Orginal Article

CHROMATOGRAPHIC ANALYSIS OF p-QUINONE REAGENT FOR SCREENING OF MONOCROTOPHOS FROM OTHER ORGANOPHOSPHORUS PESTICIDE POISONING.

Ambika Prasad Patra[1#], Sasmita Mishra[2], Kusa Kumar Shaha[1]

ABSTRACT

There is inherent rise of pesticide poisoning in India over the last decade. Organophosphorus pesticide poisoning cases has dominated the rest all types of poisoning and monocrotophos dominated the over the rest other types of Organophosphorus (OP) pesticides, e.g. malathion, parathion, methyl parathion, fenthion and methyl demeton. Unfortunately, the management is quite different for monocrotophos poisoning compared to types of OP pesticide poisoning. Hence, clinicians requires an early and prompt diagnosis of monocrotophos poisoning cases for a better outcome. Therefore, there should be a toxicological contrivance in the laboratory to delineate monocrotophos from other OP compounds. Use of p-Quinone reagent in thin layer chromatographic method has paramount significance for screening monocrotophos from rest other types of Organophosphorus pesticides. Because, the alkali hydrolysis by-product of Monocrotophos reacts selectively with p-benzoquinone to produce an amber yellow coloured compound. Nevertheless, this reagent is specific only for monocrotophos and does not react with other organophosphorus, organochlorine and carbamate insecticides. This reagent does not interfere with the constituents of viscera, e.g. amino acid, peptides, polysaccharides, etc. including vegetable constituents too. However, the sensitivity of p-benzoquinone reagent is relatively lower; because, its detection ability is limited up to 0.40 to 0.48µg of monocrotophos only. This study deals with the use of p-benzoquinone reagent in TLC applied on 144 autopsy samples of gastric-extracts from OP pesticide poisoning. We have successfully demonstrated the ability of p-benzoquinone to screen monocrotophos among other OP compounds.

Keywords: monocrotophos; p-benzoquinone; organophosphorus; pesticide; para-quinone; 1,4-benzoquinone

INTRODUCTION

1.1. Background

1,4-Benzoquinone, popularly known as para-Quinone or simply Quinone was originally prepared industrially by oxidation of aniline, for example by manganese dioxide.[1] 1,4-Benzoquinone is a constituent of tobacco smoke and by-product of many benzene containing compounds, e.g. petroleum toxicity.[2,3]

1.2. Principle of exploiting monocrotophos using p-benzoquinone reagent

Monocrotophos releases an intermediate byproduct N-methyl Acetoacetamide on alkaline hydrolysis. And, p-benzoquinone if used as reagent, it react immediately with N-methyl Acetoacetamide to form an amber yellow coloured compound (Fig.1). [1,2]

Reaction process: [2]

Monocrotophos (on Alk. Hydrolysis) = Dimethyl Phosphoric acid + N-methyl Acetoacetamide

p-Benzoquinone + N-methyl Acetoacetamide = [Yellowish compound]

1.3. Burden of OPC poisoning

A large number of cases of monocrotophos poisoning are reported compared to other Organophosphorous Compounds (OPCs), e.g. malathion, parathion, methyl parathion, fenthion and methyl demeton, etc. Monocrotophos (MCP) is widely used in agriculture sector to protect the crop from insects, particularly in Kerala and other parts of south India. The unrestricted availability and ease of accessibility monocrotophos and other pesticides dominates among the household poisons.[3] Thence, it becomes imperative for toxicologists not only

[#]**Corresponding author:** Department of Forensic Medicine & Toxicology, JIPMER. Email : dr.ambika@yahoo.co.in
[1]Department of Forensic Medicine & Toxicology, JIPMER, Pondicherry
[2] Department of Biochemistry, Aarupadai Veedu Medical College, Vinayaka Mission University, Pondicherry

to diagnose OPC poisoning cases at the earliest, but also screen monocrotophos from other OPC poisoning. The present work have reviewed the use of p-benzoquinone as a bedside reagent for detection and identification of monocrotophos by thin layer chromatography.

