ORIGINAL ARTICLE

Evaluation of Histological Changes in the Salivary Glands of Sprague-Dawley rats Following Injections of Various Forms of Areca Nut Preparations

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Abstract

Introduction: The habit of areca nut chewing impinges on the daily lives of about one-tenth of the world's population. Areca nut is used in many forms ranging from raw to commercial varieties. The consumption of areca nut is found to have important effects on salivary glands leading to an altered salivary flow rate and pH of saliva. Areca nut consumption is significantly associated with the development of oral submucous fibrosis (OSF). OSF in the early stages is characterized by an increased salivation and advanced stages by an increased dryness of the oral mucosa. **Aims and Objectives:** The present study aimed to evaluate the histopathological tissue changes in the rat submandibular salivary glands subjected to various forms of areca nut (raw, roasted, and boiled), pan masala extracts, and pure arecoline solution over a period of 36-week. **Materials and Methods:** 3-4 months old Sprague-Dawley rats were randomly selected and divided into six groups - Control, raw areca nut, boiled areca nut, roasted areca nut, pan masala, and pure arecoline groups; treated with the respective solutions. The control group was treated with distilled water. Rats were sacrificed randomly at an interval of every 6 weeks and submandibular glands dissected out and assessed for any histological changes. **Results:** Significant histological changes were observed in the rat submandibular tissues including fusion and dilatation of acini and ducts with pooling of the salivary secretions in the initial weeks of treatment and degenerative changes in the later weeks. Among all the groups, the pure arecoline treated group showed significant and early degenerative changes in comparison to the other groups. **Conclusion:**Chronic consumption of areca nut in various forms leads to deleterious effects on the salivary gland histology leading to degeneration of acini and ductal structures. This degenerative effect leads to an overall decreased salivary output reflecting as dryness of mucosa in the advanced stages of OSF and probably to a decreased local immunity and hence an increased propensity for malignant transformation in the later stages of OSF.

Key words: Areca nut, arecoline, oral submucous fibrosis, pan masala, submandibular glands, Sprague-Dawley rats

INTRODUCTION

The habit of areca nut chewing impinges on the daily lives of about one-tenth of the world's population. It is the fourth most common addictive psychoactive substance consumed after caffeine, nicotine, and alcohol. The addictive properties are attributable to the inhibition of gamma-amino butyric acid (GABA) uptake in the nervous system by the areca alkaloids.[1]

India lines among the top rankers in both production and consumption of areca nut. In India, Karnataka, Kerala, and Assam are the important states contributing to the high percentage of production of areca nut.[2]

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Areca nut is perused in many forms in the region ranging from raw to commercial varieties. Pan masala, a popular dispensation among the commercial products is a blend of areca nut powder with specified and unspecified additives. When combined with tobacco it is called gutka. Marketed aggressively, packaged attractively and the pricing made affordable, its popularity among the youth has led to its widespread use. Processed forms of areca nut are also an important and common form of consumption of the nut. The more commonly used processed forms include boiled, roasted, baked and/or soaked nuts in water.[3]

Areca nut extract contains alkaloids such as arecoline, arecaidine, guvacoline, and guvacine; flavanoids such as tannins and catechins and traces of copper. Arecoline and arecaidine alkaloids in the nut are thought to play a major role in the development of adverse effects resulting from this chewing habit. The roasted variety of nut possesses the highest tannin content (5-41%) followed by the raw (25%) and the boiled areca nut (17%). The arecoline content is highest for the sun-dried raw variety (1.35%) followed by the roasted (1.29%) and the boiled variety of areca nut (0.1%). The second International Agency for Research on Cancer (IARC) Monograph on betel quid labels areca nut as a "group one carcinogen."[3]

The consumption of areca nut has deleterious effects on almost all organs of the human body; including the oral cavity, pharynx, esophagus, brain, heart, liver, lungs, and reproductive organs with the salivary glands being no exception to this gospel truth.[4-6] The foremost effect of areca nut on salivary glands is a para-sympathomimetic action of arecoline on the glands resulting in increased salivation. Previous reports also state that an altered salivary flow rate and pH are seen in areca nut chewers, rendering the oral mucosa vulnerable to the toxic effects of areca nut.[7] Furthermore, previous experimental studies also affirm an increased incidence of parotid tumors in response to chronic exposure of high doses of areca nut.[8,9]

Rats possess the similar set of salivary glands as humans-major glands parotid, submandibular and sublingual gland, and minor salivary glands. The rat salivary glands are structurally similar to the humans except for slight structural differences in the submandibular glands; including the presence of an additional granular duct and absence of mucous acini or demilunes in the rat submandibular glands. The granular ducts in the rats are responsible for the mucous secretions.[10,11]

A notable cause-effect correlation is reported between consumption of areca nut and development of oral submucous fibrosis (OSF).[12-14] An increased salivary rate is reported in the early stages of OSF. On the contrary, the advanced stages of OSF are characterized by an increased dryness of the oral mucosa.[15]

Studies to evaluate the effect of areca nut consumption on salivary glands histology are few and far in the literature and hence formed the mainstay of our study.

