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Assessment of potential toxicity of a smokeless tobacco product (naswar) available on the Pakistani market

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ABSTRACT

Background 'Naswar' is a smokeless tobacco product (STP) widely used in Pakistan. It has been correlated with oral and oesophageal cancer in recent clinical studies. The toxic effects associated with STPs have been associated with trace level contaminants present in these products. The toxin levels of Pakistani naswar are reported for the first time in this study.

Methods A total of 30 Pakistani brands of naswar were tested for a variety of toxic constituents and carcinogens such as cadmium, arsenic, lead and other carcinogenic metals, nitrite and nitrate, and nicotine and pH.

Results The average values of all the toxins studied were well above their allowable limits, making the product a health risk for consumers. Calculated lifetime cancer risk from cadmium and lead was 1 lac (100000) to 10 lac (1000000) times higher than the minimum 10E–4 (0.00001) to 10E–6 (0.000001), which is the 'target range' for potentially hazardous substances, according to the US Environmental Protection Agency. Similarly, the level of arsenic was in the range of 0.15 to 14.04 μg/g, the average being 1.25 μg/g. The estimated average bioavailable concentration of arsenic is 0.125—0.25 μg/g, which is higher than the allowable standard of 0.01 μg/g. Similarly, the average minimum daily intake of chromium and nickel was 126.97 μg and 122.01 μg, as compared to allowable 30—35 μg and 35 μg, respectively; a 4—5 times higher exposure. However, beryllium was not detected in any of the brands studied. The pH was highly basic, averaging 8.56, which favours the formation of tobacco specific amines thus making the product potentially toxic. This study validates clinical studies correlating incidence of cancer with naswar use in Pakistan.

Conclusions This study shows that the production, packaging, sale and consumption of naswar should be regulated so as to protect the public from the health hazards associated with its consumption.

INTRODUCTION

Tobacco use in Pakistan is common. About 54% men and 20% women in Pakistan use tobacco in one form or other. It is used in the form of cigarettes and also in the form of beedis (hand-rolled cigarettes), huqqa (water pipe) and smokeless tobacco products (STPs) such as naswar.[1]

Naswar is a STP widely used in Pakistan, Afghanistan, Iran and the Central Asian Republics, and in South Africa. It is a mixture of mainly sun-dried, powdered local tobacco (*Nicotina rustica*), ash, slaked lime, and in some areas flavouring agents (eg, cardamom, menthol) and colouring agents (indigo). It is made by pouring water into a cement-lined cavity to which lime is added, followed by tobacco. Colouring and flavouring agents are then added. The ingredients are then pounded and mixed with a heavy wooden mallet. Water is added and the mixture is then moulded into various shapes and subsequently packed into small polythene bags. It is consumed by placing it in the mouth cavity, usually between the oral mucosa and gingival cavity or sometimes under the tongue (floor of the mouth). After about half an hour it is then spat out.[2]

Naswar contains various types of substances, among which nicotine is addictive.[3] About 4000 substances have been reported to be present in tobacco, including toxic substances such as tobacco-specific nitrosamines (TSNAs) specially 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN), arsenic, beryllium, cadmium, nickel, chromium, cobalt, lead, nitrate and nitrite.[4 5] The first seven have been declared by WHO's International Agency for Research on Cancer as group 1 carcinogens, and the last four as group 2 carcinogens.[6] Group 1 is the agent/mixtures that are proved carcinogens, and the exposure circumstance entails exposures that are carcinogenic to humans. Group 2 includes agents, mixtures and exposure circumstances for which the degree of evidence of carcinogenicity in humans is almost sufficient, or there is evidence of their carcinogenicity in experimental animals, but data in humans are lacking.[7] The levels of the various TSNAs depend on various factors such as curing method, pH, and nitrite and nitrate content.[7 8] Marked variations have been reported in the contents of toxic substances in tobacco products. For example toombak from Sudan has been shown to contain the highest quantities of TSNAs so far detected in these products.[9 10] Snus produced by the Swedish Match Company of Canada have been shown to contain the smallest amounts of these substances.[5]

