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Arecanut, Areca catechu L. as such is not carcinogenic in normal dose if chewed without tobacco: compilation of research work

[1] S Keshava Bhat, [2] Ashwin Devasya, [3] Mythri Sarpangala

[1] Executive Officer, Arecanut Research and Development Foundation, Varanashi Towers, Mission Street, Mangaluru, Karnataka, India
[2] Senior Lecturer, Dept of Pedodontics and Preventive Dentistry, Kannur dental college, Anjarakandy, Kannur, Kerala state, India
[3] Senior Lecturer, Dept of Periodontology, Kannur dental college, Anjarakandy, Kannur, Kerala state, India

Abstract
Review of literature on the health effects of arecanut chewing shows that it is not carcinogenic in normal dose. It was reported that an adult human being masticate up to 0.5g of arecanut/kg bw/day. Animal studies have revealed that feeding of processed arecanut (dried or boiled) at 1.0g/kg body weight/day and pan masala up to 1.67g/kg bw/day were safe for mice. Arecanut paste when applied to bare skin at 1.5g /kg bw/day was safe for hamsters. Feeding of arecoline, the physiologically most active chemical compound of arecanut, was found safe for mice at 100mg/kg bw/day. The LD50 value for arecanut extract was reported to be >15,000mg/kg bw for rats. The betel quid at a concentration of 0.1ml of 2% solution without tobacco was also found safe for mice. The arecanut and betel quid extracts without tobacco were even reported to retard the development of tumors in mice and cure breast cancer cells, gastric cancer cells and liver cancer cells in human being. Several population studies carried out in India and abroad on the effects of chewing betel quid without tobacco did not show any significant harmful effects on human health. It is really sad to note that such reports were sidelined by most of the researchers and reviewers.

Keywords: arecanut, areca catechu, betel quid, pan masala, arecoline, non-carcinogenic

1. Introduction
Betel quid chewing is an ancient, socially, ceremonially and culturally accepted practice in India and several other countries. One of the ingredients of betel quid is arecanut. It is actually the seed or endosperm of the oriental palm *Areca catechu* L. of the Palmae family. This palm is grown mainly in south and south East Asian Countries such as India, China, Bangla Desh, Srilanka, Myanmar, Malaysia, Indonesia, etc [1]. Traditionally, arecanuts are used for mastication as they are believed to have lots of medicinal properties [2]. Arecanut is misnamed as 'betel nut' by several researchers as this nut is commonly used for chewing along with the leaf or inflorescence of *Piper betle*, a tropical shade-loving perennial evergreen vine of the Piperaceae family.

In India, the use of arecanut has been mentioned as early as in 1300 BC as quoted by Sisu Mayana in 'Anjana Chaitra' [3]. In other countries such as Vietnam, its use was even noticed during the Bronze Age of human civilization [4]. Arecanut has an important place in the ancient system of Indian medicine such as Ayurveda, Unani and Homeopathy [5]. The seeds of arecanut are widely used in clinical practices in China and other south and Southeast Asian countries [6-8]. WHO has listed out as many as 25 different beneficial effects of *A. catechu* on mankind [9]. Arecanut is traditionally used to treat several ailments as it has laxative, digestive, antiulser, carminative, antidiarrhoeal, anthelmintic, antimalarial, antihypertension, diuretic, prohealing, antibacterial, hypoglycaemic, antiheartburn properties [10-12]. All the seven alkaloids (arecoline, arecaidine, guvacine, guvacoline, isoguvacine, arecolidine and homoarecoline) present in arecanut possess drug-like properties [13]. In China as many as 30 medicines, prepared using arecanut as one of the ingredients, are already in practice for the treatment of several gastrointestinal disorders and parasitic diseases of man [14].

In spite of these useful properties of arecanut, it is also labelled as carcinogenic by several researchers [15-20]. On close observation of such reports, it was seen that the carcinogenic activities of arecanut and its chewing products were highlighted by feeding in higher doses much above the normal quantity generally chewed by the common man or by giving injections or by exposing arecanut extracts on cultured cells which are not at all comparable to human habits [21]. It is also true that there are ample scientific evidences to show that arecanut and its chewing products devoid of tobacco are non-carcinogenic if chewed in normal doses [22-28]. But, it is strange that most of such reports were not properly discussed in the papers or review articles which highlighted arecanut and the chewing products containing arecanut as carcinogenic [29-31]. Hence, an attempt has been made in this report to compile all such scattered research data on the non-carcinogenic effects of arecanut and the chewing products containing arecanut but not tobacco, by searching Google scholar, Pub Med, textbooks and old journals until January 2017 and urge the researchers to discuss on these lines or look into these facts seriously while carrying out research studies on the health aspects of chewing arecanut or other products containing arecanut.

