Review Article

Chemistry and toxicology of smokeless tobacco

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Abstract

In most parts of the world, tobacco is used for smoking, whereas, in India, tobacco is used for smoking as well as in diverse smokeless forms. Absorption of toxic and carcinogenic chemicals in tobacco and other ingredients added to various products are causally associated with several non-communicable diseases including cancer, especially oral cancer, which is the leading cancer among men and the third most common cancer among women in India. This article highlights the toxicity, mutagenecity and carcinogenic effects of hazardous chemicals present in smokeless tobacco products. This endeavor was based on the extensive review of literature from various sources. The SLT products have influence on cellular metabolism, ability to cause DNA damage, and cancer in experimental animals. It is, therefore, essential to consider the collective role of chemical constituents of SLT products in the causation of adverse effect on human health.

Key words: Chemistry and toxicology, India, smokeless tobacco products

Introduction

In India tobacco is used mainly for smoking and oral use while nasal use is relatively infrequent. The term, smokeless tobacco (SLT) is used to describe tobacco that is not burned before or at the time of use as opposed to cigarettes or *bidis* that are burned to liberate smoke. SLT products range in complexity from tobacco-only, to products containing numerous chemical ingredients and additives.[1-3] This article deals specifically with the chemistry and toxic effects of smokeless tobacco products. SLT products contain a number of toxic, mutagenic or carcinogenic chemicals that can contribute to the onset of non-communicable diseases including cancer, heart disease, diabetes, and other oral pathologies. As a prelude to the chemistry and toxic products in SLT an overview of some common SLT products are given below.

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Smokeless tobacco products

Smokeless tobacco products can be grouped into those used for chewing, sucking, gargling, sniffing, and as dentifrice. Some products are commercially available or a user can prepare the desired product from ingredients.

Products chewed and sucked

- Orally used products are chewed and placed in the space between the lower lip and gums or in the space between the gums and the cheek.
- *Khaini* prepared from sun-dried tobacco and slaked lime is commonly used in the states of Gujarat and Maharashtra.
- *Zarda*, a mixture of tobacco, lime, spices, and occasionally, silver flakes is also added to *pan* and chewed
- *Khiwam*, a mixture of tobacco extract, spices, and additives is a paste-like preparation that may be added to *pan* or chewed as it is
- *Betel quid or pan* contains four main ingredients, betel leaf (*Piper betel*), areca nut, catechu, slaked lime, and tobacco. Spices and flavoring agents may also be added
- *Kharra* is a combination of tobacco, areca nut, lime, and catechu that is chewed in some parts of Maharashtra

- *Mainpuri* tobacco preparation named after the Mainpuri district of Uttar Pradesh contains tobacco, slaked lime, areca nut, camphor, and cloves[4]
- *Mawa*, a mixture of thin shavings of areca nut, tobacco, and slaked lime is widely preferred in Gujarat state
- *Gutka* or *pan masala* with tobacco is a ready-to-eat tobacco product has become extremely popular in all parts of India due to its user friendly packaging. It contains areca nut, slaked lime, catechu, and tobacco as well as flavoring agents and sweeteners that are added to improve taste.[5]

Products used as dentifrice

- *Gudakhu or gul* is a paste prepared from powdered tobacco and molasses. It is applied to the gums and teeth with a finger.
- *Masheri*, also called *mishri*, is made at home by roasting tobacco flakes on a hot griddle until it turns brown or black. It is applied to gums and teeth and retained in the mouth for variable time period.
- *Bajjar* (dry snuff) is another tobacco product used mainly by women for cleaning teeth and gums.
- *Lal dantamanjan* (red tooth powder) is a commercially available tooth powder, while creamy snuff is a tobacco-containing tooth paste.

Product used for sipping

- *Tuibur* is actually water through which tobacco smoke is passed. The water containing chemicals present in tobacco smoke is used for sipping or gargling in the northeastern states of India.

Products used for inhalation

- Nasal snuff is finely ground flavored tobacco that is placed in the nostrils and sniffed.

- *Bidi* tobacco is prepared from sun-dried *Nicotiana tabacum* leaves which are manually shredded, pounded and sieved to obtain flakes of desired size. In the work situation, *bidi tobacco* dust and volatile compounds are inhaled by tobacco processors and *bidi* rollers, inadvertently. These workers also absorb tobacco constituents via the cutaneous route.[6] Tobacco used for *bidi* manufacture is also chewed by the workers. Hence available information on *bidi* tobacco is included here.

