ORIGINAL RESEARCH

Histopathological Changes in Oral Tissues Induced by Pesticide Poisoning: A Pilot Study

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Abstract

Objective: The present study evaluated the histopathological changes in oral tissues induced by pesticide poisoning.

Patients and methods: This was a cross-sectional pilot study. The sample consisted of oral tissues obtained from deceased patients during autopsy. The study samples were obtained from 10 cases of ingested pesticide poisoning, and the control samples were obtained from road traffic accident cases. All the obtained samples were subjected to histopathological examinations. The changes observed in poisoning cases were compared to those in the road traffic accident cases.

Results: Significant degenerative changes were observed in the epithelial cells and connective tissue components, such as collagen, muscles, nerves, vasculature, adipose tissue, and salivary acini and ducts, in the poisoning cases. The oral tissues of the road traffic accident cases did not show any significant degenerative changes.

Conclusion: The degenerative changes in the study samples can be attributed to the direct contact of the pesticide with the autopsied oral tissues when the poison was consumed. There are instances in which the entire body may not be recovered or may not be in an examinable state. In such cases, an oral autopsy could provide additional evidence for determining the cause of death in suspected poison cases.

Keywords: Autopsy, Degeneration, Histopathology, Oral mucosa, Pesticide, Poisoning.

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Introduction

Suicide attempts that involve consuming pesticides and drugs have skyrocketed both in rural and urban areas, as it is an accessible and instant method. Usually, individuals with poor self-esteem are the victims of this type of suicide attempt.[1] Poisoning is the third most common causative factor for death following vehicle accidents and fire.[2] The World Health Organization (WHO) estimated that 3 million people globally consume pesticide poison, and approximately 99% of these deaths occur in India.[3,4]

Poisoning is caused by exposure to a substance that leads to injury or damage to the body and is detrimental to life upon ingestion, inhalation, or contact.[5,6] Accidental and suicidal acute poisoning has led to significant mortality and morbidity worldwide.[7] According to the WHO, more than three million global acute poisoning cases with 220,000 deaths occur annually.[8] Various factors, including the availability of and access to poison, the socioeconomic status of an individual, cultural and religious elements, etc., influence the pattern of poisoning in a region.[9] Accidental ingestion or acute poisoning is one of the causes of medical emergencies globally, accounting for 1.8 per 100,000 mortalities among children below 20 years of age.

Pesticide exposure is a global public health issue. The WHO has estimated that there are approximately 250,000 deaths due to pesticide poisoning annually.[10] More than 90% of these cases are reported in developing countries such as India.[3,4,10] The most common forms of pesticide poisoning that causes mortality in India are organophosphorus (OP) and aluminum phosphide (AlP). As the incidence of OP poisoning has increased in recent years, it is considered to be a social crisis in developing countries. Common OP compounds used in agriculture are parathion, malathion, chlorpyrifos, and dichlorvos. AlP is used as a rodenticide and

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pesticide in grain storage facilities. AlP produces phosphine gas, which results in mitochondrial poisoning. In India, this pesticide is marketed as a tablet of Celphos, Alphos, QuickPhos, Phostoxin, etc. This highly toxic chemical is cheap and is usually formulated as tablets, pellets, granules, and powders.[11]

Oral ingestion is the most common mode of exposure to pesticides, followed by inhalation and cutaneous routes.[5] As the oral cavity is the first area to come into contact with ingested pesticides, histopathological changes in the oral cavity may reveal the cause of death and act as an aid in the forensic investigation. Further, it also simplifies the pending legal proceedings, the issue of death certificates, insurance claims for the deceased, etc.[2] An oral autopsy is preferred due to its merits in regard to the ease of sampling and serves as a useful tool when key vital organs are destroyed. Hence, the present study aimed to study histopathological changes in the oral tissues of the deceased associated with ingested pesticide poisoning to provide adjunctive evidence for the cause of death.

