1.1 Introduction

Organophosphate (OP) pesticide poisoning is a major challenging public-health problem in developing countries like Pakistan, Sri Lanka, India and other developing countries of Asia. WHO estimates that three million cases of poisoning occur worldwide, mostly in the developing countries. Of the estimated 500 000 deaths from self-harm in the region each year about 60% are due to pesticide poisoning. Many studies estimate that OP pesticides are responsible for around two-thirds of these deaths—a total of 200000 a year. Deaths from unintentional OP poisoning are less common than those from intentional poisoning and seem to be more common in regions where highly toxic OP pesticides (WHO Class I toxicity) are available.¹,²

Pesticide poisoning is a significant problem in India. OP compounds are the most common cause of self-poisoning deaths in Southern India. The state of Andhra Pradesh in southern India is one of the areas of intensive agricultural production and pesticide use is very high in this state. It has one of the highest reported rates of pesticide poisoning in India.³ In the developing countries OP poisoning is the major cause of morbidity and mortality especially in patients admitted to the intensive care unit (ICU). In hospital based studies of India, mortality rates associated with pesticides have been reported to be as high as 50–70%.⁴ A study conducted in the Christian Medical College and Hospital, Vellore, reported that OP poisoning accounted for 12% of ICU admission and 29% of total poisoning admissions.⁵

Organophosphate pesticides are common household insecticides used extensively by agricultural communities in the developing countries. Seasonal variations especially in the monsoon season results in financial crisis and drive farmers to suicide by ingestion of agricultural pesticides. Most of these pesticides are sold directly from shops due to lack of special rules and regulations regarding the use and sale of pesticides in countries like India.⁶

Early identification followed by effective management in the initial stages increases the rate of survival among OP patients. Standard treatment involves initial gastrointestinal decontamination followed by intravenous administration of atropine and pralidoxime to counter act the acetyl cholinesterase inhibition. The role of atropine in OP poisoning is already well established.⁴ The benefits of oximes are not clear in these patients. Several studies conducted to study the efficacy of oximes in OP poisoned patients showed contradictory results about its efficacy in these patients.⁷ A recent study conducted by Eddleston etal., showed that even though there is a clear
evidence of reactivation of red cell acetylcholinesterase in diethyl organophosphorus pesticide poisoned patients due to oxime, there is no clear evidence that it influences the outcome in OP poisoning patients. Most of these studies don’t clearly mention either the dose of oxime used or the severity of poisoned cases at the time admission. Recently, glycopyrrolate has also been tried as an alternative for atropine. The major advantage of this drug is that it has less effect on the central nervous system when compared to atropine and has selective action on the peripheral cholinergic system.

In developing countries there is a scarcity of skilled and trained medical professionals in regard to the rational use of antidotes. There is also a scarcity of antidote which may be one of the reasons for higher mortality rates in poisoning management. OP poisoning with WHO category Ia pesticides need higher monitoring with properly trained medical professionals and a highly sophisticated intensive care unit. The majority of cases reported in India are due to methyl-parathion which is widely used by the farmers. It is a highly lipophilic compound where continuous administration of oximes may be needed for a longer time for its management. The kinetics of oximes also play an important role in the outcome of OP poisoned cases. The kinetic data of oximes suggest that continuous infusion maintained at constant plasma concentration is most important for efficacy of pralidoxime. WHO also recommends 30mg/kg bolus dose of pralidoxime followed by 8mg/kg/hr continuous infusion in moderate to severe OP poisoned patients.
1.3 Review of Literature

Acute organophosphorus poisoning

Poison is a substance that causes harm if it gets into the body. Acute poisoning is a single exposure that occurs in day or several exposures over a day or less. Acute exposure can be suicidal or accidental or homicidal. Acute poisoning continues to be an important public health problem and represents a frequent cause of admission in emergency departments of the hospital. The OP pesticides belong to a class of organic insecticides and chemically are derivates of phosphoric acid which are highly toxic to vertebrates. They are the most popular insecticides because of their effectiveness and lack of persistence in the environment due to their unstable chemical nature. Moreover they are cheaper and easily available in developing countries like India.\textsuperscript{12,22} Poisoning is a common method of suicide in India. In many Indian reports, the rates of poisoning as a suicidal method range from 20.6\% (10.3\% organophosphorus) to 56.3\% (43.8\% organophosphorus).\textsuperscript{23} A survey by Latha and Bhat reported that poisoning rates in the suicide attempters who attend hospital varies from around 40\% to over 80\% in many Indian studies and OP compounds available as pesticides are amongst the most common poisons used.\textsuperscript{24} In hospital based studies, mortality rates associated with these pesticides have been reported to be as high as 50–70\%. Most patients are young (less than 30 years) with a male preponderance. The incidence of occupational and accidental poisoning are comparatively less in India compared to suicidal poisoning.\textsuperscript{25}

Classification of OP pesticides

World Health Organization classified the OP pesticides based on LD50 for rats (WHO classification of pesticides 2001) as shown in table 1.\textsuperscript{26}

<table>
<thead>
<tr>
<th>Class</th>
<th>LD\textsubscript{50} for the rat (mg/kg body weight)</th>
<th>Oral</th>
<th>Dermal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Solids\textsuperscript{a}</td>
<td>Liquids\textsuperscript{a}</td>
</tr>
<tr>
<td>Ia  Extremely hazardous</td>
<td>5 or less</td>
<td>20 or less</td>
<td>10 or less</td>
</tr>
<tr>
<td>Ib  Highly hazardous</td>
<td>5 - 50</td>
<td>20 - 200</td>
<td>10-100</td>
</tr>
<tr>
<td>II Moderately hazardous</td>
<td>50 - 500</td>
<td>200 - 2000</td>
<td>100-1000</td>
</tr>
<tr>
<td>III Slightly hazardous\textsuperscript{b}</td>
<td>Over 500</td>
<td>Over 2000</td>
<td>Over 1000</td>
</tr>
</tbody>
</table>

Table 1. WHO Classification of pesticides

\textsuperscript{a}LD50 varies with the method of administration. The values above are for oral administration.

\textsuperscript{b}LD50 values for slightly hazardous pesticides are not well established.
Among the four classes of pesticides, class Ia and class Ib come under extremely hazardous and highly hazardous category. Whenever a patient is admitted with a history of consumption poisoning there is a great need for the first aid with trained medical expert’s team.