Common ingredients present in various SLT products/preparations

Sun-cured unprocessed or processed tobacco of *Nicotiana. tabacum* species is utilized in many SLT products. *Nicotiana rustica* tobacco that contains much higher levels of nicotine and TSNAs[3] is also used. The International Agency for Research in Cancer (IARC) has classified SLT as a Group 1 human carcinogen.[3]

Other components of SLT products of relevance

Areca nut (*Supari*), the seed from the areca palm (*Areca catechu*), is a major ingredient of *gutka*, *Mainpuri, mawa, pan*, and some forms of *zarda*. Like SLT, areca nut has been classified as a Group 1 human carcinogen (IARC, 2004).[2] The major constituents of areca nut are carbohydrates, fats, proteins, crude fiber, polyphenols, alkaloids such as arecoline, arecaidine, guvacine, guvacoline,[7-11] and minerals.[7-9] Presence of sodium, magnesium, chlorine, calcium, vanadium, manganese, bromine, and copper has been reported in areca nut, pan masala, and other areca nut-containing chewing products.[12]

Arecoline, the major alkaloid in areca nut, has been found to stimulate collagen synthesis in fibroblasts;[13] whereas, catechin, flavonoid, and tannin compounds in areca nut cross-linked collagen fibers making them less susceptible to collagenase degradation.[14] It has also been suggested that copper upregulates lysyl oxidase, leading to excessive cross-linking and accumulation of collagen in patients with oral submucous fibrosis (OSF).[15] In a study of seven Indian SLT products, high level of copper were detected in four *gutka* products as compared to *zarda*, creamy snuff, and *khaini*, which do not contain areca nut.[16] Jacob *et al*.,[17] demonstrated a definitive dose-dependent relationship between the frequency and duration of chewing areca nut without tobacco and the development of OSF in users.

Betel leaf contains phenols including chavicol, hydroxychavicol, eugenol, vitamin C, and carotenes.[18] A number of trace elements (e.g., iron, mananese, sodium) are also present.[19]

Slaked lime is composed of calcium hydroxide and is obtained from lime stone or sea shells. In addition, it also contains iron, magnesium, and a number of trace elements.[19,20] The addition of slaked lime and other alkaline agents like magnesium carbonate[21] boost the pH of a product and results in increased availability of free nicotine, the form that is most easily absorbed.

Catechu is the residue resulting from the hot water extraction of heartwood from the *Acacia catechu* tree. Catechu contains catechu-tannic acid, quercetin, and catechu red. Several trace elements have also been detected.[19]

Red tooth powder may contain plant-related materials like ginger, black pepper, long pepper (*Piper longum*),

camphor, prickly ash seeds, clove oil, peppermint, chicory, and Black Myrobalan (*Terminalia chebula*). Some of these are added for their anti-bacterial, antiseptic, anti-fungal, anti-inflammatory, and astringent properties (Dabur product website, 2012). Nicotine has been detected in some brands of red tooth powder.[22]

Toxic and carcinogenic chemicals in SLT

SLT is known to contain approximately 4,200 chemicals.[23] Chemical composition of tobacco changes as the plant grows and also during curing, fermentation, processing, and storage of processed products.[24-28] During the curing process, starch content of leaves declines, the amounts of reducing sugars increase, while polyphenols and carbohydrates in the leaves diminish during fermentation.[3]

Chemicals in SLT include alkaloids such as nicotine, nornicotine, cotinine, anabasine, anatabine, aliphatic hydrocarbons, and hundreds of isoprenoids that produce typical aroma of tobacco leaves. The alkaloid content of tobacco leaves varies greatly depending on the soil conditions, use of fertilizers, and the degree to which the plant is ripened.[29] A number of phytosterols such as cholesterol, campesterols and alcohols, phenolics, chlorogenic acid, rutin, carboxylic acids, turpenes, polyphenols, aromatic hydrocarbons, aldehydes, ketones, amines, nitrites, N- and O-heterocyclic hydrocarbons, pesticides, and alkali nitrates have been detected.[30] Toxic metals including mercury, lead, chromium, and other trace elements and several free amino are also present.[31] Nicotine, the addictive substance, exists in two forms, acid (bound) and base (free). Free or unionized nicotine is most rapidly and easily absorbed in the mouth.[20,32] Slaked lime or other alkaline additives contribute to high pH at which increased amount of free nicotine is delivered to the user.[33]