Materials and Methods

This cross-sectional pilot study was conducted by the Department of Oral Pathology, Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences, in collaboration with the Department of Forensic Medicine, MS Ramaiah Medical Teaching Hospital, after receiving the approval from the institutional ethical committee. All fatal cases of pesticide poisoning, either intentional or accidental ingestion, in any age group and gender, brought to the Department of Forensic Medicine, MS Ramaiah Medical College and Hospital, from January to July 2018 were included in the study.

Study Sample Selection

Inclusion Criteria

The study sample included individuals, irrespective of age and sex that had been deceased for fewer than 12 hours with a history of ingested pesticide poisoning.

Exclusion Criteria

Death due to reasons other than pesticide poisoning and bodies that were decomposed and autolyzed are the exclusive criteria.

Control Sample Selection

Inclusion Criteria

The inclusion criteria for the control samples are the deceased individuals, irrespective of age and sex, who arrived at the hospital due to road traffic accidents.

Exclusion Criteria

A history of ingested pesticide poisoning is the criteria for the control sample exclusion.

Consent

Individuals who fulfilled the selection criteria were included in the study only after obtaining informed consent from their relatives.

Data Retrieved from Patient Charts

History regarding the type of pesticide and the demographic details of the patients were retrieved from the patient charts (Table 1).

Selection of the Oral Autopsy Site

Oral tissues that may have come in direct contact with the pesticide during its consumption were obtained during the autopsy. The oral sites included the tongue and left and right buccal mucosa. All the autopsied oral tissues were immediately placed in formalin. After 24 hours of fixation in formalin, the tissues were subjected to routine histopathological processing. The processed tissues were stained using hematoxylin and eosin.

For the road traffic accident cases (control samples), the same oral tissues (tongue and left and right buccal mucosa) were obtained during the autopsy and subjected to 24-hour formalin fixation followed by routine histopathological processing and staining.

Hematoxylin and eosin (H and E)-stained sections of oral specimens from both the study samples and the control samples were examined for any potential histopathological changes by two independent oral pathologists. Any discrepancies in the observations were resolved by the examination by a third independent oral pathologist.

Table 1: Data retrieved from the patient charts

Case No.	*Age (in years)*	*Gender*	*Type of poison consumed*
1	38	Male	Paraquat insecticide (dipyridil)
2	60	Female	Aluminium phosphide
3	21	Female	Organophosphorus
4	22	Male	Aluminum phosphide
5	45	Male	Organophosphorus
6	32	Female	Organophosphorus
7	60	Male	Organophosphorus
8	25	Female	Organophosphorus
9	35	Male	Organophosphorus
10	58	Male	Organophosphorus

Results

The study sample consisted of six males and four females with a wide age range (from 21 to 60 years). Seven individuals had OP poisoning, two individuals had AlP poisoning, and one individual had insecticide (paraquat or dipyridyl) poisoning. The histopathological examination of all the study samples revealed significant degenerative changes both in the epithelium and in the connective tissue.

Degenerative Changes Observed in the Histopathological Examination

A summary of the specific degenerative changes noted in the study samples is elaborated in Table 2.

In the epithelial tissue of the study samples, the most distinct change noted was the vacuolar degeneration of the nucleus and cytoplasm (Fig. 1A). The muscle tissue showed the fraying and shredding of fibers (Fig. 1B). Collagen fibers showed architectural loss and fragmentation (Fig. 1C). Salivary glands exhibited the loss of lobular architecture and the vacuolization of acinar cells (Fig. 1D). The degeneration of ductal cells (Fig. 1E) and the severing of the salivary ducts (Fig. 1F) were also observed. The nerve tissue specimens showed a vacuolization in the nucleus and cytoplasm and a disruption in the perineurium (Fig. 2A). The architecture of the adipose tissue was disrupted with the degeneration of cells and the absence of nuclei (Fig. 2B). There was narrowing of blood vessels, rupture of the vessel walls, and vacuolar degeneration of endothelial cells (Figs 2C and D). Tongue specimens showed vacuolar degeneration of the cells of the papillae and taste buds

Table 2: Histopathological changes observed in the epithelium and connective tissue components of autopsied oral tissues from the study samples