The use of naswar has been associated with various complications. The incidence of cancer associated with naswar and other STPs have been reported in various studies.[11–15] Greater incidence of cancer of the oral cavity and oesophagus in individuals using naswar in Pakistan has been reported in the recent clinical studies.[16–19] Oral cancer attributable to use of STPs are about 50% of the total oral cancer cases reported in Pakistan.[19] Similarly, consumption of naswar has also been correlated with higher incidence of

Table 1 Brands of smokeless tobacco product (naswar) by company

Karachi	Bannu	Swabi	Mardan	Charsadda	Quetta	Jhob	Mohmand Agency
Chinar Gul Pellitized Sardar & Irfan Green Naswar Khi Chinar Gul Smooth Makki	Wali Zaman Haji Ghani Badshah Jan Laram Mukki International	4-Star Shaheen Lakki Bechumarka Special Khamar Laram Marka Babar Sher Shaheen Supreme Khyber Marka Khumar	Khamar Shabazgara Khamar Balagari Saifoor Naswar	Gul Chaqwar Bilal	Jam M Chocolate Green Naswar Que	Three Star	Katari Naswar Toor Naswar Gul Mohmand Lachiwala

peptic ulcer disease.[20] These complications may be due to the above-mentioned toxic substances present within this product.

To the best of our knowledge no study has so far reported the toxicity profile of naswar in Pakistan. However, there are extensive data on such products available elsewhere, specifically in the US and India.[7] Due to the above-mentioned facts we investigated the toxicity profile of this extensively used local product. In this study we have determined the basic toxic constituents such as nicotine, toxic metals, nitrate and nitrite etc. and further research is being carried out in our laboratory to determine TSNA levels such as that of 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN) and their biomarkers such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

We hope this study will help to create awareness in the general public about the health hazards associated with naswar, thus making a significant contribution to the betterment of human life.

MATERIALS AND METHODS

Materials

A total of 30 brands of naswar were evaluated (table 1). The products were picked up personally from local markets of Bannu, Mohmand Agency, Swabi, Charsadda, Mardan, Quetta and Karachi in February 2010. As there is no industry that prepares the product on a national level the samples were collected from the local small-scale manufacturers that possess certain brand names for their products, indicating their popularity. Samples were picked up from shops and were labelled with unique identifiers and stored in double-wrapped plastic bags at 4°C until they were used for analysis.

Methods

Methodologies used were based on Health Canada, Centers for Disease Control, or in-house techniques based on the most up-to-date literature available.[21 22]

The carcinogenic potential of naswar was then evaluated using the method used by Ayo-Yusuf *et al* for selected STP constituents for which the comparable carcinogenic potency data is available in the University of California (Berkeley) carcinogenic potency database and which have been proven to be human carcinogens.[23]

The formula used was: incremental lifetime cancer risk=$ADE_{lifetime}$×CPF (where $ADE_{lifetime}$=lifetime average daily oral exposure (mg/kg bodyweight/day) and CPF=cancer potency factor ((mg/kg bodyweight/day)$^{-1}$)).

$ADE_{lifetime}$ values were calculated using formula: $ADE_{lifetime}$=ADE×no. of years snuffing/average lifetime (where no. of years snuffing and average lifetime were assumed to be 30 and 70 years, respectively).

ADE values were calculated assuming 10 g dry weight of naswar per day. Cancer potency factor values for cadmium and lead were taken from Ayo-Yusuf *et al* as follows: cadmium: 46.1 ((mg/kg bodyweight/day)$^{-1}$); lead: 0.02 ((mg/kg bodyweight/day)$^{-1}$). The values obtained were then compared with the 10E–4 to 10E–6 range (1 in 10 000 to 1 in 1 000 000), which is the 'target range' for potentially hazardous substances, according to the US Environmental Protection Agency (USEPA).[23]

Carcinogens for which data was not available in the carcinogenic potency database were compared with their corresponding standards given in the scientific literature, as shown in table 2.

RESULTS

Naswar toxicity

Table 3 shows the characteristics of various brands of naswar measured in this study.