**Epidemiology**

World Health Organization (WHO) in 1990 estimated around 3 million poisoning cases, with 220,000 deaths occurring annually. About them 99% of these deaths occur in developing countries like India. According to WHO report 2002, about 849,000 people die globally from self-harm each year and the number of cases as a result of poisoning with pesticides is unknown. However, poisoning is the commonest form of fatal self-harm in rural Asia accounting for over 60% of all deaths. Among them OP pesticide self-poisoning is an important clinical problem in rural regions of the developing world and accounts for about two third of the deaths all over the world. A study conducted in Sri Lanka on 8110 poisoned patients showed that majority of admissions in the intensive care units were due to acute ingestion of OP compounds involving young adults who deliberately self-poisoned themselves with pesticides.

In India pesticide poisoning is a major problem in the agricultural group. Majority of cases have been reported from Andhra Pradesh, Southern India. A study conducted in 6 different government hospitals of Warangal showed that about 8040 reported cases had a fatality rate of 22.6%. OP poisoning constitutes 60% of the total poisonings with two-thirds of the patients aged <30 years, 57% were males and 96% of them had intentionally poisoned themselves. In a study conducted in Christian Medical College and Hospital, Vellore, OP poisoning accounts for 12% of ICU admissions and 29% of total poisoning admissions. Similarly, a study conducted in Mangalore for 2 years and 5 months showed that acute poisoning accounts for a total of 1% of the ICU admissions. OP poisoning constituted around 66.9% of the ICU admissions. Majority of the patients were 21-30 years of age with male to female ratio 2.4:1.
**Pathophysiology of OP poisoning**

The OP pesticides mainly inhibit the carboxyl ester hydrolases, particularly acetylcholinesterase (AChE) in human. There are different types of cholinesterases in the human body and they differ in their location in the body, affinity for the substrate and in their function. The acetylcholinesterase (AChE) found in the nervous system and outer membrane of red blood cells called as true cholinesterase. Another type of cholinesterase present in the plasma, liver, cerebrospinal fluid and glial cells is called psuedocholinesterase.

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**Fig 1. Mechanism of action of OP compounds**

The function of AChE is the termination of action of acetylcholine at the junctions of the various cholinergic nerve endings with their effector organs or post-synaptic sites. OP compounds are the most important AChE inhibitors and they also called as anticholinesterases. In the presence of OP compounds, AChE becomes progressively inhibited and not able to hydrolyze in to acetylcholine to choline and acetic acid. This causes acetylcholine to accumulate at cholinergic receptor sites and thus produce effects equivalent to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous systems.\(^{32, 33}\) These inhibitors bind at catalytic sites of AChE by phosphorylation and the phosphorylated enzyme is stable. The inhibited AChE enzyme can be spontaneously reactivated at different rates depending on the type of OP compound. Once these AChE enzymes are inhibited they induce overstimulation of nicotinic and muscarinic receptors in the central and peripheral nervous systems resulting in clinical signs and symptoms. There are indications that signs and symptoms only appear after 50% of AChE inhibition and death occurs at >90% of AChE inhibition, if adequate treatment is
not provided. The duration of inhibition mainly depends on the type of OP compound. AChE inhibited by OPs might undergo two different reactions; spontaneous reactivation or aging. The presence of sulphur in the OP compounds instead of oxygen greatly increases the rate of reactivation of the analog compounds. Reactivation in the presence of larger alkyl groups is much slower when compared to smaller alkyl groups. Aging is due to the loss of one of the side-chains attached to the phosphorus, leaving a negatively charged mono-substituted phosphoryl residue attached to the enzyme which is never reactivated by oximes.\(^{34}\)

Recent prospective studies suggest that the intermediate syndrome is associated with high doses or slow kinetics of the toxicant causing high and persistent AChE inhibition for up to 2-3 days. Intermediate syndrome is a distinct clinical feature developed after acute cholinergic crisis, but before the delayed neuropathy and characterized by various degrees of cranial nerve palsies and proximal muscle weakness. It develops 24-72 hours after acute poisoning. Neuropathological damages occur due to excessive cholinergic crisis or convulsions may lead to neurobehavioral changes which are very rare in OP poisoning. Certain OP compounds also cause a delayed polyneuropathy after 2-5 weeks of acute poisoning. Pathophysiology of this delayed polyneuropathy in these patients is not clearly understood.\(^{35}\)

**Role of Cholinesterase level in identification of OP poisoning**

As all the OP compounds and carbamates are potent inhibitors of cholinesterase enzymes, cholinesterase assays play an important role in identification of the poisoning of these compounds in the early stages. Even though it helps in the initial stages of poisoning, but it was not useful to assess severity or confirmatory tests for OP poisoning unless the precise pesticide ingested is known to patient.\(^{36,37}\)

Recent studies suggest that red cell AChE activity accurately correlates with AChE activity at the synapse and it will be good marker of severity in initial stages of poisoning.\(^{38,39}\) However serial measurements of cholinesterase for the prognosis of OP poisoning are still controversial. It depends on the poison load, aging of AChE enzymes, nature of OP compounds etc. A study conducted in Saint John's Medical College, Bangalore suggested that the correlation between serial measurements of cholinesterase level with prognosis of OP poisoning doesn’t have any significance.\(^{40}\) But another study conducted in Turkey in 1999 to 2001 on 32 patients showed
that serum acetylcholinesterase activity may be a useful parameter in following the acute prognosis of OP poisoning.\textsuperscript{41}

**Clinical manifestation of OP poisoning**

Clinical signs and symptoms of OP poisoning are broadly classified as 1) Muscarinic effects 2) Nicotinic effects 3) Central effects. The different signs and symptoms are tabulated in table 2.\textsuperscript{42}

<table>
<thead>
<tr>
<th>Muscarinic effects</th>
<th>Nicotinic effects</th>
<th>Central effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradycardia, Hypotension</td>
<td>Tachycardia, Hypotension, Weakness, Fasciculations, Cramps, Paralysis</td>
<td>Anxiety, Restlessness, Ataxia, Absent reflexes, Convulsions, Insomnia, Tremors, Dysarthria, Coma, Hyperreflexia, Stokes breathing, Respiratory depression, Circulatory collapse</td>
</tr>
<tr>
<td>Rhinorrhea, Bronchorrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchospasm, Cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased salivation, Nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting, Abdominal pain, Diarrhea, Fecal incontinence, Urinary incontinence, Blurred vision, Increased Lacrimation, Miosis, Excessive sweating</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Clinical signs and symptoms of OP poisoning**

Apart from this, generally OP poisoning is associated with three different neurological complications. These are recognized based on the time of occurrence and differ in their pathophysiology.