Carcinogenic compounds in SLT

Carcinogenic compounds in SLT include polycyclic aromatic hydrocarbons, lactones, coumarin, ethyl carbamate, some volatile aldehydes, volatile N-nitrosamines, nitrosamino acids, tobacco specific N-nitrosamines, inorganic compounds, radioactive Polonium 210, and Uranium 235 and 238. N-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1 (3-pyridyl)-1 butanone (NNK), and N-nitrosamino acids are quantitatively the most prevalent strong carcinogens in SLT. 4-[Methylnitrosamino]-1-[3-pyridyl] 1-butanol) (NNAL) is also carcinogenic, while NAB (N9-nitrosoanabasine) is a moderately potent carcinogen and N-nitrosoanatabine (NAT) is generally considered inactive.[30] Both NNK and NNN have been classified by the IARC as carcinogenic to humans (Group 1).[31] Carcinogenic tobacco specific N-nitrosamines are formed by nitrosation of tobacco alkaloids.[34] Hence, nitrate and nitrite levels in SLT assume importance.

Presence of NNK and NNN was reported in green leaves of *N. tabacum* and *N. rustica* tobacco species. Morover, the levels of NNK and NNN in sun-dried tobacco were several fold higher than in green tobacco.[35] *In vitro* nitrosation of BQT at pH 7.4 existing in oral cavity did not increase NNN levels significantly, while a significant increase in NNN levels occurred at pH 2.1 similar to that in the stomach.[36] High concentration of NAT at both pH values indicated that additional N-nitroso compounds may be produced in the oral cavity and stomach of tobacco chewers. Levels of preformed NNN and NAT, B (a) P, and nicotine in aqueous extracts of black and brown *masheri* were higher than in tobacco used for their preparation.[37] HCN and phenols shown to have tumor promoting and co-carcinogenic activities were detected in both brown and black *masheri*. HCN is metabolized in the liver to thiocyanate, which acts as a catalyst in the formation of N-nitrosamines at acidic pH suggesting that HCN metabolism may have contributed to increased levels of N-nitrosamines in *masheri*.[37] *Masheri* also contained a number of carcinogenic and co-carcinogenic PAH.[38]

Chewing tobacco products

Preformed NNK, NNN, and NAT were detected in chewing tobacco sold under brand name Lanka.[39] High levels of NNK, NNN, NAB, and NAT were reported in *zarda* and *khaini* and TSNA level in *gutka* was higher than the permissible limits in food.[22,40] [Table 1 from Stepanov *et al*. 2005).

Pakhale *et al*.,[41] estimated moisture content, pH, nitrite, nitrate, nicotine, and other tobacco-specific alkaloids in Pandharpuri brand of chewing tobacco, *zarda*, and three brands of *mishri* and processed tobacco used for *bidi* manufacture. In that study, pH of two *masheri* brands was found to be higher than that of other products. Nicotine content of Pandharpuri tobacco was maximum, followed by *zarda*. These two products also showed high content of nor-nicotine, which is converted to carcinogenic NNN during curing of tobacco leaves. [Table 2 adapted from Pakhale *et al*. 1997)

Reexamination of chemical composition of ten different SLT products revealed that pH was maximum in *zarda*, nicotine content was highest in *Raja khaini*, nitrate level was the highest in Dabur tooth powder, and cadmium and arsenic levels were the highest in Click Eucalyptus moist snuff (sold previously in India).[21] (see below

Table 1: Tobacco-specific nitrosamines, nitrate, nitrite, and nicotine in Indian smokeless tobacco and related products[a]