Nicotine and pH

The concentration of nicotine and pH are given in table 3. The highest concentration of nicotine was observed in Three Star (26.68 mg/g), followed by Chinar Gul Smooth (20.05 mg/g). The concentration of nicotine in 16 brands, namely Badshah Jan Laram, Wali Zaman, Bilal, Toor, Haji Ghani, Jam M Chocolate, Katari Naswar, Khamar Shabaz Gara, Makki, B-52, Gul, Sardar & Irfan, Mukki International, Babar Sher and Khumar was in the range of 15–20 mg/g. Saifoor Naswar, Chinar Gul Pelletized, Laram Marka, Shaheen Supreme, Khyber, Chaqwar, Green Naswar, Gul Mohmand Lachiwala, Green Naswar and Lakki Bechu contained nicotine in the range of 10–15 mg/g. Khamar Bala Gari, 4-Star Shaheen and Special Khamar Torlandi were found to possess nicotine in the range of 5–10 mg/g. The average nicotine content of all products was found to be 14.667 mg/g.

Similarly, pH values were observed in the range of 8.5–9.0 for 21 brands, namely Badshah Jan Laram, Mukki International, B-52, Babar Sher, Shaheen Supreme, Sardar & Irfan, Khumar, Khyber Marka, Gul Mohmand Lachiwala, Makki, Toor, Lakki Bechu, Khamar Bala Gari, Jam M Chocolate, Katari Naswar, Laram Marka, 4-Star Shaheen, Chaqwar, Green Naswar, Bilal, Special Khamar Torlandi, Haji Ghani, Three Star and Wali Zaman. Khamar Shabaz Gara, Green Naswar, Gul, Saifoor,

Table 2 Standards of comparison for chromium, nickel and arsenic

Metal	Daily recommended allowance	Source
Chromium	30–35 μg/day	Agency for Toxic Substances and Disease Registry[24 25]
Nickel	35 μg/day	Agency for Toxic Substances and Disease Registry[26]
Arsenic	0.01 μg/g	International Agency for Research on Cancer[27]

Table 3 Characteristics of various brands of naswar

Brand name	Nicotine mg/g*	Cr mg/kg†	Pb mg/kg†	As mg/kg†	Ni mg/kg†	Cd mg/kg†	Be mg/kg†	Nitrite mg/kg†	Nitrate mg/kg†	pH*
Badshah Jan Laram	17.14	8.2	21.2	0.75	15	1.35	BDL	BDL	3.31	8.63
Mukki International	15.5	8.1	16.2	1.42	18.05	0.75	BDL	BDL	3.54	8.78
B-52	15.56	9.65	26	1.19	15.7	2.15	BDL	BDL	1.70	8.7
Gul	15.22	4.25	35.65	0.59	7.15	1	BDL	BDL	1.11	8.1
Babar Sher	17.23	5.7	46.25	0.52	4.85	2.45	BDL	BDL	0.88	8.5
Saifoor Naswar	13.07	7.35	27.2	0.6	7.25	0.95	BDL	BDL	1.60	8.49
Chinar Gul Pelletized	13.73	25.8	25.05	1.4	18.8	0.95	BDL	1.30	0.846	8.24
Shaheen Supreme	13.61	12.1	35.25	0.9	9.15	1.55	BDL	1.85	0.50	8.59
Sardar & Irfan	15.92	14.45	20.65	0.15	15.15	1.55	BDL	2.11	1.308	8.5
Khumar	16.07	4.2	35.5	0.7	9.05	3.2	BDL	2.45	1.22	8.96
Chinar Gul Smooth	20.05	0.8	21.8	1.66	13.1	0.6	BDL	2.56	1.6	8.12
Khyber	13.6	3.7	16.2	0.42	5.45	1.05	BDL	2.72	2.09	8.56
Gul Mohmand Lachiwala	13.58	54.05	63.75	0.67	64.85	3.15	BDL	2.90	0.80	8.39
Makki	16.12	17.4	48.2	1.7	18.25	7.25	BDL	3.31	2.29	8.59
Toor Naswar	15.9	8.5	12.45	14.04	8.6	0.95	BDL	3.36	0.16	8.35
Lakki Bechu	7.8	10.9	111.15	BDL	4.4	9.2	BDL	4.20	3.59	8.93
Khamar Shabaz Gara	19.26	7.1	23.65	0.69	6.8	0.8	BDL	4.49	0.417	8.14
Green Naswar Que	12.16	4.5	12.4	0.63	10.75	BDL	BDL	4.5	1.16	8.48
Khamar Bala Gari	13.94	2.5	19.8	0.53	8.25	0.5	BDL	4.93	0.43	8.73
Jam M Chocolate	15.82	34.9	13.3	0.39	21.7	0.25	BDL	5.72	3.17	8.65
Katari Naswar	15.08	7.6	19.75	1.38	12.5	1.9	BDL	6.37	0.15	8.25
Laram Marka	10.22	8.55	35.7	0.85	7.65	0.7	BDL	6.55	2.23	8.75
4-Star Shaheen	9.94	15.05	20.2	0.84	9.05	1.5	BDL	6.66	2.06	8.76
Chaqwar	11.7	13.4	43.7	1.02	11.55	2.2	BDL	6.78	0.95	8.86
Green Naswar Khi	11.1	4.85	31.25	0.25	BDL	BDL	BDL	7.18	2.32	8.85
Bilal	15	11.15	16	1.35	12.15	1.3	BDL	7.62	1.84	8.54
Special Khamar Torlandi	7.35	11.9	45.15	BDL	2.2	8.85	BDL	8.32	1.63	8.5
Haji Ghani	15.72	4.4	24.85	0.87	10.9	1.35	BDL	8.53	1.63	8.63
Three Star	26.68	4.05	67.45	0.38	3.1	5.2	BDL	8.88	1.51	8.63
Wali Zaman	15.94	10.85	61.2	1.59	14.65	7.45	BDL	12.3	2.04	8.77

