क्षेत्रीय आयुर्विज्ञान संस्थान, इंफाल: मणिपुर
REGIONAL INSTITUTE OF MEDICAL SCIENCES, IMPHAL, MANIPUR
(स्वास्थ्य और परिवार कल्याण मंत्रालय,भारत सरकार के अंतर्गत एक स्वायत्त संस्थान)
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NOTICE

Imphal, the 23rd September, 2026

No. 191/RIMS-MRU/2026: It is hereby notified that the Department of Health Research, MoHFW, GoI, has scheduled the **3rd Fortnightly Research Presentation Series (Webinar)** as per the following scheme.

- **Date:** 29th September, 2026 (Tuesday)
- **Time:** 3:00 PM
- **Speaker:** Dr. Ram S. Kaulgud, Associate Professor, Department of General Medicine, Karnataka Medical College and Research Institute, Hubballi.
- **Format:** 40-minute research presentation followed by 20-minute Q&A session
- **Webinar Link:** https://teams.microsoft.com/meet/457030534652193?p=TGxP2mfa96jVV9P7PC
  - **Meeting ID**: 457 030 534 652 193
  - **Passcode**: U8tw2dq7

All faculty members of RIMS-Imphal, Dental College, College of Nursing are requested to attend the webinar and actively participate in the discussion at respective departments and attendance register to be shared with MRU for onward submission to the DHR.

The LRAC members (internal) and EC members, scientists and research staff of MRU, RIMS Imphal are requested to attend the webinar at MRU EC-Conference room.

23/9/2026

(Prof. T. Jeetenkumar Singh)
Nodal Officer
Multi-Disciplinary Research Unit,
RIMS, Imphal

Copy to:

1. The P.S. to Director, RIMS, Imphal for kind information of Director
2. The P.A. to Medical Superintendent, RIMSH, Imphal for kind information
3. The Dean (Academic), RIMS, Imphal for kind information.
4. The Principal, Dental College, RIMS, Imphal
5. The Principal, College of Nursing, RIMS, Imphal
6. All Heads of Departments, RIMS, RIMS, Imphal
7. The Chairperson/Co-Chairperson, LRAC, MRU, RIMS, Imphal
8. The Internal Member, LRAC, MRU, RIMS, Imphal .....
9. The Member, EC, MRU, RIMS, Imphal .....
10. System Administrator, RIMS, Imphal – with a request for uploading the notice in the website
11. Guard file.

R.11016/07/2026-HR

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स्वास्थ्य अनुसंधान विभाग/Department of Health Research

IRCS Building,
2nd Floor,
Red Cross Road, New Delhi - 110 001
22.09.2026

To,

The Dean/ Principal/ Director of Medical Colleges/ Institutes

Subject: Request to attend fortnightly webinar for MRU network-reg.

Sir/Madam,

Department of Health Research (DHR), is pleased to initiate a Fortnightly Research Presentation Series (Webinar) among Multidisciplinary Research Units (MRUs) to promote scientific excellence, facilitate knowledge exchange, and foster collaborative research. These webinars mark a new initiative of its kind. The objectives of the webinars are as follows:

a. To strengthen collaboration among MRUs through knowledge sharing and multicentric research.
b. To enhance research quality and translational impact by promoting best practices and innovation.

2. These webinars will be organized twice in a month, and the speakers will be nominated from the MRUs. The second webinar in the series is scheduled for 29.09.2026 (Tuesday) at 3 PM. The invited speaker is Dr. Ram S. Kaulgud, Associate Professor, Department of General Medicine, Karnataka Medical College and Research Institute, Hubballi.

3. The research paper to be discussed during the webinar is enclosed. The link for the webinar will be shared shortly.

4. The target audience of this webinar is faculty, scientists and research staff of Multi-Disciplinary Research Units in Medical Colleges and Research Institutes.

Format: Presentation of 40 minutes followed by 20 minutes of Q&A with the online attendees.