Product	Tobacco-specific nitrosamines (μg/g)[b]				NO_3 (μg/g)[c]	NO_2 (μg/g)[c]	Nicotine (mg/g)[d]
	NNN	NAT	NAB	NNK			
Khaini							
Raja	76.9	13.8	8.83	28.4	705	1020	21.3
Hans Chhap	39.4	4.83	3.78	2.34	1,090	1410	19.6
Zarda							
Goa 1000	8.36	1.98	0.48	3.09	966	2.20	14.6
Moolchand Super	6.47	0.64	0.46	1.64	1,320	ND	15.0
Sanket 999	7.77	1.51	0.36	1.99	1.910	2.08	65.0
Baba 120	4.81	1.40	0.19	1.07	1,700	1.63	44.2
Shimla	19.9	1.53	1.19	2.61	1,360	2.53	13.8
Other Tobacco							
Hathi Chhap	2.75	1.53	0.23	0.85	2,760	1.97	39.5
Gai Chhap	19.2	11.9	1.57	2.61	2,950	8.40	47.8
Miraj	1.74	0.35	0.12	0.08	1,420	13.6	15.6
Mishri							
Shahin	4.21	2.55	0.15	0.87	1,720	5.18	21.0
Gutka							
Star 555	0.47	0.07	0.02	0.13	417	1.61	6.77
Manikchand	0.38	0.05	0.01	0.12	43.9	2.00	3.22
Zee	0.32	0.05	0.01	0.08	62.3	3.42	3.31
Tulsi Mix	0.69	0.07	0.02	0.31	184	2.58	5.67
Wiz	0.31	0.04	0.02	0.13	215	2.82	1.67
Kuber	0.32	0.03	0.01	0.13	47.3	4.50	1.23
Pan Parag	0.44	0.06	0.02	0.12	332	2.84	2.67
Zatpat	1.09	0.08	0.05	0.43	171	1.99	5.48
Vimal	0.09	0.01	ND	0.04	268	1.58	6.82
Josh	0.49	0.08	0.03	0.20	252	1.74	11.4
Supari							
Goa	ND	ND	ND	ND	7.5	4.71	NA
Moolchand	ND	ND	ND	ND	8.5	2.48	NA
Rajnigandha	ND	ND	ND	ND	8.8	3.34	NA
Sanket	ND	ND	ND	ND	8.5	4.27	NA
Shimla	ND	ND	ND	ND	8.0	6.56	NA
Creamy snuff/Toothpaste							
IPCO	3.32	0.53	0.11	1.31	580	ND	4.71
Dentobac	2.52	1.49	0.07	2.16	232	ND	7.71
Snuff							
Click	0.56	0.38	0.02	0.24	2,260	ND	71.4
Tooth Powder							
Baidyanath	0.04	ND	ND	ND	48.6	ND	0.72
New Roshanjyot	ND	ND	ND	ND	11.6	1.25	0.25
Dabur	0.04	ND	ND	ND	27.6	ND	0.58
Reference Snuff							
Kentucky IS3	3.39	3.15	0.25	0.94	3.86	6.35	36.2

[a]All data per gram wet weight, ND: Not detected; detection limit 50 pmol/g tobacco; NA: Not analyzed. [b]Ean of duplicate analyses of product from one package. [c]Lean of duplicate injections of a single sample. [d]Ingle determination. *Source*: Stepanov *et al.* 2005

Table 3 from IARC Vol 89, P 45, 2007). A number of carcinogenic and cocarcinogenic PAH[39] and preformed NNK, NNN, and NAT were found in snuff samples used for inhalation.[40] High levels of nicotine[21] and TSNA were detected in creamy snuff[39] and high levels of nitrite, nicotine, and TSNA were detected in

Table 2: Moisture, pH, and alkaloid content of chewing tobacco products

Tobacco product	Moisture %	pH	Nitrate (mg/g)	Nitrite (μg/g)	UP-SP Nicotine (mg/g)	Nicotine* (mg/g)	Nornicotine* (mg/g)	Anabasin* (mg/g)	Anabatin* (mg/g)	Conitine* (mg/g)
Pandh arpuri	3.99	5.15	4.66	23.05	55.25	54.77	17.11	0.31	0.63	0.37
Zarda	11.58	5.02	5.00	30.80	25.79	26.20	10.23	0.09	0.92	0.15
Masheri Br. 1	7.69	6.33	6.49	11.07	5.52	6.02	0.46	0.05	0.04	0.10
Masheri Br. 2	5.80	7.12	2.26	9.25	18.90	23.08	3.66	0.07	0.38	0.43
Rawa tobacco	9.52	5.18	8.56	9.01	14.35	16.91	4.23	0.72	0.91	0.09
Rawa *Masheri*	4.29	5.89	4.49	16.40	5.60	4.99	0.34	0.74	0.09	0.11
Beedi Tobacco	10.26	5.09	1.15	13.43	37.70	35.15	3.41	0.10	1.53	0.16