Aims and objectives

The present study intended to evaluate the histopathological tissue changes in the rat submandibular salivary glands subjected to various forms of areca nut (raw, roasted, and boiled), pan masala extracts, and pure arecoline solution over a period of 36-week.

MATERIALS AND METHODS

3-4 months old Sprague-Dawley (SD) rats, weighing 100-200 g were randomly selected and divided into six groups - Control, raw areca nut, boiled areca nut, roasted areca nut, pan masala, and pure arecoline groups. Each group comprised six animals each. The animals were procured and housed at the animal house of the institution. The rats were maintained under standard laboratory conditions at controlled temperature (25 ± 2°C) with 12 h light/dark cycle and humidity. They received standard diet and water *ad-libitum*.

Experimental rats in the raw, boiled, roasted areca nut, pan masala, and pure arecoline groups were injected with 0.2 ml of the respective solutions of the areca nut extracts, pan masala, and pure arecoline solution on every alternate day for 36 weeks. The control group was treated with distilled water. The injections were given intra orally into the left buccal mucosa using a U-40 Insulin syringe. The study involved simulating the effects of patients chewing areca nut which causes a potentially malignant oral disorder, OSF.

Animals were sacrificed randomly at an interval of every 6 weeks. The submandibular glands were dissected out and fixed in 10% buffered formalin followed by conventional processing, sectioning, and staining with H and E staining. The histological sections were then subjected to a comparable analysis (blind version) for any structural changes. Inter- and intra- observer variations were addressed by standard protocols of all authors observing and reporting on the changes in the tissues simultaneously.

The study protocol was approved by the Institutional Animal Ethics Committee (ref nos. SRDC 462/12 and KCP/0002/13-14, respectively).

RESULTS

Control group

Salivary gland changes

The distilled water treated tissue showed no significant changes in the gland structure or architecture over a period from 6 to 24 weeks. However, the 30th and 36th week tissue showed slight dilatation of the granular ducts. The normal

Table 1: Evaluation of histological changes in rat submandibular salivary glands on exposure to various forms of areca nut

Weeks	Control	Raw areca nut	Roasted areca nut	Boiled areca nut	Pan masala	Pure arecoline
6	Architecture - Normal Normal	Architecture - Normal Normal	Architecture - Poorly preserved Acini - Fused Granular ducts - Dilated Moderate	Architecture - Normal Normal	Architecture - Normal Ducts - Increased serous and mucous secretion pooling in duct spaces Mild	Architecture - Poorly preserved Ducts - Fragmentation of ductal cells in few areas with degradation of cellular boundary Other changes - Inflammation and congested vessels Moderate
12	Architecture - Normal Normal	Architecture - Normal Normal	Architecture -Normal Ducts - Dilated without a lining Mild	Architecture - Normal Normal	Architecture - Normal Ducts - Dilated with secretions Mild	Architecture - Normal Normal
18	Architecture - Normal Ducts - Larger and prominent Mild	Architecture - Normal Acini - Fused Ducts - Dilated and fused Mild	Architecture -Normal Ducts - Fusion and more prominent Other changes - Congestion Mild	Architecture - poorly preserved Ducts - Focal degeneration Moderate	Architecture - poorly preserved Ducts - Fusion Moderate	Architecture – Normal Ducts - Fused ducts Other changes - Mild congestion Mild
24	Architecture - Normal Normal	Architecture - Normal Acini - Large acinar cells (hydropic swelling) Ducts - Fused at periphery Mild	Architecture -Normal Acini - Larger Ducts- Fused Mild	Architecture- poorly preserved Acini - Acinar cells are vacuolated Ducts - Less prominent Moderate	Architecture - poorly preserved Moderate	Architecture - Normal Acini - Edematous changes Ducts - Larger Other changes - Congestion Mild
30	Architecture - Normal Ducts - Prominent ducts Normal	Architecture - Poorly preserved Acini - Edematous changes ducts - Edematous Moderate	Architecture - Poorly preserved Ducts - Periphery - degeneration Other changes - Plasma cell infiltration and hemorrhagic areas Moderate	Architecture - Fairly normal Acini - prominent Ducts - Degenerated Moderate	Architecture - Fairly normal Ducts - Degenerated Moderate	Architecture - Poorly preserved Acini - Edematous Ducts - Eegenerated Moderate
36	Architecture - Normal Acini - Prominent Ducts - Prominent Normal	Architecture - Lost Ducts - Edematous Other changes - Congested vessels Severe	Architecture - Lost Acini and ducts - Slight degeneration at periphery Severe	Architecture - Lost Acini and ducts - edematous degenerative changes Severe	Architecture -Lost Acini and ducts - Degenerative and edematous changes in acini and ducts Severe	Architecture - Lost Acini and ducts - edematous and degenerative changes Other changes - Congestion Severe