Yours faithfully,

(धरकत आर लुइकेंग| Dharkat R. Luikang)
उप सचिव| Deputy Secretary

Copy to: The Nodal Officer of Multi-Disciplinary Research Units (MRUs)

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Serum Organophosphorus compound levels as a surrogate marker for severity assessment and management in Acute Organophosphorus Poisoning – A novel approach

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ABSTRACT

Organophosphorus (OP) compounds are the most extensively used pesticides' class worldwide; cause most self-poisoning deaths especially in India. Thus, it is utmost important for early identification and aggressive management of OP poisoning from the clinical perspective to prevent serious complications by using sophisticated LC-MS/MS approach. This was a prospective study involving 103 patients of OP cases admitted to Karnataka Institute of Medical Sciences from June 2022 to May 2023, based on the inclusion and exclusion criteria patients were subjected to study. On admission, venous blood was collected from patient with Malathion and Profenofos OP poisoning history and subjected to serum biomarker and to LC-MS/MS analysis. Out of the 103 patients, 68 patients consumed Profenofos (66%) and 35 patients consumed Malathion (34%). Pseudocholinesterase levels among the of OP cases revealed that the 33 patients had mild toxicity, 40 patients had moderate toxicity and 30 patients had severe toxicity of OP poisoning. Subsequently LC-MS/MS analysis showed that the results obtained are not in correlation with indirect serum marker pseudocholinesterase levels. On the other side, LC-MS/MS results are in correlation with the clinical outcome of the patients with respect to morbidity and mortality. Thus, LC-MS/MS approach to assess the OP levels in patients could be used as potential diagnostic and prognostic marker for the absolute quantification of OP compounds compared to indirect OP levels estimation.

1. Introduction

Pesticides are the class of chemical compounds used to control the growth of insects, which are responsible for the depletion of crops productivity. As per the WHO estimates, approximately 3 million pesticide poisoning occur worldwide and causing more than 0.2 million deaths and 1.9 million with other long term comorbidities [1–3]. Among all the pesticides studied, Organophosphate Pesticides (OPs) are considered as the largest group of pesticides used in agriculture, livestock, and other commercial purposes globally [4]. In spite of having several advantages of OPs in increasing the agricultural yield to meet the food demand of growing population, they are found to be potentially hazardous to the humans and other living beings in the environment [4, 5]. OPs are irreversible inhibitor of neurotransmitter acetylcholine esterase (AChE) and pseudocholinesterase (butyryl cholinesterase), which are involved in the reduction of acetylcholine (ACh) concentration by hydrolyzing ACh into choline and acetic acid/butyric acid. These enzymes, plays an essential role for proper functioning of the human nervous system [6]. Exposure to OPs can lead to acute poisoning, in severe cases, OPs poisoning can lead to respiratory failure, cardiac arrest which can be life-threatening if not treated promptly [7]. One of the study conducted in India showed that the incidence of suicidal poisoning mortality using OPs accounted 10–44% in the hospitalized patients [8, 9].

The clinical features of OP poisoning comprise a three phasic response such as, initial acute cholinergic phase, high mortality associated intermediate phase and nonlethal delayed polyneuropathy associated with morbidity phase [10].The cholinergic phase occurs immediately and last for hours, which usually includes excessive salivation, lacrimation, abdominal cramps, vomiting, urination, frothing from the mouth, sweating, bronchospasm, and fasciculation and sometime cause loose stools [11,12]. Thus, early identification and aggressive management of OP poisoning is significant from the clinical perspective to prevent serious complications and improve the chances of a full

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recovery. Clinicians have been used to assess the severity of OP poisoning by indirect blood markers such as serum amylase, cholinesterase and creatinine phosphokinase levels are used to determine the severity of OP poisoning. However, these markers will not give real quantification of OPs present in blood. Thus it is utmost crucial to correlate the clinical features and OPs precise quantification to anticipate the requirement for ventilators to avoid fatal outcome. Hence accurate, robust and fast diagnostic and prognostic methods would be useful for the clinicians to stratify the patients according to their risk of deterioration. Malathion and Profenofos are systemic organophosphorus pesticides which are widely used for ornamental plants, cotton and tobacco plants in this region of the country. Quantification of the Malathion and Profenofos compounds in human samples is crucial and important for the clinicians to diagnose and treat patients efficiently. Among different markers pseudocholinesterase (PChE), routinely used as a main prognosticator and is used as an indirect measure of acetylcholinesterase activity. Moreover the present clinical practice agrees that a reduced level in PChE does indicate OP poisoning; on the other side it will not indicate the severity of OP poisoning [13]. In this context liquid chromatographic-mass spectrometry (LC-MS/MS) instrument showed to be highly sensitive to detect these OPs [14,15]. Hence in the present study we have used LC-MS/MS analytical tool to quantify OP Malathion and Profenofos in human serum sample and co related with the clinical severity with respect to clinical outcome.