UV-SP: Ultraviolet spectrophotometry; *GC-FID: Gas chromatography-flame ionization detection. *Source:* Pakhale *et al.* 1997

Table 3: Chemical composition of smokeless tobacco products used in India

Constituent	Minimum value	Brand	Maximum value	Brand
pH	5.21	Baba *Zarda* 120	10.1	Lime Mix-Miraj Tobacco
Ammonia (μg/g)	4.04	Baidyanath red tooth powder	5280	Gai Chhap Zarda
Total carbonate (μg/g)	140	Dabur red tooth powder	2040	Baba Zarda 120
Nicotine (mg/g)	1.24	Raja *Khaini*[a]	10.16	Dentobac Creamy Stuff
NNN (μg/g)	ND	Click Eucalyptus[b]	7.36	Baba *Zarda* 120
NNK (μg/g)	ND	Click Eucalyptus[b]	4.88	IPCO Creamy Stuff
Benzo[*a*]pyrene (μg/g)	<0.0001	Click Eucalyptus	0.94	IPCO Creamy Stuff
Cadmium (μg/g)	0.03	Click Eucalyptus	0.5	Baba Zarda 120
Arsenic (μg/g)	0.07	Click Eucalyptus	1.53	Shahin Mishri
Nitrate (μg/g)	<0.01	Dabur red tooth powder	13.85	Lime Mix-Miraj Tobacco

ND: Not detected; NNK, 4-(*N*-methyl-*N*-nitrosoamino)-1-3 (3-pyridyl)-1-butanone; NNN: *N*-nitrosonomicotine; [a]*Tuibur* contained no detectable amounts of nicotine. [b]Click Eucalyptus and six other compounds in this report contained nitrosamines other than tobacco-specific *N*: Nitrosamines. *Source:* Gupta 2004

processed tobacco used for *bidi* manufacture.[41]

Many SLT products contain methyl or ethyl salicilates, β-citronellol, 1,8 cineole, menthol, benzyl benzoate, eugenol, and coumarin as flavouring agents.[42-46] Additives such as ammonia, ammonium carbonate, and sodium carbonate raise the pH and free form of nicotine that is readily absorbed from the oral mucosa and carried to the circulating blood.[47]

Toxic metals in SLT products include Pb, Cd, As, Cu, Hg, and Se. Daily intake of each metal was determined in 25 product samples in a study by considering the average amount of *gutka*, *zarda*, creamy snuff, *dantamanjan*, or tobacco-containing red tooth powder, *khaini*, or *masheri* consumed per day. The levels of Pb, As, Cd, and Cu exceeded the permissible daily average of consumption of these metals.[16] Arsenic was detected in all 25 different smokeless tobacco samples analysed.[16]

Biomarkers of tobacco exposure

Specific exposure to toxicants is demonstrated by their presence in the body fluids of exposed individuals, while elevated levels of xenobiotic detoxification products and detoxifying enzymes denote response to toxic agents in general. Presence of nicotine or its metabolite cotinine or tobacco specific nitosamines in body fluids denote specific exposure to tobacco. Elevated urinary thiothers and glucuronide levels indicate non-specific exposure to toxicants, while demonstration of urinary mutagenicity denotes an individual's exposure to mutagens.

TSNA was detected in the saliva of betel quid with tobacco (BQT) chewers[37,48] and also from chewers of tobacco or only *masheri*.[49] Nicotine and cotinine were found in the saliva and urine of chewers of BQT and in chewers of tobacco only.[36] Presence of urinary cotinine was reported in samples from females who used *masheri* (M) only and those habituated to BQT + M.[6,50,51] Cotinine was also detected in the gastric fluid of tobacco and lime chewers.[52] Excessive occupational exposure to tobacco was evident from a seven-fold increase in inspirable dust particles in the breathing zone of tobacco processors[53] and presence of cotinine in urine samples.[54] In a solitary study, tobacco exposure was found to elevate excretion of urinary thioethers and glucuronides among *bidi* rollers.[55] A significant reduction in glutathione s-transferase (GST) activity[56] and glutathione (GSH) level[57] was observed in the lymphocytes of male tobacco chewers female *masheri* users, respectively. Diminished GST activity was

also noted in the lung and liver tissues of rats treated with an extract of chewing tobacco.[58] An increase in the activity of cytochrome P450 and cytochrome b5 enzymes was observed in the liver of mice fed an extract of chewing tobacco by gavage.[59]

