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Gross examination and Toxicological Analysis of Gastrointestinal Tract for Dichlorvos Poisoning Caused by Nuvan Insecticide During Post-Mortem Examination: A Case Series

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Abstract

Background: "Nuvan" is a trading name for Dichlorvos (DDVP) organophosphate insecticide. Nuvan contains 76% DDVP and is accessible in India. Nuvan Insecticide is highly misused as orally ingested in suicide attempts and is one of the leading causes of poisoning deaths in Indian rural areas.

Methods: In this case study, we have diagnosed the four poisoning deaths by Nuvan-dichlorvos insecticide through Post-Mortem examination and toxicological analysis. The gastro-intestinal tract (GIT) was grossly examined. The solvent extraction method is used for withdrawing pesticide content from GIT. Toxicological analyses were performed using thin-layer chromatography (TLC) using a spraying reagent

Results: A Fluorescent turquoise blue liquid content was found throughout the stomach to the small intestine. Content had a notable odor of volatile organic compounds (VOCs). The mucosal walls of the stomach were highly congested and hemorrhagic. Toxicological analyses done for all four cases showed a confirmatory detection of dichlorvos.

Conclusions: The simple gross examination of gastrointestinal viscera during post-mortem is supporting diagnostic evidence for orally administered Nuvan insecticide. Solvent extraction method is a suitable technique for the extraction of pesticides from aqueous biological matrices. Thin Layer Chromatography is a simple and inexpensive technique for chemical confirmation and DDVP was detected.

Keywords: Pesticide poisoning; Dichlorvos (DDVP); Forensic Toxicology; Thin layer chromatography (TLC); Nuvan insecticide; Post-Mortem Examination; Clinical Toxicology.

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Introduction

Organic phosphorus pesticides (OPs) are cholinesterase inhibitors that can reversibly or irreversibly bind with the serine residue in the active site of the enzyme acetylcholinesterase and prevent the normal functioning of neurotransmitters. This action is not limited to insects but can produce toxicity in animals and humans [1][2]. OP insecticides refer to phosphorus(P) containing compounds with various structural types including phosphates, that have four oxygen atoms surrounded by central Phosphorus (P) atom as Dichlorvos (DDVP) (Fig 1) [1][3]. DDVP is the dimethylated OPs that belong to the fourth class of phosphate pesticides, bonded with two methoxy groups [3]. Phosphoryl group of OP attaches to the active hydroxyl site of the acetylcholinesterase (AChE) and inactivates the enzyme which leads to the accumulation of excessive acetylcholine (Ach) at the cholinergic junction that disrupts the normal synaptic transmissions (Reaction 1) [1]. The inactive AChE enzyme also regenerates by removing the phosphate moiety attached to it by hydroxyl ion reaction and releases back the active enzyme (Reaction 2) [3–5]. But this regenerative process is much slower than inhibition and takes hours to days depending on the chemistry of the substituted phosphate [3,4]. Enzyme inactivated by Dimethylated OPs takes 0.7 – 86 hours to regenerate back [5]. During the inactive state, the enzyme is also prone to "aging" in which one alkyl side chain of the phosphoryl moiety is removed and leaves a hydroxyl group as a substitute (Reaction 3) [3,5]. "Aged" AChE cannot regenerate and this reaction occurs faster with enzymes that have been inhibited by dimethylated pesticides as in the case with DDVP ($t_{½}$ ~ 3.3 hours) [3–5]. Therefore, oximes are only effective if given within 12 hours of poisoning by dimethylated OPs as inactive enzyme rapidly converts into aged enzyme before going into regeneration and produces aged-dimethylphosphoryl-AChE, generally resistant to oxime therapy [4,5]. Dichlorvos is defined by WHO as a class -b highly hazardous pesticide as it has a very low LD_{50} value for oral and dermal exposure[6]. Acute ingestions can become symptomatic quickly, onset the complications of aspiration and respiratory failure that causes the majority of deaths before medical assistance could be provided [3,7]. In addition to the risk of occupational and environmental toxicity, pesticides are the trending method of suicide in rural areas and kill over 2,00 ,000 people annually [8–11]. In several countries, dichlorvos is prohibited to minimize suicidal, occupational, and accidental poisonings [12]. Dichlorvos insecticide is accessed in India with different trade names such as Agrovan 76, Agro 76EC, Divisol, Nuvan, Nuvasuls 76, Bangvas, DDVP, Divap, and D-aivisol [13]. Nuvan contains 76% of dichlorvos, the most common brand available in India, accessible even after the ban, and uncontrolled selling is threatening to life [1,14]. The frequency of toxic or fatal events in India suggests that they are still easily accessed [11,15]. In developing nations, Emergency medicine in hospitals lack clinical toxicological laboratories and such pesticidal poisonings are left unnoticed. As alternative blood AChE activity can indicate cholinesterase inhibitors, but cannot confirm the xenobiotic chemical compound while medications should be followed as per the type of pesticide poisoning [3,16,17]. For instance, Carbamates (CA) also cause cholinergic crises but are clinically indistinguishable from OP poisoning. Pralidoxime is the antidote to OP intoxication but is considered to be contraindicated in CA poisoning [18]. Also, the In-vitro half-life of human AChE after poisoning with dimethoxy OPs is 3.7 hours as compared to 31 hours in cases of diethoxy OPs [16]. Hence, the oxime therapy is time bounded for dimethoxy OPs [5]. Analytical extraction of pesticides from viscera or lavage is the first crucial step for analysis. Solvent extraction method is one of the best suitable techniques for the extraction of pesticide residues from aqueous viscera (GIT) and biological fluids such as gastric lavage/vomit [19]. Solvent extraction is dependent on the hydrophobicity and miscibility of compounds, hence polar and non-polar pesticides are extracted accordingly [20][21]. This study aims to simple, rapid, and inexpensive detection of DDVP in biological matrices.