2. Animal Studies
2.1 Using arecanut
Several scientific observations made on laboratory animals have revealed that the arecanut extract was safe at normal dose. The carcinogenicity depended mainly on the dose and how it was applied to the animal. It was found unsafe only in higher doses or when applied in unusual manner such as by injection or by

direct application to cultured cells. Arecanut or products containing arecanut such as the betel quid or pan masala are generally used for mastication. Few people are reported to swallow these products but no one administers them by injection. Hence studies conducted by injecting arecanut extract or by exposing cells to these extract were kept out of the purview of the present work. Those studies which neither mentioned the correct dosages tried nor fully described the product utilized were also not entertained as the methodology adopted itself was incomplete.

There are mainly two types of arecanuts in the market [32]. The first type is called 'white supari' obtained by ripe unprocessed sundried nuts (R-UP-SD) and another type is 'red supari' obtained by boiling and drying unripe or semi-ripe dehusked nuts (UR or R-P-SD). The former type is common in Mangaluru region whereas the latter is common in Shivamogga and Sagar region of Karnataka, India. Both these types of arecanuts were reported to be safe for oral feeding for Swiss mice at a dose of 1.0g/kg bw/day [25]. They conducted two experiments. In one experiment powdered arecanut was mixed with the normal diet at a concentration of 0.25, 0.5 and 1.0% of the feed and fed to them for 12 months. In another experiment arecanut was administered orally in the form of paste at 0.25, 0.5 and 1.0g/kg bw twice daily for 12 months. In both the experiments, the dosage up to 0.5% or 0.5g x2/kg bw/day (=1.0g/kg bw/day) were found safe for these animals. It was reported that an adult person masticate up to 0.5g of arecanut per kg bw/day [25]. This quantity is much below than the safe dose reported for mice. Further, it was observed that most of the people don't consume arecanut but only masticate and spit the liquid out [29]. Hence, the actual quantity of arecanut which is going into the system will definitely be lesser than this quantity. Carcinoma was noticed in treated mice only in higher dose (1.0gx2/kg bw/day = 2.0g/kg bw /day). It was earlier reported that all the major chemical constituents of arecanut, including arecoline decrease significantly while drying, boiling, roasting and soaking[33,34]. This might be the reason why there was no carcinogenic effect in lower doses of dried or boiled arecanuts.

In another study, application of 0.1ml of arecanut paste, prepared by using 30g of dried arecanut powder in 20ml of dimethyl sulphoxide (DMSO), was applied to the buccal pouch of golden hamsters and on the interscapular region of C17 mice at tri-weekly intervals throughout the life of the test animals no tumors were developed either in the place of painting or in any of their internal organs [24]. On calculation, this dose comes to 1.5g of arecanut/kg bw for hamster and 5g/kg bw for mouse, much more than the quantity (about 0.5g of arecanut per kg bw/day) generally masticated by human being [25].

The arecanut extract was even reported to retard the development of tumors. In a study when 0.1 ml of the arecanut extracts prepared from 100g of dry arecanut using acetone and dimethyl sulphoxide (DMSO) were applied on the skin of laboratory mice thrice a week for nearly two years an inhibitory effect on the development of tumors induced by a known carcinogen, 3:4, benzpyrene (BP) was seen [23]. When the mice skin were painted with 0.1ml of arecanut extract alone or in combination with 5µg of BP for a continuous period of 39 weeks no tumors were developed, whereas in the control groups when the animals were painted with 5µg BP + DMSO 75% of animals and when painted with 5µg BP + acetone all animals showed tumors. At the end of 45th week, when all BP exposed animals showed large tumors, only 25-33% of the BP + arecanut extract treated mice showed tumors, that too significantly smaller and fewer in numbers. This clearly showed that arecanut extract was neither responsible for the initiation nor promotion of tumor growth, rather it retarded the growth of tumors induced by BP.

