Review

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Areca Nut Use and Cancer in India

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Areca nut is widely used in India and the consumption has increased over the past two decades, with availability in new dry packaged forms (*pan masala, gutka, mawa*). Recent reports of increasing mouth cancer incidence have suggested an association with areca nut consumption. Here we have reviewed the evidence for carcinogenicity of areca nut, including epidemiological studies, several animal studies and mechanistic evidence. Studies primarily from India, providing odds ratios (ORs) or relative risks for precancers or cancer with use of areca nut without inclusion of tobacco is the focus of the review. Six case-control studies on oral submucous fibrosis (OSF) had significantly elevated ORs for use of areca nut in various forms. Six case-control studies on head and neck cancers, primarily oral cancer reported elevated ORs for chewing of betel quid without tobacco. Eight case control studies on oral cancer have reported elevated and significant ORs for betel quid with tobacco. A significant risk in oral cancer was noted in *gutka* users. Animal studies confirmed correlation between development of precancers or cancers and exposure to areca nut or *pan masala* without tobacco. Mechanistic evidence shows a role for areca nut alkaloids, polyphenols and copper in promoting carcinogenesis. Our review emphasizes control policies on areca nut products and appropriate mass communication programs for awareness of hazards of areca nut with emphasis on areca nut *per se*.

INTRODUCTION

The areca nut, fruit of the oriental palm (*Areca catechu*), also called 'betel' nut in English, *supar*i in Hindi, *adike* or *betta* in Kannada, *adakka* in Malayalam, and *pakku* in Tamil, is commonly used in India (FRLHT.org, 2015) and needs no introduction. It is used in traditional quids (*beeda*) wrapped in betel leaves (*Piper betle*) or as tobacco and areca nut mixtures. Areca nut is also used *per se* and available in specialised shops and by roadside vendors, in sachets, as *pan masala* and *gutka*. A product containing areca nut chips, slaked lime and tobacco, popularised in Gujarat, is called *mawa* (Gupta, 1998), also sold in Maharashtra as *kharra* (Hazare *et al.,* 1998). *Mainpuri* tobacco containing similar ingredients is

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consumed in Uttar Pradesh, since the 1960s (Wahi, 1968). These commercial developments resulted in doubling areca nut consumption in India during 1991 to 2010 from 2.5 to 5.2 lakh tons, with about 5% increase each year (Kammardi *et al.,*2012).

A recent report of the National Cancer Registry Programme (ICMR) showed an increasing incidence of cancer of the buccal mucosa ('mouth cancer') for six to ten years up to 2009 or 2010 in five of the nine population-based cancer registries (NCDIR-NCRP, 2013), reiterated by a similar trend in a single registry (Gupta *et al.,* 2014). The cancer registries located in Bhopal, Mumbai, Delhi, Dibrugarh and Ahmedabad rural and urban, in the states or territory of Madhya Pradesh, Maharashtra, Delhi Union Territory, Assam and Gujarat, respectively have high prevalence of high areca nut use (IIPS & MOHFW, 2010). In addition to the increased incidence, patients of oral cancer are younger than 35 years of age since the mid-1990s as compared to the mid-1980s (Gupta, 1999).

Betel quid has been linked with head and neck cancers including oral cancers since the last century, although at that time tobacco and lime in the betel quid were viewed as the likely causes of the associated cancer (IARC, 2004; Orr, 1933). In the past decade, oral cancer has been diagnosed with increasing frequency in young users (< 35 years) of packaged areca nut products, bringing the potential carcinogenicity of areca nut into focus (Chaudhry, 1999; Gupta, 1999). An extensive review and evaluation of evidence was undertaken on areca nut and betel quid by the International Agency for Research on Cancer (IARC) reported in 2004. The evidence for carcinogenicity of areca nut, primarily from India and South Asia was from use of betel quid with tobacco. Relatively few epidemiological studies on precancers or cancer, in the past twenty years reported on cancer risks associated with use of betel quid without tobacco or use of industrially manufactured areca nut products. Nevertheless these few studies, along with laboratory evidence, made it possible for the monograph to conclude that areca nut by itself is carcinogenic to humans. The final evaluation by IARC concluded that betel quid without tobacco causes cancer of the oral cavity, and betel quid with tobacco causes cancer in the oral cavity, pharynx and esophagus; and emphasized that areca nut is carcinogenic to humans

(IARC, 2004). In the most recent monograph (Vol. 100E) several additional studies were reviewed and the evidence confirmed carcinogenicity of areca nut in humans and animals. However, the message has apparently not reached the masses, perhaps ignored or discounted, in view of the overwhelming evidence of carcinogenicity of tobacco.

Thus, in view of increasing consumption of areca nut products in India and reports of increasing oral cancer incidence over the past ten years, a review of currently available evidence of the carcinogenicity of areca nut was undertaken. An initial literature survey of the use of areca nut products in India, followed by epidemiological and laboratory evidence for the role of areca nut in causing oral cancer and other head and neck precancers, and an outline of the mechanisms of cancer causation are reviewed.

MATERIALS AND METHODS

Literature on carcinogenicity of areca nut and its products as used in India (areca nut, betel quid or *paan*, *gutka*, *pan masala, mawa*) was surveyed. Since use of areca nut without tobacco has been rare, earlier epidemiological studies have generally not reported separate risks for areca nut. However, since tobacco is widely recognised as carcinogenic, and areca nut has not been associated with cancer we focused on case-control studies that reported ORs for use of areca nut without tobacco. Epidemiological studies reporting on oral precancers or oral and pharyngeal cancers are included. The evaluation monographs of the IARC, volumes 85 (2004) entitled, "Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines" (2004), and 100E (2009) on *"*Betel quid and areca nut*"* were used as the basic resources, along with internet searches in Pubmed for case control studies, cohort studies, animal experiments and mechanistic studies. The more recent studies are emphasized, with a few highly informative earlier studies included. Certain studies on oral submucous fibrosis (OSF) not reviewed in the IARC Monographs are emphasized (Bathi *et al*. 2009; Mehrotra *et al*., 2013). Research conducted in India is prioritized, and additional studies in other parts of the world cited to provide evidence of areca nut as an important carcinogen globally are included. In addition, basic prevalence data on use of areca nut products were obtained from the Global Adult Tobacco

Survey for India (GATS) (International Institute for Population Sciences and Ministry of Health and Family Welfare, Government of India, 2010).

