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ORIGINAL ARTICLE

Effects of Plain and Blended Indian Pan Masala on Murine Liver

[1]Suresh Kumar Nigam, [2]Huthi Venkatakrishna Bhatt

[1]Medical Scientist (Emeritus), Laboratory and Department of Neurobehavioral Toxicology, National Institute of Occupational Health (NIOH), Ahmedabad, Gujarat, India

[2]Medical Scientist (Emeritus), Ex-Senior Grade Deputy Director, Consulting Editor, ENVIS-NIOH News Letter, Ahmedabad, Gujarat, India

Correspondence: Huthi Venkatakrishna Bhatt, Flat No. 13, Block No. 2, Suramya Apartments, Sola Road PO, Mirambika Street Ahmedabad-380063, Gujarat, India, e-mail: hvkbhatt@yahoo.co.in

ABSTRACT

Chronic consumption of pan masala plain (PMP) or with tobacco (PMT) cause liver injury. Three groups of mice of both sexes consuming PMP, PMT and control diet for prolonged period were enrolled in this study. Blood samples were collected 16, 56 and 70 weeks after study commencement and checked for serum alkaline phosphotase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Significant increased levels of all these enzymes were found in mice provided with PMP and PMT compared to control mice, however, elevation in the intermittent samples varied between PMP and PMT groups. This study has indicated the liver injury potential of pan masala and their effects should be assessed in human.

Abbreviation: PM—Pan masala; PMP—Plain pan masala; PMT—Pan masala with tobacco; ALP—Alkaline phosphatase; GOT—Glutamic oxaloacetic transaminase; GPT—Glutamic pyruvic transaminase.

Keywords: Pan masala, Liver enzymes, Mouse.

INTRODUCTION

Eating pan masala (PM), a product without betel leaf, currently, is a very popular habit among Indians. Aggressive marketing and high profile advertising developed inclination and addiction even among school children, youth, and labors and in various occupations just to elevate their mood and psychoenlightenment. Generally, it contains betel nut, catechu (kattha) and lime as major ingredients, flavorings and exotic spices. Betel nut and catechu were found toxic.[1-4] However, consumption of this mixture causes more toxicity than the individual components of PM alone. The present study deals with hepatotoxicity of 2% plain and tobacco blended PM mixed in diet for prolonged duration in mice.

MATERIALS AND METHODS

Plain PM (PMP) and PM with tobacco (PMT) (Gutkha) of a very popular brand were obtained from the market and used throughout the experiment. A total of 180 mice with an average age of 6 weeks were used. Sixty animals each of both sexes were exposed to PMP and PMT and equal number of controls were provided with normal diet. PM in powder form was mixed in diet (2%) after grinding properly in an electric mixer. The diet consisted of cracked wheat (70%), cracked Bengal gram (20%), fish meal (5%), yeast powder (4%) and shark liver oil (1%) in the form of dry mesh. Mice were given water *ad libitum*. PM was fed for 70 weeks. Autopsy was performed and liver was collected along with other organs for histological observation after 16, 56 and 70 weeks. Only four blood samples from each group were collected after 16, 56 and 70 weeks from the eye. Serum was separated and stored at –20°C until for further analysis.

Serum samples from all the mice were analyzed for levels of alkaline phosphatase (ALP),[5] glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).[6]

RESULTS AND DISCUSSION

PM intake of mice with or without tobacco produced significant alterations in all the three biochemical parameters (ALP, GOT and GPT) compared to mice fed with control laboratory chew. The data represented in Table 1 shows increased levels of GOT and GPT in serum. A rise in plasma transminase activity is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membrane due to liver toxicity. Similarly, a rise in ALP activity in serum suggests cholestatic liver disease when enzyme within the biliary tract regurgitate into plasma. Further, a decrease in the weight of testis with an increase in serum ALP, an androgen-dependent enzyme, also suggests testicular damaging effect. Similar effects of PM have been reported in rats.[7] Drug-induced liver damage also induces an increase in the activity of GOT and GPT.[8] The GOT and GPT are involved in amino acid metabolism, therefore

Table 1: Enzyme activity in mice fed with pan masala with and without tobacco

Group	*SGOT*	*SGPT*	*ALP*
Control	97.26 ± 8.28	85.64 ± 4.142	29.08 ± 1.129
Pan masala without tobacco (plain)	104.63 ± 7.55	114.33 ±12.29	29.98 ± 0.323
16 weeks exposure	p < 0.01	p < 0.001	
Pan masala without tobacco (plain)	108.94 ± 6.41	128 ± 10.54	37.58 ± 2.543
56 weeks exposure	p < 0.01	p < 0.001	p < 0.01
Pan masala without tobacco (plain)	110.98 ± 6.62	124 ± 8.24	30.08 ± 1.12
70 weeks exposure	p < 0.01	p < 0.001	
Pan masala with tobacco (gutkha)	143.74 ± 10.5	141.85 ± 10.492	36.42 ± 1.272
16 weeks exposure	p < 0.01	p < 0.001	p < 0.01
Pan masala with tobacco (gutkha)	124 ± 4.06	136.46 ± 8.796	35.72 ± 1.586
56 weeks exposure	p < 0.01	p < 0.001	p < 0.01
Pan masala with tobacco (gutkha)	136 ± 4.06	141.64 ± 8.42	32.04 ± 1.42
70 weeks exposure	p < 0.01	p < 0.001	

