review

Association of betel nut with carcinogenesis

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HISTORICAL PERSPECTIVE

THE USE OF BETEL NUT, *Areca catechu L.* (family Palmaceae), as a masticatory by humans has been known since the 4th century A. D. in different parts of the world. This includes South and South-East Asia, where it is one of the oldest known masticatories, several Pacific islands, many regions of the former Soviet Union, parts of North America, and Europe. It is estimated that over 600 million individuals consume betel nut (also called areca nut) in one form or an other world-wide. In old Indian scripts, such as Vagbhata (4th century), and Bhavamista (13th century), betel nut has been described as a therapeutic agent. Its use was recommended in many diseases, such as leucoderma, leprosy, anaemia, and obesity. It was also reported to have deworming properties. In China, it has been used as a vermifuge since the 6th century and is still employed as such in some parts. The major alkaloid of betel nut, arecoline, has been reported to lower blood pressure. Betel nut used to be a native plant of South Asian subcontinent but now it has spread to other parts of the globe. Over the course of time, the form of mastication of betel nut has undergone modifications in different parts of the world, conforming to the social and other aspects of the region. Consequently, betel nut is masticated either alone or as quid along with a large variety of ingredients, such as betel leaf (Piper betle; family Piperaceae), slaked lime, catechu, different types of tobacco, and various additives, perfumes, and stimulants (IARC, 1985). The masticated nut or quid in saliva is either spat out or swallowed. The form of betel nut is highly variable between different parts of the world. The most common type is sun-dried variety, in which unripe or ripe nuts are sun dried for several weeks. Sometimes before sun-drying, the nuts are boiled for several hours in an aqueous solution containing pieces of bark from the plant *Eugenia jambolana*, jaggery of brown sugar, and various edible oils to complete "processing" or "curing". The sun-dried, unprocessed or processed, and relatively hard, nut is cut into small pieces for mastication. The other type is raw and wet variety as used in Taiwan and whole of the North-Eastern and parts of the Southern states of India. In this case, the fruit is plucked from the trees while it is still green. After shelling the fibrous coat, raw, wet and unripe nut is used fresh for mastication. Sometimes, the nut with its green shell may be left in wet pits for a few weeks for "ripening". In either case, after shelling, the nut is usually cut into four pieces for consumption. The North-East Indian variety of betel nut, locally called kwai or tambul, is raw, wet and unprocessed betel nut consumed with betel leaf and slaked lime (Figure 1). This form of kwai consumption causes an immediate thermogenic physiological response lasting 2-3 min with significant perspiration on the forehead and reddening of ear pinnae. This effect is markedly different from that of sun-dried variety of betel nut consumed elsewhere in the world.

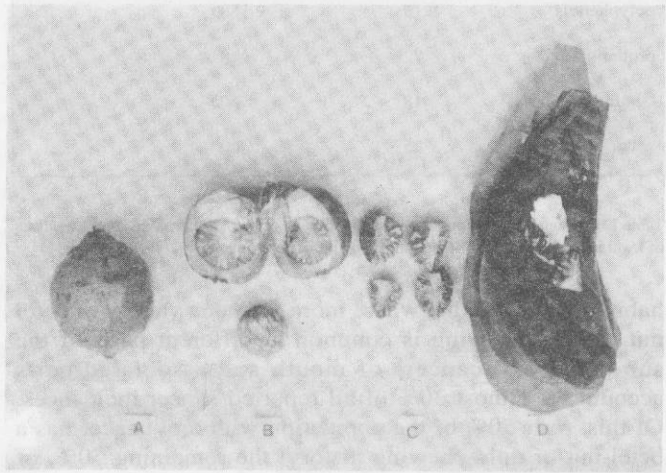

Figure 1: Wet variety of betel nut (kwai or tambul) at various stages and a typical preparation of betel quid consumed in North-East India. A: a betel-fruit; B: betel nut after shelling along with empty shell; C: pieces of betel nut for mastication; D: betel quid (kwai or tambul) consisting of a piece of wet and raw betel nut on betel leaf with slaked lime