2. Materials and methods

The present study is single centre, prospective observational study, carried out in the Department of Internal Medicine, Karnataka Institute of Medical Sciences (KIMs), Hubli, Karnataka, India. Cases were selected from patients presenting to KIMS hospital, IPD section with history of OP compound poisoning considering the inclusion and exclusion criteria.

2.1. Reagents and chemicals

External standard LC-MS grade Malathion and Profenofos were obtained from HPC-standards GmbH (Cunnersdorf, Germany). Individual pesticide standards (purity >96–98%) or stock solutions (1 mg/mL) was used for analysis. LC-MS grade organic solvents Methanol and Acetonitrile were obtained from Merck (Darmstadt, Germany). Formic Acid and ammonium formate obtained from sigma-Aldrich (Missouri, United States). 0.2 μ filters were obtained from Sartorius (Gottingen, Germany). Micro Volume QuEChERS Kit obtained from Shimadzu (Kyoto, Japan). Double distilled 0.2 μ filtered water was used throughout the study from Millipore (Burlington, USA). LC-MS/MS 8040 from Shimadzu (Kyoto, Japan) was used to analyse the analyte by using Lab Solution soft wear from the same company.

2.2. Inclusion criteria

Men and women between 18 and 80 years of age with confirmed cases of OP compound consumption with clinical features of OP poisoning.

2.3. Exclusion criteria

Patients presenting to KIMS hospital after 1–2days of hospitalization and received treatment elsewhere were excluded from the study and pregnant women visiting to the KIMS hospital were excluded from the study.

2.4. Sample size

Based on the previous year data in KIMS hospital total number of organophosphorus poisoning in KIMS is 780. After applying inclusion and exclusion criteria the sample size was 103 patients.

Sample size $=4pq/d^2$

Where p =prevalence, q = (1-p)

103 patients presenting with Organophosphorus poisoning in Department of Internal Medicine, KIMS hospital during the study period were taken for the study.

2.5. Collection of data

Patients meeting inclusion criteria were considered for the study and informed and written consent was obtained from the subjects. Ethical approval was obtained from the Institution Ethics committee. The patient's clinical severity was assessed based on history and clinical features, and the patient venous blood was collected and subjected to serum biomarker and LC-MS/MS analysis.

2.6. Laboratory investigations

Biochemical analysis of samples such as complete hemogram, renal function test, Serum electrolytes, serum pseudocholinesterase was carried using automated biochemical analyzers, on the day of admission and repeated every 48 h till discharge or death. Electrocardiogram were performed on the first or second day of admission and repeated if needed and all these test were done at Karnataka Institute of Medical Sciences, Hubli.