The extract of arecanut and its active compound arecoline were found to arrest the growth and multiplication of several human cancer cells such as MCP-7 breast cancer cells [35], Hep-2 larynx cancer cells [36] and SGC-7901 gastric cancer cells and SMMC-7721 liver cancer cells [37]. In a recent study at the Winship Cancer Institute of Emory University, Atlanta, USA, the mechanism of action of arecoline to arrest the growth of cancer cells was explained [38]. They reported that the arecoline hydrobromide inhibited the activity of the enzyme ACAT1 (acetyl-CoA acetyltransferase) which lead to attenuated cancer cell proliferation and tumor growth in mice.

Arecoline, though a minor constituent (up to 0.24%) of arecanut as far as its quantity is concerned [39], is pharmacologically very active [40]. A study was conducted on Wistar rats to find out the safe dose of arecoline hydrobromide by administering three different doses (100, 500 and 1000mg/kg bw) of the commercial preparations of this compound by gastric lavage for 14 consecutive days [28]. They reported that arecaoline hydrobromide was safe to these rats at 100mg/kg bw/day. Tannin, one of the major constituents of arecanut, is also found safe for rodents. On a dry weight basis arecanut contains up to 29.8% of tannin [39]. The carcinogenic effect of tannic acid on mice was studied by gavage feeding [17]. At a dose of 1.9mg of this compound no tumor was developed in any of the internal organs on the treated mice. Arecanut extract was found nontoxic to rats in normal doses. The LD50 value of arecanut extract for Sprague-dawley rats was reported to be >15,000mg/kg bw[41]. Based on this, they even suggested that arecanuts could safely be used in pharmaceutical preparations.

2.2 Using betel quid

The betel quid (BQ) is a common chewing form containing arecanut along with several other ingredients like the leaf or inflorescence of *P. betle*, powder or paste of catechu (*Acasia catechu*), slaked lime, leaf or leaf stalk of tobacco (*Nicotiana tabacum*) and certain condiments and sweeteners [29]. The extracts of these quids without tobacco were also found safe for rodents. The carcinogenicity of BQ used in several geographical areas of United States was studied by inserting all the BQ ingredients in pellets of bees wax into the cheek pouches of hamsters [22]. They could not observe any malignant tumor in the treated animals, whereas in the control group which received carcinogenic hydrocarbons malignant tumors were developed in the cheek pouches. The water extract of the BQ, prepared from 100g of betel leaf, 50g of dry arecanut powder and 4g of lime with 200ml of water, when applied (0.1ml of 2% solution) to the skin of laboratory mice thrice a week for nearly two years no carcinogenic activity was noticed [23]. Even in higher mammals such as baboons, exposure of BQ containing arecanut, betel leaf and lime for a continuous period of 42 weeks and more did not induce any malignant change in their buccal mucosa [42].

The Taiwan BQ, which contains fresh green arecanut sandwiched with an unripe fruit or inflorescence of *P. betle* and slaked lime, was also found safe for hamsters. In a study conducted by inserting 1.5g of Taiwan BQ as such into the cheek pouches of hamsters for a period of 52 weeks or by application of an initiator of carcinogen, DMBA at 0.1%, for 10 weeks after BQ treatment no tumor was developed [43]. In another

experiment also when the buccal pouches of hamsters were applied with 0.1ml of the extracts of all the ingredients of Taiwan BQ (450g of fresh green arecanut, 120g of unripe betel fruit and 50g of slaked lime) for 14 weeks no visible tumors were developed [26]. However, in the control treatment where carcinogenic hydrocarbon was applied tumors were noticed.

2.3 Using Pan Masala

Pan Masala (PM) is a dry packaged form of chewing product containing arecanut and several other ingredients such as lime, catechu, condiments and certain flavoring agents and artificial sweeteners. Generally PM does not contain tobacco. When tobacco is mixed with pan masala it is called as gutka, zarda or khaini[29]. Even application of common forms of PM without tobacco was not found to be carcinogenic in normal doses to animals.

The carcinogenicity of PM was studied on mouse by topical application and by gavage feeding [27]. In both the experiments, three different concentrations (12.5mg, 25mg and 50mg) of the ethanol extract of PM were tested for more than 6 months. It was found that in all the three doses there was no tumor development in any of its organs. On calculation, 50mg of PM per mouse is equal to a dose of 1.67g of PM/kg bw/day [21]. This dose is much above the quantity of 0.5g of arecanut/kg bw/day, generally masticated by an adult human being [25].