ASSOCIATION OF BETEL NUT WITH CANCER

There are strong indications for an association of the habit of betel nut or quid chewing with cancers of mouth, oropharyngeal cavity, and upper parts of the digestive tract (IARC, 1985, 1987). A rough estimate shows that approximately 15% of the world population may practise betel quid mastication, with certain geographical areas in which this habit is very widespread. Usually, men outnumber women in betel mastication habit in most parts of the world. In the South-East Asian countries, mastication of betel quid is very common. Population studies show very high incidence of oral and associated cancers in this subcontinent as compared with parts of the world where this

abbreviations: AAEBN (acetic acid extract of betel nut), AEBN (aqueous extract of betel nut), BSNA (betel nut specific nitrosamine), CA (chromosomal aberration), CHO (Chinese hamster ovary), EEBN (ethanol extract of betel nut), HEBN (hydrochloric acid extract of betel nut), Mice (Swiss albino mice), NOC (N-nitroso compound), PADPR (poly-ADP ribosylation), SCE (sister chromatid exchange).

constituents	sun dried (%)	wet (%)*
alkaloids	0.25	0.25 - 0.38
a. arecaidine	0.10	0.10 - 0.20
b. arecoline	0.15	0.18 - 0.24
c. others	trace	ND
ash content	low	very high
carbohydrates	25	30
crude fibre	15	18
fats	12	2.50
polyphenols	15	ND
proteins	7.5	18
tannins	18	ND
water	25	60

Table I: Average constitution of two main varieties of betel nut (*based on the analysis of kwai in the laboratory of the author; ND = not determined)

habit is rare. In India, where more than one variety of betel nut or quid chewing is common in different parts of the sub-continent, cancers of mouth and associated parts account for almost 30% of all reported cancer incidences. Of this, over 50% of the population with oral cancer has a betel-nut or quid-chewing history; the remaining 50%, of cancers may be attributed to genetic, nutritional and other undetermined factors. The association of betel nut or quid with oropharyngeal cancer is further substantiated by the fact that oral cancer is the most common of all cancers among men while, among women, it is the third most common cancer. This gives strong statistical data pointing to etiology of betel nut or quid mastication with oral and associated cancers. In Taiwan, 80% of total deaths due to oral cancer has been reported to be associated with the betel-chewing habit. Since metabolic absorption of ingredients of betel nut or quid directs the cancer-causing principles of betel nut or quid to other organs/tissues of the body, the evidence is growing to indicate that cancers other than oropharyngeal may also be caused by betel-nut or quid chewing. For instance, urinary tract and urinary bladder are likely to be susceptible to cancer due to betel nut or quid chewing (Trivedi *et al.*, 1995). We have observed that an aqueous extract of betel nut (AEBN) affects the high mobility group (HMG) proteins and their states of poly-ADP-ribosylation (PADPR) in mouse liver, which may lead to liver cancer (Pariat *et al.*, 1995).

EFFECTS OF BETEL NUT CONSUMPTION

Betel nut is usually consumed as betel quid (a complex mixture of betel nut, betel leaf, lime, catechu, various additives, and flavours; betel nut being predominant by weight), often with preparations of tobacco. In addition, a statistically significant proportion of the betel-nut consuming population are also smokers and/or drink alcohol. Therefore, it is difficult, if not impossible, to work out the carcinogenic potency in humans of betel nut alone. Several attempts have, however, been made to obtain a clear insight into the carcinogenic properties of different

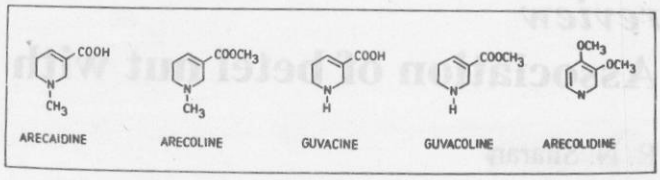

Figure 2: Chemical structures of different alkaloids of betel nut

ingredients of this mastication and thus of betel-nut-associated carcinogenesis. Long-term experiments involving dietary and oral feeding of mice with different types of betel-nut preparation showed that wet, and not dry, varieties of betel nut was able to induce low-level hyperplasia, atypia, and papilloma/carcinoma (Rao and Das, 1989).

COMPOSITION OF BETEL NUT

As the major part of betel quid, betel nut has been the main suspect for delivering carcinogenic chemicals to the masticators. A number of reports on the constituents of betel nuts show considerable variation in their contents, due to geographical and climatic conditions of growth of the plant and also because of the special treatments (processing, curing, ripening) that are applied to betel nut before it is consumed. Table I shows the average composition of the two main varieties of betel nut: namely, sun-dried and wet.
Among the alkaloids, arecoline and arecaidine predominate in both the varieties of betel nut. Carbohydrates and proteins are abundant in both. It is expected that the wet variety of betel nut would be relatively rich in polyphenol and tannin contents as compared with the dry one. Trace amounts of fluorine, sapogenin, and free amino acids have also been reported.