(i) Type-I paralysis or Acute paralysis

It occurs with the initial cholinergic crisis and usually develops within 24-48 hours. The main features include muscle fasciculations, muscle cramps, muscle twitching and muscle weakness. Muscle weakness may have upper motor neuron manifestations. Muscle paralysis usually involves the respiratory muscles leading to respiratory failure and requires ventilation.

A study conducted in China on acute OP poisoning cases showed that out of 107 acute OP poisoning cases, 33% of them got type I acute respiratory paralysis.\textsuperscript{43} In another prospective study carried out by Eddleston \textit{et al.}, showed that out of 376 acute OP poisoning cases, 52 patients got type I acute respiratory paralysis within 2 hours of ingestion.\textsuperscript{44}
(ii) Type-II paralysis or Intermediate syndrome
Intermediate syndrome usually develops after the acute cholinergic crisis, 24-96 hours after the poisoning. One of the earliest manifestations in these patients is the presence of marked weakness of neck flexion with the inability to lift the head. Its most commonly used as a reliable test to assess whether the patient is likely to develop respiratory muscle weakness. The common cranial nerves involved are those supplying the extraocular muscles. This was first described by Wadia et al., as Type-II paralysis and subsequently described and termed as intermediate syndrome by Senanayake and Karaliedde. This syndrome persists for approximately 4-18 days and most patients survive this with ventilator support unless infections complicate the course.

(iii) Type-III paralysis or OP-Induced delayed polyneuropathy
OP-induced delayed polyneuropathy (OPIDP) is a sensory-motor distal axonopathy that usually occurs after the ingestion of large doses of certain OP insecticides or after chronic exposure. Usually it develops after 2-3 weeks of acute poisoning episode and is characterized by distal muscle weakness with sparing of neck muscles, cranial nerves, and proximal muscles and recovery may also extend up to one year. The high-dose methyl prednisolone found to beneficial in animal studies.

Assessment of severity of poisoning by clinical scores
For developing uniform and effective management strategies it is necessary to assess the severity of poisoning through scientific methods of approach. Among acute OP poisoning cases there is a lack of accurate and precise laboratory methods to assess the severity and prognosis of poisoning to check effectiveness of management. Considering this, literature search was made for using effective scoring system to assess the severity and prognosis. The majority of articles effectively used GCS (Glasgow Coma Scale), APACHE-II (Acute Physiology and Chronic Health Evaluation II), PSS (Poison severity Score) scoring systems to assess the severity of poisoning.
**Glasgow Coma Scale (GCS)**

Glasgow Coma Scale is a neurological scale which gives a reliable and objective way of recording the conscious state of a person to assess the severity and as well as prognosis. It is also used as a neurological predictor in other scoring systems like APACHE, APACHE II, APACHE III, Acute Physiology Score (APS) and Simplified Acute Physiology Score (SAPS). Several studies of poisoning that have evaluated the GCS for brain injury.\(^49\) Chan et al., reported that an initial GCS score of 8 or less was found to be a useful guideline for intubation when used within specific clinical context (sensitivity = 90%, specificity = 95%).\(^49\) Emerman et al., concluded that a GCS score of less than 8 was the most sensitive predictor of serious complications (sensitivity = 86%, specificity = 89%) in tricyclic antidepressant overdose.\(^51\) Grmec et al., found a good sensitivity and specificity with GCS score in predicting respiratory failure and found it to be relatively good in predicting in-hospital mortality following OP poisoning.\(^50\)

Glasgow Coma Scale is a neurological scale which seems to give a reliable, objective way of recording the conscious state of a person, for initial as well as continuing assessment. GCS score is assigned, based on three responses, i.e. best eye response, best verbal response, and best motor response. A maximum of 15 and a minimum of 3 is assigned. The severity is assigned based on the GCS scores as Severe (GCS ≤ 8); Moderate (GCS 9 – 12); Minor (GCS ≥ 13).\(^50\)

**Acute Physiology and Chronic Health Evaluation II (APACHE-II)**

Acute Physiology and Chronic Health Evaluation II (APACHE II) is used to predict the probability of mortality through the estimate of severity of a disease. Apache II score is ideal when applied for patients aged 15 or older and is used to describe the morbidity of a patient when comparing the outcome. Predicted mortality rates are averaged for groups of patients in order to specify the group’s morbidity. Even though newer scoring systems like SAPS II have replaced APACHE II in many places, APACHE II will be the more precise when compared to the existing one. The APACHE II scores are calculated from 12 routine physiological and laboratory measurements. The parameters used for calculating APACHE II scores are body temperature, mean arterial pressure, heart rate, and respiratory rate, partial oxygen pressure (PaO2), arterial blood pH, serum sodium, serum potassium, serum creatinine, haematocrit, white blood cell count and GCS. The sums of these 12 values are called Total Acute Physiology Score.
(A). It is added to age points (B) and chronic health points (example severe organ insufficiency or immunocompromised patients), to arrive at the APACHE II score. Higher the APACHE II score more worse the prognosis of the patients. According to a study conducted by Sungurtekin K et al., there was a significant correlation between the mortality and scoring systems, like APACHE II, GCS, APACHE III and SAPS II and he recommended the use of APACHE II scores in OP poisoning patients due to its high precision and specificity. Another study by Bilgin et al., showed that the three scoring systems like GCS, APACHE II, and SAPS II had similar impacts in OP poisoning patients and GCS system has superiority over the other systems as it easy to perform and no laboratory parameters are required for estimation.