RESULTS

Prevalence of Areca nut Use in India

The report of the GATS for India showed betel quid with tobacco was used by 7.5% men and 4.9% women, and mixtures of areca nut and tobacco, without betel leaf (*gutka* and *mawa*) used by 13.1% men and 2.9% women. The report did not provide data for use of areca nut without tobacco (Table 1). The data showed that use of pre-packaged imperishable forms of areca nut have superseded the popularity of betel quid.

In rural areas, the prevalence of betel quid with tobacco was higher in urban areas (6.8% rural vs. 4.8% urban), prevalence of *gutka* and similar products in rural areas was higher than in urban areas (8.6% rural vs. 7.1% urban). The regions with high prevalence of use of areca nut products in India were the

Table 1: Prevalence of use of products containing areca nut and tobacco among persons ≥ 15 years in India, from the GATS (International Institute for Population Sciences and Ministry of Health and Family Welfare, Government of India, 2010).

Substances used	*All current users* N %	Males N %	Females N %
Gutka & other areca nut mixtures with tobacco and lime	*65,072,000* 8.2	13.1	2.9
	Range: 2.8–12.1 North to Central	Range: 4.9–18.4 North to Central	Range: 0.2–5.0 North to Central
Betel quid with tobacco:	*49,672,000* 6.2	7.5	4.9
	Range: 0.7–17.2 North to Northeast	Range: 1.1–18.9 North to Northeast	Range:0.3–15.6 North to Northeast
Pan masala or betel quid without tobacco; and/ or use of nasal snuff	*35,106,000* 4.4	3.5	5.4
	Range: 0.8–10.9 North to East	Range: 1.0–10.5 North to Northeast	Range: 2.3–15.0 West to East

Note: Total prevalence could not be calculated from these values as the categories are not mutually exclusive.
Key to regions:
North-East: Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya and Assam
East: West Bengal, Jharkhand, Odisha and Bihar;
Central: Rajasthan, Uttar Pradesh, Chhattisgarh and Madhya Pradesh;
North: Haryana and northwards including Jammu & Kashmir, Himachal Pradesh, Punjab, Chandigarh, Uttarakhand and Delhi.

Northeast (Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya and Assam), the East (West Bengal, Jharkhand, Odisha and Bihar) and the Central region (Rajasthan, Uttar Pradesh, Chhattisgarh and Madhya Pradesh). Low prevalence was found in the North (Haryana and northwards including Jammu & Kashmir, Himachal Pradesh, Punjab, Chandigarh, Uttarkhand and Delhi). In particular, prevalence of betel quid with tobacco was high in the Northeast (17.2%) and East (9.7%), and lowest in the North (5.5%). On the other hand, prevalence of *gutka* and similar mixtures was high in the Central states (12.1%). Among men, *gutka* use was concentrated among the 15 to 44 year age group, whereas women users tended to be older. Betel quid with tobacco was used mainly among the 45–65 year age groups in both men and women (International Institute for Population Sciences and Ministry of Health and Family Welfare, Government of India, 2010).

Occasional consumption of areca nut without betel leaf, lime and condiments has been a norm and a common culturally accepted practice in India (Reddy and Gupta, 2004). Areca nut consumption without tobacco and by itself has been occasionally reported as practiced by a small fraction of the population before the 1980s (Mehta *et al.,* 1972). In the last 10–15 years areca nut habits have been observed in children (Chaturvedi *et al.,* 2002; Khandelwal *et al.,* 2012).

Evidence of Carcinogenicity in Humans

In India, a quid containing areca nut is chewed and kept next to the cheek (buccal) mucosa, for hours including overnight. Blanching often appears at the site as an early sign of OSF and squamous cell carcinoma may develop. Various case-control studies on precancers and cancers associated with areca nut use are summarised in the following section.

Oral Precancers

Six case control studies on OSF, five from India in the states of Bihar, Gujarat, Kerala, Karnataka and Uttar Pradesh (Ahmad *et al.,* 2006; Bathi *et al.,* 2009; Jacob *et al*., 2004; Mehrotra *et al.,* 2013; Sinor *et al.,* 1990) and one from Sindh in Pakistan (Maher *et al.,* 1994), showed significantly elevated ORs for OSF associated with areca nut use without tobacco in various forms (Table 2). ORs for OSF for betel quid without tobacco (BQ) ranged from 1.3, the lowest, which

Table 2. Oral submucous fibrosis and use of areca nut (AN) in various forms, in case control studies conducted India and Pakistan (both men and women).