ACTIVE CARCINOGENIC PRINCIPLES OF BETEL NUT

Alkaloids and polyphenols are two groups of chemicals which could contribute to carcinogenicity (IARC, 1985; 1987). It has been shown that with ageing of the betel nut the alkaloid content increases while the content of polyphenols decreases.

(A) Alkaloids - The prime suspect for betel nut carcinogenesis is alkaloid, a group of reduced pyridine compounds (Figure 2) producing various adducts including cysteine ß-alkylation products. As apparent from Table I, arecoline (1,2,4,5,-tetrahydro-1-methyl-pyridinecarboxylic acid; molecular weight 155.19 Da) is the most abundant alkaloid of betel nut, but other alkaloids, such as arecaidine (1,2,5,6,-tetrahydro-1-methyl-3-pyridinecarboxylic acid; molecular weight 141.17 Da), guvacine (methyl ester of arecaidine), guvacoline (methyl ester of guvacine) and arecolinidine are also present in small or trace amounts. Of these, arecoline and arecaidine, due to chemical similarities, may be bio- equivalents in carcinogenesis; other alkaloids contributing only marginally. Arecaidine is reported to be more toxic than arecoline but both have similar pharmacokinetic behaviour.

(B) Polyphenols and tannins - The main polyphenols of betel nut are catechin, flavanoids, flavan-3:4-diols, leuco-

treatment group	mutation frequency
spontaneous	1.00
arecoline	1.33
AEBN	1.74
HEBN	1.74
AAEBN	7.07
EEBN	8.00

Table II: Mutation frequency of arecoline and extracts of betel nut

cyanidins, and hexahydroxyflavans. When oxidized in the presence of lime, these give the characteristic red colour to saliva, teeth and lips. The predominant tannin of betel nut is gallotannic acid. In addition, minor amounts of gallic acid, D-catechol, and phiobatannin are also present.

GENOTOXICITY AND CYTOTOXICITY OF BETEL NUT

More than three decades of consistent research has generated a significant amount of data to show that betel nut has a high potential for inducing genetoxicity and cytotoxicity. However, variability in the collected results due to divergent modes, habits and constituents of betel quid, especially the presence of tobacco, has prevented a firm conclusion on the geno- and cytotoxicities of betel nut alone. All major components of betel quid, namely, betel nut, betel leaf, and catechu, have been shown to individually enhance chromatid breaks and exchanges in the range of 12 to 37% in human cells *in vitro* (Stich and Rosin, 1985). Mouse kidney cells or human buccal epithelial cells *in vitro* showed increased DNA strand breaks induced by aqueous extract of betel nut (AEBN) or betel-nut specific N-nitrosamines (BSNA), betel leaf, and a polyphenol, catechin, as compared with arecoline, indicating that other constituents of betel quid may contribute synergistically to the geno- and cytotoxic effects (Wary and Sharan, 1988; Sundqvist *et al.*, 1991; Jeng et al, 1994). Cell proliferation activity was also enhanced under these conditions (Wary and Sharan, 1988). It was concluded that this enhancement was achieved by shortening the duration of cell cycle.

CYTOSTATIC EFFECTS OF BETEL NUT

Different extracts of betel nut, such as AEBN, acetic acid extract (AAEBN), HCl extract (HEBN), and ethanol extract (EEBN), as well as arecoline have been shown to exert different extents of cytostatic and cytotoxic effects in a dose range of 10 to 100 µg on Hep2 cells *in vitro*. While all of these treatment conditions showed a dose-dependent increase of cytostatic influences, arecoline, HEBN, and EEBN were the most potent (Sharan and Wary, 1992). Human fibroblast cells *in vitro* were also sensitive to this effect, especially for betel nut and arecoline (Jeng *et al.*, 1994).

MUTAGENESIS CAUSED BY BETEL NUT

Betel nut either alone or as a part of betel quid has been shown to be mutagenic in bacterial systems as well as in rodents (IARC, 1985). In mammalian systems, Shirname *et al.* (1984) showed that betel quid or its ingredients caused gene mutations. It has also been observed that arecoline as well as AEBN, AAEBN, HEBN, and EEBN induced variable levels of dose-dependent unscheduled DNA synthesis (UDS) in Hep2 cells *in vitro* (Sharan and Wary, 1992), pointing to genomic damage caused by these treatments. Coupled with the data on the viability of cells under these treatment conditions, the results suggest that there were qualitative differences between arecoline and extracts of betel nut in causing genomic damages; arecoline alone being more potent.

