

Long-term toxicity of pan masala in female swiss mice

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ABSTRACT

The onset of pan masala chewing occurs primarily in early adolescence, leading to addiction in adulthood, not only in males but also among females. No data on females are found, thus the present study targets the damaging effect of pan masala on female swiss mice. The studies were carried out on 15 female swiss mice and were divided into two groups: Control(C) and Pan masala treated (PMT). Lung, liver, kidney and ovary were excised periodically to measure acid phosphatase, alkaline phosphatase, and lactate dehydrogenase activity. Histology of different tissues and serum parameters were also analysed. There was a statistically significant increase in serum parameters and enzymatic activities in lung, liver, kidney and ovary of female swiss mice. Severe damage in the tissue sections was also observed. Pan masala is highly toxic and causes severe damage to the various organs of the female swiss mice. Thus there is a need to put ban on manufacture and sale of such products and the acceleration of the programmes which are aimed at their reduction or elimination from the market.

Keywords: Pan masala, Female, Toxicity

INTRODUCTION

In India, tobacco usage in women has risen in previous years. It is used orally throughout the world but mainly in South- Asian countries. Pan Masala, a popular chewing tobacco, consists of areca nut, slaked lime, catechu, tobacco, and flavoured components. Unfortunately, even the children and young adolescents have access to these products as they are freely available and at affordable costs at every nook and corner of the different states in the country. These products thus act as a gateway to drug addiction for the vulnerable population as poor section of society consumes it as a leisure product. The usage of pan masala and such other chewing tobacco products are found to be significant among females and young adults in different states. Globally, studies have associated smokeless tobacco consumption to low birth weight, preterm birth and even stillbirth in women consuming tobacco during pregnancy [1]. Apart from this, it also alters other physiological functions in females which are the main part of the present research. Thus the present study focuses on the toxicity of pan masala on various organs of female swiss mice.

MATERIALS AND METHODS

A total of 15 female swiss mice were acquired and were divided into two groups: Control(C) and Pan masala treated (PMT). Pan masala was fed orally for 9 months and the animals were sacrificed for the collection of liver, lung, kidney and ovary. Tissues were homogenized in ice cold 0.25 M sucrose and the homogenates were centrifuged at 10,000g at 4°C for 10 minutes to obtain a clear supernatant for biochemical estimation [2, 3]. Alkaline phosphatase (ALP, EC 3.1.3.1), Acid phosphatase (ACP, EC 3.1.3.2), and Lactate dehydrogenase (LDH, EC 1.1.1.27) activities were determined by their respective kits. Various serum parameters like glucose, calcium, triglyceride, protein, and creatinine were analysed to assess the damaging effect of pan masala. All the kits were of Crest Biosystems. Data

were analyzed via one-way ANOVA. The results were presented as individual values or mean \pm SD. A F-value > Fcrit was considered significant. For histological analysis, the fragments from harvested tissue were fixed in Bouin's fixative, embedded in paraffin, stained and observed under light microscope.

RESULTS

Data on the body weight for the control and experimental groups are presented in Figure 1. The mean body weight of animals fed pan masala was consistently lower than that of the controls, and the difference was particularly marked from month 7 onwards.