Material and Methods

Four fatal cases with an alleged history of Nuvan insecticide and unknown substance consumption were autopsied. Three cases were brought dead without any prior treatment and one case was hospitalized. The admitted patient was on medical

assistance, underwent gastric lavage, intubated on a ventilator, Atropine and Pralidoxime IV were injected as per treatment protocol. During Post-mortem, the gastro-intestinal tract was grossly examined, sensed for odor, and photographs were taken. Toxicological analyses were performed for the identification of xenobiotic pesticidal compounds using TLC for dichlorvos (DDVP).

Toxicological Analyses

Chemical analyses were performed in all the above four cases. Analytes were extracted from the gastrointestinal tract, mainly from stomach matrices through the solvent extraction method via acetonitrile and hexane solvent[22–24]. Acetonitrile is used as an aqueous phase (polar solvent) to extract Polar pesticides such as – DDVP [21,23–25]. Acetonitrile is highly efficient to extract hydrophobic substances containing polar groups from hydrocarbons and fatty matrices[23,25]. 50 grams of macerated tissue with content was dissolved in 100 ml of acetonitrile, evaporated on a water bath for 30 minutes, and filtered through anhydrous magnesium sulfate. Anhydrous salt absorbs the residual water and evaporation enhances the analyte preconcentration[23,24]. Extracted analyte solution was collected and further separated in a separating funnel. Adding hexane as a non-polar solvent (25ml) in the separating funnel has no influence on the acetonitrile phase in the aqueous phase, leading to decreasing the co-extraction of non-polar matrix components by salting out [22,24]. Shaking the funnel for 1–2 minutes (inverting the separating funnel approximately 5–6 times), this process allows thorough interspersion of extracted analyte to the aqueous phase, assisting mass transfer and allowing efficient partitioning[26]. After 5 minutes of the resting period, both the phases get stable and partitioned in a funnel. The Acetonitrile layer was collected and passed through anhydrous magnesium sulfate and the final analyte sample was prepared. The final sample analyte was loaded on a silica gel TLC plate(10x10cm) and ran parallelly with dichlorvos standard (IS) in the solvent system n-hexane: ethyl acetate: methanol (7:1.5:1.5) for 30 minutes [27]. The developed TLC plate is sprayed with 1% phenyl hydrazine hydrochloride solution and, a yellowish colour spot [27]. Further sprayed with 10% hydrochloric acid turns the spot to red coloured as positive confirmation for dichlorvos[27]. Toxicological analyses done for all four cases showed a confirmatory detection for dichlorvos.