Grading criteria: Normal - Normal architecture, normal acinar and ductal components, Mild - Normal/fairly normal architecture, dilatation and fusion of acini and ducts, Moderate - Poorly preserved architecture, edematous and focal degenerative changes in acini and ducts, Severe - Loss of normal architecture and/or extensive degenerative changes

gland architecture was maintained throughout [Figure 1, Tables 1 and 2].

Buccal mucosal changes

The rat buccal mucosal tissue showed an increase in the keratin and epithelium thickness at 6th week with no significant changes in the vascularity and depth of fibrosis compared to other groups. From 12th to 36th decrease in the keratin thickness, epithelium thickness and vascularity was noted. The values depth of fibrosis was highest at 30th and 36th week, but these values were not significant as compared to other test groups [Table 2].

Raw areca nut treated group

Salivary gland changes

In the initial weeks (6th and 12th week), abnormal salivary gland architecture was maintained with no significant changes. The tissue in the 18th and the 24th week showed dilatation and fusion of acini and granular ducts with the normal architecture well preserved. Degenerative and edematous changes in the acini and the ductal structures were seen in the 30th week that progressively increased toward the 36th week. The architecture appeared distorted for the 30th and the 36th week tissue [Figure 2].

Buccal mucosal changes

The buccal mucosal tissue showed a marked increase in the fibrotic tissue deposition and an abnormally low keratin and epithelium thickness with decreased vascularity as early in the 6th week. From 12th to 36th week depth fibrosis, keratin thickness, epithelium thickness, and vascularity followed an irregular trend but increased the depth of fibrosis and decreased keratin thickness, epithelial thickness, and vascularity were noted at the end of the experimental period [Table 2].

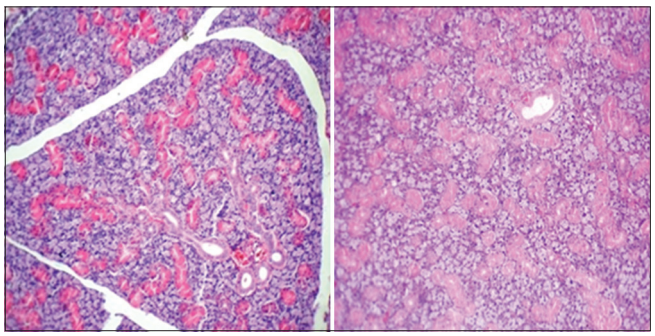

Figure 1: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the control group at 6 weeks (L) - normal architecture, acini and ducts and at 36 weeks (R) with little changes (H and E, original magnification ×10)

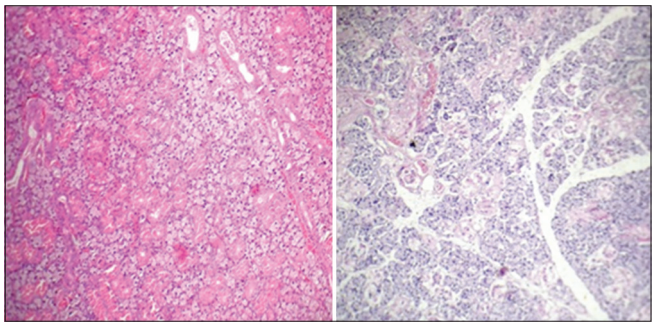

Figure 2: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the *raw areca nut* group at 6 weeks (L) - normal architecture, acini and ducts and at 36 weeks (R) with edematous and degenerative changes in ducts and acini with loss of normal architecture (H and E, original magnification ×10)

Roasted areca nut treated group

Salivary gland changes

The roasted areca nut treated tissue showed immediate hyperplastic changes as early as the 6th week consisting of fusion and dilatation of the acini and the ductal structures. The architecture appeared to be poorly preserved. Degenerative changes were notably seen especially at the periphery at the 30th and 36th week with a loss of normal architecture. Plasma cell infiltration into the tissue was evident in the later weeks [Figure 3].