Location	Chewing status	OSF Cases	Controls	OR (CI) OSF	Study type. Matching, Adjustments, Stratifications	References Notes
Bhavnagar, Gujarat, India	Non Chewer (currently)[a]	1	39	1.0 (ref.)	Dental clinic based. Age ≥15 yrs. Matched on age, sex, religion, and occupation.	(Sinor *et al.,* 1990) Significant dose response for frequency & duration
	BQ or AN (no tobacco)	4	2	78.0*		
Karachi, Pakistan	None (includes ex-chewers > 6 m)	2	82	1 (ref.)	Dental clinic based. Matched on sex and age.	(Maher *et al,* 1994) Significant dose response for frequency & duration
	BQ (no tobacco)	7	9	32 (6–177)**		
	AN alone	64	17	154 (34–693)**		
Kerala (rural), India	No chewing (currently)	9	31884	1.0 (ref.)	Population based. Age >35 yrs Adjusted for age, sex, education, smoking and alcohol drinking.	(Jacob *et al.,* 2004) Significant dose response for frequency
	BQ (no tobacco)	15	1100	47.2 (20.2–110.4)		
	Areca nut only	0	12	-		
Patna, Bihar, India	No areca nut product use	5	108	1.0 (ref.)	Dental clinic based. Matched on age, sex, religion & socio-economic status. ORs calculated by review authors	(Ahmad *et al.,* 2006)
	BQ not specified	25	13	41.5 (13.5–127.2)		
	AN alone	8	1	172.8 (18.0–1662.6)		
	Pan masala	32	5	138.2 (37.6–507.7)		
Dharwad, Karnataka, India	No habit (currently)	1	119	1.0 (ref.)	Hospital based. Matched on age, sex, and socio-economic status.	(Bathi *et al.,*2009) Dose response calculation included diverse product users, not informative
	Pan masala/BQ/areca nut	2	4	59.5 (3.1–2154)		
	BQ (no tobacco)	23	43	63.6 (8.7–1304) Confidence intervals recalculated by review authors		
Lucknow (urban, rural), Uttar Pradesh, India	Non users (currently)	NA	NA	(ref.)	Subjects from urban and rural health camps Matched on age, and socio-econ. status. Males: (89.1% cases); (82% controls).	(Mehrotra, *et al.,* 2013) Dose response seen for frequency per day for each product
	Tobaccoless products					
	BQ			1.3 (0.95–2.7)		
	Pan masala			3.0 (1.2–7.4)		
	Both			6.4 (4.3–9.5)		
	Total visitors to camps	448	2688			

CI= Confidence intervals; AN=areca nut; BQ=Betel quid without tobacco; BQT= Betel quid with tobacco.

[a]Occasional chewer of AN

*$P < 0.01$, **$P < 0.0001$

did not reach significance (Mehrotra *et al.,* 2013) to 78.0 (Sinor *et al.,* 1990). In contrast, ORs for BQ with tobacco ranged from 7.9 (Mehrotra *et al.,* 2013) to 64 (Maher *et al.,* 1994). Use of areca nut alone reported in two studies had ORs of 154 (Maher *et al*., 1994) and 172 (Ahmad *et al*., 2006). ORs exclusively for tobacco-less *pan masala* use in two studies were 3.0 (Mehrotra *et al*., 2013) and 138.2 (Ahmad *et al*., 2006).

Ors tended to be higher for users of mixtures made with areca nut and tobacco but without betel leaf, such as *mawa* (106.4) (Sinor *et al.,* 1990), or *gutka* (from 10.8 to 1142) (Bathi *et al.,* 2009; Mehrotra, *et al.,* 2013). Additionally, a cross-sectional house-to-house study showed an OR for men with OSF as 75.6 for *mawa* chewing in 11,262 men in Bhavnagar District of Gujarat (Gupta *et al*., 1998).

Studies were not adjusted for smoking, with one exception that also studied leukoplakia (Jacob *et al*., 2004). However, the report of the earliest study stated the rate of smoking in cases and controls was similar and also that smoking did not appear play a role in the development of OSF (Sinor *et al*., 1993). Another study performed multiple logistic regression on smoking and OSF and reported negligible effect of smoking (Bathi *et al*., 2009). Few smokers were found among chewers in a study and they were excluded from calculation of ORs (Maher *et al.,* 1994). Mehrotra *et al* (2013) concluded that tobacco smoking did not affect risk of OSF, whereas alcohol consumption increased the risk in chewers of tobacco-less betel quid or *pan masala* several fold.

A dose response was clearly seen for frequency per day of using areca nut preparations in four of the studies (Jacob *et al.,* 2004; Maher *et al.,* 1994; Mehrotra *et al.,* 2013; Sinor *et al.,* 1990). A clear dose response was also clearly seen for duration (Jacob *et al*., 2004; Maher *et al*., 1994; Sinor, *et al.*, 1990).

Two case control studies, one with betel quid and *pan masala* (Shah and Sharma, 1998) and the other with *pan masala, kharra,* tobacco-lime and betel quid in different combinations (Hazare *et al.,* 1998), reported significant increasing trends for frequency of use of areca nut containing substances per day ($p < 0.01$), although overall ORs for OSF was not reported. An increasing prevalence of OSF was observed between 2000 and 2004 with 77.8% of OSF patients using multiple areca nut products (Hazare *et al.,* 2007).

Table 3. Selected precancers and use of areca nut without tobacco in Kerala, India (Both men and women).

Location	Chewing status	Cases	Controls	OR (CI)	Study type. Matching, Adjustments, & Stratifications	Reference
Kerala				Leukoplakia:	Population based.	(Jacob *et al.,* 2004)
	Non chewers	176	31884	1.00 (ref.)	Aged >35 yrs.	
	BQ	27	1100	4.0 (2.7–6.1)	Adjusted for smoking &	
	AN only	1	12	12.8 (1.6–101.2)	alcohol drinking.	
				Erythroplakia:		
	Non chewers	8	31884	1.00 (ref.)		
	BQ	4	1100	12.5 (3.70–42.4)		

CI = Confidence intervals. BQ = Betel quid without tobacco; AN = Areca nut. Note: Use of lime may be inferred.

Leukoplakia

Betel quid chewing with or without tobacco has been associated with leukoplakia, a precancerous lesion, as reported in case series, case-control, cross-sectional and cohort studies (IARC, 2004). A case control study from Kerala (Jacob *et al.,* 2004), reported an OR of 4.0 for chewers of betel quid without tobacco, and OR of 12.8 (1.6–101.2) for chewers of areca nut by itself, that may include lime. Both the ORs were adjusted for smoking (Table 3). The trends for both frequency and duration were significant ($p < 0.0001$). The OR for chewers of BQ with tobacco was 10.0 (8.3–12.0) and that for tobacco only was 30.9 (13.7–69.7). The study also showed an OR of 12.5 (3.70–42.4) for erythroplakia, a rarer lesion.

Oral Cancer and Other Head and Neck Cancers

Eight case control studies on oral and other head and neck cancers, in India are summarised in Table 4, conducted in Madhya Pradesh (Dikshit and Kanhere, 2000), Maharashtra (Jussawala and Deshpande, 1971; Wasnik *et al.,* 1998), and southern Indian states of Kerala, Karnataka and Tamil Nadu (Balaram *et al.,* 2002; Mahapatra, 2015; Muwonge *et al.,* 2008; Nandakumar *et al.,* 1990; Znaor *et al.,* 2003), with two studies being multi-centric, and two studies in men only. Five of the studies adjusted for tobacco smoking, one also for oral dip products (smokeless tobacco) and four adjusted for alcohol.

