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Phyto-intruders in oral tissues: A polarized light microscopic study

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ABSTRACT

Objectives: Foreign body reactions are common in the oral cavity due to its proximity to the external environment. Rarely, foreign body of plant origin may be encountered in the histopathological sections making the diagnosis problematic. The aim of the present study was to analyze the histological features of various products of plant origin emphasizing on the pathogenesis of tissue reaction occurring in response to their implantation.

Methods: This observational study included various plant products or phytoproducts commonly consumed in South Indian diet such as rice, curry leaves, coriander leaves, spinach leaves, coconut, green chilli, onion, French beans, urad dal, lentil beans, mustard seed, ginger, and garlic. Formalin-fixed specimens were routinely processed and stained with hematoxylin and eosin. The slides were viewed under light microscope and polarized microscope and evaluated by two oral pathologists.

Results: Each of the phytoproducts had distinctive histological appearance and exhibited positive birefringence. Phytoproducts such as rice, curry leaves, French bean, onion, and green chilli resembled pathological structures such as calcifications, ghost cells, clear cells, atypical adipocytes, and fungal hyphae, respectively.

Conclusions: Plant products appear as puzzling structures in histological section posing difficulties to the pathologist. Recognition of these structures as foreign body based on their histological appearance is inevitable and their identification may avoid unnecessary delay in treatment planning. The current study serves as an atlas for the histology of extraneous material study and also as a reference for the pathologists whenever mystifying structures are encountered.

Keywords: Food implantation, foreign body, histology, phytoproducts, plant

Introduction

The foremost site for food processing is the oral cavity. It is foreseeable that the food particles, especially the plant products or phytoproducts, can be commonly encountered in the histopathological examination of oral tissues. The pathologists have a very limited knowledge about the histopathological features of such phytoproducts, causing a confusion in diagnosis. More frequently, the phytoproducts may be overlooked or may be identified as artifacts when these products remain superficial to the mucosa. Sometimes, they may get implanted into the deeper tissue, triggering a foreign body reaction. In such cases, a granuloma may be formed which may be mistaken for foreign body reaction to other living organisms such as bacteria, fungi, protozoan, or to other particulate matter such as suture material, cotton, fat, or keratin.[1] Splinters and spines of plants like cactus are the common phytoproduct confronted in the skin of the extremities.[2] Tissue reaction to the phytoproducts has also been reported in gastrointestinal specimens,[3] lungs,[4] gallbladder,[5] fallopian tube,[5] knees,[6] ovary,[7] and intrahepatic portal vein.[8] Lack of relevant clinical history and lack of familiarity with the morphologic features of these extraneous material could result in erroneous management.

In oral cavity, the phytoproducts may gain access through extraction socket, tooth left open for root canal, grossly decayed tooth, or traumatized mucosa resulting in granuloma formation. Several terms have been used in the literature to describe such granulomas such as "Granuloma induced by vegetal inoculation," pulse granuloma, and oral vegetable granuloma. Pulse granulomas can occur in the walls of the inflammatory odontogenic cysts when they are treated by marsupialization to expose the cyst to the oral cavity and not adequately irrigated.[9] Two types of pulse granuloma can occur in the oral cavity, central or peripheral based on their location. It is common in posterior region of the mandible. Histopathological features consist of granulomatous reaction containing multinucleated foreign body giant cells, inflammatory cells, and eosinophilic material surrounded by the fibroblasts. Oral vegetable granuloma differs from similar lesions in other sites in that the oral lesions have few giant cells and lack starch. The homogeneous, eosinophilic material is referred to as the hyaline ring which is thought to represent the plant material. When the plant material is present for a longer duration, it is difficult to identify the old plant material since they tend to lose their morphologic characteristics. As a pathologist, misdiagnosis may be eluded if these phyto-intruders could be accurately identified from the microscopic sections.

To circumvent the difficulties posed by these puzzling structures, it is essential that the literature contain an atlas for the histology of extraneous materials. Few studies have been done illustrating morphologic features of various plant contaminants.[10,11] The current study was done to expand our knowledge about the histology of various phytoproducts that are more likely to get entrapped in the oral tissues emphasizing on the tissue reaction occurring in response to implantation of these phytoproducts.

Methods

Samples

The phytoproducts commonly consumed in South Indian diet were included in this study which comprised rice, curry leaves, coriander leaves, spinach leaves, coconut, green chilli, onion, French beans, urad dal, lentil beans, mustard seed, ginger, and garlic. Some of the phytoproducts such as ginger, garlic, onion, and coconut were difficult to process and hence they were cooked and then processed since they are more likely to get entrapped in the tissues in the cooked form.

