

ESTIMATION OF ARECOLINE CONTENTS IN COMMERCIAL ARECA (BETEL) NUTS AND ITS RELATION TO ORAL PRECANCEROUS LESIONS

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SYNOPSIS

Areca catechu (betel) nut is widely used as a chewing agent. The nut alkaloids have been implicated in the pathogenesis of oral precancerous lesions. Quantitative analysis of the chloroform extracts by gas-liquid chromatography of ten commercial nut samples from Bombay have shown a wide variations in their arecoline contents (0% — 1.4%; mean: 0.7%). Nut samples of identical processing method also vary in their arecoline levels. These variations were suggested to be due to the difference in the raw materials and processing methods. Comparisons were made between the arecoline contents and the incidence of oral precancerous lesions from the present studies and also from those of Kerala and Mysore. It was concluded that the difference in nut arecoline contents not only reflect their appeal, potency but also influence upon the incidence of these diseases.

INTRODUCTION

Areca catechu or commonly known as betel nut is widely chewed in India and South East Asia. Betel nuts are chewed as such or after being treated by roasting, boiling or soaking in water. Chewers consume betel nuts either alone or in combination with one or more of these agents such as betel leaves (piper betel vine), tobacco, gambir, slaked lime, spices, coconut and flavouring agents. This combination is collectively known as 'pan' (1). Chewing of betel nut provides a sense of well being and self satisfaction which is equivalent to that of cigarette smoking but costs less.

The chemical compositions of betel nut have been reported in the literature since 18th century (2, 3, 4). Although a considerable number of chemical constituents are present in the betel nut, only the pyridine alkaloids and polyphenols have received particular attention; as these substances have significance clinical implications. Arecoline forms the major alkaloid and arecaidine, guvacine, guvacoline and arecolidine constitute the minor alkaloids in the betel nut.

Clinical and laboratory studies have clearly shown the adverse effects of betel nut consumptions. Oral precancerous lesions in particular oral submucous fibrosis (OSF) and leukoplakia have been reported in association with betel chewing habits (6, 7, 8, 9, 10, 11, 12, 13). Various animal studies on betel nut carcinogenesis have also been documented (14, 15, 16, 17, 18, 19, 20, 21). Neither of the earlier reports on the chemical compositions of betel nut stressed on the specific alkaloid such as arecoline; nor compared the betel nut chemical constituents in nuts from different locations where the incidence of oral precancerous conditions were being intensively studied.

This article present the investigations that have been carried out to look into these aspects. The relation between the oral precancerous conditions and the betel alkaloids are also discussed.

MATERIALS AND METHODS

Alkali extraction was suggested to be an efficient method of extracting arecoline from the betel nut (22). Alkali extracts were prepared from ten different varieties of commercial betel nuts which were originated from Bombay, India. Four samples consisted of boiled nut, three samples were roasted nut and three samples were sundried variety. Each sample was dried in oven at 50 C for 2 days prior to handground to a fine powder.

Five grams of each powder was moistened with concentrated ammonia and mixed with 50ml of chloroform in a sonification bath for 2 hours. After filtration, the filtrate was shaken with 2% sulphuric acid and the layer was retained. The arecoline salts in the acid layer were purified from lipid impurities by repeatedly shaken with petroleum ether and discarded the ether layer. The acid layer was made basic (pH 10) by adding a few drops of concentrated ammonia and extracted again with chloroform three times to ensure complete extraction of arecoline from the acid. The chloroform layer was retained and inorganic ions impurities were then removed by adding a little water. Anhydrous sodium sulphate was added to remove the water and filtered. The filtrate was collected in a known weight sample tube and then evaporated to dryness by vacuum distillation. The weight difference of the tube before and after evaporation determined the yield of the arecoline extracts.

The arecoline estimation was carried out by gas-liquid chromatographic separation method (22). For this report, the F-17 Perkim-Elmer model using 10% Carbowax 20M on chromosorb 80-100 mesh as the

liquid phase and nitrogen as the gaseous phase; was utilised. The oven temperature was set to 210 C.

The arecoline residue was diluted to 5ml. of methanol. Then 50 μ L of this solution was added to 200 μ L of methanol containing an internal standard (0.5 mg/ml. of quinoline and 1% potassium hydroxide). Using a Hamilton syringe 1 μ L of the resulting solution was injected into column B of the machine. After 20 minutes, the arecoline peak was noted on the recording chart which moved at a speed of 10 mm/min and followed by the peak of the internal standard. Then the second injection was applied so as to ensure that the peak patterns of both injections were identical. The area ratios of both the arecoline and internal standard peaks were calculated from the chart. By injecting different known concentrations of arecoline (arecoline bromide from Sigma Co.) and internal standard, a calibration curve was constructed. The value of the gradient of the curve was measured and the concentration of the arecoline in the injected sample was derived as follows:

$$\text{Conc. of arecoline} = K \frac{\text{Area peak X concentration (arecoline)}}{\text{peak area of internal standard}}$$

The value was then expressed as a percentage dry weight of the nut powder. For each sample the extraction and the estimation of the arecoline contents was carried out three times from which the average value was determined.