Six of the studies showed elevated ORs for cancer and chewing of betel quid without tobacco. In the two smallest studies, the ORs were not significant (Dikshit and Kanhere, 2000; Nandakumar *et al.,* 1990). The study from Trivandrum, Kerala, reported an elevated and significant OR on chewing of areca nut

Table 4. Areca nut chewing practices ad risk of oral and other head and neck cancers in case control studies in India.

Sex / Location/	Cancers	Chewing status	Cases	Controls[#]	Odds ratio (CI) for cancer	Study type. Matching, Adjustments, & Stratifications	References Notes on trends
BOTH MEN & WOMEN							
Mumbai, Maharashtra	Oral cavity, pharynx, esophagus, and larynx: ICD 9 codes 140–148, 150, 161.	Non chewers	129	1340	1.0 (ref.)	Population based. Matched on age, sex, and religion. Not adjusted.	(Jussawala and Deshpande, 1971)
		BQ	44	152	3.0 oral cavity* 1.0 (ref.)		
		Non chewers	106	152	3.0 oropharynx*		No analysis for trends.
		BQ	106	152			
Bangalore, Karnataka	Oral cancer: ICD 9 sites for lip, tongue (excluding base of the tongue), alveolus, and mouth	Never chewers	87	233	1.0 (ref.)	Hospital based. Matched on age, sex, and area of residence.	(Nandakumar *et al.,* 1990)
		BQ	24	45	1.7 (0.9–3.5) NS	Adjusted for smoking.	Significant trends for frequency and duration of chewing in general.
Nagpur, Maharashtra	Oro-pharyngeal cancers, ICD 9 codes not specified.	Non chewers	33	185	1.0 (ref.)	Hospital based. Matched on age and sex. Univariate.	(Wasnik *et al.,* 1998)
		Areca nut	5	14	2.6 (0.9–7.7) NS		Significant trends for frequency and duration of use.
		BQ	7	18	2.8 (1.1–7.4)		
Trivandrum, Kerala	Oral cancer: ICD 10 codes C001–C009	Never chewers	80	915	1.0 (ref.)	Population based. Matched on age & sex. Adjusted for education, religion, smoking and alcohol drinking.	(Muwonge *et al.,* 2008). Significant trends for frequency and duration of use.
		BQ	13	44	3.5 (1.7–7.1)		
Manipal, Karnataka	Oral cancer: ICD 10 codes Not specified	Supari :				Hospital based. Unmatched. Adjusted for age, sex, social class, education level, diet, other tobacco types, dip products, and alcohol.	(Mahapatra, *et al.,* 2015) No analysis for trends.
		No	114	261	1.0 (ref)		
		Yes	20	7	11.4 (3.4–38.2)		
		BQ (T):					
		No	110	257	1.0 (ref)		
		Yes	24	17	6.4 (2.6–15.5)		
		Incorporation of tobacco not specified					

Contd…

Table 4. Areca nut chewing practices ad risk of oral and other head and neck cancers in case control studies in India (Contd…)

Sex / Location	Cancers	Chewing status	Cases	Controls[#]	Odds ratio (Adj.) & CI for oral cancer	Study type, Matching, Adjustments, & Stratifications	Reference
MEN							
Bangalore, Karnataka	Oral cancer: ICD 9 sites for lip, tongue (excluding base of the tongue), alveolus, and mouth	Never chewers BQ	68 15	89 15	1.0 (ref.) 1.5 (0.6–3.8) NS	Hospital based. Matched on age, sex, and area of residence. Adjusted for smoking.	(Nandakumar *et al.,* 1990) Significant trends for frequency and duration of chewing in general.
Bhopal, Madhya Pradesh	Oral cavity & orophayrynx: ICD 9 codes 140, 141, 143–145, 146–149	Non chewers BQ	28 4	140 12	1.0 (ref.) 1.7 (0.9–3.3NS	Population based. Men only. Matched for age. Adjusted for age and smoking.	(Dikshit and Kanhere, 2000) Significant trends for frequency and duration of use
Chennai, Tamil Nadu; Bangalore, Karnataka; Trivandrum, Kerala	Oral cavity: ICD 9 codes not specified	Never chewers BQ	127 15	232 6	1.0 (ref.) 4.2 (1.5–11.8)	Hospital based, at 3 centers. Matched on center, age and sex. Adjusted for age, center, education, smoking and drinking.	(Balaram *et al.,* 2002) Significant trend for frequency per day
Chennai, Tamil Nadu; Trivandrum, Kerala	Oral cavity: ICD 9 codes 140, 141, 143–145	Non chewers BQ	122 24	1471 83	1.0 (ref) 3.4 (2.0–5.7)	Hospital based at 2 centers. Men only. Stratified: only non-smokers and non-drinkers. Adjusted for age, center, education	(Znaor *et al.,* 2003) Significant trends for frequency and duration of use.
Trivandrum, Kerala	Oral cavity: ICD 10 codes C001–C009	Never chewers BQ	64 5	561 16	1.0 (ref.) 3.3 (0.9–12.0)	Population based. Matched on age & sex. Adjusted for education, religion, smoking and alcohol drinking (never vs ever).	(Muwonge *et al.,* 2008) Significant trends for frequency and duration of use.