(A) Mutation frequencies of betel nut extracts - In order to test the mutagenic potential of arecoline and different extracts of betel nut, an Ames test was performed using *S. cerevisiae* tester strain TA 1535 and compared with the mutagenic potential of ^{60}Co γ-rays (Sharan and Balachandran, unpublished results). Mutation frequencies were calculated from these data for different exposure conditions (Table II). Applying the criteria of 2-fold increase in the mutation frequency over the spontaneous rate (Maron and Ames, 1983), the result indicate that arecoline was non-mutagenic, AEBN and HEBN were weak mutagens, and AAEBN and EEBN were strong mutagens in the Ames test. This finding suggests that the mutagenic potential of arecoline is enhanced significantly by other constituents of betel nut and the presence of other factors like the extent of alkalinity/acidity or the presence of alcohol in the extraction medium. Alkaline pH is known to enhance the potential damaging actions of alkaloids (Sharan and Wary, 1992).

(B) Sister chromatid exchange (SCE) - The frequency of lymphocytic SCE was elevated in betel-nut chewers, oral submucous fibrosis patients, and oral cancer patients in comparison to non-chewer controls (Adhvaryu *et al.*, 1991). Chinese hamster ovary (CHO) cells subjected to a 3-h exposure to urine of betel nut and tobacco chewers showed significant elevation of 11 or more SCE/metaphase cells (Trivedi *et al.*, 1995), indicating that this habit may also cause genomic damage of systems and tissues other than oral cavity and oropharyngeal tract.

(C) Chromosomal aberrations (CA) - The assay of CA represents several types of DNA damage (*viz.*, gaps, breaks, accentric fragments, interchange, ring chromosome, and dicentric chromosomes), and unrepaired or misrepaired damage sites. Thus, this assay has been used to quantify the DNA-damaging ability of betel quid, betel nut, or their constituents. The most prevalent type of CA associated with betel quid was chromatid type (Dave *et al.*, 1992). However, chromosome type CA was also found. From this it appears that most CA are induced in the S or G_2 phase, wherein the number of intrastrand crosslinkings introduced is enhanced in a dose-dependent fashion in the presence of betel quid or its constituents. In another approach, smears from mouth of humans volunteers (non-chewer controls, betel-nut chewers, oral submucous fibrosis patients, and oral cancer patients) were scored for CA and micronuclei. As compared with controls, all three groups showed significantly elevated CA frequencies; the chromatid type CA and micronuclei being predo-

minant (Adhvaryu *et al.*, 1991). Even urine samples of betel nut and tobacco chewers induced significantly high levels of different types of CA, particularly chromatid type, in CHO cells (Trivedi *et al.*, 1995).

TERATOGENESIS INDUCED BY BETEL NUT

Teratogenic effects of betel nut and its alkaloids have been reported in mice and rats chronically exposed to betel nut or arecoline (Sinha and Rao, 1985). Exposure to betel-nut extracts leads to death, enhanced resorption, and reduced weight of the foetus. Certain abnormalities, such as curved tails, and abnormal ribs were also observed. No such report for humans is available.

INFLUENCE OF BETEL NUT ON THE IMMUNE SYSTEM

Some information on the actions of betel nut or its constituents on the immune system is available. This includes effects such as suppression of phagocytotic functions, reduced antibody formation and delayed hypersensitivity responses. Chronic exposure to arecoline has been shown to cause inhibition of IgM type immunoglobulin production. The overall suppression of immunesurveillance by betel nut and its alkaloids suggest that a sub-optimal immune system may help progression of carcinogenesis in betel nut or quid chewers (Selvan, 1988).