Case Series

Case 1

A 48-year aged male was brought dead to emergency with the history and police intimation of self-ingested Nuvan insecticide. Gross examination of the GI tract revealed fluorescent turquoise blue coloured liquid content throughout the stomach and small intestine (Fig 1 A, B). Content had a notable odor of volatile organic compounds (VOCs). Stomach mucosal walls were highly congested and hemorrhagic (Fig 1C).

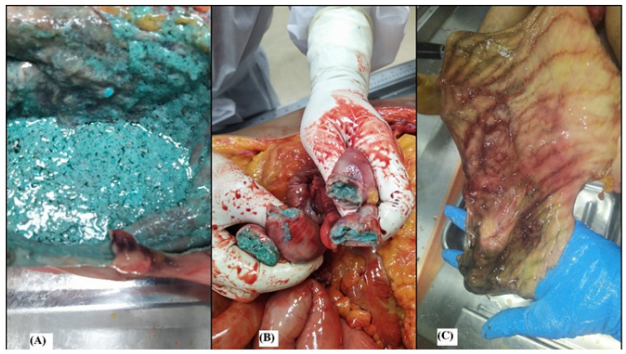

Figure 1 (Case 1): PM findings of the GI tract. (A) Stomach (B) Small intestine (C) Mucosal wall of the stomach

Case 2

A 52-year aged male was brought dead to the hospital with police intimation of insecticide poisoning. Gross examination of the GI tract revealed turquoise blue-coloured liquid content in the stomach, adhered to congested and hemorrhagic mucosal walls (Fig 2). Pungent odor of VOCs was sensed.

Figure 2 (Case 2): PM findings: The gross examined view of the stomach.

Case 3

A 38-year aged male was brought dead with a history of unknown substance uptake. PM examination showed turquoise blue coloured content in the stomach and upper abdominal cavity (Fig. 3A&B). The content was leaked to the abdomen through the perforated hole in the stomach wall (Fig. 3C). Pungent odor of VOCs was sensed.

Figure 3 (Case 3): PM findings (A) Open abdomen cavity (B) Stomach and content (C) Perforation in stomach wall

Case 4

22- year aged female was admitted to the hospital in an unconscious state with a history of unknown substance uptake. Patient suffered prolonged emesis, convulsions, and abdominal pain and died after a week on ventilatory support. The stomach and its mucosal walls were highly hemorrhagic (Fig. 4A). Dark bluish charred content was found adhered to the mucosal walls of the stomach (Fig. 4B).

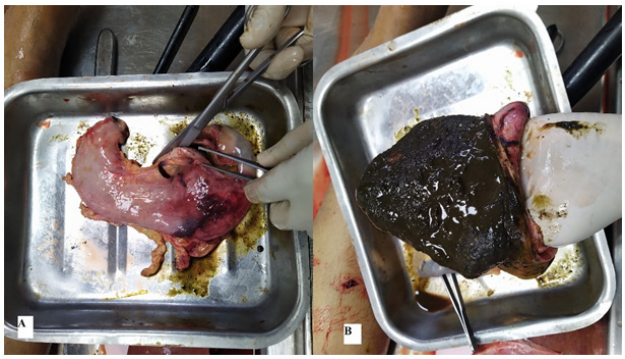

Figure 4 (Case 4): PM Gross findings of (A) Stomach (B) Adhered content to stomach inner linings of mucosal wall.