*Nicotine and pH levels are the mean of two observations.
†Values are from single observations.
BDL, below detectable levels.

Chinar Gul Smooth, Chinar Gul Pelletized had pH values in the range of 8–8.5. The average pH of all 30 brands studied was 8.56.

Group 1 carcinogens

Four group 1 carcinogens were measured in naswar in this study, as outlined below.

Cadmium

The concentration of cadmium in various brands is given in table 3. In Green Naswar Que and Green Naswar Khi cadmium was not detected. The highest concentration of cadmium was observed in Lakki Bechu Marka (9.2 mg/kg), while the range in all brands was 0.25–9.2 mg/kg. The average cadmium concentration in all brands was 2.34 mg/kg.

Chromium

Table 3 shows the concentration of chromium in all the brands studied. The highest concentration of chromium was found in Gul Mohmand Lachiwala (54.05 mg/kg), while the range was 0.8–54.05 mg/kg. The average concentration of chromium in all brands was found to be 13.70 mg/kg.

Nickel

The concentrations of nickel observed in various brands of naswar in this study are given in table 3. In Green Naswar nickel was not detected. The highest concentration was observed in Gul Mohmand Lachiwala (64.85 mg/kg). The concentration in other brands ranged from 2.2 to 64.85 mg/kg. The average nickel concentration in all brands was 12.20 mg/kg.

Arsenic

The arsenic concentration observed in various brands of naswar is given in table 3. In Lakki Bechu and Special Khamar Torlandi the arsenic level was below the detection limit. The level of arsenic was in the range of 0.15–14.04 mg/kg and the average arsenic concentration was 1.25 mg/kg.

Beryllium

Beryllium was not detected in any of the samples studied.

Group 2 carcinogens

Three group 2 carcinogens were detected in this study, as outlined below.

Lead

The levels of lead observed in various brands of naswar are given in table 3. The level of lead was alarmingly high in all the brands; the range being 12.4–111.15 mg/kg. The highest concentration was detected in Lakki Bechu Marka (111.15 mg/kg). The average concentration of lead in all the brands studied was 21.055 mg/kg. The very high level of this carcinogen is clearly putting the health of the users at risk and warrants the immediate attention of health regulatory authorities.

Nitrate and nitrite

Concentrations of nitrate and nitrite are given in table 3. Nitrate was present in the range of 0.15–3.59 mg/kg, the average being 1.78 mg/kg, while nitrite was present in the range of 1.3–12.3 mg/kg, the average being 4.18 mg/kg. The highest concentrations of nitrate and nitrite were found in Lakki Bechu