Poison severity Score (PSS)

PSS is a standardized scale for grading the severity of poisoning and allows qualitative evaluation of morbidity caused by poisoning. The PSS was adopted by the IPCS, the Commission of the European Union, and the European Association of Poison Centers and Clinical Toxicologists (IPCS/EC/EAPCCT). It is not a prognostic score but it defines the degree of severity based on the overall clinical features of poisoning. Severity grading should take into account only observed clinical symptoms and signs and it should not estimate risks or hazards on the basis of parameters such as amounts ingested or serum/plasma concentrations. It is also useful for estimating the outcome of poisoning. The occurrence of a particular symptom will be checked against the chart (Appendix III ) designed by the IPCS and severity will be graded. The severity will be graded from 0 to 4 ranging from no toxicity to severe life threatening symptoms and death is taken into consideration, clinical signs/symptoms and/or laboratory data. The four levels are represented as follows; grade 0 for no symptoms or signs, grade 1 for mild, transient and spontaneously resolving symptoms or signs, grade 2 for moderate, pronounced or prolonged symptoms or signs, grade 3 for severe or life threatening symptoms or signs, while grade 4 represents extremely severe toxicity leading to mortality. A multicenter cohort study carried out in Srilanka showed that both GCS and the IPCS/PSS were similarly effective at predicting outcome in acute OP poisoning patients.
Different management strategies used in the OP poisoning

Management of OP poisoning is still a great challenge to the treating physician and is always associated with high morbidity and mortality particularly in developing countries. Current treatment practices mainly focused on gastrointestinal decontamination followed by administration of atropine and oximes as antidotes. The general management mainly aims to control the symptoms and reverse the effect of the OP compound.

Gastrointestinal decontamination

Gastric lavage and activated charcoal are most commonly used in gastrointestinal decontamination. Gastric lavage is the first intervention and most common form of decontamination for OP poisoned patient at the initial stages. Guidelines are suggest that lavage should be considered only if the patient arrives within 1 hour of ingesting the poison. The relevance of these guidelines with respect to OP poisoning is unclear. Gastric lavage should be considered for patients who present soon after ingestion of a substantial amount of toxic pesticide who are intubated, or conscious and willing to cooperate. There no randomized controlled trials to confirm benefit of the gastric lavage in OP poisoning. Ipecacuanha-induced emesis should not be used in OP pesticide poisoning because of risk of aspiration.55, 56

Activated charcoal can also be used in the treatment of OP poisoning if the patient is cooperative or intubated. It can be administered as a single dose or in multiple doses if the patient presents to the hospital within 1-2 hours of ingestion or in cases of severe toxicity. 1gram/kg of activated charcoal can be given orally via nasogastric tube at the end of the lavage. A randomized controlled trial of single and multiple doses of superactivated charcoal in Sri Lanka failed to find a significant benefit of either regimen over placebo after testing in more than 1000 patients poisoned with pesticides.37

Role of different Antidotes in OP poisoning

Atropine and oximes are the most commonly used antidotes in the management of poisoning. Recently the addition of glycopyrrolate was found to be beneficial in the management of OP poisoning. It is administered along with atropine and reduces the total atropine requirement in the patient.
Atropine:

Anticholinergics are still the mainstay of treatment in acute OP poisoning and should be started as soon as the airway has been secured. Atropine will probably remain the drug of choice because it is available widely, is affordable, and is moderately able to penetrate the CNS. It has only antimuscarinic action, but has no effect on nicotinic receptors. No known randomized controlled trials have compared different regimens of atropine for either loading or continuation therapy. A 2004 review identified more than 30 dosing regimens of atropine, some of which would take many hours to obtain the full loading dose of atropine. The aim of early atropine therapy is to reverse cholinergic features and to improve cardiac and respiratory function as quickly as possible.\(^\text{29}\) The target end-points for atropine therapy are clear chest on auscultation, heart rate>80/min, systolic BP >80mmHg, pupils no longer pinpoint, dry axillae.\(^4\)

The recommended dose of atropine varies from patient to patient till target end point is reached. One dosage regimen says atropine is started initially as a 2-mg intravenous (IV) bolus and then at doses of 2-5 mg IV bolus every 5-15 minutes until atropinization is achieved.\(^\text{29}\) In another dosage regimen, an initial bolus of 1-2mg is recommended with subsequent doses doubled every 5 minutes until atropinisation is achieved. This regimen requires not more than 20 minutes to administer 25mg of atropine. It was found to be effective and the most appropriate therapy for OP poisoned patients who need a large dose of atropine and it also works well for patients who need even low doses of atropine as low as 1mg.\(^\text{29}\) Similarly a study conducted in South India recorded the benefit of an atropine infusion compared with repeated bolus doses, but the drawback of this study was that it used historical controls which reduced the confidence in this finding. Continuous infusion reduced the blood level fluctuation of atropine when compared to bolus dose.\(^\text{57}\)

Oximes:

Oximes are nucleophilic agents that are known to reactivate the phosphorylated acetylcholinesterase by binding to the OP molecule. Pralidoxime is the most common and widely used oxime. Use of oximes in OP poisoning remains conflicting and controversial. It is more effective when it is administered immediately after exposure. However, in some cases it was found more effective in patients who presented late, even 2 to 6 days after exposure.\(^\text{58}\) WHO recommends an initial bolus of at least 30 milligrams/kilogram followed by an infusion of more
than 8 milligrams/kilogram/hour. In some cases it can be given as an intermittent dosing with 1 to 2 grams diluted in 100 milliliters of normal saline infused over 15 to 30 minutes. The dosing may be repeated at one hour after the initial dose if muscle weakness or fasciculations are not relieved. The dose can be repeated at 3 to 8 hour intervals. The maximum dose in a day should not exceed 24 grams.

**Glycopyrrolate:**

Glycopyrrolate has also been tried as an alternative for atropine. It is a quaternary ammonium anti-cholinergic agent, with anti-muscarinic activity with high selectivity for peripheral cholinergic sites like atropine and has been suggested as a useful drug in organophosphate intoxication for controlling secretions with minimal side-effects of flushing, tachycardia, and depressed level of consciousness often experienced with atropine. Glycopyrrolate has the advantages of better secretion control, less tachycardia and inability to cross the blood-brain barrier but it is less effective in treating bradycardia and may not affect central neurological effects from organophosphates. A randomized control trial was carried out to compare the effects of atropine and glycopyrrolate on 44 OP poisoned patients and 39 patients were evaluated (22 atropine and 17 glycopyrrolate cases). The outcome was same in both the groups like fatalities, need for ventilation etc. A case report by Peter et al., supported the successful treatment of glycopyrrolate in a patient with OP poisoning. The combination of atropine and glycopyrrolate is more beneficial as it reduces the atropine requirement and thereby reduces the effect on the central nervous system.