BIOCHEMICAL CHANGES INDUCED BY BETEL NUT

Arecoline has been shown to be able to inhibit DNA, and protein synthesis in a dose-dependent manner (Wary and Sharan, 1991). This inhibition was enhanced in the presence of sodium nitrite and the S-9 mixture which promotes metabolic conversion of alkaloids to their potent carcinogenic form, N-nitroso compounds (NOC). The effect was reversible when the alkaloid was withdrawn. Several antioxidants, such as glutathione (GSH), L-cysteine (GySH), mannitol, catalase, and superoxide dismutase (SOD), have also been shown to reverse the inhibitory effects of arecoline on DNA synthesis (Jeng *et al.*, 1994). In a long term experiment recently concluded in the laboratory of the author, mice were put on diet containing betel nut or different concentrations of arecoline (1 or 3 mM) in their drinking water in a chronic exposure protocol to mimic the consumption of betel nut by habitual chewers. The level of kidney gamma-glutamyl transpeptidase (GGT), one of the marker enzymes of carcinogenesis, was monitored. GGT showed a periodicity in increase in its activity (Figure 3) over a six-month exposure period while the total protein content increased progressively as expected due to ageing (inset). Since GGT is involved with glutathione metabolism and in the transport of amino acids and peptides, it appears that betel nut and its main alkaloid, arecoline, interfere with glutathione metabolism, as has also been shown by Sundqvist *et al.*, (1991). The significance of the periodic outbursts in GGT activity, however, remains unclear. It may indicate that there is a push-and-pull situation of carcinogenesis during chronic betel nut exposure conditions. In another study, 10 µg/ml dose of arecoline in drinking water caused an inhibition of the level of total poly-ADP-ribosylation (PADPR) of chromosomal proteins in mouse spleen cells in one week

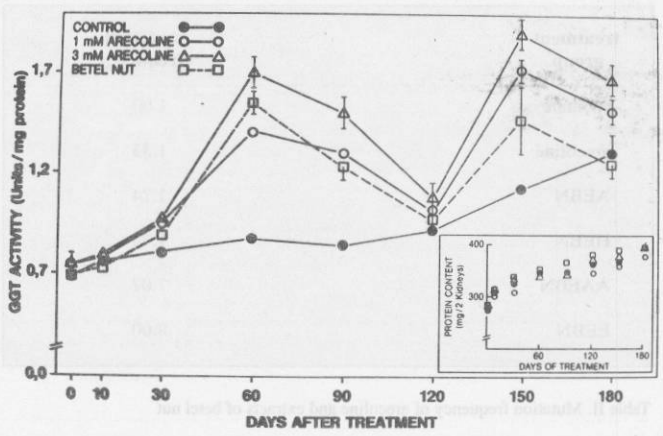

Figure 3: Activity (mean ± SD) of mouse kidney gamma-glutamyl transpeptidase (GGT) following chronic exposure of mice to either betel nut in diet or to arecoline (1 and 3 mM) in drinking water; the inset shows total protein content of kidney under the same treatment conditions. Controls were age-matched mice kept under identical conditions on normal diet and water

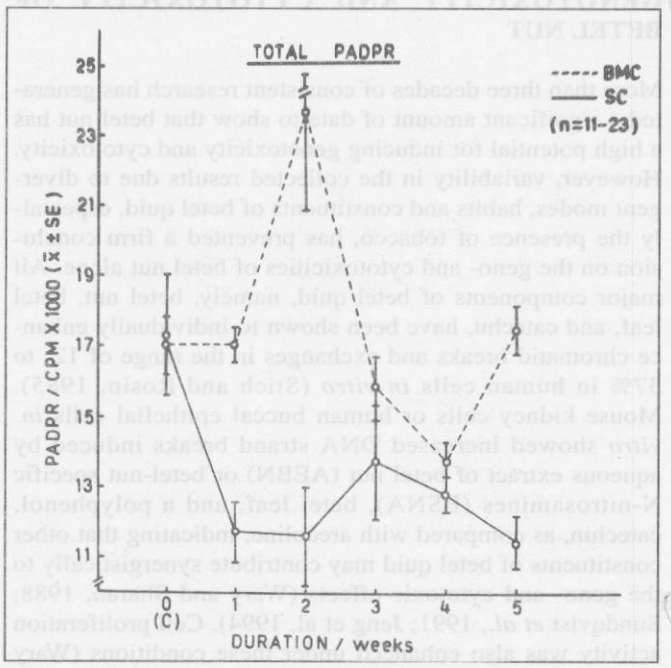

Figure 4: Total cellular poly-ADP-ribosylation (PADPR; mean ± SEM) of bone marrow cells (BMC) and spleen cells (SC) of mice chronically exposed to arecoline in drinking water (10 mmmg/ml) for up to 5 weeks

(Figure 4) and maintained at this level for the next 5 weeks (Saikia *et al.*, 1995). Bone marrow cells of exposed mice also showed a similar trend. Since the level of PADPR of chromosomal proteins is indicative of the state of chromosomal structure and its functions, these results point to condensation of chromosomes by arecoline treatment and consequent alteration in gene expression. A similar treatment protocol using AEBN caused extensive fragmentation and quantitative reduction of high mobility group (HMG) proteins in mouse liver and dose and duration-dependent inhibition of PADPR (Figure 5) of HMG proteins (Pariat *et al.*, 1995). The HMG proteins are a class of proteins believed to exert regulatory influences on chromosomal organization and function.