Site	*Epithelium*		*Muscle*		*Salivary glands and ducts*						*Nerve tissue*		*Blood vessels*		*Adipose tissue*		*Collagen*		*Papillae and taste buds*	
	Vacuolar degeneration		*Fraying and shredding of fibers*		*Loss of lobular architecture*		*Vacuolar degeneration in acinar cells*		*Severance of ducts and degeneration of ductal cells*		*Vacuolar degeneration*		*Compressed/ collapsed*		*Loss of architecture*		*Loss of organization/ fragmentation of fibers*		*Vacuolar degeneration of cells and taste buds*	
	Present	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*	*Present*	*Absent*
Tongue ($n = 10$)	10	0	10	0	8	2	10	0	7	3	10	0	10	0	10	0	10	0	9	1
Buccal mucosa ($n = 10$)	10	0	10	0	8	2	10	0	8	2	10	0	10	0	10	0	10	0	NA	NA

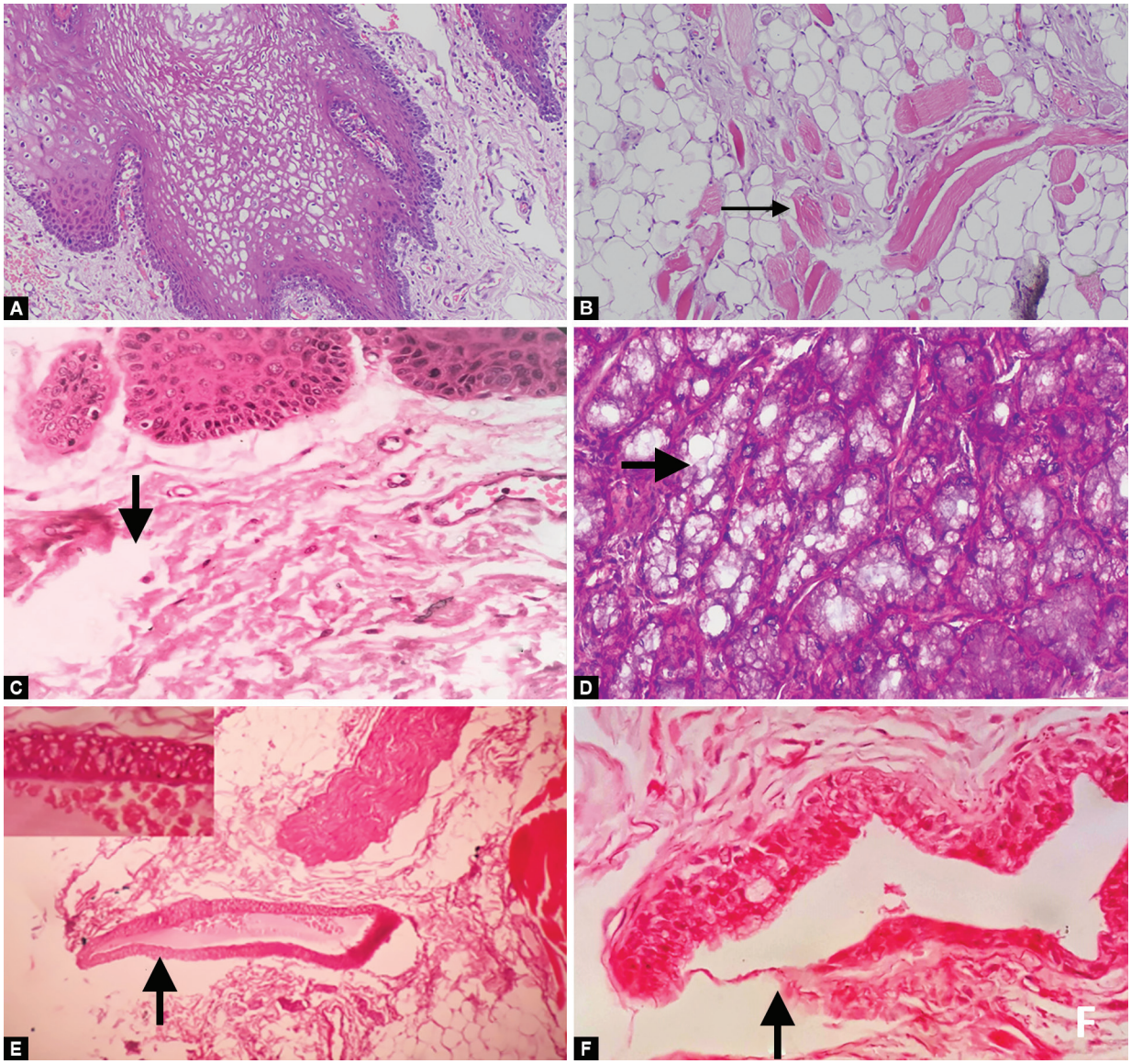
(Figs 2E and F). In contrast, the control samples did not show any degenerative histopathological changes.

