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Original Article

Correlation of oxidative stress and antioxidant status with cholinesterase in organophosphorus poisoning cases

Vinutha Bhat[1], Thejesh Srinivas[1] and Shankar M. Bakkannavar[2,*]

[1]Department of Biochemistry; [2]Department of Forensic Medicine and Toxicology,
Kasturba Medical College, Manipal Academy of Higher Education, Manipal -576104, India.

ABSTRACT

Pesticides are the substances used to kill pests which adversely affect human health or agricultural yield. They usually alter certain physiological or reproduction cycles of pest's life finally focusing to get rid of them. In the present study the levels of pseudo cholinesterase (PChE) in 53 organophosphorus (OP)-poisoned cases were obtained from the clinical laboratory. Serum was collected for the determination of malondialdehyde (MDA), ferric reducing ability of plasma (FRAP), glutathione-S-transferase (GST) and total thiols which were estimated spectrophotometrically. The results revealed the significant Correlation of PChE with FRAP, MDA, GST & total thiols, whereas β glucuronidase showed no significant correlation. Correlation of MDA with PChE, FRAP, GST and total thiols was significant, whereas β glucuronidase showed no significant correlation. MDA, used as a conventional biomarker for oxidative damage, showing moderate negative correlation with GST, FRAP and total thiols suggests that the oxidative damage occurred has been counter stroked by antioxidant pool majorly consisting of GST, FRAP and total thiols. There are various studies that support the same. Correlation of PChE with MDA showing highly significant negative correlation gives us evidence that severe oxidative damage (with elevated levels of MDA) occurs in OP poisoning cases due to generation of reactive oxygen species (ROS). The current study concludes that, OP poisoning along with collapsing cholinergic system at neural synapses accounts for the generation of reactive oxygen species which leads to neurological complications coupled with severe multiorgan breakdown.

KEYWORDS: oxidative stress, antioxidant status, cholinesterase, organophosphorus poisoning.

INTRODUCTION

Pesticides are substances made up of a single compound or mixtures of multiple compounds, basically with chemical or biological origin that are implemented by human community to kill or keep pests away such as mites, nematodes, rodents, molluscs and other organisms which adversely affect agricultural yield and/or human health. Pesticides usually alter or deteriorate certain physiological or reproduction cycles of pest's life, finally focusing to get rid of them [1].

The knowledge of pesticide is way too old. In 1000 B.C. sulphur was used in fumigating houses and around 900 B.C., Arsenic was used to control garden pests by Chinese. Pesticides have greatly contributed towards the upliftment of agricultural traditions by inhibiting pest action, resulting in good crop yield. Apart from agricultural areas, pesticides are also used in indoor activities. About 50% of world's population is indulged in agricultural practices [2]. Pesticides have major useful functions but parallelly they are reaching destinations other than targets. Toxicity may be found due to improper handling, disposal or by over use [3]. Pesticides have negative effect to human health as well as other animals due to its accumulation in body, food and water [4].

*Corresponding author : shankar.mb@manipal.edu

Pesticide poisoning has become a huge issue in developing countries [2, 3]. The main occupation of the people in rural regions of developing countries like India is agriculture. Easy availability of pesticides for this purpose is responsible for the increase in morbidity and mortality due to acute pesticide poisoning. According to World Health Organization (WHO) around 3 million pesticide poisoning cases occur annually in India [2, 5].

In India organophosphorus, carbamate and pyrethroids are the widely used pesticides [5, 6]. The chronic and acute exposure of pesticide is mainly through its residues in crops and vegetables [7]. Due to this, farmers and agricultural workers are more prone to chronic or acute pesticide poisoning. This may happen through various routes such as dermal contact, inhalation or ingestion [8]. Organophosphates are used in India for more than 50 years as it is easily available and cost efficient too [8]. Carbamates, the other class of pesticides which is extensively used in farming has the same effect as of organophosphates [9]. Accidental ingestion or inhalation is the major cause for pesticide poisoning in the agricultural oriented country like India. OPs being easily reachable is opted or preferred mostly in suicidal attempts and accidental ingestion by infants is too common in rural areas [2, 5, 10].