Buccal mucosal changes

Buccal mucosal tissue showed a continuous decrease in the keratin thickness and epithelium thickness, increase in depth fibrosis, and decreased vascularity from 6th to 18th week. The rat buccal mucosal tissue showed a minimum amount of keratin thickness in 30th week compared to other groups. The maximum changes in vascularity and depth of fibrosis were noted in the 36th week [Table 2].

Boiled areca nut treated group

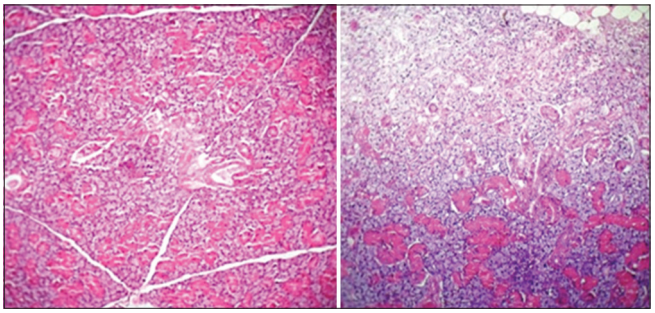

Figure 3: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the *roasted areca nut* group at 6 weeks (L) - showing fusion of ducts and acini with a poorly preserved architecture and at 36 weeks (R) with degenerative changes in acini and ducts in the periphery with loss of normal architecture (H and E, original magnification ×10)

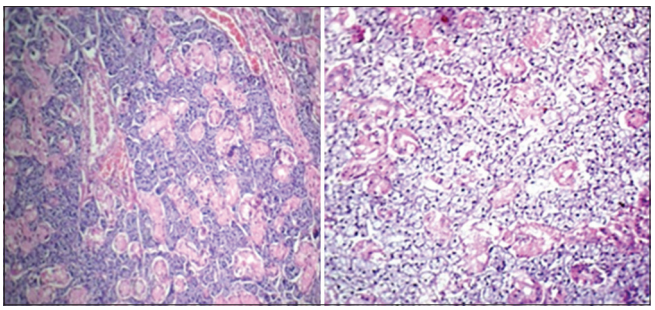

Figure 4: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the *boiled areca nut* group at 6 weeks (L) - normal architecture, acini and ducts and at 36 weeks (R) with edematous and degenerative changes in the acini and ducts with loss of normal architecture (H and E, original magnification ×10)

Table 2: Evaluation of histological changes in rat buccal mucosa on exposure to various forms of areca nut

Weeks	Control	Raw areca nut	Roasted areca nut	Boiled areca nut	Pan masala	Pure arecoline
6	Hyperplastic epithelium Normal vascularity Grade I OSF	Atrophic epithelium Decreased vascularity Grade I OSF	Normal epithelium and vascularity Grade I OSF	Hyperplastic epithelium Normal vascularity Grade I OSF	Atrophic epithelium Minimal vascularity Grade I OSF	Hyperplastic epithelium Normal vascularity Grade I OSF
12	Hyperplastic epithelium Increased vascularity Grade I OSF	Atrophic epithelium Normal vascularity Grade I OSF	Atrophic epithelium Normal vascularity Grade II OSF	Hyperplastic epithelium Decreased vascularity Grade I OSF	Atrophic epithelium Decreased vascularity Grade II OSF	Normal epithelium and vascularity Grade II OSF
18	Normal epithelium Increased vascularity Grade II OSF	Atrophic epithelium Normal vascularity Grade II OSF	Atrophic epithelium Normal vascularity Grade II OSF	Atrophic epithelium Normal vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade II OSF	Atrophic epithelium Decreased vascularity Grade II OSF
24	Normal epithelium and vascularity Grade II OSF	Atrophic epithelium Decreased vascularity Grade III OSF	Atrophic epithelium Normal vascularity Grade II OSF	Atrophic epithelium Normal vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade II OSF	Atrophic epithelium Normal vascularity Grade III OSF
30	Atrophic epithelium Decreased vascularity Grade II OSF	Atrophic epithelium Decreased vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade III OSF	Atrophic epithelium Minimal vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade III OSF
36	Atrophic epithelium Decreased vascularity Grade II OSF	Atrophic epithelium Minimal vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade III OSF	Atrophic epithelium Minimal vascularity Grade III OSF	Atrophic epithelium Minimal vascularity Grade III OSF	Atrophic epithelium Decreased vascularity Grade III OSF