Discussion

Though the systemic effects of various types of pesticides have been studied extensively, limited data are available regarding their local effects. Therefore, the current pilot cross-sectional study aimed to investigate the local effects of pesticides on the oral cavity following the accidental or suicidal ingestion of pesticides. To the best of our knowledge, the current article is the first to examine the microscopic changes of autopsied tissue samples from subjects with ingested pesticide poisoning.

The present study examined histopathological changes in oral specimens from patients who succumbed to pesticide poisoning. The control samples consisted of oral tissues obtained from patients who were involved in road traffic accidents. At present, toxicology reports on poison cases are based on qualitative and quantitative tests of biological tissues, for example, the lung, liver, and kidney, where the poison is usually absorbed and eliminated.[2] However, there are instances wherein the entire body may not be recoverable or may be heavily damaged and is not suitable for examination. In such cases, it may be necessary to identify the cause of death using the tissues that are available. Thus, in our present study, we aimed to examine the histopathological effects of pesticide poisoning in oral tissues. Our hypothesis was that during the consumption of poison, the poison would come in direct contact with oral mucosa, causing toxic changes. Thus, it is possible that the histopathological examination of these oral tissues may provide clues regarding the consumption of poison.

As mentioned in the results, in our present study, seven individuals had OP poisoning, two individuals had AlP poisoning, and one individual had paraquat insecticide (dipyridyl) poisoning. OPs act as irreversible cholinesterase inhibitors, leading to the accumulation of acetylcholine at synapses. This results in the overstimulation and disruption of impulse transmission in the central and peripheral nervous systems. Subsequently, the hypersecretion and paralysis of respiratory muscles occur. OP compounds are chiefly absorbed by inhalation, ingestion, and mucosal penetration, undergo bioactivation, and detoxification in the liver and are eliminated through the kidneys.[12] AlP is a phosphine-generating pesticide that is highly toxic. Phosphine gas is released in contact with moisture or with the hydrochloric acid of the stomach, which is responsible for its toxic effects.[13] Toxicity occurs either due to the ingestion of AlP, the inhalation of phosphine, or after absorption through the skin or mucosa. Phosphine is soluble in organic solvents and water, whereas it reacts with OH radicals in the air and is eliminated. Phosphine gas is rapidly absorbed by the gastric mucosa, leading to toxic systemic effects involving the heart, lung, kidney, and liver. Paraquat is a widely used contact herbicide used for weed control and as a preharvest defoliant in India. Paraquat causes direct damage on contact with the mucosal lining of the mouth, stomach, or intestines. Paraquat causes intracellular toxicity via the liberation of reactive oxygen and nitrite species that nonspecifically damage the lipid membrane of cells, inducing cellular toxicity and death.[14] Beydilli et al.[15] evaluated the histopathological and biochemical effects of silibinin in diazinon-induced liver damage. These authors observed that an acute, high dose of diazinon, an organophosphate insecticide, caused the hypertrophy and swelling of hepatocytes, the vacuolization of the cytoplasm, and macrovascular steatosis.