RESULTS

Ten samples of the commercial betel nuts showed a wide variations in their arecoline contents (0%-1.4%; mean: 0.7%). Nuts having undergone identical commercial processing methods also differed from each other in relation of their arecoline contents. The sundried nut samples contained arecoline from 0% to 1.4% (mean: 0.5%); nuts from boiled varieties varied from 0.4% to 1.3% (mean: 0.8%) and those roasted samples contained arecoline from 0.4% to 1.3% (mean: 0.9%), figure 1.

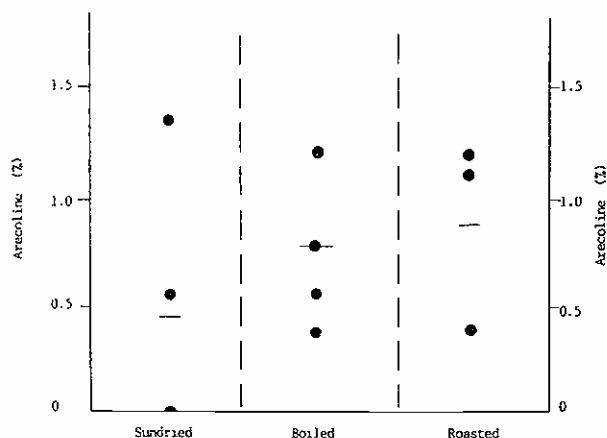


Figure 1: Arecoline contents of Commercial Areca Nuts.

DISCUSSION

It appears that commercial areca nuts not only vary in terms of their morphological characteristics but also differ in terms of their arecoline contents. These variations were also reported in nuts from Kerala and Mysore (5). However, the present study showed relatively higher contents of arecoline in sample from Bombay. The variation in the arecoline contents in the commercial betel nuts may be due to the variations in the raw materials and the methods of processing the nuts.

The addictive nature of areca nut has been suggested to be due to the inhibition of GABA uptake in central nervous system by the areca alkaloids, arecaidine and guvacine (23). Slaked lime which is commonly included in the betel nut quid is able to hydrolyse arecoline to arecaidine. Thus the incorporation of slaked lime in the quid inevitably potentiates this effect with nuts from the present study.

The adverse effects of the betel nut alkaloids on oral tissue have been constantly reported in the literature (14, 16, 18, 19, 20). Many epidemiological studies have showed a constant association of betel chewing with oral submucous fibrosis (8, 13, 24, 25). The excess fibrous tissue, which is the characteristic of this disease was suggested to be due to the stimulation of collagen synthesis by the arecoline and arecaidine (6, 26). Comparing the incidence of oral submucous fibrosis in Bombay (0.5%) and those of Kerala (0.1%) and Mysore (0.2%) correlates with the contents of arecoline in nuts from these locations (5, 11, 24, 27). Although incidence of oral submucous fibrosis in other parts of the world were also being documented, the above comparison could not be made due to the lack of reports on the arecoline contents in nuts from these regions. Further investigations would be required with well controlled variable factors such as intensity of chewing. At present it may be suggested that the difference in the arecoline contents may influence the incidence of this disease.

The trend is again observed in relation to the incidence of oral leukoplakia. This disease was also reported to be associated with the betel chewing habits (9, 28, 29). The incidence of oral leukoplakia in Bombay was reported as 2.8% whereas those of Kerala and Mysore where nut arecoline levels were lower were reported as 2.4% and 1.6% respectively (1, 30).

CONCLUSION

Areca Catechu (Betel nut) has been implicated in the pathogenesis of oral precancerous lesions such as oral submucous fibrosis and leukoplakia. The role of the betel nut alkaloids, the arecoline and arecaidine in relation to these diseases was discussed. The analysis of ten commercial betel nut samples from Bombay, India have shown wide variations and higher average levels of arecoline contents than those reported from Kerala and Mysore. The variations in arecoline contents of betel nuts were suggested to be due to the differences in the raw materials and the processing techniques. Incidence of oral submucous fibrosis and leukoplakia in Bombay, Kerala and Mysore were compared with their respective arecoline levels in the betel nuts. It was concluded that the variations in the arecoline levels in the betel nuts influence the incidence of these diseases. These variations may not only reflect the potency of the nuts but also the appeal to the chewers.

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