OSF: Oral submucous fibrosis

Salivary gland changes

Tissues in the initial weeks were characterized by a normal architecture and structure of the glands. The 18th weeks tissue showed initial signs of vacuolar degeneration in granular ducts and acini. Progressive degenerative changes were obtained in the subsequent weeks with the complete loss of architecture toward the 30th and 36th weeks [Figure 4].

Buccal mucosal changes

The rat buccal mucosal tissue showed a slight decrease in the keratin thickness epithelium thickness and vascularity with a mild increase in depth of fibrosis was noted in the 6th week. In the following weeks, keratin thickness, epithelial thickness, and vascularity were found to decrease with increase in the duration of exposure to the areca nut. The minimum values for keratin thickness were found at 36th week compared to other groups. The most atrophic epithelium with decreased vascularity and maximum value for depth of fibrosis was seen at 36th week compared to other weeks [Table 2].

Pan masala treated group

Salivary gland changes

An initial reaction (6th and 12th week) to pan masala solutions was characterized by an increased pooling of secretions in side the ducts. The normal glandular architecture was however maintained. This reaction was followed by progressive dilatation of the acinar and the ductal structures in the 18th and 24th week with a slight distortion of the architecture. Degenerative changes of the acini and ducts were seen at the 30th and 36th week tissues [Figure 5].

Buccal mucosal changes

At 6th week rat, buccal mucosa showed dramatic changes with decreased keratin thickness, atrophic epithelium, increase in the depth of fibrosis, and severe reduction in the vascularity. There was an increase in depth of fibrosis, decreased keratin and epithelium thickness, and severe reduction in vascularity over a period of time from 12th to 36th week [Table 2].

Pure arecoline treated group

Salivary gland changes

An initial damaging effect of pure arecoline solution characterized by inflammation and fragmentation of ducts was seen in the 6th week tissue. This was followed by a hyperplastic reaction of the tissue showing fusion and dilatation of ducts in the 18th and 24th week. 30th and the 36th week were characterized by progressive degenerative changes in the acini and ducts with the loss of normal architecture [Figure 6].

Buccal mucosal changes

The rat buccal mucosa showed variations in the keratin thickness, epithelial thickness, depth of fibrosis, and vascularity from 6th to 36th week. Although this group showed an irregular trend with relation to keratin thickness, epithelial thickness, and vascularity, a significant decrease was noted in these histological parameters was noted from 6th to 36th week.

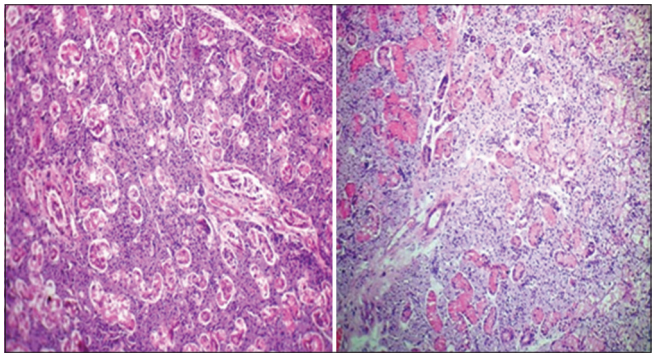

Figure 5: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the pan masala group at 6 weeks (L) showing normal architecture with increased pooling of secretions in ductal spaces and at 36 weeks (R) with edematous and degenerative changes in the acini and ducts with loss of normal architecture (H and E, original magnification ×10)