Table 4 Assessment of potential toxicity of naswar

Brand name	ADE lifetime Pb	ADE lifetime Cd	Lifetime cancer risk Pb (100% transfer)	Lifetime cancer risk Cd (100% transfer)	Lifetime cancer risk Pb (6% transfer)	Lifetime cancer risk Cd (6% transfer)	Total lifetime risk Pb + Cd (100%)	Total lifetime risk Pb + Cd (6%)
Green Naswar Que	53.32	BDL	1.0664	–	0.063	–	–	–
Green Naswar Khi	134.37	BDL	2.6874	–	0.16	–	–	–
Laram Marka	153.5	3.01	3.07	13	0.184	0.78	0.964	1.744
Jam M Chocolate	57.19	1.07	1.1438	49.32	0.068	2.95	3.018	5.968
Khamar Bala Gari	85.15	2.15	1.703	99.11	0.102	5.94	6.042	11.982
Chinar Gul Smooth	93.74	2.58	1.8748	118.93	0.112	7.13	7.242	14.372
4-Star Shaheen	86.86	6.45	1.7372	138.76	0.103	8.32	8.423	16.743
Mukki International	69.66	3.22	1.3932	148.44	0.083	8.9	8.983	17.883
Khamar Shabaz Gara	101.69	3.44	2.0338	158.58	0.121	9.51	9.631	19.141
Toor Naswar	53.53	4.08	1.0706	188.088	0.064	11.28	11.344	22.624
Chinar Gul Pelletized	107.71	4.08	2.1542	188.08	0.129	11.28	11.409	22.689
Saifoor Naswar	116.96	4.08	2.3392	188.08	0.14	11.28	11.42	22.7
Gul	153.3	4.3	3.066	198.23	0.183	11.89	12.073	23.963
Khyber	69.66	4.51	1.3932	207.911	0.083	12.47	12.553	25.023
Bilal	68.8	5.59	1.376	257.69	0.082	15.46	15.542	31.002
Badshah Jan Laram	91.16	5.8	1.8232	267.38	0.109	16.04	16.149	32.189
Haji Ghani	106.8	5.8	2.137	267.38	0.127	16.04	16.167	32.207
Sardar & Irfan	88.79	6.66	1.7758	307.02	0.106	18.42	18.526	36.946
Shaheen Supreme	151.4	6.66	3.029	307.02	0.181	18.42	18.601	37.021
Katari Naswar	84.92	8.17	1.6984	376.63	0.101	22.59	22.691	45.281
B-52	111.8	9.24	2.236	425.96	0.134	25.55	25.684	51.234
Chaqwar	187.9	9.46	3.7582	436.1	0.225	26.16	26.385	52.545
Babar Sher	198.8	10.5	3.9774	485.43	0.238	29.12	29.358	58.478
Gul Mohmand Lachiwala	274.1	13.54	5.4824	624.19	0.328	37.45	37.778	75.228
Khumar	152.65	13.76	3.053	634.33	0.183	56.05	56.233	112.23
Three Star	290.03	22.36	5.8006	1030.79	0.348	61.84	62.188	124.02
Makki	207.2	31.1	4.1452	1436.93	0.248	86.21	86.458	172.66
Wali Zaman	263.1	32.0	5.2632	1476.58	0.315	88.59	88.905	177.55
Special Khamar Torlandi	194.1	38.0	3.8828	1754.1	0.232	105.24	105.50	210.72
Lakki Bechu	478.0	39.6	9.5588	1823.71	0.573	109.42	109.99	219.43

ADE, average daily exposure; BDL, below detectable levels.

Marka (3.59 mg/kg) and Wali Zaman Bannu (12.3 mg/kg), respectively.

Potential toxicity of naswar

Tables 4 and 5 give the potential toxicity of naswar. The ADE for cadmium and lead was calculated assuming 10 g dry weight naswar exposure. Lifetime cancer risk for cadmium and lead was calculated for 100% transfer, and then reduced cancer risk, based on the bioavailability of the carcinogens (which is 6%[23]), was evaluated. Total lifetime cancer risk was calculated for individual brands by combining the risk from cadmium and lead. Similarly, daily exposure from 10 g of naswar was calculated for chromium and nickel. Arsenic was compared with allowable concentration in ppm (μg/g).