Urine samples from females who used *masheri* or *masheri* + BQT were mutagenic in *Salmonella typhimurium* strain TA 100 directly and upon nitrosation with sodium nitrite,[50] while, in another study, samples from *masheri* habitués were mutagenic in strains TA 98 and TA 102 upon metabolic activation with rat liver microsomal fraction (S9).[51] Gastric fluid from chewers of tobacco and lime was directly mutagenic in *S. typhimurium* strains TA 98, TA 100, and TA 102.[52] Occupational exposure to *bidi* tobacco was found to result in direct urinary mutagenicity in strain TA 98 and upon treatment with B glucuronidase among *bidi* rollers, while the samples from tobacco processors exhibited direct mutagenicity in strains TA98, TA100, and TA102.[53]

Toxic effects on cells: Treatment of Syrian golden hamster tracheal epithelial cell line (HTE) with a non-toxic dose of an aqueous extract of *bidi* tobacco induced cellular hypertrophy, widening of intercellular spaces, and stimulated ornithine decarboxylase activity and the rate of DNA synthesis. However, inhibition of cell growth was associated with a significant increase in cell doubling time. Reduction in cell number was associated with blocking of cells in S phase. Growth suppression of cells was reversed after repeated exposure of cells to the *bidi* tobacco extract.[60]

In an *in vivo* study in DMBA-initiated hairless mice, increased epidermal mitotic activity, mild epidermal hyperplasia, increase in epidermal, and dermal thickness were induced by a single application of two increasing doses of an aqueous extract of *bidi* tobacco. These changes persisted upon multiple treatment of mouse skin with the tobacco extract.[61]

Genetic damage caused by SLT products

Mutagenicity of SLT products

A crude ethanolic extract of chewing tobacco[62] and *masheri*[63] were mutagenic in *S. typhimurium* strain TA 98 in the presence of rat liver S9 fraction. Nitrosated extracts of *masheri* and chewing tobacco without lime elicited mutagenic response in strains TA100 and TA102 and TA98 and TA100, respectively.[62] The extract of chewing tobacco and lime was directly mutagenic in all the three tester strains[62] and upon nitrosation in the strain TA102 only. Aqueous and organic extracts of a tobacco blend used for making *bidi*s were not directly mutagenic in any of the tester strains. However, nitrosated aqueous extract elicited mutagenic response in strains TA98 and TA100, while the aqueous ethanolic extract was highly mutagenic in strain TA 98.[62]

Micronucleus formation

Micronucleus assay detects genotoxic effect of exposure to hazardous agents resulting in the formation of small membrane bound DNA fragments or micronuclei in the cytoplasm of interphase cells.

The frequency of micronucleated cells (MNC) was significantly elevated in exfoliated buccal epithelial cells from chewers of BQT and tobacco + lime,[64-66] tobacco and areca nut mixture,[67] regular users of *masheri* alone or *masheri* and BQT,[53,67] people who used *gudakhu* for more than 5 years[68] and in patients with oral cancer.[67] A step-wise increase in MNC frequency was observed in samples from subjects with no tobacco habit, healthy SLT habitués, and those with oral leukoplakia.[69]

The MNC frequency in exfoliated oral mucosal cells from *bidi* rollers and tobacco processors who did not use tobacco in any form, but were exposed occupationally to *bidi* tobacco was significantly elevated as compared to controls with no tobacco exposure.[6,60]

MNC frequency was also elevated in cultured peripheral blood lymphocytes (PBL) from subjects who used *masheri* as a dentifrice.[57]

Cytogenetic alterations

In a study that assessed toxic effects on *allium*, or onion bulbs treatment with *tuibur* was found to reduce root growth. Onion root tip cells exhibited reduced mitotic index, formation of micronuclei, lagging chromosomes, and c-mitosis.[70]

Chromosomal aberrations in PBL: Increased frequency of chromatid breaks, gap-type aberrations, and an increase in sister chromatid exchanges were observed in cultured PBL of male tobacco chewers,[65] female *masheri* users,[57,71] and mawa chewers with normal buccal mucosa, oral sumucous fibrosis, or oral cancer.[67]

A high proportion of tobacco processors with no tobacco habit and those with *masheri* habit exhibited a significantly elevated frequency of chromatid breaks and deletion fragments as compared to respective controls.[71] SCE frequency was also elevated in the lymphocytes of habit-free tobacco processors.[57] A significant increase in the frequency of chromosomal aberrations was observed in PBL from tobacco processors with or without smoking habits[72] with the frequency of chromosomal aberrations increasing with the number of years of exposure.