Contd…

Table 4. Areca nut chewing practices ad risk of oral and other head and neck cancers in case control studies in India (Contd…)

Sex / Location		Chewing status	Cases	Controls[#]	Odds ratio for oral cancer	Study type. Matching, Adjustments, & Stratifications	Reference
WOMEN							
Bangalore, Karnataka	Oral cavity: ICD sites for lip, tongue (excluding base of the tongue), alveolus, and mouth	Never Chewers BQ	19 9	144 30	1.0 (ref.) 2.2 (0.7–6.5)	Hospital based. Matched on age, sex and area of residence. Hospital based. Adjusted for smoking.	(Nandakumar *et al.,* 1990) Significant trends for frequency and duration of chewing in general.
Chennai, Bangalore & Trivandrum	Oral cavity: ICD codes not specified	Never Chewers BQ	29 14	251 5	1.0 (ref.) 16.4 (4.8–56.5)	Hospital based. Matched on center, age and sex. Adjusted for age, center, education, smoking and drinking.	(Balaram *et al.,* 2002) Significant trends for frequency. Starting chewing at an early age has higher risk only for women.
Trivandrum, Kerala	Oral cavity: ICD 10 codes C001–C009	Never chewers BQ	16 8	354 28	1.0 (ref.) 5.4 (2.1–14.1)	Population based. Matched on age & sex. Adjusted for education, religion, smoking and alcohol drinking (never vs. ever)	(Muwonge *et al.,* 2008) Significant trends for frequency and duration of use.

CI= confidence intervals. BQ=Betel quid without tobacco. Note: Use of lime may be generally inferred even if not mentioned.
BQ= Betel quid; T=tobacco; AN= Areca nut; NS=Not significant; [#]Controls, from voters' list; † Some smokers among chewers.
* $p < 0.001$

without tobacco for men and women combined (Muwonge *et al*., 2008). One study reported an OR of 11.4 for *supari* (areca nut) chewing for men and women combined (Mahapatra *et al.,*2015).

All eight studies had significantly elevated ORs for cancer for chewing of betel quid with tobacco. Trends for frequency were analysed in all but two studies and were significant. Trends for duration analysed in all but three studies and were significant. It is notable that in one study the OR for cancer for past users of any type of betel quid was 11.9 (7.0–20.4), higher than for current users, 4.3 (3.1–6.1) (for men and women combined) (Muwonge *et al.,* 2008), suggesting an accumulation of risk over time before the users quit.

For betel quid with tobacco (BQT), available ORs for men and women combined ranged from 4.8 to 14.6 (Jussawala and Deshpande, 1971; Nandakumar *et al.,* 1990); for men only ORs ranged from from 3.4 to 9.3 (Muwonge *et al.,* 2008; Znaor *et al.,* 2003) and for women only ORs ranged from 30.4 to 45.9 (Nandakumar *et al.* 1990; Balaram *et al.,* 2002), all significant. All of the studies, but one, were matched on age and sex (Table 4). Five studies were adjusted for smoking, and three for alcohol drinking; one study was stratified for smoking and drinking and was of high significance (Znaor *et al.,* 2003).

ORs for areca nut, lime and tobacco use without betel leaf, for men and women combined ranged from a non-significantly elevated 2.4 to a significant 10.2 (Muwonge *et al.,* 2008; Wasnik *et al,* 1998). For women, the only available OR for areca nut, lime and tobacco was 9.1 (Muwonge *et al.,* 2008). An OR for *gutka* for men and women combined was 5.1 and highly significant (Mahapatra *et al.,* 2015).

Animal Experiments

Studies in animals carried out to investigate the carcinogenicity of areca nut, its constituents and its products and have helped to validate the results of epidemiologicial studies. Two sets of studies with areca nut (Table 5) and with *pan masala* (Table 6) are reviewed in the following section.

Areca nut studies

Three different animal experiments were designed for simultaneous testing of the carcinogenicity of areca nut, in 2–3 months old inbred Swiss mice (n = 65),

Table 5. Selected animal experiments on the carcinogenicity of areca nut.

Animals	Treatment	Route of administration	Frequency	Durations	Cancerous and other changes observed		Authors, Year, (Country), Notes
25 control Swiss Mice (13M+12F) Aged 2–3 months	Group 1: Pure distilled water	Subcutaneous injections	Once weekly	10 weeks Then allowed to live their full life span up to 27 months	0/25; No local tumour		Ranadive et al., 1976, (India)
40 Experimental Swiss Mice (10 Males & 10 Females) Aged 2–3 months	Aqueous extracts of areca nut: Group 1: cold aqueous extract Group 2: hot aqueous extract	Subcutaneous injections	Once weekly	Life span up to 27 months	Fibrosarcomas at the site of injection: (first after 8 months): Group 1 (cold) : 10/20 Group 2 (hot) : 14/20 (Equal numbers in both sexes)		
30 control Golden Syrian hamsters Aged 2–3 months; Sex not recorded	Untreated	Untreated	NA	Killed in two age groups: 6–12 and 13–21 months (e.g. duration from about 3–9 and 10–18 months)	Cheek pouch: 0 atypia 0 precancers 0 cancer	Fore-stomach: 0 atypia, 1 precancer 0 cancer 0 glandular stomach ulcerations (GSU)	Ranadive et al., 1979, SEE P 152 IARC (India) Results for the two age groups are combined
21 Golden Syrian hamsters; Aged 2–3 months	Aqueous extract of areca nut	Cheek pouches painted inside	Tri-weekly	Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 12 atypia, 2 precancers 1 cancer	Fore-stomach: 5 atypia 6 precancers, 4 cancers 5 GSU	
20 Golden Syrian hamsters; Aged 2–3 months	Polyphenol fraction of areca nut (aqueous)	Cheek pouches painted inside	Tri-weekly	Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 13 atypia 6 precancers 1 cancer	Fore-stomach: 6 atypia 2 precancers 4 cancers 5 GSU	

Contd…

Table 5. Selected animal experiments on the carcinogenicity of areca nut. (Contd…)

