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Isolation and detection of poison- A case study

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Abstract

Pesticides, a class of compounds comprising mainly of Organophosphorous, Organochloro and Carbamate are a cause of major social concern worldwide because of their high toxicity, easy availability and affordable cost, making them the most potent and common poisoning agents. Pesticides are the most common poison for suicides. Poisoning by pesticides also comprise a significant number of homicide cases and accidental poisoning too. These are also used as chemical weapon in warfare. Though a global estimate is not possible even today, according to WHO about one million serious accidental cases and two million suicidal cases due to insecticidal poisoning occur worldwide of which nearly 2,00,000 cases lead to death. In India too pesticides are the most prevalent poison of death out of suicidal, accidental and homicidal poisoning.

Though the mechanism of pesticides interactions in human body is neither the focus nor the scope of present study some discussion on this is necessary. Organophosphorous and Carbamate group of pesticides act as acetyl cholinesterase inhibitor that affects the central and peripheral nervous system,muscles,liver,pancreas and brain resulting in death due to non-functioning of vital organs. Organophosphorous insecticides are potent inhibitors of serine active site enzymes including esterases, proteases and lipases. These can also form adduct on other cellular proteins. It has also been observed that other proteins i.e. other than cholinesterase are also important in case of certain Organophosphorous insecticides that cause neurotoxicity without significant cholinesterase inhibition while some others also interact with albumin, amino acids and have nonspecific interaction with proteins. Organochloro insecticides mostly affect lipid metabolism in the adipose tissues and change glucose pathways in other cells.

The present study is focused on isolation, purification and identification of Organophosphorous insecticides in a simple yet efficient way. It relates to a case study of 1990s where three school boys aged about 12-13 yrs. developed complications after their free meal i.e. lunch provided by the school and were admitted to a hospital but died after some hours. Their viscera (biological matrices) comprising portions of heart, liver, kidney, stomach, brain etc. were received along with the leftover food and food items like rice, pulses etc. The case history and post-mortem examination report revealed nothing particular but to be a case of suspected poisoning. All the three viscera samples were subjected to solvent extraction by solvent mixture Hexane: Acetone:: 4:1 after mincing of viscera and adding Ammonium Sulphate which were shaken thoroughly and kept overnight. The food items were extracted directly with Acetone. The solvent of all samples were decanted and evaporated to dryness at room temperature. The residue left were dissolved in Acetone and subjected to Thin Layer Chromatography(TLC) using Silica gel-G as adsorbent and Hexane: Acetone::4:1 as eluent .The plate was sprayed with chromogenic reagent Palladium Chloride. An Organophosphorous insecticide was detected in the sample pulses but was found negative in all the three viscera samples. Other plates were also developed for Carbamate and Organocloro insecticides and found to be negative. It may be mentioned here that as a routine procedure the first step adopted in the laboratory was to check the possibility of presence of any pesticides which were the most prevalent poison in the region.

This being a very important and sensitive case it was decided to opt for different methods of isolation of the insecticide in the viscera samples. Other solvent extraction method by instrument where 20 gms of viscera samples with the solvent were subjected to extraction at a desired temperature and pressure and the extracts analysed in the same way as discussed but result was negative. Since Ammonium Sulphate act as a protein precipitator rather than a denaturant, metal salt i.e. Copper Sulphate was used as a denaturant and analysed in the same way but ended with negative result again. In view of this and tremendous work pressure it was decided to go for complete deproteinization instead of trying other denaturants.

With limited scope for references in those years, it was decided to use Hydrazine hydrate, a strong base for deproteinization. Thus Hydrazine hydrate was added just enough to cover the viscera, shaken properly along with the solvent mixture of Hexane and Acetone as discussed earlier and subjected to Thin Layer Chromatography. This time the result was positive and the same insecticide as detected in the pulses sample was detected in all the three viscera samples as per Rf. value but was masked by fatty materials and artefacts. So to eliminate the problem a suitable solvent i.e. Acetonitrile was used. Acetonitrile was added to dissolve the extracted residue and passed through column containing anhydrous Sodium Sulphate .This was evaporated to dryness at room temperature and subjected to Thin Layer Chromatography as described earlier. This time the result was distinctly positive in all the three viscera samples. In absence of a suitable instrument this was evaluated semi quantitatively by applying approximately same amount of extracts of viscera samples and the pulses on the plate and it was observed that extraction of the insecticide was nearly complete as visualized from the area and intensity of colour of spots.

Result and discussion:

Both the first and second method of extraction yielded no result because no free or excess of the insecticide was left for extraction as the deaths occurred several hours after ingestion and being hospitalized, their stomach were thoroughly washed. Thus all the poison ingested was not only assimilated but interacted to different sites of enzymes, nerves and proteins.

Identification of the specific insecticide was not possible without an authentic control sample and non-availability of suitable instrument. However there is no legal necessity to identify or to quantify the particular insecticide in India.

The most important factor in this method of isolation is that Hydrazine hydrate is able to completely degrade the protein and other adducts releasing the insecticide for isolation to the solvent and that it seems to have no action on the insecticide as revealed in this study. However this needs further study applying instrumentation technique to ascertain it.

Above all reporting these procedure decades after its use was felt because **(i)** for the first time Hydrazine hydrate has been used for isolation of poison like insecticide from viscera sample and found not reported yet. **(ii)** Though Acetonitrile was used as solvent for elimination of fats in other works as cited in literature, it is the solvent of choice in viscera

analysis also particularly in case of pesticides poisoning .Presently some laboratories use this solvent for the purpose.

(iii) The method is very simple and less cumbersome yet quite efficient and can be adopted as a routine procedure especially in those cases where free insecticide is not expected for extraction as in this case.

However in view of the highly toxic nature of Hydrazine hydrate it must be used with extra precaution not to get exposure to its vapour or contact to it. Further study is required to standardize the method and judge it's efficacy by instrumental technique including the interaction if any with the pesticides released. Similarly further study is also required if this method can be applied for isolation and purification of other poisons like drugs since there is no common procedure so far available for a Forensic Toxicologist.