Organophosphates (OPs) are a class of organophosphorus compounds with the general structure $O{=}P(OR)_3$. They are the esters of phosphoric acid [11]. OPs usually associated with agricultural practices are extensively used as insecticides which repel or kill the insects (pests) by inhibiting their neuromuscular enzymes that adversely affect the nervous system leading to paralysis and death of pests [8, 12]. OPs strongly inhibit the paraoxonase (liver and serum enzyme) which in turn inhibits acetylcholinesterase (AChE) in synapse and on red blood cell (RBC) membrane and butyryl cholinesterase (BChE) in plasma to very little extent. Pesticides like OPs have anti-cholinesterase property where it inhibits and lowers the level of cholinesterase in body. Acute OP poisoning leads to nervous system breakdown and affects respiratory system. Other cholinesterases like pseudo cholinesterase (PChE), found in serum and cerebrospinal fluids (CSF), are also altered in case of pesticide poisoning. PChE being more predictive and easily estimated enzyme, it is used as the predominant biomarker in OP poisoning cases. The levels of PChE determines the severity of poisoning; lower its level, higher is the severity of poisoning [12, 13].

Pesticide toxicity especially OP poisoning may lead to oxidative damage of cells specifically leading to RBC membrane damage which ultimately leads to anaemia and this is mostly seen in women than men. OP poisoning may also adversely affect vital organs like liver, lungs and brain. The current study was conducted to show the degree of oxidative damage that occur due to OP poisoning as well as to look for anti-oxidant status in the same [14, 15]. Metabolites of urine and blood cholinesterase are used as the predominant biomarker for pesticide poisoning [13]. In recent decades, there is an increased interest in discovering new biomarkers that are thought to be more efficient. In this view, here, in OP-poisoned cases β-glucuronidase, a liver enzyme, is tested parallelly to look for its agreement with PChE (a conventional biomarker of OP toxicity) which gives an idea about its efficiency to be used as a better biomarker in OP toxicity. Also, oxidative stress and antioxidant status are correlated with cholinesterase level.

MATERIALS AND METHODS

After receiving approval from the Institutional Ethics Committee, (IEC 681/2017) the study was conducted at the Manipal Poison Detection Centre of a tertiary care teaching hospital in South India.

Study subjects: 53 confirmed OP-poisoned cases were included in the study. The anonymized serum samples of the OP cases were collected from the Manipal Poison Detection Centre, attached to the teaching hospital. The OP poisoning was confirmed by detection of the compounds using thin layer chromatography (TLC).

Laboratory investigations: In the current study, levels of PChE were obtained from the clinical laboratory. Serum was collected for the determination of malondialdehyde (MDA), ferric reducing ability of plasma (FRAP), glutathione -S- transferase (GST), total thiols and β glucuronidase. The serum

samples were stored at -80 °C to maintain stability. MDA, FRAP, GST and total thiols were estimated spectrophotometrically.

Statistical analysis: As per the statistical analysis, the current study consisted of non-parametric data and they were not normally distributed; therefore, Spearman's correlation was conducted to correlate between the parameters. p value <0.001 was significant. The correlation range determines the extent of correlation. IBM Statistical Package for Social Sciences (SPSS) software (version 16) was used for statistical analysis.

RESULTS

Correlation of PChE with FRAP, MDA, GST & total thiols was significant with p value <0.01 as depicted in Table 1 and Graph 1, 2, 3, & 4 respectively whereas β glucuronidase showed no significant correlation.