Tissue processing

The samples were fixed with 10% formalin and then dehydrated by placing them in graded alcohol solution 70%, 90%, and 100% for 1 h each. Following dehydration, the samples were placed in three changes of 100% xylene for 1 h each, then placed in three changes of melted paraffin wax at a temperature of 60°C for 1 h each, oriented, and finally blocked. From the blocks, 4 µm thick sections were obtained which were placed on plain glass slides then dewaxed, rehydrated in descending concentration of alcohol solution 70%, 90%, and 100%, and stained. The sections were stained with hematoxylin and eosin using the standard routine protocol and mounted permanently using DPX. The slides were covered with coverslip and left to dry at room temperature.

Microscopic analysis

The slides were examined under bright field transmitted light microscope and polarized microscope at 200–400× using Olympus BX 52 microscope. The slides were microscopically evaluated by two pathologists and validated by a botanist.

Results

On light microscopic examination, the histopathological observations were recorded as follows:

Rice (*Oryza sativa*): On longitudinal section, rice appeared as scattered hematoxyphilic masses in the periphery, polygonal structures with eosinophilic outlines and colorless centers in the center. Hematoxyphilic areas in the periphery resembled calcifications. When observed under polarized microscope, the cellular outlines exhibited positive birefringence [Figure 1a].

Curry leaves (*Murraya koenigii*): Ovoid cells with eosinophilic outlines and pale center were seen and they appeared similar to ciliated structures in some areas. The xylem and phloem were birefringent when viewed under polarizing microscope [Figure 1b].

Coriander leaves (*Coriandrum sativum*): Birefringent, eosinophilic cluster of cells with faint outline were seen [Figure 1c].

Spinach (*Spinacia oleracea*): Birefringent, hexagonal structures resembling xylem and phloem were seen [Figure 1d].

Coconut (*Cocos nucifera*): Birefringent, hexagonal to polygonal structures with empty centers were seen [Figure 1e].

Green chilli (*Capsicum annuum*): Outer layer of flattened cells with eosinophilic outlines and inner polygonal cells with pale centers were seen. The cellular outlines exhibited positive birefringence [Figure 1f].

Onion (*Allium cepa*): Polygonal, birefringent eosinophilic structure with nutrient material inside was seen [Figure 2a].

French bean (*Phaseolus vulgaris*): Eosinophilic, ovoid structures were seen with internal compartmentalization and empty center mimicking adipocytes [Figure 2b]. Some of the cells resembled adipocytes. The outer lining layer of cells was birefringent.

Urad dal (*Vigna mungo*): Cluster of birefringent, eosinophilic cells were seen giving a honeycomb appearance [Figure 2c]. It resembled salivary gland acini.

Lentil beans (*Lens culinaris*): Sieve tubes and granular cells with nutrient material inside were observed [Figure 2d]. The cellular outlines were birefringent. Few cells appeared like foamy macrophages.

Mustard (*Brassica juncea*): Outer pericarp with epidermal layer, inner cotyledon with few cells containing nutrient material and remaining cells appeared empty [Figure 2e].

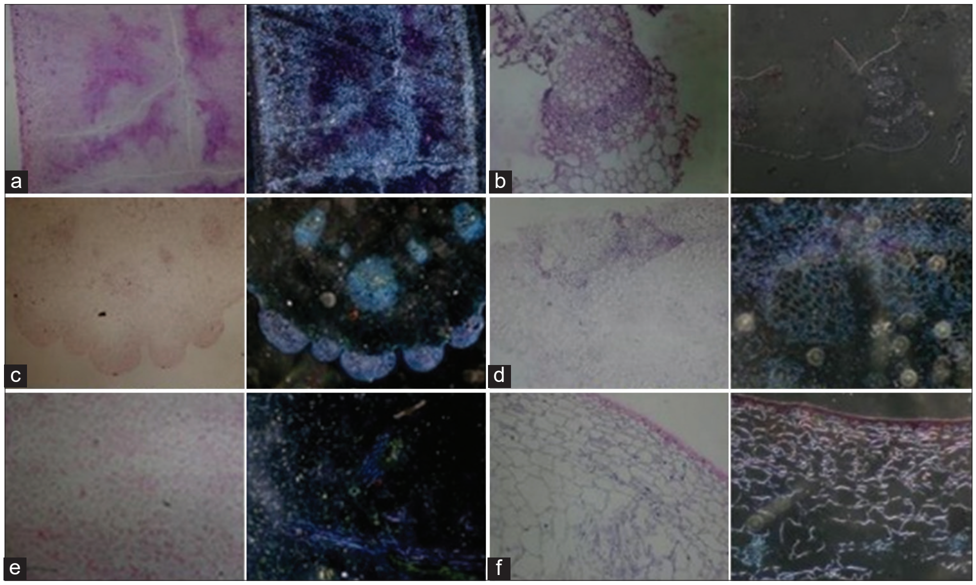


Figure 1: Light microscopic and polarized microscopic appearance of rice (a), curry leaves (b), coriander leaves (c), spinach leaves (d), coconut (e), and green chilli (f)