**Role of other experimental therapies in management of OP poisoning**

**Benzodiazepines**

Patients poisoned with OP frequently develop agitated delirium and some patients also develop seizures. Diazepam is the first-line therapy and it is used to reduce the agitation in OP poisoning. Diazepam is given at a dose of 10 mg by slow IV push and repeated as necessary up to 30–40 mg per 24 hours. Diazepam is preferred over haloperidol because of high doses of haloperidol may be needed for the patients receiving the atropine.
Magnesium Sulphate

No systematic review or randomized clinical trials, or observational studies of sufficient quality are available to prove benefit of magnesium sulphate in OP poisoning. Magnesium sulphate is an inhibitor of acetylcholine release in the central nervous system and at peripheral sympathetic and parasympathetic synapses. The use of magnesium in acute OP poisoning in humans has been reported in two small studies.\textsuperscript{63, 64}

Sodium bicarbonate

Alkalization of the serum to pH 7.5 with sodium bicarbonate may be useful in the management of OP poisoning. Clinicians in Iran reported the successful management of OP-intoxicated patients using infusions of sodium bicarbonate.\textsuperscript{65} A Cochrane review concluded that insufficient evidence exists at present to establish whether sodium bicarbonate should be used in humans poisoned with OP compounds.\textsuperscript{66}

Clonidine

The alpha2-adrenergic receptor agonist clonidine also reduces acetylcholine synthesis and release from presynaptic terminals. Animal studies show the beneficial effect of clonidine treatment, in combination with atropine and effects in human still not known.\textsuperscript{67}

Fresh frozen plasma

Use of fresh frozen plasma in people with OP poisoning is that it may reduce high blood pesticide concentrations by increasing levels of butyrylcholinesterase. Butyrylcholinesterase is generally sensitive to OP pesticides and it is rapidly used up in moderate to severe poisoning. Replacement of butyrylcholinesterase can possible by administration of fresh frozen plasma or by plasmapheresis. This will increase the level of enzyme in the blood and neutralize some pesticide. But increase in the level of butyrylcholinesterase enzymes and its clinical significance is not clear. A small controlled study which compared two arms, one arm treated with plasma (n=12) and other arm without plasma (n=21) showed benefit with fresh frozen plasma. But it was not a randomized trial and allocation of decisions was unclear.\textsuperscript{68}
**Role of pralidoxime in organophosphorus poisoning**

The principal pharmacologic effect of pralidoxime is reactivation of cholinesterase which has been inactivated by phosphorylation as a result of exposure to organophosphates. Pralidoxime removes the phosphoryl group from the active site of the inhibited enzyme by nucleophilic attack and regenerates active cholinesterase forming a soluble complex with oximes. Pralidoxime also detoxifies certain OPs by direct chemical reaction and probably also reacts directly with cholinesterase to protect it from inhibition. Pralidoxime must be administered before aging of the inhibited enzyme. After aging is completed, phosphorylated cholinesterase cannot be reactivated. Pralidoxime does not equally antagonize all anticholinesterases because the aging of the inhibited enzyme varies and it mainly depends on the specific organophosphate bound to the cholinesterase. Pralidoxime also reactivates the cholinesterase inhibited by the carbamates which is having a faster reactivation than the phosphorylated compounds. The reactivation of anticholinesterase mainly occurs at the neuromuscular junction and there by inhibition of paralysis of respiratory and other skeletal muscles.42, 69

**Controversies of oximes used in OP poisoning**

There are many controversies regarding the usage of pralidoxime in OP poisoning. A total of 7 clinical trials and 2 meta-analyses concluded doubtful opinion regarding the efficacy of oxime use in OP poisoning. But recent studies supported that continuous infusion of high dose of oximes showed a better outcome when compared to intermittent dosing.11, 19, 20 Similarly a study conducted by Eddleston et al., concluded that despite reactivation of red cell acetylcholinesterase in organophosphorus pesticide poisoned patients, there is not enough clinical evidence to show that continuous infusion has a better outcome in OP poisoned patients.8 But this study was also unable to conclude effectiveness of continuous infusion in OP poisoning. Many kinetic studies conducted showed that continuous infusion maintained a constant plasma concentration which is most important for efficacy of pralidoxime.11

Though there are several studies conducted, no studies were able to conclude whether pralidoxime can be used as an effective antidote or what dose is effective and how much duration this drug has to be administered. Presently two dosage regimens of pralidoxime are commonly used in OP poisoning viz. intermittent bolus dose and continuous infusion.
(i) Intermittent bolus dose

Initial dose for adults is 1 to 2 grams diluted in 100 milliliters of normal saline infused over 15 to 30 minutes. In cases where intravenous administration is not possible, pralidoxime can be given intramuscularly at an initial dose of 1 gram or up to 2 grams in cases of very severe poisoning. The dosing may be repeated at one hour after the initial dose if muscle weakness or fasciculations are not relieved. The dose can be repeated at 3 to 8 hour intervals.  

(ii) Continuous infusion

WHO recently recommended an initial bolus of at least 30 milligrams/kilogram of pralidoxime followed by an infusion of more than 8 milligrams/kilogram/hour. Pawar et al, used high doses of pralidoxime with 2g loading dose followed by 1g/hr for 48 hours and then 1g/4th hourly till weaned from ventilator.  

For children, initial dose of 20 to 50 milligrams/kilogram (maximum 2 gram/dose) infused over 30 minutes as a 5% solution in normal saline. This was followed by administration of 10 to 20 milligrams/kilogram/hour of a solution containing 10 to 20 milligrams of pralidoxime/milliliter.  

The duration of administration should be continued for at least 24 hours after cholinergic manifestations have resolved. Prolonged administration may be necessary in severe cases, especially in the case of poisoning by lipophilic OP compounds like phosphorothioates.  

Pharmacokinetics of pralidoxime

The initial response of the drug is seen after one hour of IV administration of pralidoxime in acute OP poisoning patients. It can be administered as a single repeated bolus dose or continuous infusion.