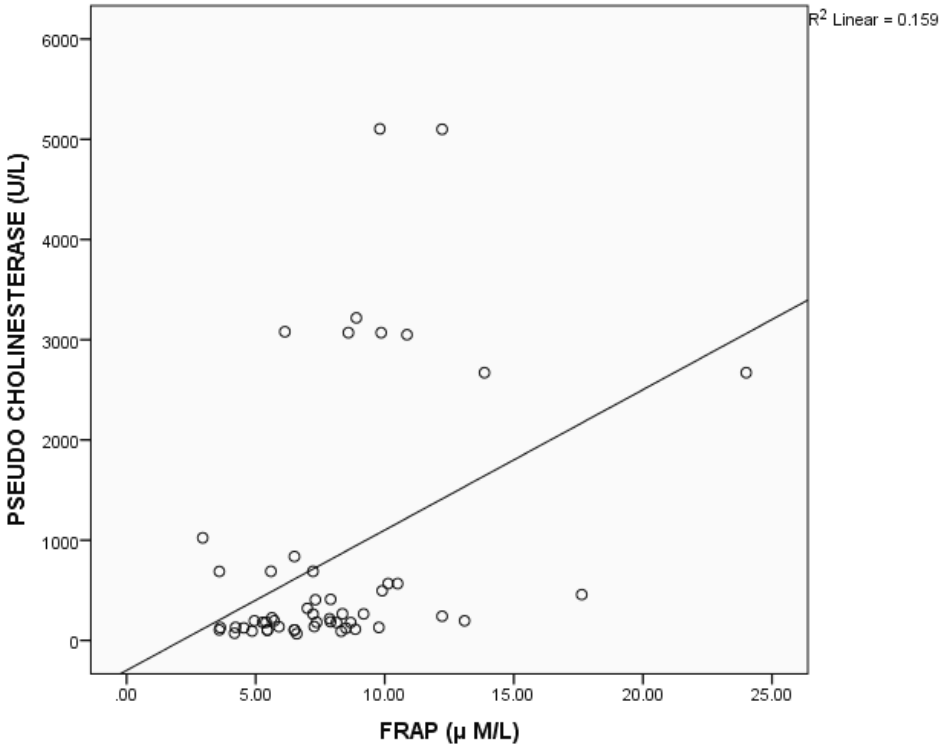
β Glucuronidase did not correlate with any of the parameters as depicted in Table 2.

MDA showed moderately negative correlation with GST, FRAP and total thiols with p value <0.01 as depicted in Table 3 and Graph 2, 5, 6, & 7 respectively whereas β glucuronidase showed no significant correlation.

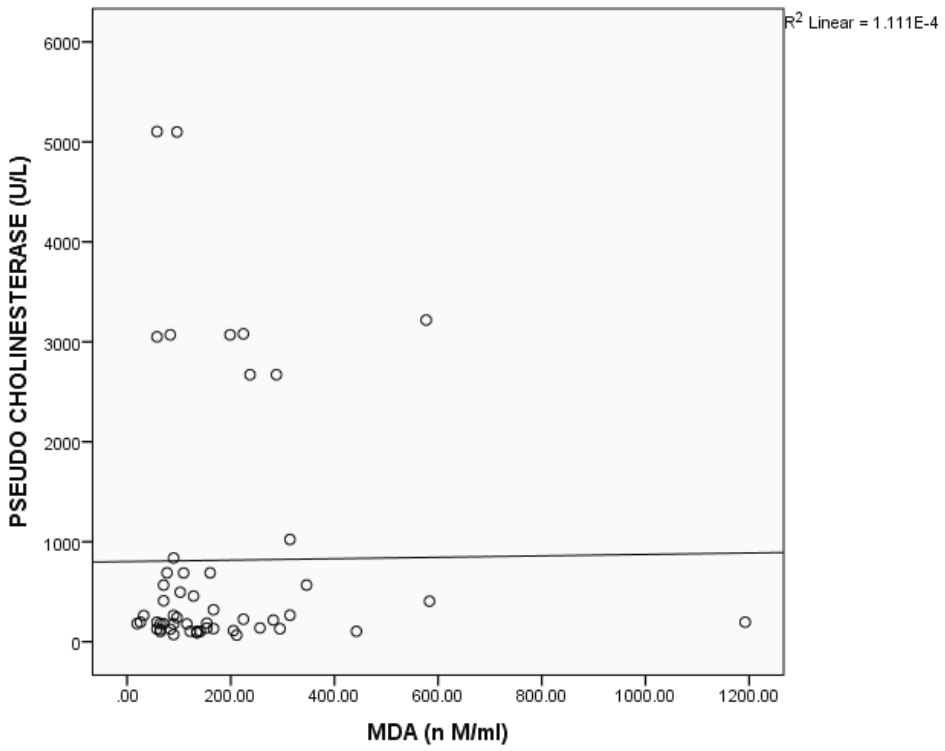
Table 1. Correlation of PChE with the following parameters.

