ABSTRACT
Swiss albino mice in three groups (control, experimental I and experimental II) of 60 each were fed staple diet (control), diet composed with 2% pan masala plain (PMP) and its blend (tobacco-PMT) for a period of up to 70 weeks with intermittent postmortem at 16, 56 and 70 weeks for assessing histological changes of the gut histopathology of mice. Mice of experimental groups I and II showed progressive advancement of structural changes of stomach during the study period. Fibrosis, thickening of prickle cells and also horney layered cells indicating lesions of acanthosis and hyperkeratosis initially and later prominently ulcers and papillomas which transformed into a peptic tumor occurred. Spleen was found enlarged in mice fed with PMT.

Animal feeding, hygiene, exploration and environmental care and health protocols were as per the standard guidelines and approved by National Institute of Occupational Health ethical committee. Here, chronic exposure of mice has been consistent in respect of feed and environment, unlike habitual PM users whose consumption is inconsistent depending on the body demand and choice on the variety of the blended PM product. This study endorses generating critical clinically designed case control, cohort data for awareness programs and PM substitutes as well as treatment to PM addicts.

Keywords: Pan masala, Gut changes, Mice.

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INTRODUCTION
Pan masala (PM) is a substance of dependence consumed like alcoholics, drug (opiates, cannabis, benzodiazepines) addicts. The PM is used as substitutes or adjuvants to psychoactive substances. Chronologically, over three decades several popular masticator products were composed, starting with areca nut, catechu, lime as betel quid, and added cardamom, permitted spices, unspecified flavoring, sweetening agents, etc. are sold as PM² which further mixed with tobacco are known as ‘Guthka’ or PM-Tobacco.³ To go partially inert to weariness of manual and nonmanual stress PM sachets are consumed occasionally to avoid halitosis and for relaxation and as mood elevator among working population.⁴ Thus, gradually dose-tolerance develops resulting in frequent (within 2-3 months) PM consumption and as a further reinforcement tobacco blended PM is consumed.⁵ Thus, PM addicts categorically show craving and substance seeking behavior (Guthka or Areca Nut Chewer’s syndrome).⁶ The spurious blends of PM and high-flown advertisement and brisk marketing and their psychoactive stress ameliorating nature and usage by young and old as well as labor and executive class may immediately cater satisfaction; aphasia ensures zeal to do over work.⁷ To clear halitosis (freshen the breath),⁸ as analgesic,⁹ carminative, bactericidal, i.e. gastric digestion after a strong spicy food PM enables spontaneous strength from weariness.⁹,¹⁰ But over threshold limits and consistent and excessive consumption for long period results in functional and structural damage of organs, i.e. liver,⁸,¹¹ lungs, testis,¹² stomach,⁹ oral cavity, accessory organs, etc. and auditory, speech and visual deformities.

Fibrosis¹³ is common in chronic PM, PMT chewers, beginning with inflammation¹⁴ progressive submucous fibrosis of oral mucosa, lungs, papillomas of stomach, liver, gut, testis, etc. The areca nut of PM has clastogenic, mutagenic, genotoxic and carcinogenic properties. The structural changes of gut on long-term exposure PM and its blend are hereby presented.

MATERIALS AND METHODS
Inbred Swiss mice with an average age of 6 weeks were set in three groups. Sixty animals each of both sexes in each group were exposed to PM (plain-PMP) and blended PM (with tobacco-PMT) and equal numbers of controls were provided with normal diet. Throughout the study period, pure inbred Swiss mice were used. The PMP, PMT fine powder in 100 gm quantity was mixed thoroughly with 4,900 gm of feed given to mice routinely besides control animals which received only staple diet. The protocols of the experiments and maintenance of animals and their usage during this study were described in an earlier study.¹¹ Exposed and control (Fig. 1) groups of animals were sacrificed by cervical dislocation in different time intervals. Postmortem in detail for gross pathology (Fig. 2) and for histology of liver, kidney, heart, spleen, stomach, esophagus
and testis were collected. Organs were fixed in 10% buffered neutral formalin and processed routinely and 5 µm thick paraffin sections were stained by hematoxylin and eosin. Tumor development was compared with control and evaluated by Students t-test.

RESULTS

The fore-stomach, apart the upper alimentary tract of the mouse, is apparently morphologically and physiologically akin to man. Fore-stomach of one animal fed with PMT (Gutkha) for 12 months showed mild thickening of prickle cells and also horny layer cells, thus indicating early hyperkeratosis (Fig. 3), lesions and squamous papilloma (Fig. 4) which is characterized by marked acanthosis, with infolding proliferation of squamous epithelium of the stomach (Fig. 5). Similarly liver exposed to sada as well as with Gutkha showed disrupted hepatocytes with fatty globular infiltration coalesced as fat cysts replacing total parenchyma and further reticular cell hyperplasia, hemangioma and hemangioendothelioma. Spleen and liver were found enlarged (Figs 6 to 8) and damages other organs too, i.e. testes, lung and stomach in mice exposed to PMT.

DISCUSSION

In a preliminary report, we have shown that PMP and PMT longer time fed mice showed early biochemical changes and hepatocarcinogenesis. In the present study, analysis
Carcinogenic activity was observed by pasting ethanol extract of PM on mice for 40 weeks which showed skin papillomas and enhanced its rate of conversion to carcinomas and at higher dose, promoted effectively the development of fore-stomach and esophageal papillomas and carcinomas. Prolonged usage of PMP, PMT in the feed of mice is a simulating condition to humans except dosage as PM user’s quite often unlimited frequent consumption by chewing and swallowing the saliva and enables PM carcinogenic sites other than oral cavity, unlike tobacco chewers who often spit out the chewed stuff. In the present study, spleen and gastric damage (and to multiple organs) in mice, after chronic feeding of PM and gutkha (PMT) and even ultimately leading to gut cancer suggest that PM especially its blend may exert carcinogenic or even cocarcinogenic influence in man, specially in those who are habitual users of such products.

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ABOUT THE AUTHORS

Suresh Kumar Nigam
ICMR Emeritus Scientist, ES’s Laboratory and Department of Neurobehavioral Toxicology, National Institute of Occupational Health (NIOH), Ahmedabad, Gujarat, India

Huthi Venkatakrisghna Bhatt (Corresponding Author)
ICMR Emeritus Scientist, Ex-Senior Grade Deputy Director, Consulting Editor, ENVIS-NIOH Newsletter, ES’S Laboratory and Department of Neurobehavioral Toxicology, National Institute of Occupational Health (NIOH), Ahmedabad, Gujarat, India, e-mail: hvkbhatt@yahoo.co.in