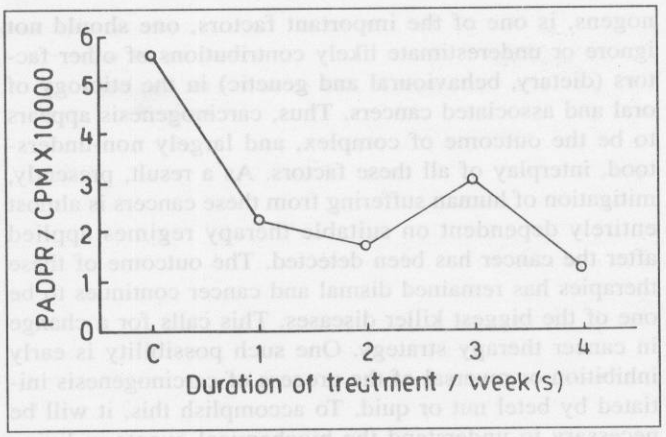

Figure 5: Poly-ADP-ribosylation (PADPR) of liver high mobility group (HMG) proteins of mice chronically exposed to aqueous extract of betel nut (AEBN; 0.5 mg/ml) in drinking water and normal diet for up to four weeks

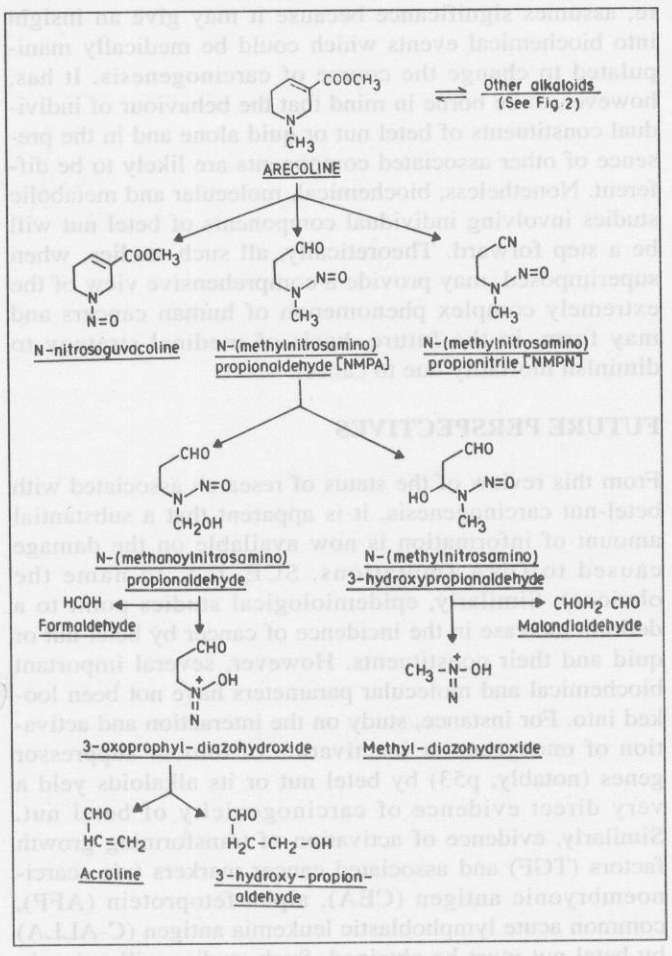

Figure 6: Chemical structures of different betel-nut specific nitrosamine (BSNA) of arecoline. Further metabolic products of one of the BSNA, NMPA, and their chemical structures are also shown

POSSIBLE MECHANISMS OF CARCINOGENESIS

The prime suspected carcinogens in betel nut are the alkaloids. However, they are only potentential carcinogens. Their conversion to ultimate carcinogens depends on their metabolic activation which is strongly influenced by physiological conditions and the presence of other factors. It

