Bull. Environ. Contam. Toxicol. (2003) 70:1184–1188

DOI: 10.1007/s00128-003-0107-0

Bulletin of Environmental Contamination and Toxicology

Effect of Pan Masala on Sperm Morphology of a Mouse

S. Kumar, S. K. Nigam, S. A. Shaikh, H. N. Saiyed

Division of Reproductive Toxicology and Histochemistry, National Institute of Occupational Health (ICMR), Meghani Nagar, Ahmedabad 380016, India

Received: 2 May 2002/Accepted: 13 March 2002

The habit of chewing pan masala is comparatively new worldwide, but it is prevalent in Indian sub-continent. The aggressive manner in which pan masala is being advertised through the electronic and print media, and the social acceptance of the use of pan masala under the pretext of being safe, has increased alarmingly. The habit of chewing pan masala is quite popular among the teenagers and housewives. Even those who generally refrain from smoking and tobacco chewing are consuming the pan masala. Pan masala generally consists of arecanut (betelnut), catechu (*Acacia catechu*), lime, cardmom (*Eletteria cardomonum*) and unspecified flavoring agents with or without tobacco. Pan masala along with tobacco is consumed as an alternate form of tobacco. Stich and Rosin (1985) reported that one of the greatest exposures of mankind to carcinogens occurred during the chewing of tobacco and betelquid. They also suggested that the use of smokeless tobacco appears to pose the highest risk for the development of oral cancer.

Few reports are available, especially on mutagenicity and clastogenicity of pan masala *in vivo* and *in vitro* (Dave et al. 1991; Patel et al 1994). Dave et al. (1991) demonstrated a significant increase in chromosomal aberrations and micronucleated cells in the peripheral blood cells of pan masala chewers as compared to control. Further, Ramchandani et al. (1998) evaluated carcinogenic and co-carcinogenic activity of pan masala in mouse skin, stomach and oesophagus and reported that habitual pan masala use may exert carcinogenic and co-carcinogenic influence. However, data on the effect of pan masala on reproductive system are scanty (Mukherjee et al.1991). Usually, most of the tobacco chewers spit out macerated quid or macerated quid juice, whereas plain pan masala chewers swallowed the chewed material during the chewing. Thus, in addition to the local effects on the oral and esophageal mucosa, these substances may also likely to have affect on other organ system also.

Keeping in view the mutagenic potential of the pan masala (Dave et al. 1991; Patel et al. 1994; Ramchandani et al. 1998) and available toxicity data on various component of betelquid (IARC, 1985; Kumar and Saiyed, 1999) as well as an earlier report about low birth weight of the offspring of tobacco chewing mothers

Correspondence to: S. Kumar

(Verma et al. 1983), we have conducted preliminary work on male reproductive system of mouse, especially on sperm head morphology, while studying the carcinogenic potential of pan masala in a long term study (Nigam et al. 2001). Further, a report indicating adverse effects of tobacco chewing and lower percentage of motile sperm and total sperm count of tobacco chewers as compared to control (Banerjee et al. 1993), also prompted us to take up this study. The purpose of this study was to investigate the effect of pan masala plain and pan masala with tobacco on sperm head morphology of Swiss albino mice.

MATERIALS AND METHODS

The pan masala plain and pan masala with tobacco locally known as sada and gutkha respectively were procured from the local market. Pan masala both plain and tobacco at the dose of 2% in mouse feed was used in this study, which simulate to the human exposure if consider total diet intake/day of human and mouse. Generally, habitual chewer consumes 10-15 pan masala pouches/day and each pouch contains about 2 gm of pan masala. Hence, human beings consume about 20-30 gm/day, which is more or less comparable to the pan masala, consumed through feed by the mouse. However, if we consider the pan masala consumption by body weight then comparatively higher dose of pan masala was used in the present study.

Adult Swiss albino mice (inbred strain), 8-10 weeks old weighing about 23±3 gm. was selected from Institute's animal house colony. The animals were maintained on standard mice feed and water *ad libitum* and were divided into three groups. The animals of the first group received the pan masala plain (PMP) orally in diet (2% of PMP mixed in feed) while animals of group second received 2% of pan masala with tobacco (PMT) through diet in similar manner. The animals of third group were maintained on standard mice feed, which served as control.