2.7. Samples preparation and LC-MS/MS acquisition

Patients presenting with history, characteristic clinical signs and symptoms of OP compound consumption were taken into the study and LC-MS/MS was done in the selected patients as per the method developed by Shin et al. [16] with few changes. Patients serum sample (200 μL) was mixed with Micro Volume QuEChERS Kit (Quick, Easy, Cheap, Effective, Rugged, and Safe), obtained from Shimadzu, which contained MgSo4 (160 mg) and CH_3COONa (40 mg) and further serum was diluted with distilled water (1:2) and protein precipitation was done by adding 600 μL of acetonitrile (100% v/v). The Patient sample was mixed thoroughly by vortex and the mixture was incubated at room temperature for 12 min. After incubation, centrifuged at 8000 x *g* for 10 min and the supernatant was separated. The supernatant was filtered through a 0.2-μm membrane and then 5 μL of the extracted solution was injected into a UHPLC Nexera X2, coupled to a Shimadzu LCMS-8040 triple quadrupole mass spectrometer (Kyoto, Japan), with a reversed-phase column (Shim-pack GISS C18, 1.9 um, 3.0 mm×150 mm) at 40 °C oven temperature. The mobile phase consisted of (A) water with 5 mM Ammonium formate and Formic Acid (0.1%) and (B) 100% Methanol with 5 mM Ammonium formate and 0.1% Formic Acid. The gradient was initiated with 20% B for 2 min, increasing to 80% B in 5 min, kept isocratic at 80% B for 5 min and decreased to 20% B in 2 mins with 0.5 mL/min flow rate and the total run time was 15 min. In the mass spectrometer study, ionization of analytes was performed by electrospray ionization (ESI) with positive mode. The interface, desolvation line (DL), and heat block temperature were 300, 250, and 400 °C, respectively. The, nebulizing (nitrogen), and drying gas (nitrogen) flow were 3 and 15 L/min, respectively. The collision-induced dissociation (CID) gas was argon with 230 kPa. For MS/MS analysis, each standard solution (0.1 μg/mL; 5 μL) was injected obtains a full scan spectrum (*m/z* 50–500). A precursor ion (e.g., [M+H]+) was selected from the spectrum data and subjected to collision with several collision energy (CE) voltages to find the product ions. Lab Solutions (Version 5.97SP1) as LCMS software was utilized for data processing. With the optimized analytical method, method validation was carried out. Accuracy and precision was assessed by analyzing OP compounds in six replicates with three different concentrations at different time intervals. Accuracy was obtained by expressing mean of measured concentration as percentage and precision was represented by percentage relative standard deviation (RSD).

2.8. Statistical analysis

ANOVA test were used to know if statistically significant association between the groups, with $P < 0.05$ was taken as statistically significant.

3. Results and discussion

In our study of 103 patients subjected, majority of the patients (36) were of 30–40 years age group, followed by 28 patients in 20–30 years, 21 patients in 40–50 years and 9 patients each in <20 and >50 years age groups, respectively and 66% of them were male (Fig. 1a &b). Profenofos and Malathion were the most commonly consumed OP compound. In the present study, 68 patients consumed Profenofos (66%) which was the major OP poison consumed followed by 35 patients consumed Malathion (34%). Most of the patients were brought to the Karnataka Institute of Medical Sciences (KIMS) hospital within 2–6 h of OP consumption. The patients admitted to KIMS hospital had vomiting was the primary presenting symptom. Age distribution among sex of the patient showed that the 4, 14, 22, 20 and 8 males among <20 years, 20–30 years, 30–40 years, 40–50 years and >50 years age groups respectively. Similarly 5, 14, 14, 1 and 1 females among <20 years, 20–30 years, 30–40 years, 40–50 years and >50 years age groups respectively and the observed chi-square values are (15.547) statistically significant ($P < 0.04$) (Table 1). Distribution of comorbidities among study population showed that out of 103 patients, 4 patients had Diabetes mellitus, 5 patients had hypertension, 15 patients had history of smoking, 46 patients had history of alcohol consumption and 42 patients had history of tobacco consumption irrespective of OP consumed (Table 2). Further, distribution of severity among study population with mean pseudocholinesterase levels revealed that the 33 patients had mild toxicity, 40 patients had moderate toxicity and 30 patients had severe toxicity of OP poisoning. On the other hand the mean pseudocholinesterase levels among the severity of OP poisoning is statistically not significant ($P < 0.0601$) (Table 3) as there were not significant difference between the mild and moderate OP patients. Moreover, hematological parameters with severity showed no statistically significant correlation with TLC, Hb, Serum creatinine, serum potassium, and serum magnesium the observed results are in agreement with the previous findings by Nejatifar group [17]. Primary action of OP poisoning is directly leads to respiratory suppression and inhibits acetylcholinesterase (AChE). This may not result in alteration of biochemical parameters. However, there can be secondary infection, sepsis and multiple Organ dysfunctions in OP poisoning patients, which can change biochemical parameters. But this is observed on few patients only. Hence, biochemical parameters may or may not correlate with clinical outcome.