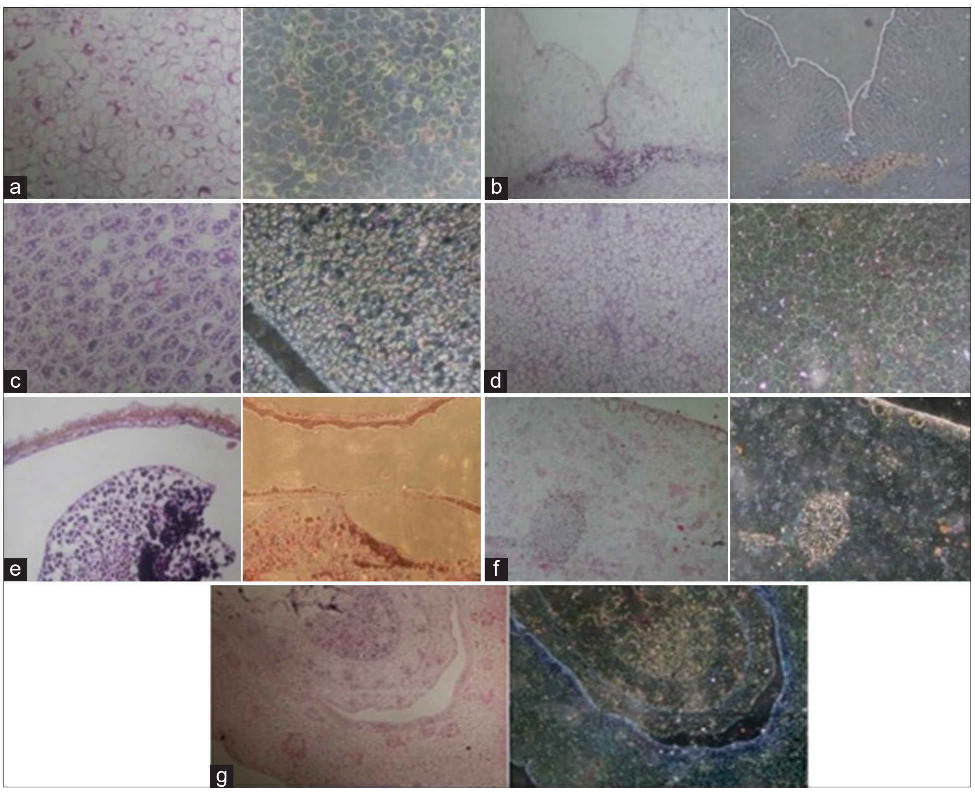


Figure 2: Light microscopic and polarized microscopic appearance of onion (a), French beans (b), urad dal (c), lentil beans (d), mustard seed (e), ginger (f), and garlic (g)

The outer layer was found to exhibit positive birefringence. It resembled parasitic cyst.

Ginger (*Zingiber officinale*): 2–4 layers of flattened peripheral cells and underlying eosinophilic structures with empty center were seen. Diffuse masses of birefringent, eosinophilic structures with nutrient material were also seen. Concentric structures were present in areas corresponding to buds [Figure 2f]. Some of the cells were mimicking the hyaline globules.

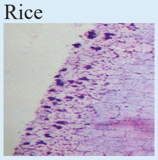
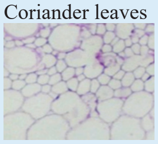
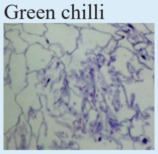
Garlic (*Allium sativum*): On cross-section, it revealed a central core of cells bounded by layer of cuboidal to flat cells. Granular cells were seen along the periphery amidst multiple floret-like eosinophilic structures [Figure 2g].

Phytoproducts such as rice, curry leaves, French bean, onion, and green chilli resembled pathological structures such as calcifications, ghost cells, clear cells, atypical adipocytes, and fungal hyphae, respectively [Table 1]. The sequelae of reaction of the host when these phytoproducts enter the tissue are represented in Figure 3.