Carcinogenicity of SLT

Oral administration: Swiss mice fed an extract of

chewing tobacco in diet developed lung adenocarcinoma and hepatocellular carcinoma,[58] while Swiss mice, Sprague–Dawley rats, and hamsters fed black or brown *masheri* developed forestomach papillomas.[73] Gavage feeding of extracts of chewing tobacco or *gutka* alone induced forestomach and esophageal papillomas in a small number of animals, while similar treatment increased the tumor yield in animals initiated with diethylnitrosamine. Histopathology revealed microscopic papillomas in some oral mucosa tissues of tobacco or *gutka*-treated animals.[74] Topical application of an extract of *Banarasi* chewing tobacco extract to the cheek pouch mucosa of Syrian golden hamsters induced leukoplakia only.[75] However, in another study, forestomach tumors developed the following application of snuff to the hamster cheek pouch mucosa.[76]

Skin papillomas were induced following application of black or brown *masheri* extract to the back skin of nude Swiss mice,[63] although topical application of a black *masheri* extract did not induce skin tumors in Swiss mice. Similar treatment, however, resulted in skin papilloma development in hairless Swiss mice and the extract promoted skin papilloma formation in DMBA initiated Swiss mice.[77] Prolonged application of an aqueous extract of chewing tobacco and lime or *gutka* to initiated or uninitiated Swiss Bare mouse skin failed to induce skin tumors, while topical application of *gutka* extract in tumor promotion and progressor stage increased the yield of papillomas and the rate of conversion of papilloma to carcinoma.[74]

Topical application of *bidi* tobacco extract to the back skin of Swiss Bare mice for 40 weeks or only once as a tumor initiator failed to induce skin papillomas in mice receiving TPA as a tumor promoter. However, application of the extract to skin papillomas induced by initiation promotion protocol increased the rate of conversion of papilloma to carcinoma.[61]

Genetic determinants of oral cancer risk associated with smokeless tobacco use

Human susceptibility to cancers caused by tobacco carcinogens is determined by genes involved in metabolism of carcinogens and repair of damaged DNA. In a study on polymorphism at GST gene loci, Buch *et al*.,[78] reported that the GST M1 null genotype is a major risk factor for the development of oral cancer among tobacco chewers. The finding was substantiated in further studies.[79-82] An association was detected between GSTM1 null, GSTT1 null, polymorphic CYP2E1 alleles, and increased risk for oral cancer[80] among SLT users. In a pooled analysis of data, an association between head and neck cancer risk and variations in MGMT and XRCC1 genes involved in DNA repair, alcohol dehydrogenase gene variants, and GSTM1 null genotype was found among tobacco users.[81] A meta and pooled analysis evaluated interactive effect of two genotypes on cancer risk. People with GSTM1 null genotype and the CYP1A1 m1m2 variant allele were reported to be at greater risk for oral and pharyngeal cancer.[82]

Conclusion

In India, where tobacco use includes, not only smoking, but also the use of a variety of SLT products, users of SLT are exposed to a number of toxicants, carcinogens, co-carcinogens, and tumor promoters, SLT use is causally associated with high incidence of oral cancer in India. Another aspect is occupational tobacco exposure of millions of workers in *bidi* industry.

NNN and NNK in SLT and areca nut, the major ingredient of many SLT products, are classified as definite human carcinogens by the IARC. Hence, it should be mandatory to list the contents of nicotine, TSNA and areca nut on every commercial SLT product, along with their cancer causing properties. Product labels should also display the metal contents and their known associations with diseases, e.g., of copper, and its association with oral submucous fibrosis. Such information along with strong health warnings, will highlight potential dangers of SLT use. Along with strong health warnings. Additionally, acceptable limits of heavy metals in SLT products should be determined and enforced.

A battery of biomarkers with strong association for cancer risk needs to be established in large population studies for early detection of individuals with very high cancer risk. Every tobacco processing factory should be mechanized and dust filters fitted into the factories to reduce tobacco dust exposure to workers. Finally, all efforts are needed to reduce the harmful agents present in SLT products.

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