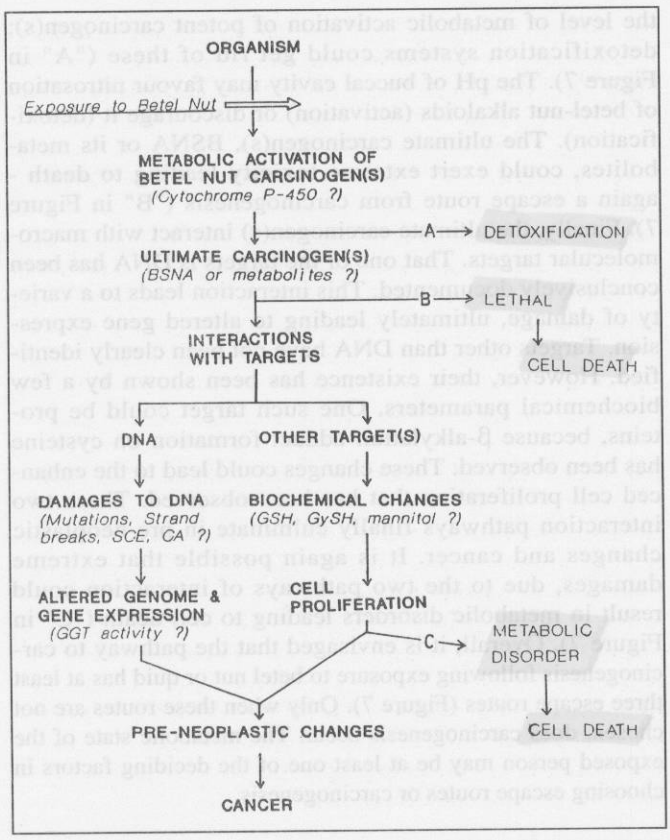

Figure 7: A schematic model of possible intermediate steps in the complex pathway of betel-nut carcinogenesis

has been suggested that metabolic activation may involve the cytochrome P-450 system (Wary and Sharan, 1991; Sundqvist *et al.*, 1991). The nitrosation of arecoline, which contains a 3- ethylenic bond at the 3-4 position on the pyridinium ring, may produce a variety of betel-nut-specific nitrosoamines (BSNA). The BSNA interact with DNA, proteins or other targets forming adducts to exert its carcinogenic activity. Three major NOC of arecoline (Figure 6), namely, N-nitrosoguvacoline, 3-(methylnitrosamino)-propionitrile (MNPN) and 3-(methylnitrosoamino)propionaldehyde (MNPA) have been identified in betel-nut chewers (Wenke *et al.*, 1984). Of these, the last, MNPA, is likely to produce a large number of metabolites in the organism (Figure 6), several of which have been detected in saliva/urine samples of betel nut chewers (Sundqvist *et al.*, 1991). The interaction of BSNA or their metabolites with cellular targets may initiate of carcinogenesis. One of the most important targets is DNA. The interaction of BSNA or its metabolites with DNA is likely to be weak and non-covalent in nature since the interaction was reversible (Wary and Sharan, 1991). Arecoline and arecaidine react with cysteine, both *in vivo* and *in vitro*, forming cysteine ß-alkylation adduct. These interactions with cellular targets may act synergistically towards carcinogenesis. Rao and Panigrahi (1984) have suggested that arecoline may be converted to arecaidine which upon epoxidation at alkaline pH, produces 3,4-epoxide as the ultimate carcinogen. Based on the overall understanding of events associated with betel-nut-associated carcinogenesis, a schematic pathway is proposed in Figure 7. It is evident that the first critical point in betel-nut-associated carcinogenesis is at

the level of metabolic activation of potent carcinogen(s); detoxification systems could get rid of these ("A" in Figure 7). The pH of buccal cavity may favour nitrosation of betel-nut alkaloids (activation) or discourage it (detoxification). The ultimate carcinogen(s), BSNA or its metabolites, could exert extreme toxicity leading to death - again a escape route from carcinogenesis ("B" in Figure 7). Finally, the ultimate carcinogen(s) interact with macromolecular targets. That one of the targets is DNA has been conclusively documented. This interaction leads to a variety of damage, ultimately leading to altered gene expression. Targets other than DNA have not been clearly identified. However, their existence has been shown by a few biochemical parameters. One such target could be proteins, because β-alkylation adduct formation on cysteine has been observed. These changes could lead to the enhanced cell proliferation that has been observed. These two interaction pathways finally culminate in pre-neoplastic changes and cancer. It is again possible that extreme damages, due to the two pathways of interaction could result in metabolic disorders leading to cell death ("C" in Figure 7). Overall, it is envisaged that the pathway to carcinogenesis following exposure to betel nut or quid has at least three escape routes (Figure 7). Only when these routes are not chosen, does carcinogenesis occur. The metabolic state of the exposed person may be at least one of the deciding factors in choosing escape routes or carcinogenesis.

