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Genotoxic Effects of Pan Masala and Gutkha: A Review

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Abstract: Chewing habits of plant products have been prevailed in Indian sub-continent since antiquity. A number of chewing products containing betel quid and its ingredients are available in the market. Chewing mixes without tobacco are termed as 'Pan masala' and those containing tobacco as 'Gutkha'. The International agency for research on cancer (IARC) regards the chewing of betel leaf and arecanut to be a known human carcinogen, which have a role in multistage progression of oral cancer. In the present review an attempt has been made to focus on the studies carried out till date on the genotoxicity of Pan masala and gutkha among chewers.

Key words: Pan masala · Gutkha · Genotoxicity

INTRODUCTION

Chewing habits are very common in India and abroad from a longer period. Presently a number of chewing products are available in the market having contents of betel quid, i.e, areca nut, chatechu and lime. Chewing mixes without tobacco are termed as 'Pan Masala' and with tobacco as 'Gutkha'. India is facing a big problem due to industrially manufactured smokeless tobacco product, "Gutkha". Areca nut is a main component of gutkha, which is able to causes oral submucous fibrosis (OSMF) [1]. OSMF is uncurable disease and finally leads to oral cancer [2]. After long time of smoking, adverse effects are seen but in case of gutkha users, OSMF develops within a very short span of time [3]. The intake of gutkha and OSMF is very common in young person [4]. Pan masala contains arecanut as one of its ingredients and is also unfit for health due to its mutagenic, genotoxic and carcinogenic properties. Areca nut increases the chances of formation of Pre-cancerous lesion and oral submucous fibrosis. The composition of Pan masala and Gutkha and their genotoxic agents are mentioned in the table 1.

Harmful Effects of Pan Masala and Gutkha Ingredients

Catechu: Tannin and polyphenols are the main constituents of catechu. Foods those are rich in tannins, have high incidence of oesophageal cancer [6]. Mutagenic property of catechu has been shown by Stich *et al.* [7] and clastogenicity by Giri *et al.* [8].

Lime: Reactive oxygen species generation in oral cavity is favoured by alkaline condition build up by $Ca(OH)_2$ in slaked lime. Lime is responsible for causing irritation and hyperplasia of the oral mucosa [9].

Arecanut: It contains a number of phenolic compounds, which are responsible for the development of proliferative lesions [10].

Tobacco: The leaching of various nitrosamines have been reported from tobacco when kept in mouth [11].

Mechanism of Carcinogenicity in Gutkha and Pan Masala: The genotoxic effects are most likely caused by tobacco and areca nut- specific nitrosamines. The nitrosamines leaches out in the saliva of Pan masala and Gutkha chewers. Secondary and tertiary amines of areca nut and tobacco undergoes to nitration during betel quid chewing when it reacts with nitrite in presence of catalysts [11-12]. Tobacco- specific nitrosamines may undergo metabolic activation by cytochrome P450s and may lead to the formation of N'- nitrosonornicotine (NNK), a major carcinogen [5]. Its further activation lead

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Table 1: Composition of Pan masala and Gutkha [5]

Composition	Ingredients	Agents responsible for genotoxic effects
Pan masala	Catechu - 10% + limeLime - 10% + ArecanutArecanut - 80%	OS, Arecoline, MNPN
Gutkha	Pan masala component +Tobacco	NNN, NNK

NNN: N-nitrosornicotine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

MNPN: 3-(methylnitrosamino) propionitrile; ROS: reactive oxygen species.