Studies on human population

Several studies have reported that chewing arecanut or products containing arecanut such as betel quid or pan masala are closely associated with the occurrence of oral submucous fibrosis (OSF) and oral cancer [44-49]. It is strange to note that most of them neither studied the effects of other ingredients of such chewing products nor took care to verify the quality of such products found in the market but simply blamed arecanut for all the ill effects. It was already reported that several samples of arecanut and the chewing products like pan masala available in the market in India and several other countries were either contaminated with cancer causing fungi (*Aspergillus* spp.) or adulterated with dangerous chemicals, pesticides and heavy metals which are known to cause several health problems on human being [50].

In India, people who chew BQ without tobacco are very few in numbers [47, 51]. In a cross-sectional study conducted at Mangalore, India on 250 cases of OSF it was noticed that people chewing arecanut alone or in combination with betel leaf only were nil [52]. It was estimated that in Delhi and Punjab, people were reported to chew up to 30.0 to 48.0g of BQ or PM / individual / day [25, 53]. No direct study was conducted on the carcinogenicity of arecanut on human beings so far. Most of the inferences were drawn from the data collected from the cancer patients who visited hospitals for treatments and in majority of the cases control groups were not there. Direct field trials involving large cohort of people with different food habits and financial background were also very scanty. However, there were some reports which showed that arecanut and the chewing forms containing arecanut but without tobacco were safe.

In a study conducted on 206 patients with cancer of the cheek and floor of the mouth at Cancer Institute, Madras it was reported that there was no significance of BQ chewing without tobacco on the prevalence of cheek cancer [54]. They reported that 85% of cheek carcinoma patients were with chewing habits of BQ with tobacco as against 8.7% for chewers of BQ without tobacco. However, the percentage of cheek cancer in non-chewers was not mentioned in this report.

In a case-control study conducted on 696 people (including 348 cases of oral cavity cancers and 348 control cases) in Bangalore city in India, it was noticed that pan chewing without tobacco did not show any significant increase in the occurrence of oral cancer (RR was 1.7; P value 0.114), whereas chewing with tobacco showed significant increase (RR was 14.6; P value < 0.001) in the prevalence of mouth cancer [55]. A new finding of this study was that there was marked elevated risk of oral cancer in persons consuming ragi (*Eleusine coracana*) or wheat (*Triticum aestivum*) as staple food compared to those consumed rice (*Oryza sativa*) as their staple food. When the RR for rice consuming people was 1.0 it was 29.3 for ragi and 15.0 for wheat consuming people. This shows that the food habits of people have a great influence on carcinogenicity. In spite of this, it is strange to note that most of the researchers who reported arecanut, BQ or PM as carcinogenic did not consider these aspects which might have vitiated their results.

As far as esophagus cancer is concerned, even chewing pan with tobacco was reported to be safe. In a case-control study carried out on 267 patients in the Regional Cancer Centre, Trivandrum, India with cancer of the esophagus, no significant difference was observed between pan-tobacco chewers and non-chewers [56]. When the relative risk was 1.0 for non-chewers, it was 0.64 to 1.03 for males and 0. 50 to 1.20 for females depending on the chewing frequencies ranging from <5 to >10 pan (with tobacco) per day. One interesting observation was that in people, chewing durations of between 11 and 30 years conferred a lower risk (RR was <0.51for males and <0.68 for females) for esophageal cancer than never chewers. This might be due to the spitting habit of people who chew the pan rather than swallowing it. Rice is the staple diet in Kerala. It was earlier reported that RR for cancer was less in rice eating people rather than in those eating other cereals [55]. This also might be another reason for the less frequency of cancers in pan-tobacco chewers in Trivandrum.

Studies conducted in Assam, India (including all the seven states of north east India) it was reported that BQ chewing with green or red arecanuts without tobacco was not a risk factor for oral cancer [57]. The subjects consisted of 502 (358 men and 144 women) confirmed patients of oral cancer. The adjusted OR's for betel quid chewing associated with just green or red arecanut was 1.9 for males and 0.5 for females. Both the figures were not found to differ significantly from that of control group. The adjusted OR's for BQ chewing with different types of tobacco products ranged from 2.2 to 7.1 and all the figures were found to be significantly more than that of control.

In a case-control study conducted in Chennai and Trivandrum cities in India on the prevalence of cancers in oral cavity, pharynx and esophagus, it was found that the unadjusted odds ratios in these cities for people who chewed BQ without tobacco were 2.11, 1.36 and 1.51, respectively [58]. However, it was not mentioned whether these figures differ significantly or not from that of control group.