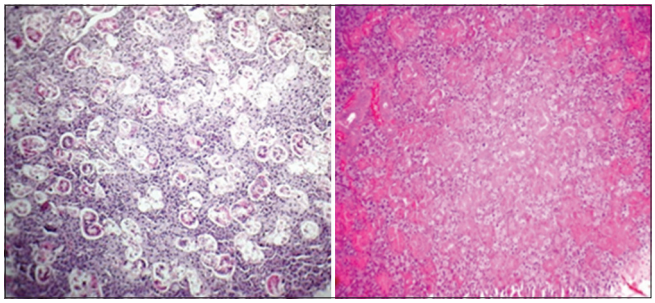

Figure 6: Histopathological images of Sprague-Dawley rat submandibular salivary gland in the pure arecoline group at 6 weeks (L) showing slight degenerative changes in the ductal cells with a poorly preserved architecture, inflammation, and congested vessels and at 36 weeks (R) with edematous and degenerative changes in the acini and ducts with loss of normal architecture and congestion of vessels (H and E, original magnification ×10)

The depth of fibrosis was consistently increased from 6th to 36th weeks with a maximum depth of fibrosis noted in the 36th week [Table 2].

DISCUSSION

The habit of chewing areca nut is a habit of great antiquity and presently impinges on the daily lives of about one-tenth of the world's population. It is the 4th most common addictive psychoactive substance consumed after caffeine, nicotine, and alcohol. The addictive and anxiolytic properties of the nut are attributable to the inhibition of GABA uptake in the nervous system by the areca alkaloids.[1,2]

Areca nut is the seed of the fruit of the oriental palm Areca catechu. The plant is native to India, Malaysia, Polynesia, Micronesia, and most places in the South Pacific Islands.[3] The current production of areca nut in the world is about 0.613 million tons from an area of 0.476 million hectares. India ranks first in both area (58%) and production (53%) of areca nut.[2,16]

Areca nut is perused in many forms in the region ranging from raw to commercial varieties. Pan masala is a popular dispensation among the commercial products. It is a blend of areca nut powder with specified and unspecified additives. When combined with tobacco, it is called gutka. Marketed aggressively and packaged attractively, the pricing made affordable, its popularity among the youth has led to its widespread use. Processed forms of areca nut are also a common form of consumption of the nut and could include sun-drying, boiling, roasting, baking, and soaking in water of the nuts. These treatments change the chemical composition, astringency, and the flavor of the nut.[3]

Areca nut extract contains alkaloids such as arecoline, arecaidine, guvacoline, and guvacine; flavanoids such as tannins and catechins and traces of copper. Arecoline and arecaidine alkaloids in the nut are thought to play a major role in the development of adverse effects resulting from this chewing habit. The roasted variety of nut possesses the highest tannin content (5-41%) followed by the raw (25%) and the boiled areca nut (17%). The arecoline content is highest for the sun-dried raw variety (1.35%) followed by the roasted (1.29%) and the boiled variety of areca nut (0.1%).[3]

The second IARC Monograph on betel quid labels areca nut as a "group one carcinogen." Its genotoxic and mutagenic effects being attributed to its content of polyphenols, alkaloids, nitrosamines such as N-nitrosoguvacoline, 3-(methylnitrosamino) propionitrile, and reactive oxygen species.[3]

The consumption of areca nut has deleterious effects on almost all organs of the human body; including the oral

cavity, pharynx, esophagus, brain, heart, liver, lungs, and reproductive organs with the salivary glands being no exception to this gospel truth.[4-6] There are reports stating that there exists an increased incidence of parotid tumors in response to chronic exposure of high doses of areca nut.[8,9] Salivary flow rates and pH of saliva are altered in areca nut chewers, rendering the oral mucosa vulnerable to the toxic effects of the nut. The raw form of areca nut was found to induce the highest increase in the salivary flow rate as compared to other forms of areca nut.[7]

Furthermore, previous experimental studies also affirm the fact that arecoline has para-sympathomimetic properties leading to an increased salivation from the salivary glands. The para-sympathetic impulses cause an increased fluid secretion along with contraction of myoepithelial cells leading to vasodilatation with varying degree of expulsion from the acinar cells.[7] On the contrary, single study reports reduced salivary outflow with continuous usage of areca nut attributed to fibrosis around the Stenson's duct opening leading to its blockage and hence a decreased salivary output. Chronic obstruction in its most severe form leads to parotid gland atrophy.[17]

The study resorted to using SD rats as the experimental study model because of the difficulty encountered in getting human salivary glandular tissue specimens. Furthermore, time and bound it has been proved that SD rats are a sustainable and reproducible model for studies involving areca nut and OSF.[18,19] This fact is further shored up by previous work in our own laboratory. Moreover, these animals are easy to handle, inexpensive, and universally available at all animal laboratories.