A minimum of four animals from each pan masala treated (PMP and PMT) groups were sacrificed after 3, 9, 12 and 13 months of feeding. Similarly representative animals from control group were also sacrificed for comparison. The cauda epididymides were cleanly dissected out sperms were collected in physiological saline and processed for head shape morphological study. The sperms were stained with 0.5 % of aqueous eosin Y solution for about 30 minutes. A minimum of 2000 sperm was scored in each group in order to note the sperm head shape changes using the criteria of Wyrobek and Bruce (1978) and Kumar et al. (1992, 1999). The data of sperm abnormalities were expressed in percentage of total abnormalities. The data were analysed using Students' 't' test to note the significance between exposed and control groups.

RESULTS AND DISCUSSION

A significant elevation in sperm head shape abnormalities was noticed in both PMP and PMT treated groups as compared to control (Table 1). Significant

elevation in sperm head shape abnormality was observed in the PMT treated group i.e. 9 months after the treatment as compare to 12 months in the PMP treated group.

The present study clearly indicates that both PMP and PMT are having adverse effects on the male reproductive system of mouse as revealed by the elevation in

Table 1. Sperm head shape abnormalities (mean ± SE in percentage) in mouse after chronic exposure to pan masala.

Groups	Autopsy intervals in months			
	3	9	12	13
PMP	1.73 ± 0.18 (3000) NS	1.81 ± 0.31 (2200) NS	2.10 ± 0.18 (2800) P < 0.05	2.50 ± 0.29 (2200) P < 0.01
PMT	1.85 ± 0.24 (2800) NS	2.04 ± 0.26 (2100) P<0.05	2.42 ± 0.30 (2600) P < 0.02	2.65 ± 0.32 (2600) P < 0.01
Control	1.43 ± 0.20 (2300)	1.22 ± 0.25 (2700)	1.50 ± 0.19 (2400)	1.36 ± 0.29 (2200)

PMP – Plain pan masala (without tobacco); PMT – Pan masala Tobacco
Figures in parenthesis are the total number of sperm observed in each group.

the sperm head shape abnormalities. This study also indicates that the long-term chronic exposure may be responsible for the adverse effect on sperm head shape morphology as present study indicated a non-significant elevation in sperm head shape morphology between pan masala treated and non-treated groups after three months of exposure at this dose level. The higher number of sperm head shape abnormalities in the present study may be due to the adverse effects of various ingredients of pan masala especially arecanut alkaloids and tobacco specific N-nitrosamine (TSNA) on the reproductive system.

The results of the present study corroborated with earlier finding of Mukherjee et al. (1991). They have conducted cytogenetic analysis of meiotic metaphase germ cells in the male mice following oral feeding of pan masala and identified abnormalities of head morphology of spermatozoa and also a significant increase in the frequency of breaks at high dose of pan masala treated animals. Further, teratogenic effects of betelnut (main component of pan masala) and its alkaloids have been reported in mouse and rats chronically exposed to betelnut or arecoline (Sinha and Rao 1985). They reported that exposure to betelnut extracts leads to death, enhanced resorption and reduced weight of the foetus etc. The arecanut alkaloids and tobacco specific N-nitrosamine (TSNA) and other components of pan masala those are having cytotoxic and clastogenic potential may be responsible for the elevated level of sperm head shape abnormalities in the present study. The various ingredients of pan masala such as arecanut (a major ingredient of pan masala) and lime has clastogenic and mutagenic potential reported by various investigators (Tanaka et al. 1983; IARC 1985; Panigrahi and

Rao, 1986; Dave et al. 1992). Further, arecoline, the major alkaloid of arecanut has also been shown to inhibit DNA and protein synthesis (Wary and Sharan, 1991). The lower number of sperm head shape abnormalities in PMP treated group as compared to PMT treated group in the present study may be due to the presence of arecanut alkaloids along with other ingredients in PMP while PMT are having both arecanut alkaloids and tobacco specific N-nitrosamine (TSNA) along with other ingredients.