MATERIAL AND METHODS

The aim of this study is to screen out monocrotophos from other types of pesticides using viscera-extracts from known pesticide poisoning cases. This is an autopsy based cross-sectional study. The sample population was – the fatal hospitalized cases who were died due to known pesticide poisoning and admitted during the period May 2016 up to April 2017. This study is in accordance with our institutional ethical guidelines.

2.1. Sample selection

We have collected 144 number of gastric-content samples during autopsy and the samples were processed for Thin layer chromatography (TLC) analysis. The gastric-content samples were segregated according to the type of OPC pesticide consumed by the deceased. During the study period we have identified the following OPC compounds among the pesticides poisoning cases, i.e. monocrotophos, malathion, parathion, fenthion, and dimethoate. We have selectively excluded the following samples from the study, i.e. unknown or undiagnosed pesticides poisoning cases, pesticide poisoning cases other than organophosphorous insecticides (e.g. monocrotophos, malathion, parathion, fenthion, dimethoate), organochlorine insecticides, carbamate insecticides and synthetic pyrethroids. As the later hardly affects the screening of OPCs in pesticide samples.

2.2. Sample extraction from gastric-contents

The insecticide from the gastric-content samples was extracted using ethyl acetate and evaporated at room temperature to get rid of solvent; further, the residue was dissolved in ethanol for purification. Then, a comparative trial run was made using gastric-content extracts of monocrotophos with standard stocks (solutions of monocrotophos) using activated TLC plates.

2.3. pre-Chromatography preparations

The p-benzoquinone reagent (0.5% w/v) was prepared by dissolving p-benzoquinone (0.5g) in 100ml of acetone.[1] Care was taken to use analytical grade of reagents and chemicals. We have used standard Glass TLC plates (10 cmX15 cm) coated with slurry of silica gel G (Acne chemicals, Mumbai) in water (1:2 w/v) to produce uniform layers of 0.25 mm thickness, and were dried at room temp. The TLC plates were activated by heating at 100^0C before the use. The TLC analysis is a semi-qualitative test based on a very simple and fundamental principle of movement of compounds in a solvent phase. Different analytes travel at different rates due to difference in their attractions to the stationary and mobile phase, e.g. strong solvents run far upwards on TLC plate while weaker elutants move lesser distance on the TLC plate.[4]

2.4. Screening of Monocrotophos from other OPCs

The gastric-extract solution of monocrotophos and other OPCs (e.g. malathion, parathion, fenthion, dimethoate), and standard solution of monocrotophos were spotted on activated TLC plate during the trial run. The intensity of coloured spot (amber yellow) developed for gastric-extracts were compared with those of the known standards.

RESULTS

It was observed that, the spot movements on TLC plate for both - the standard technical grade monocrotophos and monocrotophos extracted from gastric-contents were same, i.e. at the same level Rf (retardation factor). Alternatively, the spots corresponding to the test and the control samples of monocrotophos were the same. Simultaneously, the retardation factor values were different for OPCs other than monocrotophos (e.g. malathion, parathion, fenthion, dimethoate). The mean Rf values for standard technical grade monocrotophos (control) and monocrotophos extracted from gastric-contents were 0.48; and the medians Rf values for parathion, malathion, fenthion, dimethoate were 0.41, 0.34, 0.00, 0.00 respectively. (Fig 2)

DISCUSSION

Over a decade the numbers of pesticide cases were increased and now they are at the peak of their incidence.[5] Near about 550 cases of monocrotophos poisoning were reported to our hospital at Pondicherry in the year 2016. In south India, monocrotophos poisoning cases are dominating over the rest all types

Fig 1: showing commercial preparations of p-Benzoquinone (yellow crystalline powders) used commonly by the photographers.