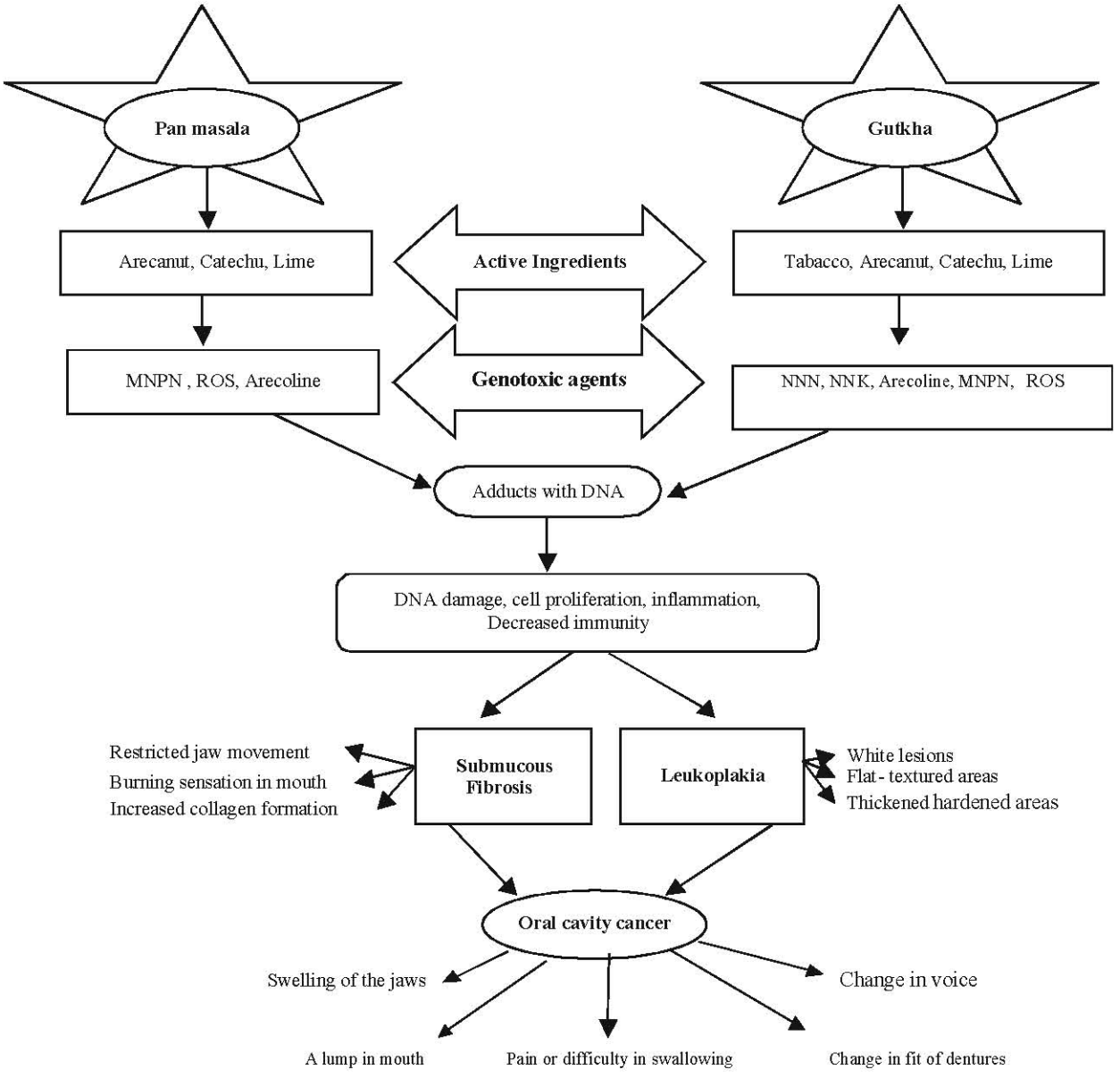

Fig. 1: Possible mechanism of action of the ingredients of Pan masala and Gutkha.

to the DNA damage. The possible mechanisms for the progression of oral cavity cancer among pan masala and gutkha chewers is shown in the Fig 1.

Genotoxicity Studies among Pan Masala and Gutkha Chewers: Micronuclei (MN) are small chromatin bodies that appear in the cytoplasm by the condensation of

Table 2: Studies on the genotoxicity of Pan masala and Gutkha

S.No.	Study	Sources	End- points	References
1.	Slaked lime	PBM	HE	Sirsat and Kandarkar [23]
2.	Betel nut and tobacco	BMC	MN	Stich *et al.* [14]
3.	Arecanut	STS	Mutagenicity	Shirname *et al.* [24]
4.	Arecoline	BMC	SCE	Panigrahi and Rao [25]
5.	Arecanut	BMC	Ames assay	Shirname *et al.* [26]
6.	Catechu extract	Mice	SCE	Giri *et al.* [27]
7.	Arecanut and AC	MBMC	SCE	Panigrahi and Rao [28]
8.	Catechu	Liver tissue	Ames assay	Nagabhushan *et al.* [29]
9.	Pan masala	Ovary cells	SCE and CA	Adhvaryu *et al.* [30]
10.	Arecanut	HBEC	CFFA, NRA, TBA	Sundqvist *et al.* [17]
11.	Pan masala	PBP	Ames assay	Bagwe *et al.* [31]
12.	Pan masala	PBL	SCE, CA and MN	Dave *et al.* [32]
13.	Pan masala	Mice	CA	Mukherjee *et al.* [33]
14.	Pan masala	BMC	SCE	Mukherjee and Giri [34]
15.	Lime	EOMC	CT and MN	Nair *et al.* [18]
16.	Pan masala	CC	CA, SCE and MN	Jaju *et al.* [35]
17.	Arecanut,	Ovary cell	SCE and CA	Dave etal [36]
18.	Pan masala	Rats	MEA	Sarma *et al.* [37]
19.	Pan masala, Arecanut	AEPS	STA	Polasa *et al.* [38]
20.	Tobacco products	EBM	MN	Kayal *et al.* [39]
21	Tobacco products	ME, CTLE	Ames assay	Niphadkar *et al.* [40]
22.	Arecoline	HBF	C and G assays	Chang *et al.* [41]
23.	Tobacco, Pan masala	HOK	NHOKs	Bagchi *et al.* [42]
24.	Pan masala	PBL	CA, SCE and MN	Yadav and Chadha [21]
25.	Tobacco	VTF	DTT	Chadda and Sengupta [43]
26.	Pan masala	Mice	MA	Kumar *et al.* [44]
27.	Pan masala/ Betel quid	TRP	QBT	Gandhi *et al.* [45]
28.	Arecanut	HP	Users and non users	Benegal *et al.* [46]
29.	Tobacco	SNT	DTT	Singh *et al.* [47]
30.	Pan masala/ Gutkha	BMC	MN	Siddique *et al.* [48]
31.	Betel leaf, Arecanut and tobacco	BC and PBL	CA and MN	Sellappa *et al.* [49]
32.	Tobacco	EBC and PBL	MN and CA	Patel *et al.*. [50]
33.	Tobacco	EOEC	MN	Jadhav *et al.* [51]
34.	Pan masala/ Gutkha	BC	MN	Fareed *et al.* [22]