DISCUSSION

The aim of this study was to assess for the first time the potential toxicity of the STP naswar that is widely consumed in Pakistan, especially by the Pathan (also known as Pashtun) population. Total lifetime cancer risk calculated for cadmium and lead according to USEPA for all the brands studied ranges from 1.744 to 219.43 (2.19E-2). The average lifetime cancer risk is 55.785 (5.5785E-1), which is about 1 (10E5) to 10 lac (10E6) times higher than the minimum 10E-4 to 10E-6, which is the 'target range' for potentially hazardous substances, according to USEPA. This risk is besides that posed by the other carcinogens present, namely arsenic, chromium and nickel. Arsenic is present in the range of 0.15 to 14.04 μg/g, the average being 1.25 μg/g. Oral bioavailability of arsenic in the indigenous cynomolgus monkey is 10% to 20%.[28] Thus the estimated average bioavailable concentration of arsenic is 0.125–0.25 μg/g, which is higher than the standard of 0.01 μg/g. Similarly, the average minimum daily intake of chromium and nickel was 126.97 and 122.01 μg, as compared to 30–35 μg and 35 μg, respectively; a fourfold to fivefold higher exposure. The oral bioavailability of chromium in rats is 40%,[29] while that of nickel is 27% (in water) and <10% (in meal) in humans.[30] The very high levels of these established carcinogens are clearly putting the health of users at risk.

The levels of TSNA depends on various factors such as curing methods, pH and nitrite and nitrate contents.[31] Nitrate is reduced to nitrite that then reacts with nicotine and other alkaloids to produce nitrosamines such as NNN and other carcinogens. This formation of carcinogen is favoured by the presence of high concentrations of nitrate and nitrite along with nicotine in a basic environment.[32] Larger amounts of nitrates are reduced to nitrite by certain microbial agents present in STPs, resulting in high levels of formation of TSNAs.[32] In the current study the levels of nicotine (7.35–26.68 mg/g, average 14.667 mg/g), nitrate (0.15–3.59 mg/g, average 1.78 mg/g), nitrite (1.3–12.3 μg/g, average 4.18), basic pH (8.1–8.96, average 8.56), and unhygienic handling during manufacturing and other processing are all indicative of the larger amounts of nitrosamines (not determined directly in the current study); further research is being carried out in our laboratory to determine NNN, NNK, NAB etc. levels, and also their biomarkers in the body such as NNAL.

Table 5 Assessment of potential toxicity of naswar

Brand name	Cr μg/10 g	As μg/g	Ni μg/10 g
Badshah Jan Laram	531.77	0.75	150
Mukki International	81	1.42	180.5
B-52	96.5	1.19	157
Gul	42.5	0.59	71.5
Babar Sher	57	0.52	48.5
Saifoor Naswar	73.5	0.6	72.5
Chinar Gul Pelletized	258	1.4	188
Shaheen Supreme	121	0.9	91.5
Sardar & Irfan	144.5	0.15	151.5
Khumar	42	0.7	90.5
Chinar Gul Smooth	8	1.66	131
Khyber	37	0.42	54.5
Gul Mohmand Lachiwala	540.5	0.67	648.5
Makki	174	1.7	182.5
Toor Naswar	85	14.04	86
Lakki Bechu	109	BDL	44
Khamar Shabaz Gara	71	0.69	68
Green Naswar Que	45	0.63	107.5
Khamar Bala Gari	25	0.53	82.5
Jam M Chocolate	349	0.39	217
Katari Naswar	76	1.38	125
Laram Marka	85.5	0.85	76.5
4-Star Shaheen	150.5	0.84	90.5
Chaqwar	134	1.02	115.5
Green Naswar Khi	48.5	0.25	–
Bilal	111.5	1.35	121.5
Special Khamar Torlandi	119	BDL	22
Haji Ghani	44	0.87	109
Three Star	40.5	0.38	31
Wali Zaman	108.5	1.59	146.5

In addition it has been shown that the carcinogenic effect of STPs such as naswar is augmented by certain factors like nutritional deficiencies, alteration in oxidative enzymes[32] and microbial contamination of the product.[19] These factors are highly probable to be present in the war-affected and flood-affected population of Pakistan.