Parameters	Correlation coefficient	P value
β glucuronidase	-0.171	0.22 (NS)
FRAP	0.454	0.001*
MDA	-0.861	0.00*
GST	0.598	0.00*
Total thiols	0.659	0.00*

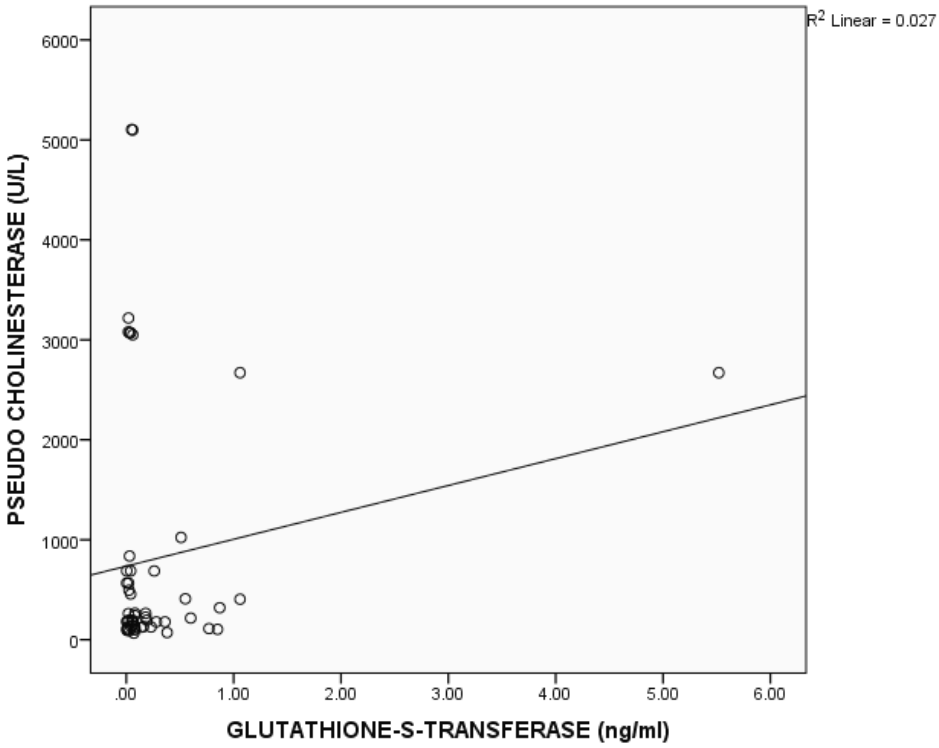
Spearman's Correlation, N = 53; NS: Not Significant, *Significant



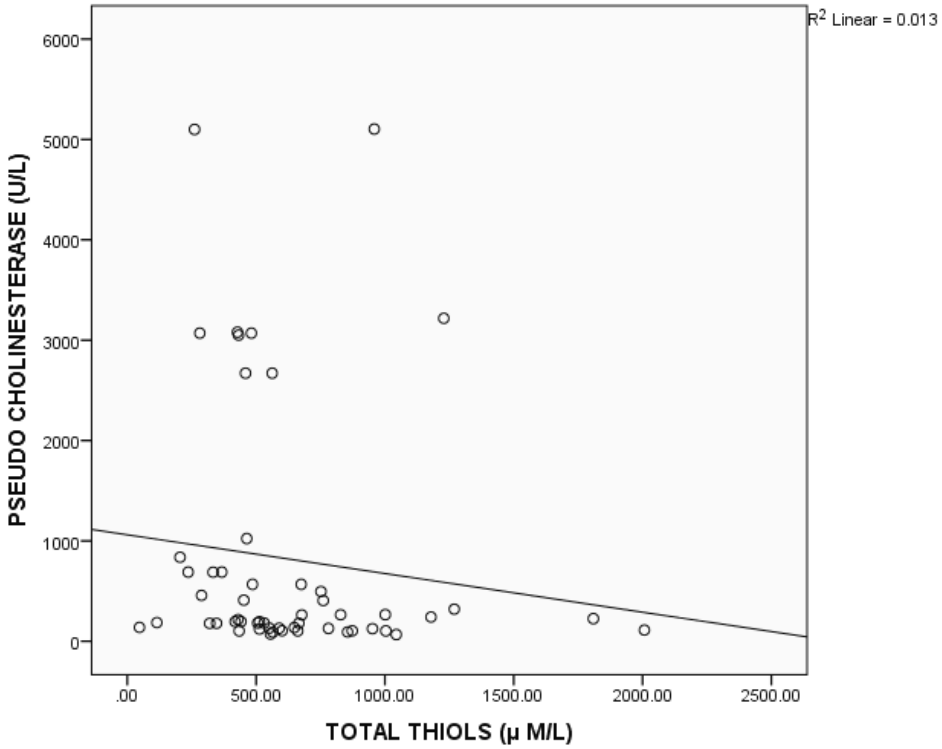
Graph 1. Correlation of pseudocholinesterase with ferric reducing ability of plasma (FRAP).