Fig 2: TLC plate showing Rf values for monocrotophos and other organophosphate compounds.

Note: spots (from left to right) for standartd Monocrotophos stock solution (control), Monocrotophos extratcted from gastric-content, Malathione, Fenthione and Dimethoate respectively.

of Organophosphorus Compounds. Wide agricultural usage, unrestricted availability and ease of accessibility monocrotophos and other pesticides dominates among the household poisons.[6] The burden of monocrotophos poisoning cases is exceptionally high, both in terms of diagnosis and management. Because, in majority of cases (mostly suicidal monocrotophos poisoning) the chemical nature of poisoning remains unclear, though it might have diagnosed provisionally as OPC poisoning by the clinicians.[6,7] The nature of poison (as monocrotophos) should be diagnosed as early as possible to hope for a better prognosis.[8,9] Quinone is a chemical compound with the formula $C_6H_4O_2$. In a pure state, it forms bright-yellow (Fig.1) crystals with a characteristic irritating odour, resembling that of chlorine, bleach, and hot plastic. Impure samples are often dark-coloured due to the presence of quinhydrone. When heated it sublimates at atmospheric pressure. In large scale, it is produced by direct hydroxylation of phenol by acidic hydrogen peroxide.[10] Quinone is mainly used to produce hydroquinone in large commercial scale, which is used in photography and rubber manufacture as a reducing agent and antioxidant.[10] Benzoquinonium-a Skeletal muscle relaxant, ganglion blocking agent that is made from benzoquinone is often used in anaesthetic medications.[11] In toxicology, 1,4-Benzoquinone metabolites in body is used to screen toxicity due to exposure to petrol (or, other petroleum products containing benzene or benzene compounds).[12] In diluted concentration, 1,4-Benzoquinone is able to stain skin dark brown, cause erythema (redness, rashes on skin); and the crystals may cause, localized tissue necrosis if keep in contact for prolonged period. It is particularly irritating to the eyes and respiratory tract. Its ability to sublimate at little higher than room temperature allows for a greater airborne exposure risk. It is unfortunate that, 1,4-Benzoquinone is having carcinogenic tendency, i.e. it can depress bone marrow production in mice and can inhibit protease enzymes involved in cellular apoptosis.[13] 1,4-Benzoquinone is a constituent of tobacco smoke.[14][15] The routine toxicological screening of organophosphorus insecticides samples are done by thin layer chromatography (TLC) because of its simplicity and rapidity. However, many other chromogenic reagents have been used for the detection and identification of monocrotophos insecticides, e.g. mercurous nitrate[10], potassium iodate starch[15], sodium carbonate chloranil acetone[12], mercuric nitrate-diphenyl carbazone[11] , palladium chloride[13], vanillin acetone[14] and benzyl acetone[16], etc. Nevertheless, these reagents

are usually shows lower sensitivity and/or specificity to detect monocrotophos; and hence, many were abandoned by the modern toxicology labs. However, p-benzoquinone is able to detect MCP in lowest quantities and is having selective affinity to detect MCP compared to other OPCs.[16,17] Therefore, this property of p-benzoquinone is exploited to develop chromogenic reagents which can be used for selective diagnosis of MCP poisoning cases.

CONCLUSION

The utility of p-benzoquinone reagent to screen out monocrotophos from pesticide samples against other organophosphate compounds has been proven beyond the reasonable doubt. This reagent may be exploited by the toxicologists to guide the clinicians for accurate diagnosis of monocrotophos poisoning and to give a specific treatment thereof. This way the overall prognosis may be improved and the patient's hospital stay may be minimised. However, the teratogenicity and carcinogenic tendencies of p-benzoquinone reagent, though noticed in experimental animals only may limit its widespread usage. But, under carefully designed laboratory protocols, p-benzoquinone reagent may be used for detection of monocrotophos. Nevertheless, further human experiments or prospective cohort studies may be undertaken to rule out the actual teratogenicity and carcinogenic properties of p-benzoquinone reagent.