Further, LC-MS/MS analysis was carried out for Profenofos and Malathion (Supplementary Fig1a &b) and the results revealed that the OP compounds are elevated and it was correlated with clinical features (Fig. 2 and Fig. 3) in selected patients (4 mild, 3 moderate and 3 severe patients in Profenofos and 2 mild 2 moderate and 2 severe) admitted at the KIMS hospital. Further, the results showed that, the some of the mild cases showed elevated levels of OP compounds by LC-MS/MS compared to indirect pseudocholinesterase levels in the serum (Fig. 4a & b). Moreover, the observed LC-MS/MS results are in good correlation with the clinical outcome of the patient. On the other side, the outcome of the study showed 2, 4 and 9 deaths among mild, moderate and severe Profenofos consumed 68 patients and it accounted 15 patients mortality with a mortality rate of 22.05%. Whereas, in Malathion consumed 35 patients, 2, 3 and 5 deaths occurred among, mil, moderate and severe toxicity conditions accounting for 28.6% mortality. In one of the study author found the mortality rate of 25–33% in patients consumed Profenofos and Difenofos and showed no significant association between the compounds consumed and clinical outcome [10].

Further to validate the method; we have used serum samples from

Table 1
Age distribution among sex of the patient.

		SEX					
		Male		Female		Total	
		N	%	N	%	N	%
Age Distribution	<20 years	4	5.9%	5	14.3%	9	8.7%
	20–30 years	14	20.6%	14	40.0%	28	27.2%
	30–40 years	22	32.4%	14	40.0%	36	35.0%
	40–50 years	20	29.4%	1	2.9%	21	20.4%
	>50 years	8	11.8%	1	2.9%	9	8.7%
	Total	68	100.0%	35	100.0%	103	100.0%

The chi-square statistic is 15.547 and p value is 0.04

Table 2
Distribution of comorbidities among study population.

	Absent		Present		Total	
	N	%	N	%	N	%
DM	99	96.1%	4	3.9%	103	100.0%
HTN	98	95.1%	5	4.9%	103	100.0%
Smoking	88	85.4%	15	14.6%	103	100.0%
Alcohol history	57	55.3%	46	44.7%	103	100.0%
Tobacco history	61	59.2%	42	40.8%	103	100.0%

Table 3
Comparison of mean Pseudocholinesterase levels among patient severity.

Total number (n-103)	Severity	Pseudocholinesterase Levels U/L Mean±SD	P value
33	Mild	6457±1046	<0.0601
40	Moderate	5950±1246	
33	Severe	3905±952	

*Significant: $P < 0.05$

Further to validate the method; we have used serum samples from

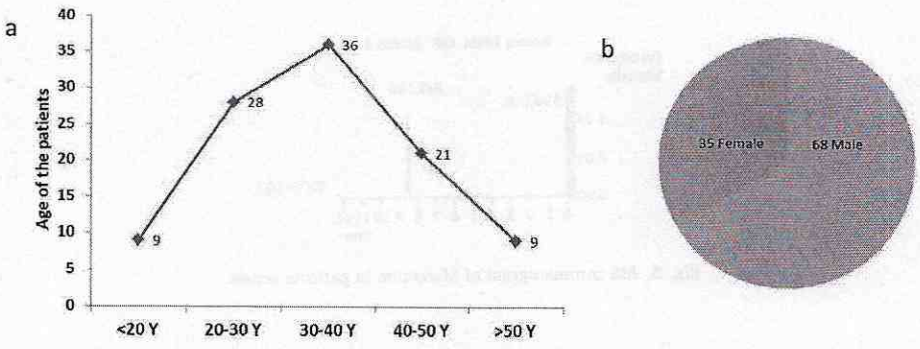

Fig. 1. a &b. Age and gender distribution among study population.