Results and Discussion

All four cases were reported from rural areas. The first three cases died before reaching the hospital and medical assistance could be provided. The prominent cause of death is hypoventilation and pulmonary dysfunction, which can onset within two minutes after DDVP poisoning [28]. DDVP is a direct-acting OP compound that quickly inhibits acetylcholine enzymes without being metabolized in the body[3]. The inhibited AChE converts rapidly ($t_{½}$ ~ 3.3 hours) into "aged" AChE (permanently irreversible) [5]. Clinically, this suggests that patients who admit to a hospital after 4 hours of poisoning with a DDVP, have 50% of their AChE irreversibly inhibited and after 14 hours, the patients become completely resistant to oxime treatment [16]. Therefore, Oximes should be given early within 2-4 hours and are effective only within 12 hours after intoxication [16] [10]. Therefore, cases 1,2, and 3 were brought dead as reported to the hospital after 14 hours of ingestion. Also, in case 4, the patient was brought after 4 hours of poisoning and did not survive, even intubated on a ventilator and medically treated. Also, the treatment doses of oxime are selective for dimethyl OPs. The reactivating potential of the oximes should be efficient and preventive for aged AChE. Reactivating potential of obidoxime for dimethylphosphoryl-AChE is considered superior to pralidoxime. Obidoxime is approximately 10-90 times more reactive than pralidoxime. Pralidoxime is effective as obidoxime if given at least five times a higher dose. In-vitro studies have shown that seven times as much pralidoxime as obidoxime is required for reactivation of dimethyl-OP inhibited AChE [29][30][5][4][16]. During Autopsy, stomach examination showed significant features of Nuvan insecticide poisoning in brought dead cases 1,2,3. The fluorescent turquoise blue coloured liquid content was found throughout the stomach to the small intestine was ingested Nuvan-dichlorvos insecticide. Therefore, an Orally ingested insecticide should be rapidly washed out from the stomach to inhibit further absorption in the body. In Case 4, the insecticidal content was found adhered to mucosal walls of the stomach, this suggested that lavage was incomplete and the patient suffered excruciating conditions on a ventilator for 7 days due to persistence of the OP that led to rapid re-inhibition of the reactivated enzymes [5]. Stomach mucosal walls were highly congested and hemorrhagic in all four fatal cases. Case 3 had a rare finding of stomach perforation, which suggests DDVP may

cause vascular endothelial dysfunction. Perforation was caused due to microvascular thrombosis by the contact of toxins with the mucosa for a longer duration[31]. Solvents in the insecticide also contribute to mucosal erosions [31]. Hence, the content found had an odor of volatile organic compound as of insecticide. Co-formulated solvents create toxicity and vary with OP and insecticidal brands[3]. In the unavailability of Toxicological laboratories, all anti-cholinesterase poisonings are treated as the same, despite wide variation in their toxicity, fat solubility, metabolism, AChE selectivity, and ageing rate, which affect poisoning severity and response to treatment[5]. Chemical analyses performed to detect dichlorvos using TLC is a simple inexpensive technique, and the solvent extraction method is efficient to withdraw pesticides from aqueous viscera and biological fluids (stomach wash and vomiting). Confirmation of OP compound is more reliable than plasma cholinesterase level for effective treatment [10]. Development of Toxicology laboratories for such evaluation is essentially important for hospitals and forensic departments. Ministry of Agriculture and welfare, India (GOI) has prohibited the manufacture, import, and use of dichlorvos pesticide by year 2021-22 as it is highly hazardous, and uncontrolled regulations have led to deaths all over India [32]. As small farmers and domestic users keep the stock they had before prohibiting laws were approved [15]. Hence, there is possibility to encounter poisoning and fatal cases by banned pesticides, as the most cases reported by "Nuvan" insecticide. In all four cases, the Nuvan insecticide was orally ingested, and the gross examination and toxicological analyses of Gastro-intestinal tract is diagnostic evidence for insecticide poisoning.

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Conflicts of Interest: None

Ethical Clearance: The study is conducted under AIIMS institution and involves general medical duties, followed by routine procedures during post-mortem examination as per the work ethics of the institution. No further ethical clearance was required.

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