Drug concentration level

The original study by Sundwal et al., indicated that 4 µg/mL was the minimum effective level, with a range of 4 to 12 µg/mL. Most of the studies reported therapeutic concentration of 4 µg/mL. A study conducted in a healthy individual showed that pralidoxime chloride administered through IV at 7.5–10 mg/kg produced a plasma concentration of 4 µg/mL or greater after 1 hour of administration.
Jovanovic conducted a study to compare the plasma concentration of pralidoxime in human volunteers and in OP poisoning patients after 1g of pralidoxime chloride given through IM route was done. He observed that there was 1.5 times more elevation in plasma concentrations of pralidoxime mainly due to prolongation of its half life in poisoned individuals. These changes were attributed to the disturbed general hemodynamics and reduced renal blood flow during OP intoxication. He concluded that prolongation of effective half life would provide therapeutic benefit in OP poisoning patients. The study conducted by Willem et al., on OP poisoning patients showed that the required plasma concentration of 4mcg/dl was also obtained after a loading dose of 4.42 mg/kg followed by maintenance dose of 2.14 mg/ kg/hour after 5-6 half lifes. A study conducted by Schexnayder et al., on OP poisoned children treated with pralidoxime loading dose of 15-50 mg/kg given intravenously over 30 minutes, followed by a continuous infusion of 10-20 mg/kg/hour showed that the serum concentration of pralidoxime was maintained greater than 4mcg/dl (22±12 mg/L, mean standard deviation, range 6.9–47, n = 11). This was found to be more than the value reported by Willems et al. They concluded from the study that pharmacokinetics of pralidoxime in children with OP poisoning differs from those reported for healthy adults and for poisoned adults.

When the drug is given intramuscularly it attains a peak concentration within 30 minutes. When administered by IV route peak plasma concentration is reached at 16 minutes. Intramuscular doses of 7.5 to 10 mg/kg every 4th hourly produced peaks greater than 5 to 6 µg/mL and maintained levels greater than 4 µg/mL for approximately 0.75 to 1.0 hour. A single 1 g IM dose (mean 12.5 mg/kg) provided peak whole blood levels of greater than 8 µg/mL, with levels above 4 µg/mL for about 1.5 hours.

**Distribution**

Following intravenous injection, pralidoxime distributes in most of the body fluids and is not significantly bound to plasma protein or hemoglobin. Pralidoxime penetrates the red blood cells both in vivo and in vitro by simple diffusion which follows first order kinetics with a t1/2 of about 4.5 hours. Data for the apparent volume of distribution at steady state (Vdss) for pralidoxime chloride 5 mg/kg IV was 0.7 ± 0.1 L/kg in human volunteers. This result was a little contrast with Willems and coworkers who did a study on OP poisoning patients. They administered a loading dose of pralidoxime methylsulfate 4.4 mg/kg IV followed by a
continuous infusion at 2.1 mg/kg/h and they found that volume of distribution (Vd) in the beta-phase was $2.77 \pm 1.45$ L/kg in OP-poisoned patients.\textsuperscript{72} Hence, it appears that the volume of distribution of pralidoxime in OP-poisoned patients is markedly higher than in healthy people.

**Metabolism and Excretion**

Most of the drug is excreted from urine without any metabolism. However in some volunteers 1-methyl-2-cyanopyridinium ion was detected in urine in people who received oral pralidoxime methanesulfonate 3g, suggesting some cyanogenic metabolism.\textsuperscript{77}

Pralidoxime is mainly excreted through kidney. The renal clearance (rate) of the drug is 600 to 700 mL/min. Renal clearance ratio of 4:1 indicates active secretion by renal tubular cells.\textsuperscript{76} Around 20\% to 99\% of the drug is excreted through renal secretion. The elimination half-life varies from 1.2-2.6 hours. Willems et al., showed that the renal clearance markedly increased in OP poisoning cases when compared to healthy volunteers.\textsuperscript{72} Different studies is listed in table 3 showing the elimination t1/2 of pralidoxime with healthy volunteers and OP poisoning patients.

**Literatures on blood level of pralidoxime**

Different dosage regimens of pralidoxime were proposed in OP poisoning patient’s viz intermittent and continuous infusion. Most of the recent studies supported that the latter approach was appropriate.\textsuperscript{19, 20, 42, 78} A study by Medicis et al., showed that short-term intermittent infusion of 1g pralidoxime within 30 minutes resulted in high plasma levels up to 30 mg/L pralidoxime and it is associated with a highly significant increase in diastolic blood pressure of 20mm Hg in healthy volunteers. This is followed by a deep trough within a short period. But in continuous infusion group a constant plasma concentration was maintained throughout the infusion and no adverse effect was seen.\textsuperscript{79}
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Num. of subjects</th>
<th>Dose of PAM chloride</th>
<th>Route of Adm.</th>
<th>Elimination t1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creasey &amp; Green (1959) (^{74})</td>
<td>Healthy volunteer</td>
<td>6</td>
<td>2.5-10 mg/Kg</td>
<td>IV</td>
<td>73.7 ± 15.2 min</td>
</tr>
<tr>
<td>Sidell et al., 1972 (^{76})</td>
<td>Healthy volunteer</td>
<td>6</td>
<td>5 mg/Kg</td>
<td>IV</td>
<td>78.6 ± 7.8 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>84.6 ± 14.4 min</td>
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<td>(For PAM. chloride)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(PAM methane sulfonate)</td>
</tr>
<tr>
<td>Jovanovic 1989 (^{21})</td>
<td>Healthy volunteer</td>
<td>9</td>
<td>1000 mg</td>
<td>IM</td>
<td>148.9 ± 65.7 min</td>
</tr>
<tr>
<td></td>
<td>OP poisoning Pt.</td>
<td>6</td>
<td>1000 mg</td>
<td>IM</td>
<td>174.4 ± 70.9 min</td>
</tr>
<tr>
<td>Willems et al, 1992 (^{72})</td>
<td>OP poisoning Pt.</td>
<td>9</td>
<td>2.14 mg/kg/h.</td>
<td>IV</td>
<td>206.4 ± 54 min</td>
</tr>
</tbody>
</table>