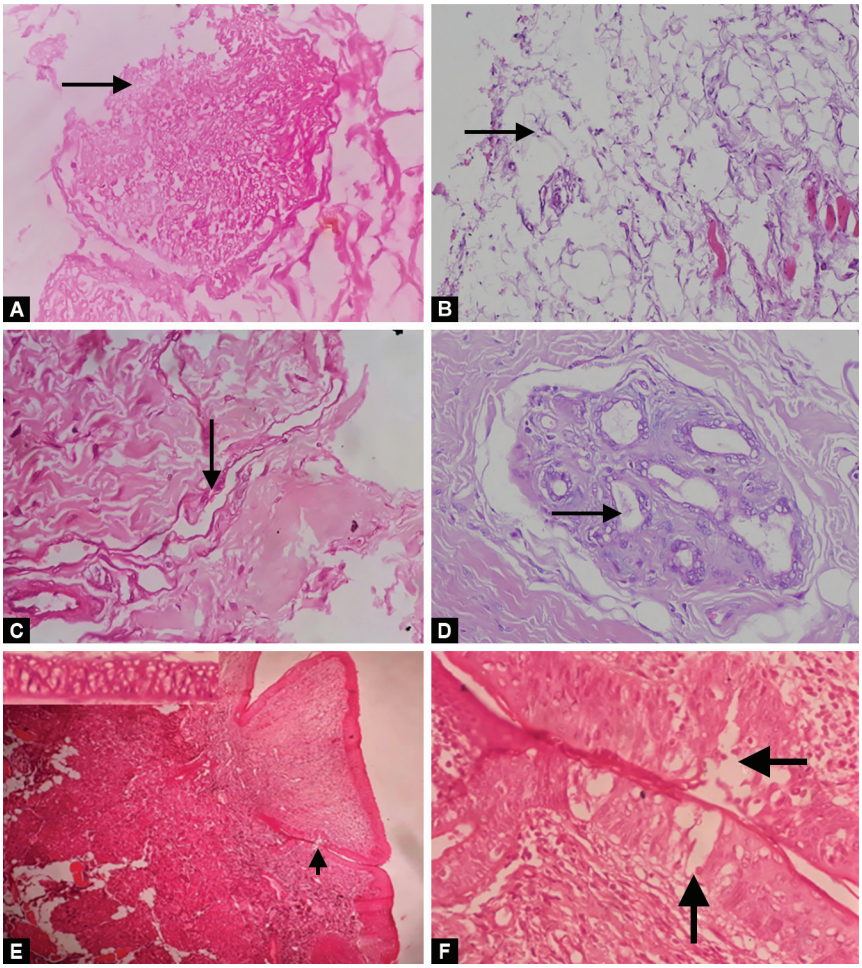


Figs 1A to F: Photomicrographs of autopsied tissues showing degenerative changes in epithelium, muscle, collagen, salivary gland acini, and ducts (H and E staining). (A) Vacuolar degeneration of epithelial cells (X100); (B) Shredding and fraying of muscle fiber bundles (black arrow) (×100); (C) Fragmentation of collagen fibers (black arrow) (×400); (D) Vacuolated appearance of acinar cells (black arrow) (×400); (E) Vacuolar swelling of salivary ductal epithelial cells (black arrow) (×100), inset (×400); (F) Disruption of salivary duct (black arrow) (×100)

Hepatocellular toxicity was induced by the release of nitrites and nitrates due to increased oxidative stress.[15]

In the current study, there was nuclear and cellular vacuolar degeneration and the severe fraying and shredding of the muscle fibers in all the study sample tissues. The degeneration of epithelial cells and the necrosis of the muscle could have been due to the myotoxic nature of pesticides. A similar hypothesis was proposed by Beydilli et al.,[15] who found degeneration and necrosis in the skeletal muscle and liver tissues of pesticide-poisoned albino rats.