Discussion

Foreign bodies are commonly encountered in the oral cavity, the site which is in immediate contact with the external environment. Entrapment of foreign bodies in the oral cavity can be either traumatic or iatrogenic. Most of the plant products enter the digestive tract as insoluble structures in the form of plant cell wall fragments and starch.[12] Rarely, vegetal material may appear in the histopathological sections making the diagnosis troublesome. Plant cells have a tough cell wall composed of cellulose and glycans embedded in pectin polysaccharides. Cellulose is composed of glucose units held together by beta-acetal linkages which make it difficult for the human digestive enzymes to digest cellulose. While the starch components present in the phytoproducts gets digested, it is this undigestible cellulose that contributes to the fiber component essential for proper functioning of the gastrointestinal tract. Phytoproducts may inadvertently

Table 1: Phytoproducts showing resemblance to pathological structures

Photomicrograph of phytoproducts	Resembling pathological structures	Photomicrograph of phytoproducts	Resembling pathological structures
Rice 	Calcifications as seen in peripheral giant cell granuloma, peripheral ossifying fibroma	French bean 	Clear cells as seen in clear cell lesions
Curry leaves 	Ghost cells as seen in dentinogenic ghost cell tumor, calcifying cystic odontogenic tumor	Urad dal 	Cribriform type of adenoid cystic carcinoma
Coriander leaves 	Adipocytes as seen in lipoma	Lentil beans 	Destruction of salivary acinar units with preservation of lobular architecture as seen in Sjogren syndrome
Spinach leaves 	Adipocytes as seen in lipoma	Mustard 	Parasitic cystic spaces as seen in hydatid cyst
Coconut 	Adipocytes as seen in lipoma	Ginger 	Rosette pattern as seen in adenomatoid odontogenic tumor
Green chilli 	Fungal hyphae and spores	Ginger 	Microcysts identical to glandular odontogenic cyst
Onion 	Atypical lipomatous tumor	Garlic 	Floret-like multinucleated giant cells as seen in neurofibroma, granular cells as seen in granular cell tumor of tongue

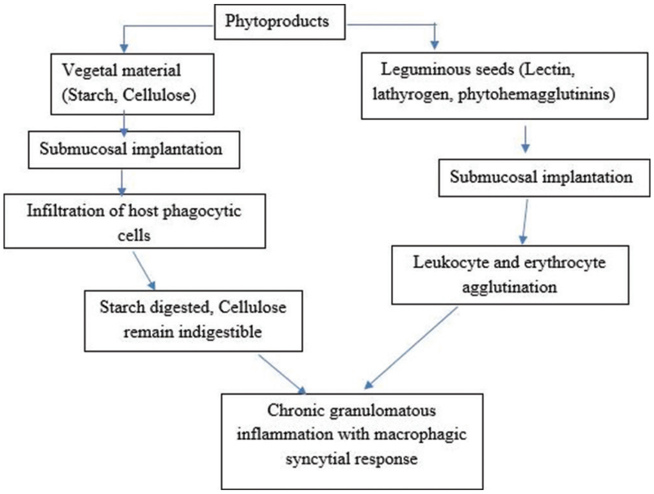


Figure 3: Flowchart depicting the sequelae of submucosal implantation of phytoproducts

enter the oral tissues through extraction socket, periodontal pocket, open root canals, or decayed teeth.[13]

When the phytoproducts gets implanted into the submucosa, the immune system is triggered and there is focal infiltration of the immune cells. The host immune cells attempt to phagocytose the phytoproducts as a result of which the cellulose moiety remains resistant to digestion evoking an inflammatory response. Animal experiment conducted in the lungs of guinea pigs, cats, and rabbits have concluded that cellulose present in the lentils is the granuloma inducing substance and not the starch.[14] Chronic granulomatous inflammation is associated with macrophagic syncytial response resulting in foreign-body giant cell formation.[15]

Lentils belong to the legumes family and it consists of an outer shell surrounding the cotyledon. The main component of the lentils which makes it indigestible is the seed coat. The presence of lectin, lathyrogens, and phytohemagglutinins in legumes is responsible for leukocyte and erythrocyte agglutination. Leguminous seeds are also rich in protein which is suggested to trigger immune granulomas.[16] It has been suggested that cellulose present in some vegetables such as carrot and onion is not as resistant as legumes to digestion. Hence, these vegetables are not capable of producing long-lasting granulomas.[14] As the tissue response progresses, the morphological characters of the plant cells are lost and the degenerated products of starch may form an amorphous debris.