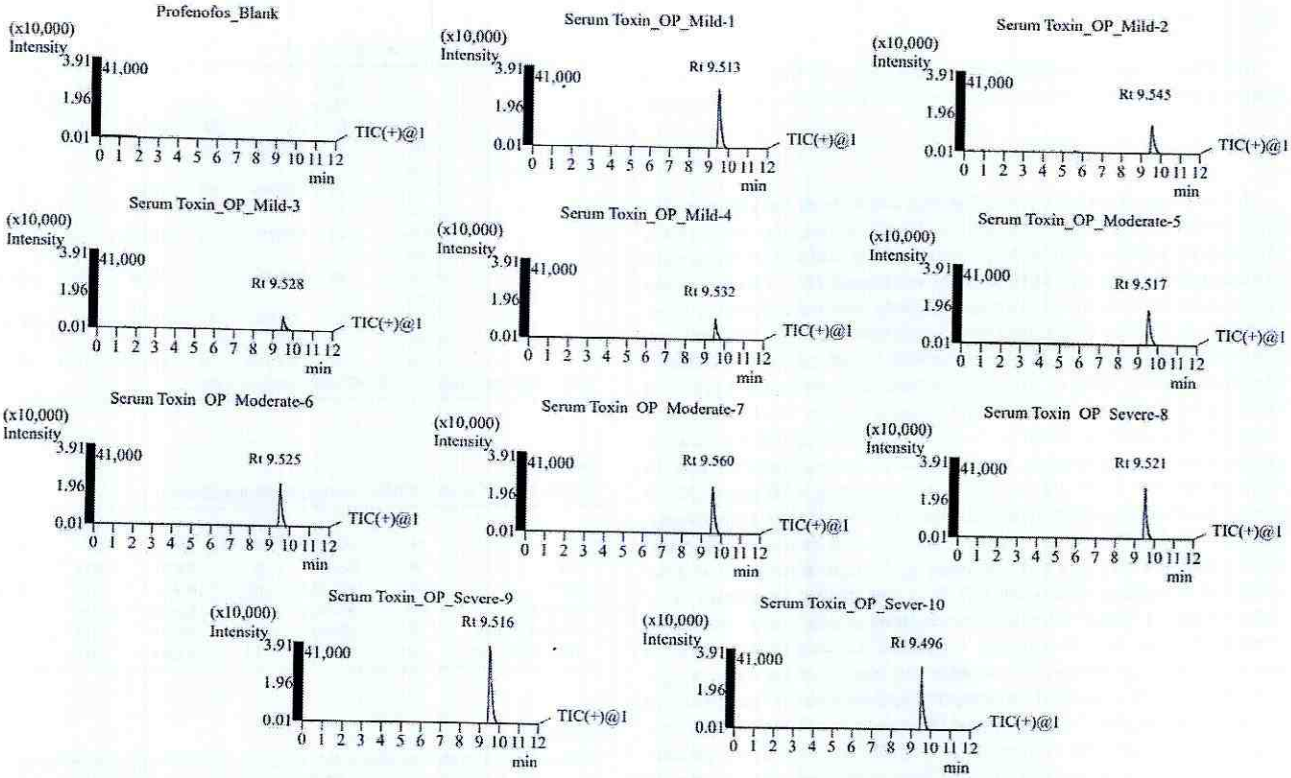

Fig. 2. MS chromatogram of Profenofos in patients serum.