SCE=Sister chromatid exchange, CA= Chromosomal aberrations, CFFA= Colony forming efficiency assay, NRA= Neutral red uptake assay, TBA= Trypan blue exclusion assay, MN= Micronucleus assay, CT= Chemiluminescense Technique, STA= *Salmonella typhimurium* assay, C and G= Cytotoxicity and Genotoxicity assays, NHOKs= Normal human oral keratinocytes cells. HBEC =Human buccal epithelial cells, PBP=Popular brand of pan masala, PBL =Peripheral blood lymphocytes, BMC = Bone marrow cells, EOMC = Exfoliated oral mucosal cells, CC = Culturel cells, AEPS = Aqueous extracts of different brands of pan masala and scented supari, EBM =Exfoliated buccal mucosa, ME =Aqueous extract of masher, CTLE =Chewing tobacco and lime, HBF= Human buccal fibroblast, HOK =Human oral keratinocytes, VTF =Various tobacco forms, TRP = Tobacco and its related products, SNT =Smoking and non smoking forms of tobacco, BC = Buccal cells, EBC = Exfoliated buccal mucosal cells, EOEC = Exfoliated oral epithelial cells, PBM =Palate and buccal mucosa, HE = Histological examination, AC = Arecanut tannins, MBMC = Mouse bone marrow cells, DTT = Different types of tobacco products, QBT = Questionnaire are filled about betel quid type, MEA = Marker enzyme activities, STS = *Salmonella typhimurium* strains, MA = Morphological abnormalities, HP = Human population.

acrocentric chromosomal fragments or by whole chromosomes, lagging behind the cell division. Thus, it is the only biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells, which are easily detected in interphase cells [13]. An elevated micronucleated cell frequency was found in the buccal mucosal epithelium of areca nut chewers [14]. MN assay has been used as a biomarker of genetic damage in buccal mucosa cells [15-16]. The aqueous extract of N-nitroso compound related to areca nut i.e, 3-(N-nitrosomethylamino) propionaldehyde was highly cytotoxic and genotoxic to cultured human buccal epithelial cells and potentially import in the induction of tumors in betel quid chewers [17]. The role of lime in the formation of reactive oxygen species (ROS) from betel quid components has been reported by Nair *et al.*. [18]. An experiment on Swiss mice of S/RV Cri strain was conducted to evaluate the carcinogenic influence of life time exposure to a popular brand of Pan masala [19]. Pan masala intake causes acute increase in pulse and blood pressure [20]. The study on the genotoxic effects of Pan masala using chromosomal

aberration (CA) and sister chromatid exchanges (SCEs) as a parameters were studied in the peripheral blood lymphocytes and tissue directly exposed to pan masala i.e. in exfoliated buccal mucosal cells, conclude that the use of pan masala has possibility of oral cancer epidemic in near future. The findings also suggested that the use of betel leaf in the ingredients of PM may reduce the genotoxic effects [21]. Gutkha and pan masala chewers may be at high risk for the development of oral cancer [22]. A brief description of the studies carried out till date on the genotoxicity of Pan masala and Gutkha chewers are listed in the table 2.

CONCLUSION

Genotoxicity assays such as micronucleus, chromosomal aberrations, sister chromatid exchanges etc. are important in the research field of cancer prevention and therapeutics. These assays play an important role in predicting precancerous stage. MN test is better indicator for genotoxicity damage than chromosomal aberrations or sister chromatid exchange. Increased micronuclei frequency has a higher risk for the development of oral cancer. Therefore, genetic composition of micronuclei must be studied to determine if they contain specific genes associated with oral carcinogenesis. Results of such studies could have a significant impact on the future use of micronuclei as a biomarker due to the Gutkha and Pan masala chewing is expected to increase in future. The alarming scenario demands that federal regulatory and health agencies and non governmental organizations should launch awareness programmes to inform and educate the public regarding the adverse health consequences and possible cancer risk associated with gutkha and pan masala.

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