Oral pulse granulomas are common in denture bearing areas followed by non-edentulous areas with a history of endodontic therapy or pericoronitis or periodontal surgery. It has also been documented in walls of cysts such as dentigerous, residual, nasopalatine, and odontogenic keratocyst.[17] Peripheral oral pulse granulomas occur in lower lip, gingiva, and dorsum of tongue and often present as painless submucosal swellings. Radiographically, there may be erosion of the alveolar crest. Central pulse granuloma is usually asymptomatic and appears as irregular radiolucent area with well-defined borders. Differential diagnosis for oral pulse granuloma includes infectious granulomas, inflammatory, and vascular diseases.[18]

Their histological appearance in lesions may vary depending on the type of food implanted, extent of mastication, and response of the host. The tissue reaction is frequently visualized as pale stained eosinophilic structure-less material in the form of rings, ovoid, filamentous, or amorphous masses referred to as hyaline ring.[19] This hyaline material was propounded to be the collagen degraded by macrophages and giant cells.[20] However, some authors believe hyaline rings to be the plant cell walls since collagen fibrils resemble cellulose ultrastructurally.[21] Several theories have been put forth concerning the origin of hyaline material. According to the exogenous theory, immune cells infiltrate the tissues after the plant material get entrapped and the indigestible cellulose remains as hyaline rings are formed. According to the endogenous theory, hyaline rings represent blood vessel wall which could have hyalinized due to acute vasculitis or coagulation of extravasated serum proteins or degenerated collagen. Hyaline rings enclose blood vessels, giant cells, and inflammatory cells which in due course may be degraded and become unrecognizable. Apart from the hyaline rings, birefringent foreign body of plant origin may also be seen. At times, the plant particles may undergo progressive mineralization which appears histologically as punctate, basophilic calcifications.[15] These calcified basophilic granules can sometimes be mistaken for fungal forms of Coccidioides.[18]

The basic structure of all vascular plants consists of the vascular tissue comprising the xylem and phloem cells enclosed in ground tissue, surrounded by the dermal tissue. In the study conducted by Chang *et al*., it was found that vegetable and fruit contaminants mimicked infectious parasites or fungal spores and also contributed to necrotic background.[10] In our study, rice had histological features similar to calcifications seen in peripheral ossifying fibroma and peripheral giant cell fibroma. Ghost cell-like structures were seen in sections from curry leaves resembling dentinogenic ghost cell tumor, calcifying cystic odontogenic tumor. Sections of coriander leaves, spinach leaves, onion, and coconut were identical to fat cells as seen in lipoma, well-differentiated lipomatous tumors. Sections from green chilli resembled fungal spores and hyphae and French beans had features of clear cells as noticed in various clear cell lesions. Typical cribriform pattern of adenoid cystic carcinoma like histopathological feature was observed from sections of urad dal. Lentil beans had features of destruction of salivary acini with preservation of lobular architecture as observed in Sjogren syndrome and the mustard section was identical to hydatid cystic spaces. Fragmented parts of the pigmented pericarp present in the mustard seed may appear similar to the cuticle of the maggot seen in oral myiasis.[22] Buds from ginger mimicked rosette pattern of adenomatoid odontogenic tumor. Garlic sections were akin to multiple pathological structures such as floret-like giant cells in neurofibroma and granular cell tumor of tongue. Superficial layer of ginger was analogous to glandular odontogenic cyst. Under polarized microscopy, all the phytoproducts included in the study showed positive birefringence. Similar birefringence of the plant materials has been observed in other studies as well.[11,23] The cellulose content of higher plants is the probable cause for the positive birefringence.[24] Since many phytoproducts resembled pathological structures, contextual interpretation of the histological findings with the clinical history is indispensable to arrive at an accurate diagnosis.

Although the histological features of the plant products understudy seem to be unambiguous, they may lose their original characteristics and may appear histologically incongruous when implanted in the host tissues due to the time-dependent degradation or degeneration that they undergo as a result of tissue response. The current study may serve as a reference for the pathologists to identify the plant products whenever mystifying structures are encountered in the tissue sections.

Conclusions

Cellulose moiety present in the plant products has granuloma stimulating effect when they get implanted in the oral mucosa. The resultant foreign body reaction is usually characterized by the presence of giant cells, inflammatory cells, and eosinophilic hyaline material histopathologically. Since hyaline ring formation may not be always present in this type of granuloma, recognition of the plant structures based on their histological appearance is inevitable and their identification may avoid unnecessary delay in treatment planning.

Authors' Declaration Statements

Ethics approval and consent to participate

Not applicable.

Competing interests

Nil.

Funding statement

Nil.

Authors' Contributions

BD and VV involved in concepts, study design, defining of intellectual content, literature search, experimentation, data analysis, and manuscript preparation; RR, ARK, and RK involved in concepts, study design, defining of intellectual content, literature search, and manuscript preparation; KKR involved in concepts, study design, defining of intellectual content, and manuscript preparation.

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