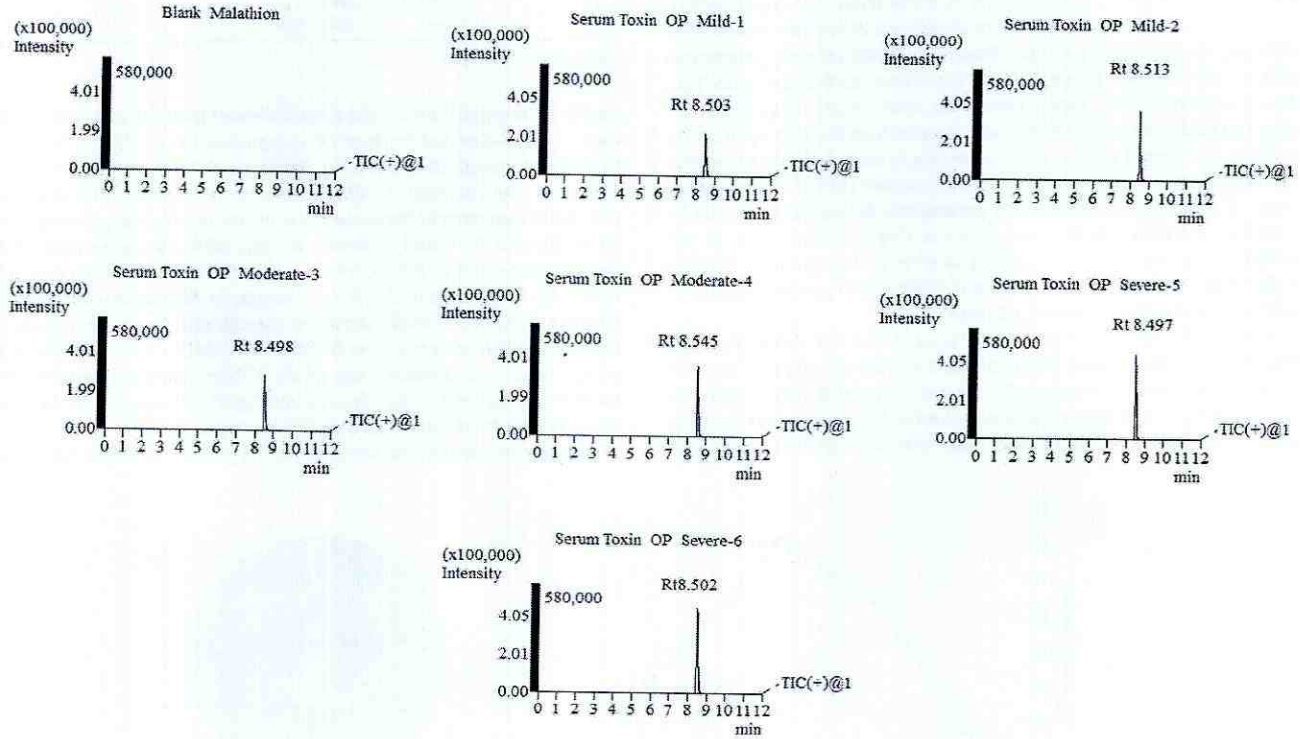

Fig. 3. MS chromatogram of Malathion in patients serum.

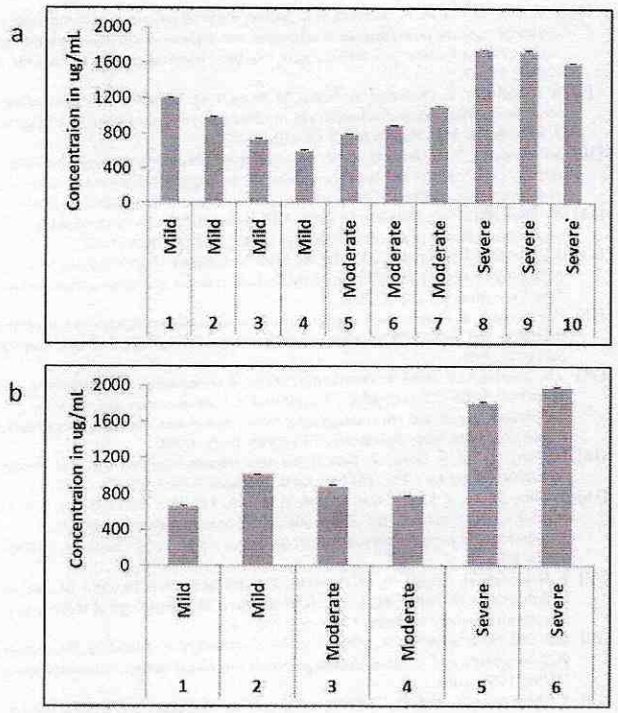

Fig. 4. Concentration of (a) Profenofos and (b)Malathion in patients serum with respect to clinical observation.