Data are shown as mean ± standard deviation
SGOT – Serum glutamate oxaloacetate transaminase
SGPT – Serum glutamate pyruvate transaminase
ALP – Alkaline phosphatase

an increase in these enzymes together with ALP in serum indicates tissue damage or toxic effect in liver.[9,10]

Animals exposed to plain PM as well as gutkha (PMT) showed alterations in the liver histology (Figs 1 and 2). The parenchymal cells around central vein had disaggregated basophilic bodies resulting in a diffuse basophilic cytoplasm. However, animals fed with gutkha showed clear cells around the peripheral region, which started appearing with dislocated bodies. The nuclei pushed on the sides of the cytoplasm. Liver cells also showed generalized fatty infiltration. Microvesicular fatty changes usually were diffused seen as myriads of tiny fatty droplets which surround the centrally located nucleus. Further fat droplets were seen as large round empty vacuoles which displace the hepatocyte nucleus to the periphery of the cell. In extreme cases these vacuoles coalesce to create cleared spaces. At places, these contiguous cells ruptured and the enclosed fat globules coalesced to produce fat cysts. Dilation of sinusoids and prominent Kupffer cells were other interesting findings. Severe fatty changes as seen in PM exposure may impair cellular function and can cause irreversible impairment of some intracellular process, such as decrease of protein synthesis, which is essential for conversion of triglycerides to lipoprotein excretion as seen in CCl_4 poisoning. A marked polymorphism or atypia with cords of liver cells covered with single layer of endothelium with separate spaces filled with blood, vascular spaces which are separated by irregular sheet of solid areas of endothelial cells showing signs of polymorphism suggesting

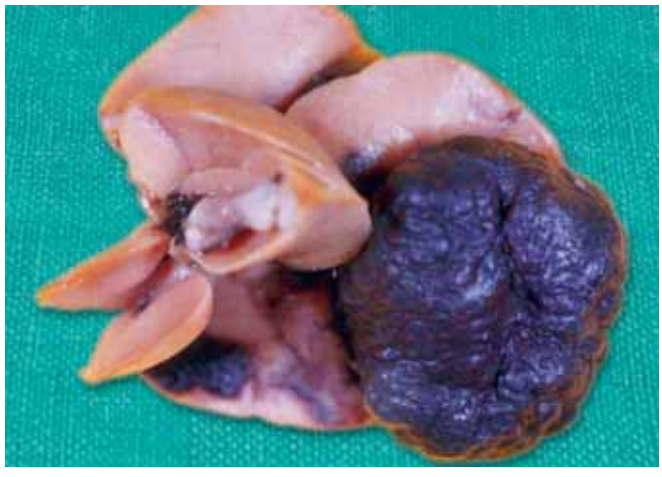

Fig. 1: Extricated liver showing gross tumor in chronic PMT fed mice

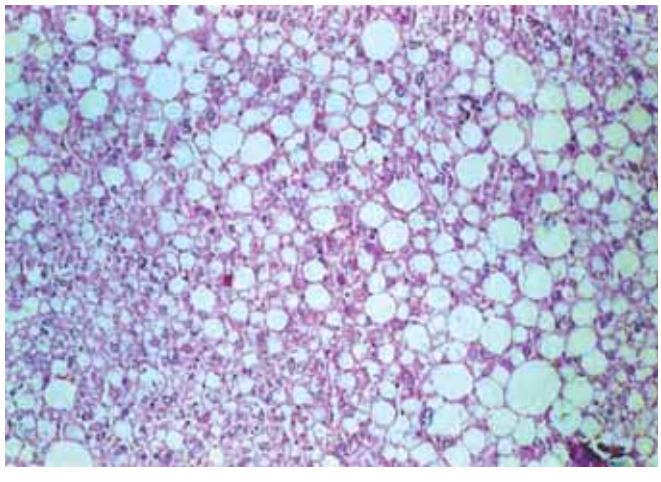

Fig. 2: Section of liver of mice receiving PMT showing fatty infiltration almost replacing the liver parenchyma (H&E 96×)

hemangiomas were also recorded in liver exposed to PM. Further a case of liver cell carcinoma consisting of cords surrounded by sinusoidal cells with marked cell polymorphism was also seen in PM exposed group was observed. It is known that there is an endogenous initiation of hepatocarcinogenesis in rodents. This occurs by increasing the intracellular production of H_2O_2 which causes DNA strand breakage as well as chemical alterations of DNA bases. Damage to DNA generally regarded as the initial biochemical alteration leading to neoplastic transformation in the case of the majority of chemical carcinogens which may be the prime factor even in this case for the induction of liver cancer.

In the present study, extensive damage to the liver in mice, after chronic feeding of PM and gutkha and even ultimately leading to liver cancer suggest that PM may exert carcinogenic or even cocarcinogenic influence in man, specially in those who are habitual users of such products.

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