SOME OPEN QUESTIONS

Although some direct evidence from human studies, and a significant amount of circumstantial and indirect evidence primarily from mice (*in-vivo* system) and several animal and human cell lines (*in-vitro* system) are available, the carcinogenesis of betel nut in humans remains an open question. One fact emerges: betel nut and its components, especially alkaloids, are potential mutagens. Since mutagenicity and carcinogenicity have a very high degree of correlation (Maron and Ames, 1983), it may be reasonable to say that exposure to betel nut in any form increases the risk of mutation. Therefore, it may also be carcinogenic. It will, however, be relevant to look for direct evidence of carcinogenicity of betel nut in humans, since extrapolation of animal studies to humans may not be possible due to inherent metabolic differences. The study of betel-nut-associated carcinogenesis assumes significance in light of recent arguments that (a) naturally occuring carcinogens cause a significantly higher body burden to humans than industrially produced ones, and (b) mutagens increase the rate of mutations by direct adduct-driven mutagenesis and/or by enhancing cellular turnover (mitogenesis). These arguments are supported by fragmentary experimental evidence. Thus, so far there is no consensus on the general molecular mechanism and pathway of carcinogenesis. The state of betel nut carcinogenesis in humans is even more complex, since the betel-nut or quid chewer is under the influence of several additional factors.

The culmination of effects of betel-nut or quid chewing is an enhanced rate of cancer incidence in humans, as evident from the epidemiological data. To say that this enhancement is due to betel-nut or quid consumption alone would be an over-simplification. Notwithstanding the fact that betel nut or quid, a complex mixture of several carcinogens, is one of the important factors, one should not ignore or underestimate likely contributions of other factors (dietary, behavioural and genetic) in the etiology of oral and associated cancers. Thus, carcinogenesis appears to be the outcome of complex, and largely non-understood, interplay of all these factors. As a result, presently, mitigation of human suffering from these cancers is almost entirely dependent on suitable therapy regimes applied after the cancer has been detected. The outcome of these therapies has remained dismal and cancer continues to be one of the biggest killer diseases. This calls for a change in cancer therapy strategy. One such possibility is early inhibition or reversal of the process of carcinogenesis initiated by betel nut or quid. To accomplish this, it will be necessary to understand the biochemical events in living systems triggered by individual constituents of betel nut or quid (or, for that matter, other carcinogens). The study of components of betel nut and their metabolism and interactions with macromolecules/cells/tissues/organism, therefore, assumes significance because it may give an insight into biochemical events which could be medically manipulated to change the course of carcinogenesis. It has, however, to be borne in mind that the behaviour of individual constituents of betel nut or quid alone and in the presence of other associated components are likely to be different. Nonetheless, biochemical, molecular and metabolic studies involving individual components of betel nut will be a step forward. Theoretically, all such studies, when superimposed, may provide a comprehensive view of the extremely complex phenomenon of human cancers and may form, in the future, basis of medical strategy to diminish mortality due to cancer.

FUTURE PERSPECTIVES

From this review of the status of research associated with betel-nut carcinogenesis, it is apparent that a substantial amount of information is now available on the damage caused to DNA (mutations, SCE, CA, to name the obvious). Similarly, epidemiological studies point to a definite increase in the incidence of cancer by betel nut or quid and their constituents. However, several important biochemical and molecular parameters have not been looked into. For instance, study on the interaction and activation of oncogenes or inactivation of tumour suppressor genes (notably, p53) by betel nut or its alkaloids yeld a very direct evidence of carcinogenicity of betel nut. Similarly, evidence of activation of transforming growth factors (TGF) and associated cancer markers (*viz.*, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), common acute lymphoblastic leukemia antigen (C-ALLA) by betel nut must be obtained. Such studies will not only provide clear understanding betel-nut carcinogenesis but will also pave the way for control of this dreadful killer of humans. In all such studies involving betel-nut consumption with respect to human carcinogenesis, the effect of diet should not be overlooked. Recent findings that populations on diets rich in fats and salts are more prone to carcinogenesis than populations without illustrate this. Even animal studies show this trend. A comprehensive study on all these aspects will be able to provide clear picture of betel-nut-associated carcinogenesis and may also provide clues to preventive measures. ❐

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