Graph 2. Correlation of pseudocholinesterase with malondialdehyde (MDA).



Graph 3. Correlation of pseudocholinesterase with glutathione-S-transferase (GST).



Graph 4. Correlation of pseudocholinesterase with total thiols.

Table 2. Correlation of β glucuronidase with the following parameters.

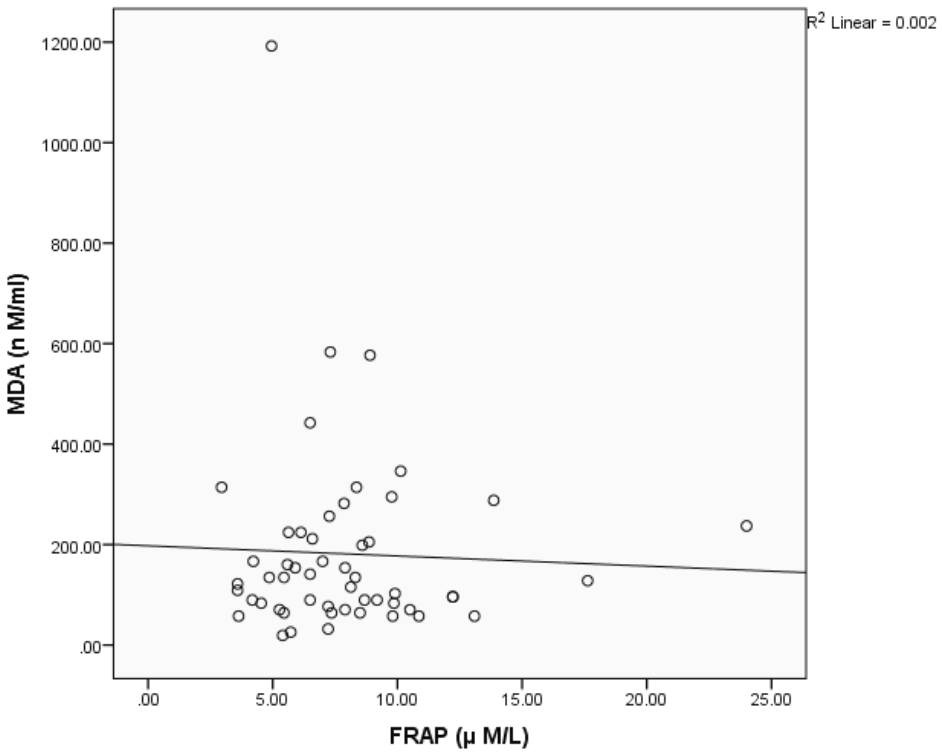
Parameters	Correlation coefficient	P value
PChE	-0.171	0.22 NS
FRAP	0.019	0.89 NS
MDA	0.134	0.33 NS
GST	0.057	0.683 NS
Total thiols	-0.265	0.056NS