The submandibular glands were used for the study, as these glands are responsible for the major contribution toward the daily output of salivary secretion. The rat submandibular salivary glands are almost similar in histology to the human glands except for a few structural differences. These differences include the lack of mucous acini, demilunes unlike in human submandibular salivary glands who have both the mucous and serous acini and demilunes with the acini being slightly larger in size in comparison to the rats. An additional structure called as the "granular duct (granular convoluted tubule)" is located between the intercalated duct and the striated duct in the rat submandibular gland and is often mistaken for mucous acini. This duct wall is composed of a simple columnar epithelial lining containing many secretory granules in its supra-nuclear cytoplasm with a few 'pillar cells- narrow lumen and wide base' sandwiched between principal columnar cells. These ducts are responsible for the mucus component of the saliva. A sexual dimorphism with the granular ducts exists; the ducts being less well-developed in the female rats. The other ductal system is similar for the humans and the rats.[10,11]

A notable cause-effect correlation is reported between consumption of areca nut and development of OSF.[12-14] OSF is a chronic insidious disease affecting any part of the oral cavity, sometimes the pharynx and is characterized by subepithelial and submucosal myofibrosis leading to stiffness of the oral mucosa with progressive limitation in the opening of the mouth.[20] An increased salivary rate is reported in the early stages of OSF. On the contrary, the advanced stages of OSF are characterized by an increased dryness of the oral mucosa.[15]

There have been no reports on the histological assessment of effects of areca nut and its derivatives on the salivary glands to the best of our knowledge and hence formed the basis of our study. The study had an added advantage of evaluation of the salivary gland histology over a period of time as a continuum in response to these toxic deleterious agents.

When a comparative evaluation of the effects of the various areca nut solutions on the buccal mucosa and salivary glands was made interesting parallels were discovered. The control groups showed no changes in the submandibular gland while fibrotic reactions in the later stages are consistent with the reaction of the tissue to continued irritation of the mucosa. All the groups where areca nut preparations were injected showed severe changes in the glands at the end of the study period and concomitant Grade III fibrosis. This is significant and indicates continuous increased assimilation of the areca nut that probably utilizes vascular channels and enters the glands. The parallel changes also indicate an almost simultaneous action of the areca nut on the buccal mucosa and the glands [Figures 7-9 and Table 3].

The results obtained in our study evidently showed fusion

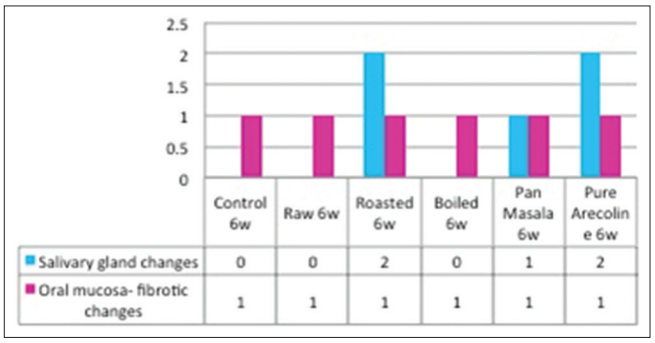

Figure 7: Comparative evaluation of the changes in the buccal mucosa and salivary glands at 6 weeks

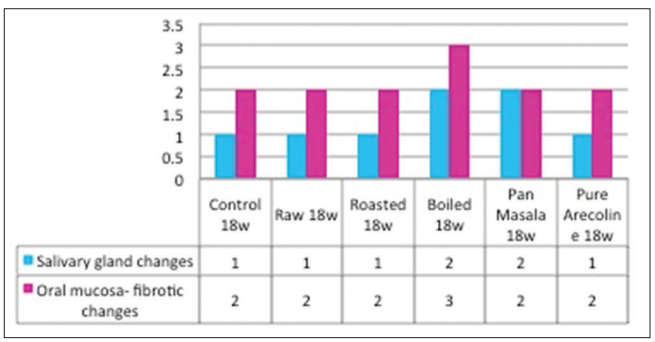

Figure 8: Comparative evaluation of the changes in the buccal mucosa and salivary glands at 18 weeks