Animals	Treatment	Route of administration	Frequency	Durations	Cancerous and other changes observed		Authors, Year, (Country), Notes
20 Golden Syrian hamsters; Aged 2–3 months	Whole betel quid aqueous extract	Cheek pouches painted inside	Tri-weekly	Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 11 atypia 1 precancers 0 cancers	Fore-stomach: 2 atypia 5 precancers 6 cancers 4 GSU	Ranadive et al., 1979, SEE P 152 IARC (India) Results for the two age groups are combined
13 Golden Syrian hamsters; Aged 2–3 months	Arecanut pieces (to inducet rauma) + aqueous extract of areca nut	Insertion into cheek pouch followed by painting		Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 8 atypia 2 precancer 0 cancer	Fore-stomach: 1 atypia 2 precancers 6 cancers 3 GSU	
27 Golden Syrian hamsters; Aged 2–3 months	Market processed suparis (pieces) of two brands (showing combined results)	Insertion into cheek pouch		Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 5 atypia 8 precancers 5 cancers	Fore-stomach: 5 atypia 5 precancers 10 cancers 4 GSU	
25 Golden Syrian hamsters; Aged 2–3 months	Wax pellet control	Insertion into cheek pouch	Assume every fortnight	Killed in two age groups: 6–12 and 13–21 months	No cancerous changes/lesions		
18 Golden Syrian hamsters; Aged 2–3 months	Wax Pellets containing Betel quid	Insertion into cheek pouch	Every fortnight	Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 3 atypia 1 precancers 4 cancers	Fore-stomach: 3 atypia 1 precancers 8 cancers 4 GSU	

Contd…

Table 5. Selected animal experiments on the carcinogenicity of areca nut. (Contd…)

Animals	Treatment	Route of administration	Frequency	Durations	Cancerous and other changes observed		Authors, Year, (Country), Notes
9 Golden Syrian hamsters; Aged 2–3 months	Gelatine capsule control	Insertion into cheek pouch	Assume every fortnight	Killed in two age groups: 6–12 and 13–21 months	No cancerous changes/lesions		Ranadive et al., 1979, SEE P 152 IARC (India)
19 Golden Syrian hamsters; Aged 2–3 months	Gelatine capsules containing Areca Nut powder	Insertion into cheek pouch	Every fortnight	Killed in two age groups: 6–12 and 13–21 months	Cheek pouch: 5 atypia, 7 precancer, 4 cancers	Fore-stomach: 4 atypia 1 precancers 6 cancers 1 GSU	Results for the two age groups are combined
15 Golden Syrian hamsters; Aged 2–3 months	DMBA wax pellet Standard carcinogen control	Cheek pouch	Assume every fortnight	Killed after 6–12 months	Cheek pouch: 0 atypia, 3 precancer, 12 cancer	Fore-stomach: 4 atypia 1 precancers 6 cancers 2 GSU	
20 control albino BALB-C mice	Normal saline solution	Buccal mucosa via pipette	Twice daily; 6 days a week	Group 1: 300 days Group 2: 350 days Group 3: 450 days Group 4: 600 days	None (normal status) Mean body weight at 600 days: 49.3 g ± 4.7 g		Perera et al., 2007, (Sri Lanka)
20 albino BALB-C mice in 4 subgroups of 5 mice each; Aged 12 weeks	Aqueous extracts of areca nut	Buccal mucosa via pipette	Twice daily; 6 days a week	Group 1: 300 days Group 2: 350 days Group 3: 450 days Group 4: 600 days	Cellularity, inflammation and muscle atrophy increased from normal to mild by 300 days and remained so up to 600 days. Compared with controls at 600 days the difference was significant (Wilcoxon statistic 15; P = 0.03) Mean body weight at 600 days: 44.5 g. ± 2.8 g		The lower average body weight of the exposed mice compared to controls was noted.

DMBA = 7, 12 - dimethylebenz (a) anthracene.

Table 6: Selected animal experiments on the carcinogenicity of *pan masala*, a processed from of areca nut.

Animals	Treatment	Treatment Location	Frequency	Durations	Changes observed	Authors, Year, (Country), Notes
14 Albino Wistar rats (Controls)	No treatment	---	---	8 months	No significant changes in biopsies of buccal mucosa taken at beginning and end of 8 month study period	Khrime et al., 1991 (India)
21 Albino Wistar rats	Paste made of *pan masala*	Oral cavity	Alternate days	6 months	Dysplasia in 65% of animals; and thickened & condensed submucosal collagen seen in 88% biopsies	
20 Swiss mice (10 Males; 10 Females) of S/RVCri strain, Aged 6–7 weeks (Controls)	Normal diet	Oral consumption	Daily	For intermediate period: killed at 6, 12 and 18 months	No neoplastic lesions	Bhisey et al., 1999 (India)
40 Swiss mice (30 M; 30 F) of S/RVCri strain, Aged 6–7 weeks	2 dose groups: Powdered *pan masala* mixed in feed at concentrations of 2.5% or 5%	Oral Consumption (10/ gender/ dose group)	Daily	For intermediate period: killed at 6, 12 and 18 months	2.5% *pan masala* group: • No tumors. • Forestomach hyperplasia in 10 out of 60 mice 5.0% *pan masala* group: • Adenocarcinoma of the lung in 1 male and 1 female. • Forestomach hyperplasia in 5 out of 60 mice	
108 Swiss mice (54 Males, 54 Females) of S/RVCri strain, Aged 6–7 weeks (Controls)	Normal diet	Oral Consumption (54/gender/ dose group)	Daily	For lifetime: killed when moribund or at 24 months	No neoplastic lesions	
216 Swiss mice (108 M; 108 F) of the S/RVCri strain, Aged 6–7 weeks	2 does groups: *Pan masala* finely powdered, mixed with feed at concentrations of 2.5% & 5% and pelleted. (54 mice of each sex allocated to each dose = 108)	Oral Consumption (54/gender/ dose group)	Daily	For lifetime: killed when moribund or at 24 months	2.5% *pan masala* group: • 5 malignant lesions 5.0% *pan masala* group: • 7 malignant lesions Overall 15 benign lesions and 12 malignant lesions Decrease in survival as shown by log rank test (p = 0.02). Lung adenocarcinoma, showed a 2-fold increase in the higher dose group compared to lower dose group.	