Spearman's Correlation, N= 53; NS: Not Significant

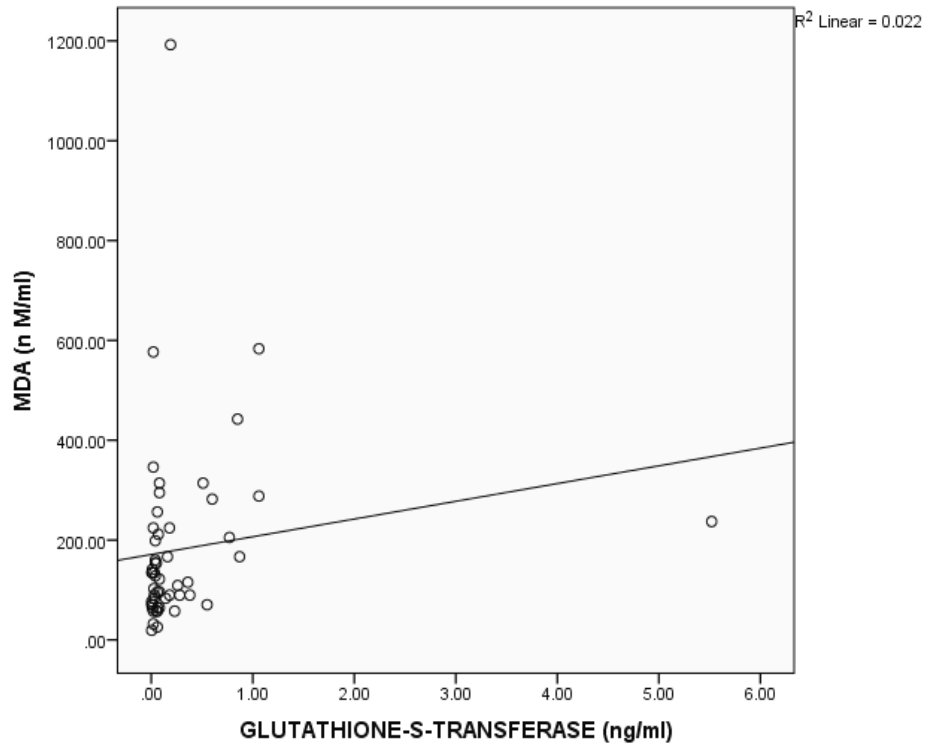
Table 3. Correlation of MDA with the following parameters.

Parameters	Correlation coefficient	P value
PChE	-0.861	0.00*
β glucuronidase	0.134	0.337 NS
FRAP	-0.425	0.002*
GST	-0.692	0.00*
Total thiols	-0.757	0.00*

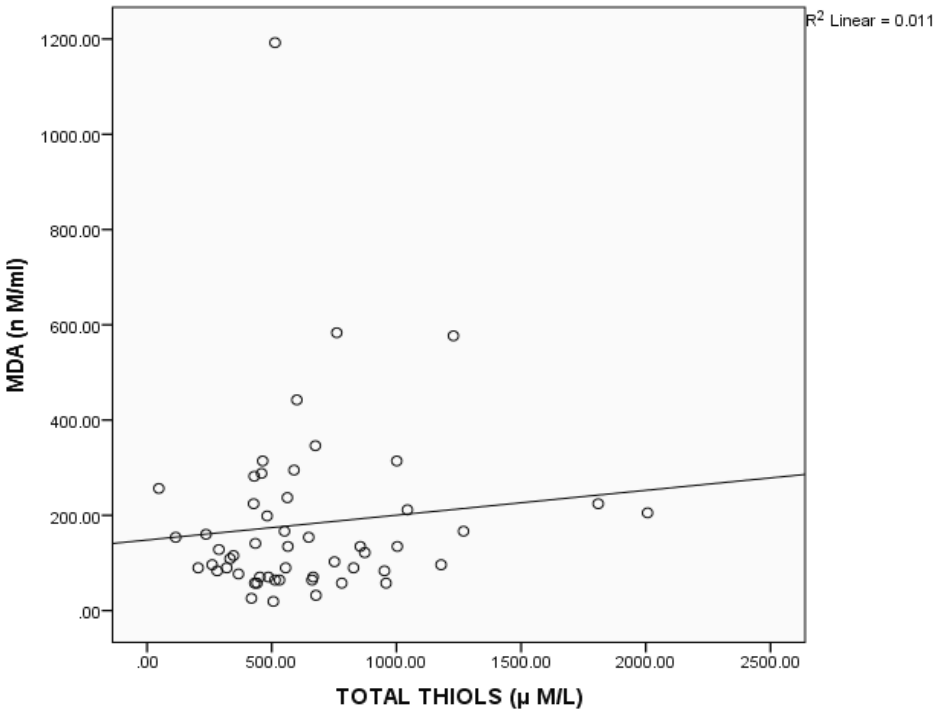
Spearman's Correlation, N= 53; NS: Not Significant; *Significant



Graph 5. Correlation of malondialdehyde (MDA) with ferric reducing ability of plasma (FRAP).



Graph 6. Correlation of malondialdehyde (MDA) with glutathione-S-transferase (GST).



Graph 7. Correlation of malondialdehyde (MDA) with total thiols.