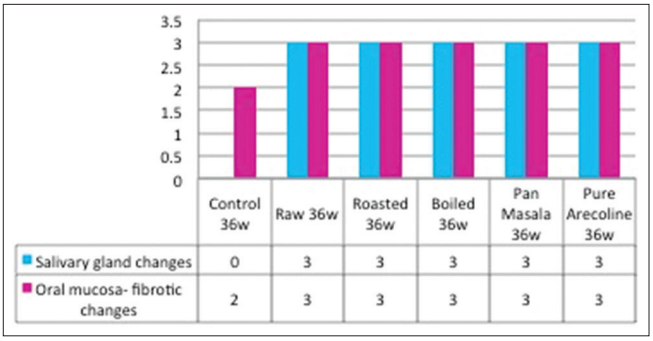

Figure 9: Comparative evaluation of the changes in the buccal mucosa and salivary glands at 36 weeks

and dilatation of acini and ducts with pooling of the salivary secretions in the initial weeks of treatment with various forms of areca nut. Later weeks were well characterized by degenerative changes in the glandular structure. These degenerative changes were first seen in the 30th week of treatment for all the groups except for the pure arecoline and the boiled areca nut group that showed degenerative changes as early as the 6th and the 18th week progressing further till the 36th week. These histological changes coincide well with the physiology, i.e., increased salivary output in initial weeks associated with fusion and dilatation of acini and ducts and a decreased output in the later weeks associated with the destruction/degeneration of the glandular structure.

Among all the groups, the pure arecoline treated group showed significant and early degenerative changes in comparison to the other groups. This is consistent with the fact that arecoline is the major alkaloid responsible for the deleterious effects of the nut on the tissues; the tissues here being the submandibular salivary gland.

The average life span of humans and rats is about 70 years and 3 years, i.e., equivalent to 3640 weeks and 156 weeks. On calculating the ratio of the life spans of SD rats and humans a value of 1:23 is obtained, i.e., 1 week of SD rats corresponds to 138 weeks in humans. The degenerative changes in the submandibular glands were seen beginning in the 30th week with a gradual progression till the 36th week. The 30 weeks of treatment of rat with areca nut equates to 690 weeks (14 years) of areca nut consumption in humans. On extrapolation of this data obtained from our animal study to humans; it can be said that chronic exposure beyond periods approximately 14 years, the salivary glands show degenerative changes amounting to decreased salivary output. This decreased output would correlate with the dryness of oral mucosa seen in the advanced stages of OSF.

A decreased salivary output associated with chronic usage of areca nut would, in addition, lead to decreased protective action of the saliva in the oral cavity, i.e. decreased buffering action and decreased immunoglobulins in saliva. This could be correlated to the increased rate of malignant transformation seen in OSF in the advanced stages. Furthermore, associated

Table 3: Comparative evaluation of the histological changes in the salivary gland and the oral mucosa

Group (weeks)	Salivary gland changes	Oral mucosa- OSF grading (Pindborg Sirsat- 1966)
Control		
6	Normal	Grade I
12	Normal	Grade I
18	Mild	Grade II
24	Normal	Grade II
30	Normal	Grade II
36	Normal	Grade II
Raw areca nut		
6	Normal	Grade I
12	Normal	Grade I
18	Mild	Grade II
24	Mild	Grade III
30	Moderate	Grade III
36	Severe	Grade III
Roasted areca nut		
6	Moderate	Grade I
12	Mild	Grade II
18	Mild	Grade II
24	Mild	Grade II
30	Moderate	Grade III
36	Severe	Grade III
Boiled areca nut		
6	Normal	Grade I
12	Normal	Grade I
18	Moderate	Grade III
24	Moderate	Grade III
30	Moderate	Grade III
36	Severe	Grade III
Pan masala		
6	Mild	Grade I
12	Mild	Grade II
18	Moderate	Grade II
24	Moderate	Grade II
30	Moderate	Grade III
36	Severe	Grade III
Pure arecoline		
6	Moderate	Grade I
12	Normal	Grade II
18	Mild	Grade II
24	Mild	Grade III
30	Moderate	Grade III
36	Severe	Grade III

OSF: Oral submucous fibrosis

with decreased salivation and chronic areca nut usage would be an alteration in the normal microbial oral flora leading to a reduced level of the commensals that would, in turn, make the oral mucosa more susceptible to increased infections and other harmful diseases.

CONCLUSION

Chronic consumption of areca nut and its various forms leads to deleterious effects on the salivary gland histology leading to degeneration of acini and ductal structures. This degenerative effect leads to an overall decreased salivary output reflecting as dryness of mucosa in the advanced stages of OSF. Furthermore, the reduced salivary secretions would lead to reduced levels of immunoglobulins leading to decreased local immunity and hence an increased propensity for malignant transformation in the later stages of OSF.

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