In the present study, a loss of the organization and fragmentation of collagen fibers with a triple-helical structure was observed in all the study samples. The observed breaks and fragmentation could be explained by the ability of organophosphates to prevent cross-linking in collagen and elastin. This postulation was proposed by Tuna et al.,[16] who observed disorganization, breaks, and fragmentation in collagen and elastin fibers in the aortic wall of rats following acute and chronic toxicities from OP poisoning. The present study also observed the loss of lobular salivary gland architecture in 8 of the study samples and the vacuolization of acinar cells in all 10 specimens. Seven specimens showed severed ducts with ductal cell degeneration. In contrast to the degenerative changes observed in the present study, Saltmiras et al. observed the hypertrophy of salivary acinar cells due to the repeated dietary effect of an organic acid, glyphosate, on salivary glands.[17] This hypertrophy was believed to be an adaptive response to organic acids and glyphosate.[18] The acute nature of poisoning in the present study would not have provided sufficient time for an adaptive response to occur. Thus, gross degeneration was noted in the salivary gland tissues.



Figs 2A to F: Photomicrographs of autopsied tissues showing degenerative changes in nerve, adipose tissue, blood vessels, circumvallate papillae, and taste buds (H and E staining). (A) Vacuolization of nucleus and cytoplasm of nerve cells (black arrow) (×100); (B) Fragmented appearance of adipose tissue (black arrow) (×100); (C) Congestion of blood vessels (black arrow)(×100); (D) Vacuolar degeneration of endothelial cells (black arrow) (×200); (E) Vacuolar swelling of epithelial cells in the circumvallate papilla (black arrow) (×40), inset (×400); (F) Vacuolar degeneration of taste buds (black arrows) (×400)

The nerve tissue in all 10 specimens showed vacuolization in the nucleus and cytoplasm and a disruption of the perineurium (Fig. 2A). Harith et al.[19] found similar changes in nerve tissues when investigating the pathological changes caused by malathion toxicity, an OP insecticide, in wild pigeons. He found the vacuolation of nerve fibers in the spinal cord and sciatic nerve, meningitis associated with lymphocytic infiltration in the brain, myocardial fibrosis, lymphocyte aggregation, and hepatic septal fibrosis.[19]

In the present study, the architecture of the adipose tissue was lost, with degenerative changes in all the specimens. Budin et al.[20] evaluated the morphological changes in the lungs due to the ingestion of OP pesticide and found the highest concentration in fat tissues followed by other organs, such as the lung.

The current study showed congested blood vessels with ruptured vessel walls and vacuolar swelling in the endothelial cells in all the specimens. These findings are similar to those in a study by El-Bendary et al.,[21] who studied the histopathological effects of the synthetic OP pesticides, profenofos, and chlorpyrifos, on the liver, kidney, brain, and spleen tissues in mice. They found that the congestion of blood vessels, necrosis, and hemorrhages were due to degeneration and damage to the endothelial cells.

The present study showed the vacuolar degeneration of the papillary cells and taste buds in nine specimens. OP exposure has been shown to cause a garlic-like taste or odor with hypersalivation.[22] However, there are only a limited number of studies available that have analyzed the effect of pesticide poisoning on the tongue and its papillae. Although the present study revealed significant degenerative changes in the histomorphological examination of oral tissues, further studies with a larger sample size will be needed to confirm our results. Similar studies should be conducted to investigate the histopathological effects of all ingested fatal poisons and to examine the local effects in other gastrointestinal tract structures in the future.

Conclusion

The diagnosis of pesticide poisoning generally depends on the characteristic clinical features, a history of exposure to a known pesticide compound, and chemical analysis for confirmation. As the oral cavity is the first area to encounter ingested poison, the histopathological changes observed in these tissues may provide adjunctive evidence for the determination of the cause of death

in forensic investigations. The present study showed significant degenerative alterations in epithelial cells and connective tissue components, such as collagen, muscle, nerve, vascular channels, adipose tissue, salivary acini, and ducts, thereby aiding forensic examinations. Further, in suspected poison cases in which vital organs are not in an examinable state or are not recoverable, an autopsy of oral tissues could provide valuable insights into the cause of death.

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