All these findings validate the clinical data that correlate cancer occurrence with naswar.[16–19]

The above-mentioned facts necessitate the regulation of naswar. The manufacturing should be regulated, and local small-scale manufacturing in shops should be discouraged. Toxin standards should be established for naswar and there should be proper packaging that should be labelled with proper warning slogans, and sale to teenagers should be banned. In this regard the Gothiatek technique developed by Swedish Match for the manufacture of naswar can be adopted, at least to reduce the major toxin levels in naswar to as low a level as possible, although this will not bring the toxin levels into an acceptable range.[23] High levels of toxic heavy metals may be due to the use of ashes and lime in naswar. Thus their levels could be minimised by replacing ash with other binding agents, and the pH can be adjusted with other suitable agents instead of lime. Reducing the basicity will also indirectly decrease the formation of TSNAs, thus further reducing the product's potential for harm. Microbial contamination could be minimised during manufacturing and storage etc., by adopting hygienic practices. These measures will reduce the potential toxicity of naswar.

The results of this study require that awareness should be raised in the general public regarding the use of naswar. Awareness campaigns in the form of conferences, seminars, presentations and talks should be organised by governmental and non-governmental organisations using mass media to make the population aware of its adverse health effects. Similarly, health authorities should arrange naswar cessation programmes.

All these measures will help protect the general public from the adverse effects of naswar.

What this paper adds

- This study has for the first time evaluated the potential toxicity of naswar used in Pakistan.
- It is evident from this work that this smokeless tobacco product is a potential health risk and must be regulated (to date there is no regulation whatsoever) to avoid its adverse health effects.

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REFERENCES

1. **Pakistan Medical Research Council.** *National Health Survey of Pakistan 1990–94*. Islamabad, Pakistan: Pakistan Medical Research Council, Network Publication Service, 1998:50–2.
2. **Voges E.** The pleasures of tobacco — how it all began and the whole story. *Tob J Int* 1984:80–2.
3. **Tomar SL.** Is use of smokeless tobacco a risk factor for cigarette smoking? The U.S. experience. *Nicotine Tob Res* 2003;**5**:545–53.
4. **Hoffmann D,** Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. *Chem Res Toxicol* 2001;**14**:767–90.
5. **Rickert WS,** Joza PJ, Trivedi AH, *et al*. Chemical and toxicological characterization of commercial smokeless tobacco products available on the Canadian market. *Regul Toxicol Pharmacol* 2009;**53**:121–33.
6. *International Agency for Research on Cancer IARC monographs 1–100* last updated list 26 May 2010. (http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf (accessed 26 May 2009).
7. *International Agency for Research on Cancer. Monograph 89: Smokeless Tobacco and Some Tobacco specific N-Nitrosamines*, 2007. http://monographs.iarc.fr/ENG/Monographs/vol89/index.php (accessed 16 Aug 2009).
8. **Richter P,** Spierto FW. Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content. *Nicotine Tob Res* 2003;**5**:885–9.
9. **Hoffmann D,** Djordjevic MV, Fan J, *et al*. Five leading U.S. commercial brands of moist snuff in 1994: assessment of carcinogenic *N*-nitrosamines. *J Natl Cancer Inst* 1995;**87**:1862–9.
10. **Idris AM,** Nair J, Ohshima H, *et al*. Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (toombak). *Carcinogenesis* 1991;**12**:1115–18.
11. **Prokopczyk B,** Wu M, Cox JE, *et al*. Improved methodology for the quantitative assessment of tobacco-specific *N*-nitrosamines in tobacco by supercritical fluid extraction. *J Agric Food Chem* 1995;**43**:916–22.
12. **Jafarey NA,** Mahmood Z, Zaidi SH. Habits and dietary pattern of cases of carcinoma of the oral cavity and oropharynx. *J Pak Med Assoc* 1977;**27**:340–3.
13. **Goud ML,** Mohapatra SC, Mohapatra P, *et al*. Epidemiological correlates between consumption of Indian chewing tobacco and oral cancer. *Eur J Epidemiol* 1990;**6**:219–22.
14. **Wasnik KS,** Ughade SN, Zodpey SP, *et al*. Tobacco consumption practices and risk of oro-pharyngeal cancer: a case–control study in central India. *Southeast Asian J Trop Med Public Health* 1998;**29**:827–34.