NA = Not applicable; DMSO= Dimethyl Sulphoxide; DMBA = 7-1 2-Dimethyl-benz(a)anthracene

C17 mice (n = 78), and golden hamsters (n = 45) (Table 5) (Ranadive *et al.,* 1976). Hot and cold aqueous solutions of areca nut were injected subcutaneously in Swiss mice, once a week. Control groups of animals were treated with distilled water. In C17 mice and golden hamsters, dimethyl sulfoxide (DMSO) solutions were used with the aim of enhancing the dermal absorption of the areca nut components from the extract. DMSO areca nut solutions were applied on the skin of the backs of the C17 mice thrice weekly between the shoulder blades. Control groups of animals were treated with 100% DMSO. The hamsters received DMSO areca nut solutions, painted inside the cheek pouch three times a week.

By the end of the lifespan of the Swiss mice (≤ 27 months), ten of 20 mice subcutaneously injected with cold water areca nut extract developed transplantable fibrosarcomas (50%) at the site of injection and 14 of 20 injected with hot water areca nut extract developed fibrosarcomas (Table 5). Tumours were not observed in the internal organs of control and experimental Swiss mice. Skin applications of DMSO areca nut extracts in C17 mice up to 27 months resulted in some mild to moderate hyperplasia but no skin lesions. Cheek pouches of golden hamsters painted with DMSO extract of areca nut showed some early malignant changes (atypia) up to 24 months. The authors concluded that areca nut demonstrated a carcinogenic principle using aqueous extracts (Ranadive *et al.,* 1976).

With the insights gained, a subsequent set of experiments was conducted in Golden Syrian hamsters. Hamster cheek pouches were painted with aqueous areca nut or betel quid extracts or distilled water for controls. Besides, either wax pellets or gelatin capsules containing betel quid or areca nut powder, or pieces of commercially processed supari were inserted into the cheek pouches and compared to controls with distilled water filled wax pellets or empty gelatine capsules on a triweekly basis. In contrast to the control groups, all treated groups developed numerous malignant changes and cancers (Table 5), a majority occurring in the forestomach (Ranadive *et al.,* 1979).

In a recent study in Sri Lanka, 20 BALB-C mice treated with aqueous extract of fresh areca nut for a maximum of 600 days, developed OSF-like condition in the buccal mucosa with 20 mice treated with normal saline solution as control

groups (Table 5). The changes observed in oral tissues of the mice included proliferation of fibroblasts (increased cellularity), abundance of collagen fibres, increased thickness of the *lamina propria*, infiltration of inflammatory cells (mainly lymphocytes and plasma cells) in the connective tissue, and atrophic epithelium and muscle atrophy in the submucosal layer. These changes closely resembled the human oral mucosa affected with OSF (Perera *et al.,* 2007).

Pan masala

The histopathological changes due to *pan masala* were depicted in a study on painting a paste of a well-known brand of *pan masala* in the oral cavity of 21 albino rats on alternate days for six months. Mild to moderate loss of nuclear polarity and increased keratosis and parakeratosis, inflammatory cell infiltration and vascularity were observed (Table 6). Nearly eight out of nine biopsies showed thickened and condensed sub-mucosal collagen. Thus, histopathological changes observed were similar to OSF in humans (Khrime *et al.,* 1991). Further, carcinogenicity of *pan masala* was studied in six groups each of 54 Swiss mice (three groups of males and three groups of females, 6–7 weeks of age). The mice were fed diet containing either dry finely powdered *pan masala* (2.5% or 5%), or normal diet (control group) either for life or an intermediate period. The animals were sacrificed when moribund or after 24 months, whichever was earlier. In the intermediate period group, no tumours were seen in the group fed with 2.5% *pan masala*, but two mice in the 5% *pan masala* group developed adenocarcinoma of the lung. In the lifetime group, a total of 15 benign and 12 malignant tumours were observed in the treated mice, while no tumours were found in controls. Most of the malignant tumours occurred in the liver (n = 13), lung (n = 8) and stomach (n = 3). The most common lung neoplasm was lung adenocarcinoma. The mice fed *pan masala* also lost weight after six months and lived a significantly shorter life span compared to the control mice. Thus, the authors have demonstrated evidence of the carcinogencity of *pan masala* in different mouse tissues, indicating that *pan masala* should be considered a potential human carcinogen (Bhisey *et al.,* 1999).

Mechanistic Evidence of Carcinogenicity

The causal biochemical and molecular mechanisms of oral submucous fibrosis and oral cancer in areca nut chewers are broadly summarized here. During chewing, certain areca nut components, including the alkaloids (mainly arecoline and arecaidine) and polyphenols (tannins, flavonols and catechins) are absorbed through the oral mucosa into the tissues and blood stream (IARC, 2004). These components promote simultaneous abnormal changes in the two main layers of the oral mucosa. A role of in areca nut metabolites in stimulating collagen synthesis in oral mucosa was suggested by tissue culture studies on human fibroblasts from the oral mucosa (Canniff and Harvey, 1981; Harvey *et al.,* 1986; Murti *et al.,* 1995). In the presence of slaked lime (aqueous calcium hydroxide), arecoline, the principal alkaloid, is hydrolysed into arecaidine resulting in irritation and induction of inflammatory mediators (Feller *et al.,* 2013), followed by inflammation. This inflammation stimulates fibroblast proliferation in the *lamina propria*, the connective tissue layer of the mucosa. The stimulated fibroblasts then synthesize excess collagen fibres, resulting in dense fibrosis, leading to stiffening of the mucosa and eventually to palpable fibrous bands. The increasing atrophy of the overlying epithelium, leads to a burning sensation, impaired vasculature and ulcerations (Angadi and Rao, 2011; Khan *et al.,* 2012). Leukoplakia caused by areca nut may cause atrophy (Borle, 2014). Impaired vasculature is initially responsible for the whitish appearance or blanching of the mucosa due to reduced blood supply, occurring from an early stage of the disease prior to fibrous bands appearance (Ekanayaka and Tilakaratne, 2013). The polyphenols and arecoline react in the presence of slaked lime, forming reactive oxygen species, such as the hydroxyl radical (Nair *et al.,* 1995), resulting in inhibition of collagenase enzymes and phagocytosis, preventing collagen degradation and increasing fibrosis. The high copper content of areca nut participates in promoting fibrogenesis (Angadi and Rao, 2011; Khan *et al.,* 2012).