Table 3. The elimination t1/2 of pralidoxime from different studies

A study by Williem et al., showed that the target plasma concentration of 4 mg/L, calculated from the data from volunteers, was approximately obtained in OP-poisoned patients (between 2.1 and 9; n = 9) with pralidoxime methylsulfate by a loading dose of 4.42 mg/kg and a maintenance dose of 2.14 mg/kg/hr. There was a variation (also intra individually) observed among the study group and it was explained by changes in the clinical condition of the patients. The total clearance in patients was very similar to the values found in healthy volunteers. But there was a significantly longer t1/2 seen in patients due to large Vd of pralidoxime.\(^{72}\)

Another study by Casey et al., on OP poisoned patients showed that pralidoxime methane sulfonate given either by bolus 30 mg/kg IV every 4 hours or by intravenous infusion at 10
mg/kg/hour showed concentrations of pralidoxime in blood between 4.31 mg/L and 145 mg/L (trough and peak level) for patients receiving bolus dose and in the range of 22.4 mg/L to 36.26 mg/L for patients receiving continuous infusion respectively. The concentrations found during steady-state are significantly higher than the 13.8 mg/L (level of pralidoxime corresponding to dosage regimen of 0.5 g/hour pralidoxime chloride in a 70kg person). The altered level was due to diminished clearance in the intoxicated patient which may have caused some accumulation. A large variation in the steady-state serum concentrations (22 ± 12 mg/L, mean standard deviation, range 6.9–47, n = 11) was also found in OP-poisoned children receiving pralidoxime chloride 14 mg/kg/hour.

**Blood level of pralidoxime and adverse effect**

Higher blood levels are associated with some of the minor side-effects in healthy individuals. Usually higher doses of pralidoxime for a shorter duration are associated with some of the minor effects in healthy individuals. A study on human volunteers by Medicis et al., 1996 showed that traditional short term infusion of 16 mg/kg over 30 minutes produced dizziness or blurred vision and occurred in all subjects when compared to continuous infusion 4 mg/kg of pralidoxime intravenous over 15 minutes followed by 3.2 mg/kg/h for 3.75 hours (for a total dose of 16 mg/kg). This is because of high blood level of pralidoxime in a short term infusion. Blurred vision, diplopia and dizziness were also caused by injection of pralidoxime methane sulphonate, lasting a few minutes following administration of 30 mg/kg IV. Administration of pralidoxime chloride 30 mg/kg IM resulted in ECG changes with T-wave elevation and increased blood pressure.

Other studies by Sidell et al., showed that IM injection of pralidoxime chloride upto 600mg caused no changes in heart rate or blood pressure but only mild pain at the site of injection. Burning and stinging at the injection site following 0.5–1.0g pralidoxime chloride IM was also reported by others. A serious adverse effect occurred in a coumaphos-intoxicated patient after pralidoxime iodide infusion of 0.4g over 2 minutes and the patient experienced a cardiac arrest. He responded rapidly to intravenous sodium bicarbonate and adrenaline. Rapid injection was also associated with tachycardia, laryngeal spasm, muscle rigidity and transient neuromuscular blockade. Hence it appears that excess of plasma concentration associated with short term infusion resulted in more adverse effects.
Similarly in another study by Schexnayder et al., on OP poisoned children treated with a pralidoxime loading dose of 15-50 mg/kg given intravenously over 30 minutes, followed by a continuous infusion of 10-20 mg/kg/hour did not produce any side effect at mean serum concentration of 22 ± 12 mg/L.  

Overall conclusion of these studies showed that the patients with continuous infusion of pralidoxime not experienced any adverse drug reaction when compared to bolus dose.

**Different methods of HPLC used for estimation of blood level of pralidoxime**

The reported analytical methods using analytical technique HPLC for estimation of serum pralidoxime are summarized below in table 4.

<table>
<thead>
<tr>
<th>Analytical Technique</th>
<th>Study</th>
<th>Author</th>
</tr>
</thead>
</table>
| HPLC                | Measurement of serum pralidoxime methyl sulfate (Contrathion®) by high-performance liquid chromatography with electrochemical detection. | Houze, P., et al., 2005  
85 |
| HPLC                | The Pharmacokinetics of Continuous-Infusion Pralidoxime in Children with Organophosphate Poisoning | Schexnayder et al., 1998  
11 |

Table 4. Reported analytical methods for pralidoxime in serum

**Kinetics of methyl parathion in OP poisoning patients**

The majority of OP insecticides are lipophilic and they are not ionized. But they are absorbed rapidly following inhalation or ingestion. The dermal absorption is slower, but severe poisoning may occur if exposure is for a long period. Following absorption, OP compounds accumulate in fat, liver, kidneys and salivary glands. The phosphorothioates (P=S), for example diazinon, parathion, and bromophos, are more lipophilic than phosphates (P=O), for example dichlorvos, and are therefore stored extensively in fat which may account for the prolonged intoxication in humans. Phosphates (P=O) are biologically active as AChEs inhibitors, whereas phosphorothioates (P=S) need bioactivation to their phosphate analogues (oxons) to become biologically active. So intoxication features of phosphorothioates (P=S) are usually delayed
unless aerial oxidation has already occurred to generate traces of oxon. OP compounds other than phosphates (P=O) are metabolically activated to their corresponding oxon by oxidative desulfuration mediated by P₄₅₀ isoforms. OP compounds also undergo other transformations mediated by cytochrome P₄₅₀ including oxidative dealkylation and dearylation, ring hydroxylation, thioether oxidation, deamination, alkyl and N-hydroxylation, N-oxide formation and N-dealkylation which does not result in active metabolites.

Oxons inhibit AChE by phosphorylation of the serine hydroxyl group in the substrate-binding domain of the enzyme. Inhibition of AChE activity occurs in blood, brain and other tissues in a time-dependent manner. The extent of inhibition of AChE depends on the rate constant for the reaction and the time that the enzyme is exposed to the oxon. Inhibition of AChE results in accumulation of acetylcholine (ACh) at autonomic ganglia and post-ganglionic nerve endings, some central synapses and at neuromuscular junctions resulting in stimulation of both muscarinic and nicotinic receptors by Ach. AChEs inhibited by OPs might undergo two different reactions; spontaneous reactivation or aging. The presence of sulphur instead of oxygen greatly increases the rate of reactivation of the analog compound. Reactivation in the presence of larger alkyl groups is much slower or almost non-existent.