The result of this study is preliminary one and need further investigations on reproductive systems and their markers using other species of mammals with low doses of pan masala. At this movement we are not aware any reproductive effects of pan masala on human being as reproductive dysfunction if any due to pan masala habit may go unnoticed due to lack of apparent clinical sign and symptoms. Our study on carcinogenic potential of pan masala in mouse indicated that pan masala in both plain as well as with tobacco in 2% concentration in feed for 16 weeks could not produce any tumour, but after 56 weeks of exposure tumours were observed in both types of pan masala treated groups and it was more in PMT group as compared to PMP (Nigam et al. 2001). Therefore, a comprehensive epidemiological study on pan masala chewers is required to note the various health problems associated with the use of this substance.

Acknowledgments. We thank Mr.S.N. Yadav for technical co-operation.

REFERENCES

Banerjee A, Pakrashi A, Chatterjee S, Ghosh S, Dutta SK (1993) Semen characteristics of tobacco users in India. Arch Androl 30:35-40

Dave BJ, Trivedi AH, Adhvaryu SG (1991) Cytogenetic studies reveal increased genomic damage among pan masala consumers. Mutagenesis 6:159-163

Dave BJ, Trivedi AH, Adhvaryu SG (1992) *In vitro* genotoxic effect of arecanut extract and arecoline. J Cancer Res Clin Oncol 118:283-288

IARC (1985) Monograph on evaluation of carcinogenic risk of chemical to human: Tobacco habits other than smoking: Betelquid and arecanut chewing and some related nitrosamine. IARC Scientific Publication, Lyon, France 37:141-200

Kumar S, Patel KG, Gautam AK, Agarwal K, Shah BA, Saiyed HN (1999) Detection of germ cell genotoxic potential of carbon disulphide using sperm head shape abnormality test. Hum Exp Toxicol 18:731-734

Kumar S, Saiyed HN (1999) Role of betelnut chewing in cancer its toxicity and chemoprevention - An overview. Indian J Environ Toxicol 9:5-11

Kumar S, Zinyu R, Chetia M, Dutta LP (1992) Genotoxic effect of smoke dried meat extract in Swiss albino mice using sperm head shape abnormality test. Cancer Lett 64:51-53

Mukherjee A, Chakraborti J, Chakraborti A, Banerjee J, Sharma A (1991) Effect of pan masala on the germ cells of male mice. Cancer Lett 58:161-165

Nigam SK, Kumar A, Sheikh SA, Saiyed HN (2001) Toxicological evaluation of pan masala in pure inbred Swiss mice a preliminary report on long term exposure study. Curr Sci 80:1306-1309

Panigrahi GB, Rao AR (1986) Study of genotoxicity of the total aqueous extract of betelnut and its tanin. Carcinogenesis 7:37-39

Patel AK, Jaju RJ, Bakshi SR, Trivedi AH, Dave BJ, Adhvaryu SG (1994) Pan masala a genotoxic menace. Mutat Res 320:245-249

Ramchandani AG, D'Souza AV, Borges AM, Bhisey RA (1998) Evaluation of carcinogenic / co -carcinogenic activity of a common chewing product, pan masala in mouse skin, stomach and oesophagus. Int J Cancer 75:225-232

Sinha A, Rao AR (1985) Embryo toxicity of betelnut in mice. Toxicology 37:315-326

Stich HF, Rosin MP (1985) Towards a more comprehensive evaluation of a genotoxic hazards in man. Mutat Res 150:43-50

Tanaka T, Mori M, Fujii M, Takahashi Hirono I (1983) Carcinogenicity examination of betelquid II effect of vitamin A deficiency on rat fed semipurified diet containing betelnut and calcium hydroxide. Nut Cancer 4: 260-266

Verma RC, Chansoriya M, Kaul KK (1983) Effect of tobacco chewing by mothers on fetal outcome. Indian Pediatr 20:105-111

Wary KK, Sharan RN (1991) Cytotoxic and cytostatic effects of arecoline and sodium nitrate on human cells *in vitro*. Int J Cancer 47:396-400

Wyrobek AJ, Bruce WR (1978) The induction of sperm shape abnormalities in mice and human. In : Hollander A, Deserres FJ (eds.) Chemical mutagens. Principles and methods for their detection. Vol 5. Plenum Press; New York, p. 257