DISCUSSION

The present study majorly focusses on the correlation among PChE, β glucuronidase, MDA, GST, FRAP and total thiols.

As MDA is used as a conventional biomarker for oxidative damage [16, 17], an attempt has been made to evaluate the same. In our study, MDA showed moderately negative correlation with GST, FRAP and total thiols with p value <0.001, which suggests that the oxidative damage occurred has been counter stroked by antioxidant pool majorly consisting of GST, FRAP and total thiols. Similar observations were made by other studies [18-21].

Correlation of PChE with MDA showed highly significant negative correlation with p value <0.001 which gives us an evidence that severe oxidative damage (with elevated levels of MDA) occurs in OP poisoning cases due to generation of reactive oxygen species (ROS). According to recent studies lower the PChE levels, higher is the complications of OP poisoning, where a serine residue consisting a free hydroxyl group in the AChE active site covalently reacts with acetylcholine (ACh) molecule. OP's in the oxon (phosphate) form can similarly react with the serine residue; however, the process of dephosphorylation is much slower, along the orders of hours to days, than that for deacetylation. Therefore, the serine residue on the phosphorylated AChE is unavailable to breakdown ACh which builds up levels of ACh leading to overstimulation of nervous system [12, 13]. On the other hand, PChE also showed moderate positive significant correlation with FRAP, GST, and total thiols with p value <0.001 which again confirms that the oxidative damage occurred with elevated levels of MDA collapses the antioxidant pool (FRAP, GST, Total thiols) [18-20].

Although cholinesterase monitoring has advantage of depicting the measure of physiological response, it has disadvantages too. Interpretation of PChE levels are complicated by inter and intra-individual variations in enzyme activity. Also, factors such as suppression of cholinesterase due to health conditions or exposure to any other cholinesterase inhibiting pesticides like carbamates may give false positive values. Another limiting factor of PChE is that large doses of pesticide are required to show significant AChE inhibition. Hence, PChE acts as a toxicity indicator only at high exposure

and is rather insensitive at low exposure levels [12]. To overcome this, β glucuronidase (a liver microsomal enzyme) was chosen to check its efficiency to be used as an alternate biomarker for OP toxicity, where β glucuronidase is loosely bound to egasyn in the liver microsomes which is one of the carboxylesterase isoenzymes. The oxon formed from OP in the liver microsomes, covalently binds to egasyn and causes the cleavage of the egasyn-glucuronidase complex, leading to the release of β glucuronidase into the blood [22].

In the current study correlation of β glucuronidase with the above parameters were not significant. Also, the levels of β glucuronidase did not show significant deviation from its normal range with respect to OP poisoning cases and hence the hypothesis of using it as an efficient alternative biomarker for OP poisoning is ruled out. However, there is discrepancy among the results obtained from previous studies [22, 23] in accordance to the current study. There could be various reasons for this including the severity or the duration of exposure. Further studies in this regard are to be encouraged so that a standard can be set while dealing with pesticides poisoning cases.

CONCLUSION

The current study concludes that, OP poisoning along with collapsing cholinergic system at neural synapses accounts for the generation of reactive oxygen species which leads to neurological complications coupled with severe organ breakdown like respiratory collapse, cardiac dysfunction, etc., leading to downfall in the revival chances of the patient. Hence, along with oxime therapy, doctors should also look for the extent of oxidative stress occurred and should administer patients with accurate dosage of efficient antioxidants such as vitamin E, vitamin K, etc., which can bring down the extent of organ damage and ultimately adding another higher step in reviving patient.

Further studies to determine the interferences in evaluation of the above-mentioned parameters in OP poisoning cases with a much higher sample size and long duration/dosage of exposure can be encouraged. Also, an intense study is required to bring out an efficient standardised method to clinically estimate the extent of oxidative damage occurred in OP-poisoned cases which can accurately guide doctors in their treatment and bring about a good prognosis.

The discrepancy occurred with respect to previous studies regarding efficiency of β glucuronidase as a biomarker of OP toxicity should be further studied in depth for a better conclusion and also researches to discover many other efficient biomarkers for OP toxicity should be encouraged.

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ETHICAL APPROVAL

Obtained from Institutional Ethics Committee.

CONFLICT OF INTEREST STATEMENT

None to declare.

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