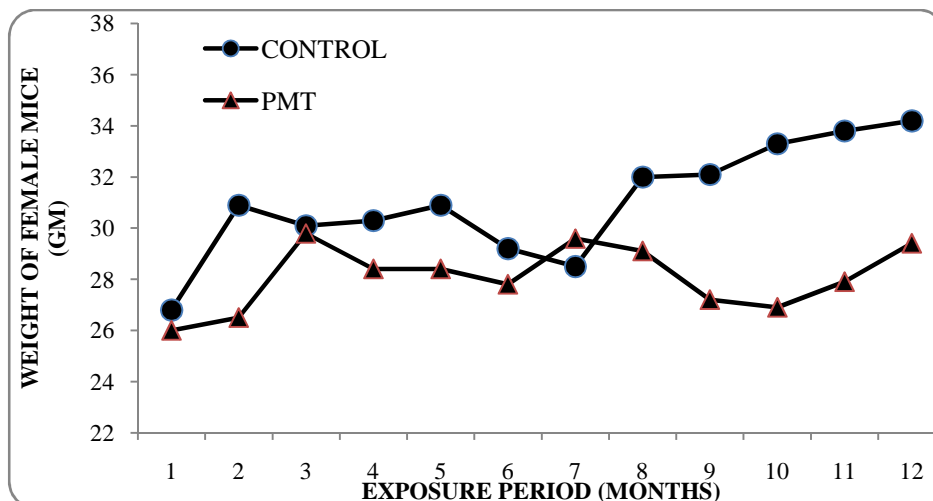


Figure1 Body weight of female Swiss mice during the complete exposure period

Table 1 presents the increasing trend of enzymatic activities in lung of female swiss mice after 3, 6 and 9 months of pan masala exposure as compared to control. There was also a significant increase in the enzymatic activities in liver, kidney and ovary of swiss mice after pan masala treatment as compared to control as evident from table 2, 3 and 4 respectively.

Table 1: Effect of pan masala on different histochemical parameters in lung of female mice

TREATMENT PERIOD (MONTHS)	ACP (U/L)		ALP (U/L)		LDH (U/L)	
	Control	PMT	Control	PMT	Control	PMT
0-3	41.25 \pm 3.01	114.1 \pm 3.68	86.75 \pm 9.35	148.7 \pm 22.33	243.80 \pm 2.62	613.66 \pm 38.43
0-6	58.88 \pm 2.58	138.5 \pm 3.39	96.85 \pm 6.88	162.5 \pm 11.29	248.64 \pm 2.15	720.48 \pm 30.95
0-9	70.75 \pm 2.93	178.3 \pm 1.84	102.4 \pm 6.36	222.6 \pm 17.53	258.25 \pm 5.22	820.81 \pm 9.30

Each value is a mean of 5 determinations \pm SD. C, Control; PMT, pan masala treated group; *F>Fcrit compared to control group

Table 2: Effect of pan masala on different histochemical parameters in liver of female mice

TREATMENT PERIOD (MONTHS)	ACP (U/L)		ALP (U/L)		LDH (U/L)	
	Control	PMT	Control	PMT	Control	PMT
0-3	137.88 \pm 4.21	201.00 \pm 6.15	48.65 \pm 8.83	117.50 \pm 12.59	249.75 \pm 5.92	520.95 \pm 17.06
0-6	139.88 \pm 1.48	238.25 \pm 10.11	59.21 \pm 18.44	173.96 \pm 5.45	259.69 \pm 15.71	696.60 \pm 7.07
0-9	159.00 \pm 4.21	278.13 \pm 6.72	72.52 \pm 9.61	221.24 \pm 13.11	309.08 \pm 27.94	725.76 \pm 22.08

Each value is a mean of 5 determinations \pm SD. C, Control; PMT, pan masala treated group; *F>Fcrit compared to control group

Table 3: Effect of pan masala on different histochemical parameters in kidney of female mice

TREATMENT PERIOD (MONTHS)	ACP (U/L)		ALP (U/L)		LDH (U/L)	
	Control	PMT	Control	PMT	Control	PMT
0-3	54.50 \pm 2.93	81.50 \pm 5.98	43.15 \pm 3.64	104.19 \pm 18.95	235.59 \pm 7.73	307.47 \pm 2.75
0-6	63.38 \pm 7.21	101.25 \pm 8.13	69.77 \pm 9.61	172.58 \pm 5.45	246.09 \pm 2.20	362.85 \pm 21.65
0-9	68.13 \pm 3.89	128.38 \pm 7.46	127.60 \pm 5.97	196.91 \pm 6.23	257.47 \pm 5.03	420.96 \pm 22.85

Each value is a mean of 5 determinations \pm SD. C, Control; PMT, pan masala treated group; *F>Fcrit compared to control group

Table 4: Effect of pan masala on different histochemical parameters in ovary of female mice

TREATMENT PERIOD (MONTHS)	ACP (U/L)		ALP (U/L)		LDH (U/L)	
	Control	PMT	Control	PMT	Control	PMT
0-3	53.13 ± 1.24	75.88 ± 5.27	40.39 ± 7.01	93.18 ± 5.58	244.41 ± 2.74	515.84 ± 15.82
0-6	62.38 ± 2.02	138.50 ± 8.10	59.21 ± 15.19	133.11 ± 7.14	251.53 ± 3.87	667.43 ± 11.39
0-9	67.63 ± 4.84	193.50 ± 6.58	71.60 ± 14.54	196.61 ± 14.28	362.07 ± 6.00	815.59 ± 7.13

Each value is a mean of 5 determinations ± SD. C, Control; PMT, pan masala treated group; *F>Fcrit compared to control group

The enzymatic activity in various tissues in PMT group increased after 3 months of pan masala treatment which kept on rising till 9 months showing severe abnormalities in the respective tissues. Biochemical studies also indicated significant increase in glucose, calcium, triglyceride, protein, creatinine in the serum of mice fed with pan masala containing diet as shown in Table 5. The level of glucose, triglyceride and protein got doubled in PMT after 9 months as compared to control while there was almost 3 fold increase in the calcium and creatinine values.