Genetic damage is observed in the oral mucosa of areca nut chewers. Areca nut-specific nitrosamines, or their precursors, and reactive oxygen species generated in the saliva during betel quid chewing are implicated in causing various forms of

genetic damage in the keratinocytes of the basal layer. The copper content promotes formation of cross linkages between the fibrous bands (Angadi and Rao, 2011; Khan *et al*., 2012). Betel leaf contains substances, including beta carotene that functions as scavenger of reactive oxygen species and help prevents DNA breakage, thus lowering the risk of cancer among *pan* chewers, compared to those who chew areca nut or its products without betel leaf (Jeng *et al.,* 2002). Genetic damage is indicated by micronucleated cells in the exfoliated oral epithelial cells of chewers of areca nut products and OSF patients (Desai *et al.,* 1996). Micronucleated cells in chewers are in excess ($p < 0.0001$) of those in non-chewers (Joshi *et al.,* 2011). Further genetic alterations in the keratinocytes followed by increased proliferation may lead to malignant phenotypes. A higher percentage of cells with karyolysis (dissolution of chromatin or nuclear contents) has demonstrated in OSF ($p < 0.05$) compared to non-chewers (Joshi *et al.,* 2011). Interactions between the fibroblasts and the keratinocytes appear to promote malignant transformation in OSF (Ekanayaka *et al.,* 2013).

Nitrosation of the areca nut alkaloids occurs in saliva in the presence of bacterial enzymes, particularly in individuals with poor oral hygiene. The resulting areca nut specific nitrosamines are mutagenic and form DNA adducts in experimental systems, indicating cancer risk (IARC, 2004). Aflatoxins, in areca nut due to fungus infection, form DNA adducts (IARC, 2004). The various genetic lesions (adducts, breaks, etc.) that form with the use of areca nut may progress to cancer over longer time periods (Shah *et al.,* 2012).

DISCUSSION

There is convincing evidence that betel quid or areca nut chewing without tobacco is a cause of oral cancer. A meta-analysis of case-control studies over the last 50 years, on oral/oropharyngeal cancers concluded that overall estimate of relative risk (RR) for use of betel quid without tobacco in the Indian subcontinent was 2.6 (95%CI: 2.0–3.3) (Guha *et al.,* 2014). The frequency of use per day was a more important factor than duration of the habit was unequivocally shown in OSF. The chewing of betel quid containing tobacco confers a greater risk than chewing betel quid without tobacco, besides the added carcinogenicity of tobacco.

Comparing ORs for use of different products and duration of use showed significant differences in risk for OSF. The betel quid chewers were diagnosed after 6–10 years of chewing, whereas *pan masala* and or *gutka* chewers presented with OSF after 2–3 years of use. Thus, chewing of *pan masala* and/or *gutka* causes progression to OSF faster than betel quid. The possible reasons considered were absence of betel leaf and higher consumption by weight of areca nut (Babu *et al.,* 1996).

In the Mumbai Cohort Study the RRs for mortality due to oral and pharyngeal cancers for areca nut or betel quid chewing without or with tobacco did not show significantly elevated RRs, although an RR was significant for other forms of smokeless tobacco use (Gupta *et al.,* 2005). The analysis of incident cancers in the Mumbai Cohort Study (Pednekar *et al.,* 2011), RRs reported for all cancers combined were elevated but not significantly for use of betel quid or areca nut; while RR for cancer of the oral cavity and pharynx for all smokeless tobacco use combined was significant (RR 1.48, 95% CI: 1.03–2.13). These results may in part be due to the rare use of areca nut and tobacco without betel leaf, protective effect of betel leaf among betel quid users, and number of person years in the cohort yielding a small number of cancer cases during the study period. In contrast, in case-control studies, cancer patients come to specialised treatment centres from very wide geographical areas, home to very large populations.

Risk estimates for precancerous lesions and cancer among the exposed are significantly elevated in case control studies, showing strength of association and a temporal relationship. Most case control studies on OSF or cancer show a dose response relationship with higher frequency per day and greater duration of use. The observed changes in exposed animals and humans fit broadly within known pathways for carcinogenesis, including chronic inflammation and genetic damage, showing plausibility and coherence of findings. Changes in the cheek mucosa occur where the quid is kept by areca nut chewers, implying a direct association. OSF shows specificity to areca nut use, almost always preceding mouth cancer in areca nut users. Alternate explanations, such as the consumption of chillies alcohol or tobacco are not causally related to OSF or cancer. Data showing a positive correlation in OSF and current

users of only tobacco are not verified and past use of areca nut not known. Thus the evidence described in this review is abundantly clear and unequivocally fits the Bradford Hill criteria for causality (Hill, 1965). Policy decisions by the Indian government to control the use of areca nut for the benefit of public health are the need of the hour.

The increasing prevalence of use of areca nut products containing tobacco such as *gutka, mawa,* and *pan masala* coincides with rise in OSF and oral cancer primarily at the site of placement in the buccal mucosa. Hospitals in India have noticed increase in admissions for OSF and oral cancer from patients using areca nut products. Thus, convincing evidence on the carcinogenicity of areca nut and tobacco, common use and consequent hazards are obvious in the Indian context. Besides, it is alarming that areca nut products are increasingly exported (40 countries and more), with official quantities of export tripling since 1991 (Kammardi *et al.,* 2012). While tobacco has been widely recognised as a carcinogen, carcinogenicity of areca nut has not been widely communicated or acknowledged. It is mandatory to dispel ignorance of the hazards of areca nut and recognize the importance of increasing awareness of the carcinogenic potential of areca nut.

CONCLUSIONS

In view of the elevated risk of cancer posed by use of areca nut and the rising incidence of OSF and oral cancer in India, control of areca nut and its products, through banning, is justified in order to contain the adverse health effects on the population and improve public health in the affected individuals. In addition, appropriate communications programmes on the harmfulness of areca nut are strongly recommended.

CONFLICT OF INTEREST

The authors acknowledge no conflict of interest.

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