In Taiwan and Papua New Guinea, the BQ is chewed mostly without tobacco and hence the observations made on such people could provide a better understanding on the association of cancer with BQ chewing without tobacco. In a cohort study conducted on 6,503 people (including 917 BQ chewers and 5586 non chewers as control) in Taiwan it was noticed that there was no significant difference in mortality due to cancer of the oral cavity or esophagus between BQ chewers and control group [59]. The mortality due to cancer in BQ chewers was 7.7% and in

control group who never chewed betel quid it was 7.5%. In a case-control study in Papua New Guinea, the adjusted odds ratio for the risk of oral cancer in previously BQ chewers was reported to be 0.57, in current occasional chewers 0.98 and in daily chewers 1.29 as against 1.0 for non-chewers [60]. According to them, this minor difference could be due to other ingredients of betel quid such as slaked lime which was already reported to increase the formation of reactive oxygen species which is mainly responsible for accelerating cell proliferation in buccal mucosa of BQ chewers [61]. Increasing epithelial atypia was already noticed in laboratory animals treated with slaked lime [62-65].

In a hospital based study conducted at Nagpur, central India on the prevalence of OSF in 192 people (94 females and 98 males) having exclusive habits of chewing arecanut, Kharra, Gutkha and Tobacco, females were significantly more (52.35%) in numbers than males (2.40%) in exclusive arecanut chewing habits, but the prevalence of OSF was significantly less in them than in males (1:4.9) who were habituated (76.84%) for chewing Chara and Gutkha which contained tobacco and several other substances apart from arecanut [46]. This clearly shows that chewing of arecanut is more safer than the products which contained tobacco and other ingredients. One lacuna in this experiment is that the authors did not give the data for non-chewers for comparison.

Two large cohort studies conducted till today to find out the effects of BQ chewing on pregnant women and child birth did not reveal any adverse effects [66, 67]. The largest population study conducted so far was on 7,685 pregnant women who attended antenatal clinics along the Thai-Myanmar border during 1997 to 2006 [66]. Of the 7,685 women 2,284 (29.7%) never used BQ nor smoked, 2,484 (32.3%) used only betel quid, 438 (5.7%) only smoking (not commercial cigarettes but cheroots) and 2,479 (32.3%) used both BQ and smoking. The BQ was made using ripe arecanut pieces wrapped in betel leaf with lime without tobacco. No adverse pregnancy effects were observed in such a large cohort of arecanut chewers compared with non-chewers. Smoking alone had a dose-related effect on miscarriage. The miscarriage experienced by pregnant women with BQ chewing habit alone was only 7.5%, whereas it was 7.7% for non-chewers. The figure for smokers was 13.7% and smokers who also chewed it was 13.2%. The figures for neo natal death were same (1.4%) for both BQ chewers and non-chewers, whereas they were 1.9% for smokers and 1.6% for smokers who also chewed BQ. These observations even showed that chewing BQ without tobacco slightly decreased the risk of smoking.

The second largest population study was conducted on 2,700 pregnant women in Papua New Guinea [67]. They also reported that there was no change in pregnancy loss or congenital abnormalities between BQ chewers and non-chewers. Analysis of 1,769 infant birth weights showed that it was 2.996 kg in betel quid chewers and 2.966 kg in non-chewers. Prevalence of malaria in pregnant women was 5.3% in heavy BQ chewers, whereas it was 6.7% in non-chewers. However, only difference between these two observations was that in PNG the BQ chewers were found to be more prone to become anemic than non-chewers, but in the study conducted at Thai-Myanmar border there was no significant difference between chewers and non-chewers on the prevalence of anemia [66]. This minor difference in these two observations might be due to the change in the composition of BQ used in these two populations. In Thai-Myanmar, the BQ contained mature arecanut, betel leaf and slaked lime, whereas in PNG it was a mixture of green arecanut, betel inflorescence and slaked lime. However, all these observations clearly show that BQ chewing without tobacco does not induce any adverse effect in pregnant women. This also supports the view that arecanut chewing is not at all harmful.

4. Conclusion

Arecanut is well known for chewing since time immemorial as it is believed to have lots of medicinal values. The research studies have shown that the arecanut or the chewing products which contained arecanut as one of the ingredients without tobacco such as betel quid or pan masala are not harmful in normal doses. The harmful effects of arecanut and other chewing products containing arecanut might be due to their high doses or application in unusual manners like injection, direct exposure to cultured cells, etc or due to several other factors such as contaminations and adulterations, the effects of other ingredients of betel quid or pan masala, the food habits of individuals, etc. The researchers should consider all these factors before tagging any one component as harmful.

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