Methyl parathion, a toxic organophosphorus ester was originally developed by the German pesticide company Bayer in 1940. According to WHO report it is relatively insoluble in water but readily soluble in organic solvents. The World Health Organization classifies methyl parathion as a class Ia 'extremely hazardous' pesticide. It is highly toxic when it is inhaled or consumed and moderately toxic when it is absorbed through skin. The oral LD₅₀ in rats was found to be 2.9 mg/kg, in mice was 33.1-119.5 mg/kg, in rabbits 19-420 mg/kg and dogs 50 mg/kg. Absorption of methyl parathion in humans is rapid and complete when consumed orally when compared to dermal or inhalation.

The toxicity of methyl parathion is mainly due to the active metabolite methyl paraxon which binds to acetylcholinesterase to form dimethylphosphorylcholinesterase complex which degrades rapidly and is unresponsive to oximes.

The oral ingestion study conducted by Rider et al. concluded that acute toxicity of methyl parathion was not observed in those patients with erythrocyte cholinesterasae activity reduced to as little as 45% of their pre-exposure baseline. More over methyl parathion follows two
compartment model kinetics in humans when it is absorbed through oral or dermal route. Though the t1/2 of methyl parathion is 51 minutes because of high volume of distribution, it is deposited in peripheral compartments like liver, brain, skeletal muscle, diaphragm etc., at 2-5 fold higher concentration when compared to plasma and a prolonged suppression of acetylcholinesterase was observed in the patients even after administration of oximes. Rapid and marked inhibition of blood cholinesterase activity following oral administration of methyl parathion suggests that a significant portion of methyl parathion is converted to methyl paraxon by first pass metabolism of the liver.92,93

According to Rao et al. methyl parathion is widely used in agriculture in Southern India and is most common cause of death amongst the agriculturists. There are not many clinical studies available in the literature with respect to its clinical outcome or pharmacokinetic properties.3
1.2 Need for the Study

In developing countries like Srilanka and India, pesticide poisoning causes more death than infectious diseases. Pesticide use is poorly regulated and often dangerous; their easy availability also makes them a popular method of self harm and thousands of death continues till this date. Unfortunately, a lack of adequate government regulatory control over the use and distribution of pesticide predisposes the agriculturists to accidental and suicidal poisoning. The WHO recommends that access of highly toxic pesticide has to be restricted.\textsuperscript{7}

Management of OP poisoning is still a great challenge to the treating physician and associated with number of complication and high death rate particularly in developing countries like. Current treatment strategies of OP poisoning are mainly focusing on reversing the effect of the OP compounds by using the antidotes like atropine and oximes. Oximes play an important role in reactivating acetyl cholinesterase, that is inhibited by the OP compound.\textsuperscript{12} Use of oximes in OP poisoning is still controversial and varies in opinion between physicians with regards to dose, regimen and duration of treatment. Very limited studies are available to prove the clinical benefits of oximes in OP poisoning.\textsuperscript{7,12}

Four different randomized clinical trials on a total of 266 OP poisoned patients showed doubtful efficacy and safety of oxime use in OP poisoning. Similarly, other nonrandomized trials failed to prove the benefits of pralidoxime in OP patients.\textsuperscript{7,12} But the main drawback of all these studies are that they used low dose of pralidoxime which is not sufficient enough to reactivate the acetylcholinesterase.\textsuperscript{12} Moreover no efforts were taken to support the clinical outcome with the kinetic analysis and dosage regimen. The study design was also not clearly defined.\textsuperscript{7,12} Earlier animal kinetic studies of pralidoxime showed that blood concentration of 4 µg/ml (range between 4 to 12 µg/ml) was the minimum level required to reactivate the inhibited acetyl cholinesterase.\textsuperscript{13,14} But in vitro studies suggest that higher concentrations are required to reactivate acetyl cholinesterase at initial stages of poisoning and will be useful even after 24 hours.\textsuperscript{15} A study by Willem \textit{etal.}, concluded that reactivation of acetyl cholinesterase not only depends on the pralidoxime concentration but also on the blood concentration of OP compounds.\textsuperscript{16} Even though various meta-analysis expressed doubtful efficacy regarding use of oximes, they suggested to conduct more randomized clinical trials (RCTs) to prove the efficacy.\textsuperscript{17,18}
WHO recently recommended an initial bolus dose of 30 mg/kilogram of pralidoxime followed by an infusion of more than 8 milligrams/kilogram/hour.\textsuperscript{7} Meta-analysis by Eddleston \textit{et al.}, suggested that large, high quality RCTs comparing the current WHO-recommended regimen with placebo is required to definitively assess the utility of pralidoxime in acute OP poisoning.\textsuperscript{4,7} Recent studies support that better outcomes can be achieved with higher doses of pralidoxime.\textsuperscript{11,19,20} There are no sufficient randomized clinical trials with pharmacokinetic evidence to support the efficacy of pralidoxime as a continuous infusion supporting the WHO regimen. Considering the above facts, a prospective observational study is planned to identify the most appropriate dosage regimen of pralidoxime that can be recommended for the management of OP poisoning. Moreover efficacy of oxime is influenced by toxicokinetic and toxicodynamic property of the OP compound, poisoning load, time of initiating therapy and severity of poisoning which is not completely understood. The specificity of oximes in reactivation of AChEs inhibited by particular OP compound and effective concentration are not fully understood. Similarly kinetics of oxime in OP poisoned patients are entirely different from human volunteers. Elevation of pralidoxime in blood along with prolongation of effective half life may occur in the OP poisoning patients when compared to human volunteer.\textsuperscript{21} No newer agent has been introduced for the past 30 years which has a good efficacy against OP poisoning. Moreover properly designed clinical trials with kinetic study have not been carried out to prove the efficacy of WHO regimen.
1.4 Objective

Evaluation of treatment pattern of organophosphorus poisoning with emphasis on pralidoxime dosage regimen.

Specific Objectives

1) To study the demography, clinical characteristics, severity, treatment and outcomes of patients with acute organophosphorus poisoning.
2) To assess the clinical benefits and risks for various dosage regimen of pralidoxime therapy in organophosphorus poisoning.
3) To study the pharmacokinetics of pralidoxime in organophosphorus poisoned patients and compare the blood levels of pralidoxime in different dosage regimen with clinical benefits and risks.
4) To compare the blood level of methyl parathion with its clinical severity and outcome.