Table 5: Effect of pan masala on different serum parameters in female mice after 9 months of exposure

GROUPS	Glucose (mg/dl)		Calcium (mg/dl)		Triglyceride (mg/dl)		Protein (g/dl)		Creatinine (mg%)	
C	170.59	± 9.98	9.87	± 1.06	141.54	± 9.14	60.45	± 3.73	2.77	± 0.39
PMT	344.12	± 4.99*	31.47	± 0.87*	376.92	± 10.88*	159.09	± 7.07*	9.60	± 0.57*

Each value is a mean of 5 determinations ± SD. C, Control; PMT, pan masala treated group; *F>Fcrit compared to control group

Under the light microscope, lung of control female mice showed the normal alveolar architecture and open intra-alveolar spaces while in the section of PMT mice, papillary formation was observed. (Figure 2a and Figure 2b)

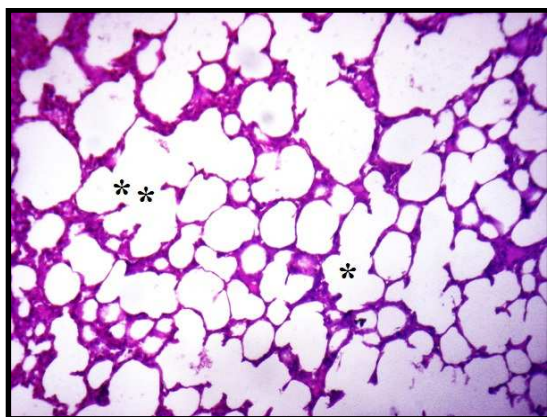


Figure 2a A photomicrograph of section of control lung of female mice; alveolar architecture (*); intra-alveolar spaces (**). (HE stain, X100).

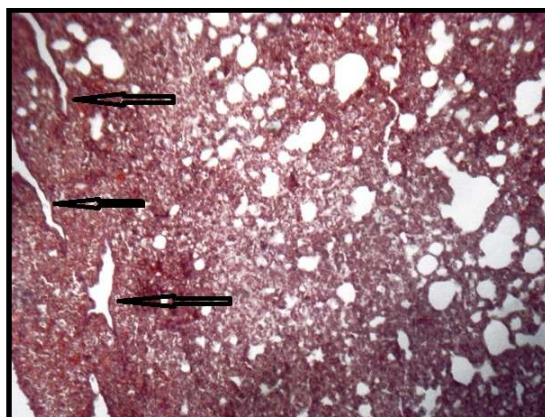


Figure 2b A photomicrograph of section of PMT lung of female mice; Papillary formation (arrow). (Mallory's triple stain, X100).

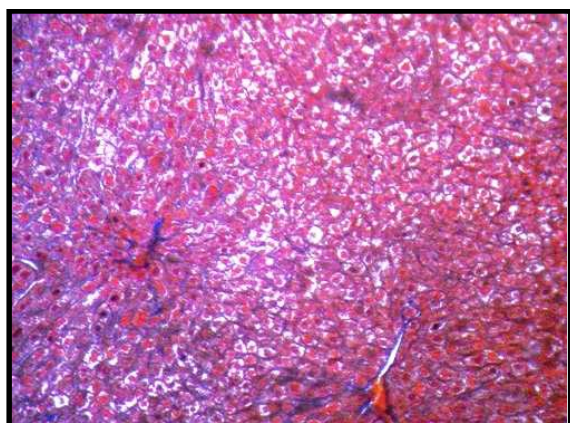


Figure 3a A photomicrograph of section of control liver of female mice. (Mallory's triple stain, X100)