Conflict of Interest and funding:

Declared none.

REFERENCES

1. Chandegaonkar VR, Shinde BD, Mane DV. Thin-layer Chromatographic detection and identification of Insecticides in Biological materials. J Planar chromatogr 2009;22: 459-60.
2. Sherma J. Recent Advances in the Thin-Layer Chromatography of Pesticides: A Review. Journal of AOAC International 2003;86(3):602-610.
3. Eddleston M, Karalliedde L, Buckley N. Pesticide poisoning in the developing world—a minimum pesticides list. Lancet. 2002;360:1163–1167.] [National Crime Records Bureau. Crime in India 2015 Statistics. New Delhi: Govt of India; 2015. Available at: http://ncrb.gov.in.
4. Morlock GE. Preparative Layer Chromatography. Eds. Kawalska T, Sharma J. Boca Raton, FL: CRC Press Taylor & Francis Group; 2006.
5. Nollet LML, Rathore HS. Handbook of Pesticides: Methods of Pesticide Residues Analysis. In: Pesticides, past, present and Future. Boca Raton, FL: CRC Press, Taylor & Francis; 2010. p51.
6. Kegley, SE, Hill BR, Orme S, Choi AH. National Toxicology Program acute toxicity studies for Monocrotophos., North America: PAN Pesticide Database; 2016. Available at: http:www.pesticideinfo.org.
7. Clark RF. Goldfrank's Toxicological Emergencies. 7th edn. McGraw-Hill Professional; New York: 2002. Insecticides: organic phosphorus compounds and carbamates; pp. 1346–1360.
8. Buckley NA, Karalliedde L, Dawson A, Senanayake N, Eddleston M. Where is the evidence for the management of pesticide poisoning—is clinical toxicology fiddling while the developing world burns? J Toxicol Clin Toxicol. 2004;42:113–116.
9. Srinivas Rao CH, Venkateswarlu V, Surender T, Eddleston M, Buckley NA. Insecticide poisoning in south India—opportunities for prevention and improved medical management. Trop Med Int Health. 2005;10:581–588.
10. Phillip M. Hudnall "Hydroquinone" in Ullmann's Encyclopedia of Industrial Chemistry 2002, Wiley-VCH, Weinheim. 2005. doi:10.1002/14356007.a13_499.
11. Yang TK, Shen CY. 1,4-Benzoquinone. In L. Paquette. Encyclopedia of Reagents for Organic Synthesis. New York: J. Wiley & Sons 2004. doi:10.1002/047084289X.rb033.
12. Lin YS, McKelvey W, Waidyanatha S, Rappaport SM. Variability of Albumin Adducts of 1,4-Benzoquinone, a Toxic Metabolite of Benzene, in Human Volunteers. Biomarkers 2006;11 (1): 14–27. PMID 16484134.
13. Anderson D, Yu TW, Schmezer P. An investigation of the DNA-damaging ability of benzene and its metabolites in human lymphocytes, using the comet assay. Environ. mol. Mutag 1995;26:305–314.
14. Chapman DE, Namkung MJ, Juchau MR. Benzene and benzene metabolites as embryotoxic agents: effects on cultured rat embryos. Toxicol. appl. Pharmacol 1994;128:129–137.
15. Talhout R, Schulz T, Florek E, et al. Hazardous Compounds in Tobacco Smoke. International Journal of Environmental Research and Public Health 2011;8 (12): 613–628. PMC 3084482. PMID 21556207. doi:10.3390/ijerph8020613.
16. Dhanlal De Lloyd Chem. Trinidad: Dept. UWI St. Augustine Campus [created June 1998; updated January 2000]. Available at: http://delloyd.50megs.com.
17. Lorentz K, Flatter B. Staining of amino acids with benzoquinone in paper chromatography. Analytical Biochemistry 1970; 38(2): 557-559.