15. **Merchant A,** Husain SS, Hosain M, *et al*. Paan without tobacco. an independent risk factor for oral cancer. *Int J Cancer* 2000;**86**:128—31.
16. **Jamal S,** Ahmad M, Khan AH, *et al*. Carcinoma esophagus - a clinicopathological study of 50 cases at Armed Forces Institute of Pathology, Rawalpindi. *Pak J Patho* 1997;**8**:24—8.
17. **Roohullah,** Khursheed AK, Burdey GM, *et al*. Cancer of esophagus: ten years experience at CENAR, Quetta. *J Ayub Med Coll Abbottabad* 2001;**13**:4—7.
18. **Khan AM,** Khan MS, Haq N. Oral snuff and carcinoma oesophagus. *Gomal Jour Med Sci* 2009;**7**:58—61.
19. **Bofetta P,** Hecht S, Gray N, *et al*. Smokeless tobacco and cancer. *Lancet Oncol* 2008;**9**:667—75.
20. **Afridi MA.** Tobacco use as contributory factor in peptic ulcer disease. *J Coll Physicians Surg Pak* 2003;**13**:385—7.
21. **Tobprac.** http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/side-second/nicotine-eng.php (accessed 7 Jul 2009).
22. **Saeed M,** Muhammad N, Khan H, *et al*. Analysis of toxic heavy metals in branded pakistani herbal products. *Jour Chem Soc Pak* 2010;**32**:471—5.
23. **Ayo-Yusuf OA,** Connolly GN. Applying toxicological risk assessment principles to constituents of smokeless tobacco products: implications for product regulation. *Tob Control* 2011;**20**:53—7.
24. **IOM.** *Dietary Reference Intakes for Vitamin A, Vitamin K, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington D.C: National Academy Press, 2001;290—442.
25. **Agency for Toxic Substances and Disease Registry (ATSDR).** *Department of Public Health and Human Services*. Atlanta, GA, U.S: Public Health Service, 2008.
26. **Agency for Toxic Substances and Disease Registry (ATSDR).** *Toxicological Profile for Nickel (Update)*. Atlanta, GA, 2005. http://www.google.com.pk/search?hl=en&source=hp&biw=1003&bih=567&q=Agency+for+Toxic+Substances+and+Disease+Registry+%28ATSDR%29.+ToxicologicalProfile+for+Nickel+%28Update%29.+Atlanta%2C+GA%2C+2005&rlz=1W1ADRA_en&oq=Agency+for+Toxic+Substances+and+Disease+Registry+%28ATSDR%29.+ToxicologicalProfile+for+Nickel+%28Update%29.+Atlanta%2C+GA%2C+2005&aq=f&aqi=&aql=f&gs_sm=e&gs_upl=2712l43795l0l2l2l0l1l0l0l215l215l2-1 (accessed 10 Apr 2010).
27. *Iarc Monographs On The Evaluation Of Carcinogenic Risk To Humans: Report On Smokeless Tobacco And Some Tobacco-Specific N-Nitrosamines*. Lyon, France: International Agency for Research on Cancer, 1985: 37—136.
28. **Roberts SM,** Munson JW, Lowney YW, *et al*. Relative oral bioavailability of arsenic from contaminated soils measured in the cynomolgus monkey. *Toxicol Sci* 2007;**95**:281—8.
29. **Witmer CM,** Park HS, Shupack SI. Mutagenicity and disposition of chromium. *Sci Total Environ* 1989;**86**:131—48.
30. **Sunderman FW Jr,** Hopfer SM, Sweeney KR, *et al*. Nickel absorption and kinetics in human volunteers. *Proc Soc Exp Biol Med* 1989;**191**:5—11.
31. **Agency for Toxic Substances and Disease Registry (ATSDR).** *Toxicological Profile for Arsenic (Update)*. Atlanta, GA: Department of Public Health and Human Services, Public Health Service, 2007. http://www.google.com.pk/search?hl=en&source=hp&biw=1003&bih=567&q=Agency+for+Toxic+Substances+and+Disease+Registry+%28ATSDR%29.+Toxicological&rlz=1W1ADRA_en&oq=Agency+for+Toxic+Substances+and+Disease+Registry+%28ATSDR%29.+Toxicological&aq=f&aqi=&aql=f&gs_sm=e&gs_upl=2095l2095l0l1l1l0l0l0l0l3l3l1 (accessed 10 Apr 2010).
32. **Ammigan N,** Nagabhushan M, Nair UJ, *et al*. Effect of nutritional status on mutagenicity of urine excreted by rats treated with standard/experimental carcinogens. *Indian J Exp Biol* 1990;**28**:711—13.

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