28, 30]. CAT in untreated pesticide sprayers were significantly affected. The increased activity of CAT seen in the poisoning cases coupled with an increase in MDA level suggests an insufficient antioxidant defense, it is an enzyme that transforms hydrogen peroxide into hydrogen and oxygen, plays an antioxidant role and its activity increasing in acute poisoning submitted to oxidative stress [28]. Glutathione peroxidase in mild, moderate and severe groups were significantly lower than their healthy control. GPx were affected by OP poisoning, where the acute poisoning lead to decrease activity of GPx. Our study agreed with Sidhu et al., Ahmed et al., and Omurtag et al., [27, 29, 35], and disagreed with Hundekari et al., [30], who reported that increased GPx level protect mammalian cells against oxidative damage.

The antioxidant mineral Zinc in mild, moderate, and severe groups was significantly higher than their healthy control. These results disagreed with Mansour & Mossa, and Goel et al., [25, 26] they stated that Oral Zn treatment to the chlorpyrifos treated animals significantly improved the activity of lipid peroxidation, SOD, CAT enzymes, as well as the total leukocyte, neutrophil and lymphocyte counts, in the rats treated with chlorpyrifos.

Vitamin C in mild, moderate, and severe groups was insignificantly higher than their healthy control. Vitamin E in mild, and moderate groups were insignificantly higher than their healthy control, while it insignificantly decreased in severe group. Our results disagreed with Zhou et al., [23] who reported decreased activity of Vit E & Vit C at acute OP poisoning. El-Shenawy et al., [24] reported that the treatment with Vit E after OPs toxicity can reduce lipid peroxidation and liver function.

In our point of view the elevation of vitamins in the study may reflect the environmental changes, especially in the small village. High levels of Zn can normalize the raised levels of lipid peroxidation to within normal limits. The elevation in Zn & TAC levels with elevation of MDA suggests an insufficient antioxidant defense, where the exposure to free radicals should exceed the protective capacity of the antioxidant defense system. This insufficient antioxidant defense decreased in the severe state due to the insignificant increase of MDA in severe group.

The oxidative stress biomarker; MDA in mild, moderate groups was significantly higher than their healthy control, while it insignificantly increased in severe group. This increase in lipid peroxidation as reflected by elevated levels of MDA indicates oxidative stress. Our results are in agreement with Sidhu et al., Mishra et al., Ahmed et al., [27–29], they also found higher MDA level in acute OP poisoned patients.

Nitric oxide in mild, moderate, and severe groups were significantly lower than their healthy control, where NO is utilized with PChE compensation, in addition to individual variation. This suggests an insufficient antioxidant defense with the increase in the antioxidant system. Our study disagreed with Tripathi et al, and Kumar et al. [31, 34], they reported increased activity of NO. Cypermethrin increased nitrite content in the plasma, platelets which suggest that it induces toxicity in the peripheral system that could possibly be correlated with oxidative stress indicators.

The neurotransmitter NE; in mild, moderate and severe groups was insignificantly lower than their healthy control. NE were affected by OP poisoning, inhibition activity of NE in acute OP poisoning of moderate and severe states was observed. These results in accordance with Stallones and Beseler [36], they reported significantly decreased NE levels. The neurotransmitter DA in mild, and moderate groups were significantly higher than their healthy control, while it insignificantly increased in severe group. Exposure to OP poisoning showed elevated DA activity in the study. These results in agreement with Ahmed et al., [29], who reported increased activity of DA concentration. Our study disagreed with Tripathi et al., and Rodríguez et al., [31, 37], they reported reduced activity of DA level. The pyrethroid cypermethrin decreased DA content by altering the status of oxidative stress indicators and antioxidant defense system; this mechanism of oxidative stress might be of value for predicting the nigrostriatal dopaminergic neurodegeneration and the nigrostriatal toxicity related to neurological disorders [37].

The neurotransmitter 5-HT in mild group was significantly higher than their healthy control, and insignificantly increased in both of moderate and severe groups. Exposure to OP poisoning showed elevated 5-HT activity in the study. Our study agreed with Judge et al., [38] . In the other hand these results disagreed with Rodríguez et al., [37], who reported reduced activity of 5-HT level, where Serotoninergic and dopaminergic neurotransmission is affected by exposure to cyfluthrin and may contribute to the overall spectrum of neurotoxicity caused by this pyrethroid.

Liver enzymes ALT & AST in mild, moderate and severe groups were significantly higher than their healthy control. Increased activity of ALT & AST in the study was observed. Our results agreed with Ganesan and Moorthy, Sidhu et al., and Raghu et al., [21, 27, 39], they reported a significant elevation in liver enzymes. The elevated liver enzymes due to midzonal necrosis are associated with acute intoxication. Albumin in mild, moderate, and severe groups was significantly lower than their healthy control. Our study showed a significant inhibition in Alb. concentration in liver due to exposure to OP poisoning.

Concerning the kidney function tests; Urea showed insignificant increase in mild, and moderate groups compared to the healthy group, while it showed a significant increase in severe group. Creatinine showed insignificant increase in mild group, while it showed a significant increase in moderate, and severe groups. In our study; exposure to OP poisoning showed increased activity of the Kidney Function Urea & Creatinine. These results agreed with Ganesan and Moorthy, Raghu et al., and Cavari et al., [21, 38, 39], they reported elevation of Kidney Function in OP poisoning. The symptoms, abnormal enzymes and elevated renal parameters are associated with acute intoxication [40].

Conclusion

Our findings indicated that OP poisoning patients were under oxidative stress condition, where OP poisoning caused inhibition of PChE, leading to generation of free radicals, and alterations in antioxidants.

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