the OP patients, as serum has a minor or negligible amount of interaction with column matrix compared to whole blood, and also serum will reduce cleanup step before injecting into LC-MS [16,18,19]. In our studies, the extraction efficiency (n-6) results showed 88.27% -89.99% for Malathion at 25, 50 and 75 ng/mL and at same concentration 87.16% -90.32% was observed for Profenofos. The linearity of calibration was evaluated (n=5) by the correlation coefficient (r2) of the calibration curve from 20 ng/mL-100 ng/mL for both pesticides and results revealed the linear curve generated with external standards have linear regression (r^2) of 0.997% and 0.999% for Malathion and Profenofos respectively. Accuracy is usually defined as the closeness of the unknown value to the known true value. Quantification accuracy tests in our study was performed using external standards at 25, 50 and 75 ng/mL levels and the observed results showed 100.39–101.33% accuracy with precision of 1.14–3.87% (RSD) for Malathion and 96.15–101.89% (n=6) accuracy with precision of 3.78.to 5.5% (RSD) for Profenofos respectively (Table 4) and the observed results are in correlation with previous findings [16]. Subsequently recovery rate (%) of OP compounds was determined by using calibration curve of standard Malathion and Profenofos. At 25, 50 and 75 ng/mL spiked Malathion showed 82–84% recovery rate intraday and 72–82% recovery rate at interday analysis. Whereas, at 25, 50 and 75 ng/mL of spiked Profenofos showed 79–84% recovery rate at intraday and 72–77% recovery rate at interday analysis. However, greater recovery rate could increase the sensitivity of target analytes and also less matrix effect and a recovery rate of 70–120% is an acceptable trueness range as per the European commission guideline [20].

Overall in this study out of 103 patients, 72 patients were discharged and 5 patients left the hospital against medical advice and 25 patients had mortality accounting for a mortality rate of 26%. Thus the outcome the study emphasize that the serum organo phosphorus compound levels by LC-MS/MS analysis could be one of the promising diagnostic and prognostic tool to assess the severity and could be used to prognosticate OP poisoning cases.

4. Conclusion

LC-MS analysis method is a robust and sensitive tool to quantity absolute serum toxin levels of Profenofos and Malathion in patients' blood and also LC-MS/MS showed that the mild OP patients especially in young individuals showed elevated levels of OP in blood compared to Pseudo cholinesterase levels. Further, LC-MS/MS approach to assess the OP levels in patients, gives absolute quantification of toxins compared to indirect serum marker Pseudocholinesterase. Indeed, comprehensive studies are needed with large number of samples to use LC-MS/MS as a diagnostic or prognostic marker to detect and quantify OP poisoning in patient.

Ethical approval

This research design was approved by the Ethics Committee of Karnataka Institute of Medical Sciences with the ethics code number ECR/486/Inst/KA/2013/RR-20.

CRediT authorship contribution statement

Ram S. Kaulgud and H.B. Nidish conceived the study. H.B. Nidish collected samples from the hospital, Mahantesh M. Kurjogi, S. Veeresh and Gulamnabi L. Vanti did LC-MS/MS analysis. Gulamnabi L. Vanti, Mahantesh M. Kurjogi wrote the manuscript. Ram S. Kaulgud and helped to correct manuscript and Gulamnabi L. Vanti analyzed the data.

Author Statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Gulamnabi Vanti reports financial support, administrative support, and

Table 4
Intraday and Interday accuracy (%) and Precision (RSD %) of OP compound in human serum (n=6).

Analyte	Conc.(ng/mL)	Intra-day			Inter-day		
		Accuracy (%)	Precision (RSD %)	% of Recovery	Accuracy (%)	Precision (RSD %)	% of Recovery
Malathion	25	101.048	3.87	82.93	100.48	4.69	78.16
	50	101.33	1.73	85.39	100.84	2.46	82.64
	75	100.39	1.14	84.32	0.00	4.66	72.24
Profenofos	25	101.892	3.79	84.02	100.48	5.48	77.73
	50	100.236	4.46	84.00	100.64	4.74	77.23
	75	96.15866667	5.55	79.67	98.93	4.66	72.526

equipment, drugs, or supplies were provided by Karnataka Institute of Medical Sciences Hubballi. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Author would like to thank Multi-Disciplinary Research Unit, Karnataka Institute of Medical Sciences, Hubli, Karnataka, for providing finical and LC-MS/MS facility to carry out this research.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2024.116237.

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