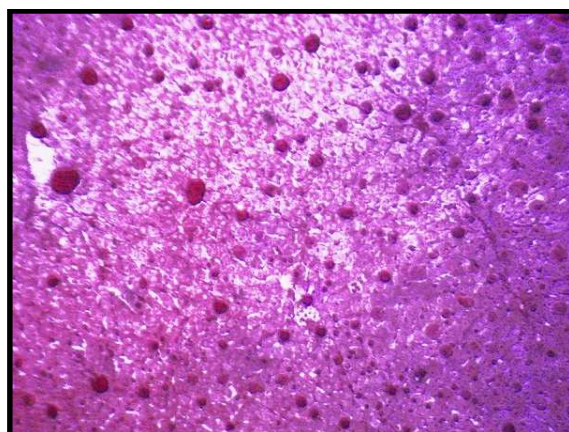


Figure 3b A photomicrograph of section of PMT liver of female mice. (Mallory's triple stain, X100)

Figure 3a shows the liver of control female displaying normal architecture, where typical aspects, i.e., lobular pattern with prominent central vein and sinusoids were evident. Figure 3b shows liver cirrhosis caused by consumption of pan masala. This case is characterized by solid sheet appearance with indistinct cell borders and poorly developed sinusoid structure. Each cell has eosinophilic cytoplasm, their nuclear morphology being greatly variable.

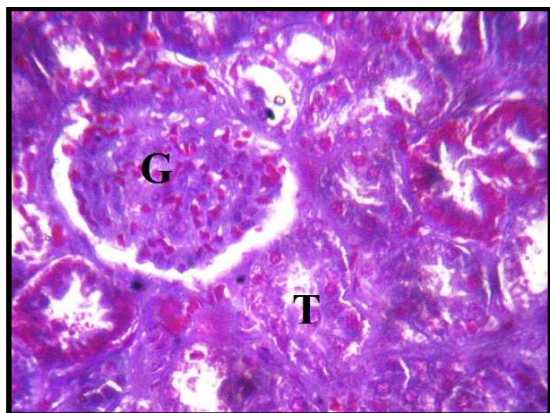


Figure 4a A photomicrograph of section of control kidney of female mice; glomeruli (G); tubules(T). (Mallory's triple stain,X400)

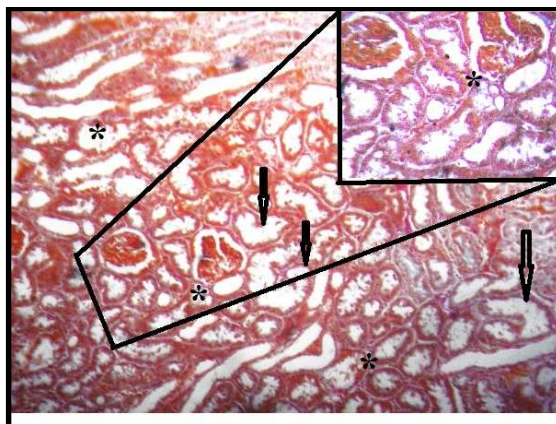


Figure 4b A photomicrograph of section of PMT kidney of female mice; Tubular necrosis (*, in inset also), swelling of tubules (arrow). (HE stain, X100)

In the section of kidney, control group showed the renal cortex of female Swiss mice with glomeruli and tubules while tubular necrosis and swelling with few numbers of glomeruli was seen in PMT. (Figure 4a and Figure 4b)

DISCUSSION

The essence of long term bioassay is to simulate the human situation in the laboratory animals to focus on the toxicity produced by the environmental agents. In the present study, the animals treated with pan masala showed a decline in their body weight as compared to control thus showing signs of deterioration in their condition. There was a significant increase in the acid phosphatase, alkaline phosphatase and lactate dehydrogenase activity of various organs in PMT mice after 9 months. The increased acid phosphatase activity can be correlated with cellular injury, inflammation, tissue damage and progression of fibrosis in chronic interstitial disorders [4,5]. The significant increase in the tissue ALP activity following the administration of the pan masala may be due to increased functional activity of the various organs, probably leading to *de novo* synthesis of the enzyme molecules [6]. A significant increase in the activity of the LDH also depicted cellular injury that may be attributed to the loss of membrane integrity due to pan masala exposure. It also caused an abnormal rise in serum parameters like creatinine which is an indicator of renal damage, protein like gamma globulins which is an indicator of inflammation. Other serum parameters like glucose, triglyceride and calcium also showed a significant rise in PMT group as compared to control. Thus, it can be concluded from the present study that pan consumption is damaging to various organs of female swiss mice.

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