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Histochemical studies on the induced toxicity of Pan masala on various organs of Swiss mice and the protective effect of *Elettaria cardamomum* (L.) Maton

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ABSTRACT

Significant increase in the histochemical parameters and serum variables was observed in the pan masala treated mice. The target organs in the present study were liver, kidney and testes which all showed severe damage as observed by the abnormal rise in Acid phosphatase, Alkaline phosphatase and Lactate dehydrogenase activity. The level of GOT and GPT was measured in liver as well as serum where its elevated value marked liver impairement. There was a significant rise in serum protein, calcium, triglyceride, glucose and creatinine after 36 weeks exposure of pan masala in Swiss mice. Elettaria cardamomum (L.) Maton *showed a promising effect by lowering down the elevated abnormal rise in enzymatic activity and serum parameters.*

Keywords: Pan masala, toxicity, cardamom, amelioration.

INTRODUCTION

Pan masala is a commercial preparation containing areca nut, slaked lime, catechu and condiments, with or without powdered tobacco. This preparation has been marketed since 1975, and aggressive advertising targeted at the middle class and youth, is believed to have enhanced the sales of this product. It is exported to well over 22 countries and is common among migrant populations from South Asia worldwide [1]. In Tanzania, the age of onset of smokeless tobacco was 12 years or less (57%), with 28.5% being less than 10 years of age [2]. In India, street childrens and some adult have been known to consume 30 packets a day and 13.5% of males and 0.5% of females have been reported to use pan masala in Karachi and Pakistan [3]. Long term harmful effects of pan masala have been established in the form of adenoma of the liver, stomach, prostate and sebaceous glands, also fore stomach papilloma, liver hamartoma, hepatoma and hemangioma, carcinoma of the fore stomach, adenocarcinoma of the lung and liver, and testicular lymphoma[4]. In vitro studies have also shown that it is highly carcinogenic due to precence of areca nut and tobacco[5][6] and can be a gateway to smoking [7].

In this regard herbal component was studied which is promising and effective and cardamom is one of them having anti-inflammatory, anti-mutagenic and anti-carcinogenic property [8]. Aqueous suspensions of cardamom have been shown to enhance the level of detoxifying enzyme[9].The primary mission of our research was to study the protective effect of cardamom against pan masala induced abnormal rise in certain enzymatic parameters and serum constituents in various organs like liver, kidney, and testes.

MATERIALS AND METHODS

A total of 45 male Swiss mice (22± 5g, obtained from B. N. Ghosh and Company, CIT Road, Kolkata) with an average age of four weeks were used in the experiment. The investigation was cleared by the Ethics committee, Ranchi University, Ranchi, for conducting research on Swiss mice and other strains of albino mice. 15 animals were

exposed to pan masala and next group was exposed to cardamom along with pan masala and an equal number of controls were provided with normal diet. Pan masala in powdered form was mixed in the diet (2%) after grinding properly in an electric mixer and 0.2% of cardamom was given to check its ameliorating property[4] [9]. The diet consisted of cracked wheat 70%, cracked Bengal gram 20%, fish meal 5%, yeast powder 4%, and groundnut oil 1% in the form of dry mash. Animals from each group were sacrificed after 36 weeks of exposure to pan masala and a combination of pan masala with cardamom mixed in feed. Autopsy was performed and liver, kidney, and testes were examined histochemically.

Tissues were homogenized (0.15g tissue/mice) in ice cold 0.25 M sucrose (1:10, w/v) [10] after infusion with formalin and the homogenates were centrifuged at 10,000g at 4ºC for 10 minutes to obtain a clear supernatant for biochemical estimation[11]. 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 the method of pNPP kinetic method, α-napthylphosphate kinetic method and IFCC method respectively. The assays were done using Acid phosphatase kit, Alkaline phosphatase kit (DEA) and LDH (P-L) kit. The enzymatic activity was expressed in U/L. Various serum parameters like glucose (GOD/POD method), calcium (OCPC method), triglyceride (GPO/PAP method), protein (Biuret method), creatinine (Alkaline picrate method), glutamic oxaloacetic transaminase (GOT/Reitman and Frankel method), and glutamic pyruvate transaminase (GPT/ Reitman and Frankel method) were analysed to assess the damaging effect of pan masala and protecting effect of cardamom. All the kits were of Crest Biosystems. Data were analyzed via one-way ANOVA. The results were presented as individual values or mean±SD. A F-value > F_{crit} was considered significant.

RESULTS

There was no significant difference between the survival rates of the animals in the control and experimental groups. Signs of pan masala intoxication such as loss of fur, ruffled skin, loss in weight, and dermal lesions were observed in animals having 9 months of exposure. Ameliorated mice showed clear signs of improvement in weight and skin structure. Oral administration of pan masala caused significant increase in the ACP, ALP and LDH levels in liver,kidney and testes. However administration of cardamom along with pan masala significantly decreased their activities in the respective organs. Table 1 presents a comparison between liver acid phosphatase, alkaline phosphatise and lactate dehydrogenase activity. There was a two fold increase in the ACP, LDH activity and four fold increase in ALP activity which was considered significant (F > F_{crit})when compared to control.

Table 1: Effect of Cardamom on different histochemical parameters against pan masala induced toxicity in liver, kidney and testes of mice after 36 weeks of exposure

Enzymatic Parameters	Group	Liver	Kidney	Testes
ACP (IU/L)	CNT	90±6.68	63.5±6.05	49.88±1.34
	PM	257±8.34*	109.25±1.7*	126.13±4.28*
	PM+C	174±20.65*	82.63±2.76*	74.88±5.59*
ALP(IU/L)	CNT	59.21±8.83	84.46±6.1	116.59±10.78
	PM	238.68±41.54*	190.49±22.07*	239.6±13.76*
	PM+C	123.47±11.42**	126.23±11.68**	175.34±10.13*
LDH(IU/L)	CNT	267.25±11.58	169.29±12.45	272.69±10.86
	PM	707.04±29.92*	333.19±28.22*	684.26±46.84*
	PM+C	543.67±101.64**	239.81±14.52*	318.88±18.64**
GOT(IU/L)	CNT	265.46±14.28	-	-
	PM	574.89±32.7*	-	-
	PM+C	433.2±40.07*	-	-
GPT(IU/L)	CNT	29.8±3.56	-	-
	PM	312.8±10.86*	-	-
	PM+C	207.2±6.05*	-	-

*Each value is a mean of 5 determinations ±SD. CNT, Control; PM, pan masala treated group; PM+C, group with co-administration of pan masala and cardamom; *F>F_{crit} compared to control group; **F<F_{crit} compared to control group*

Excessive rise in the level of GOT and GPT was also observed in the liver (Table 1). These are the two commonly measured transaminases which leak out into the blood stream due to increased permeability of the hepatocyte cell membrane after severe liver damage. Kidney and testes also showed a higher activity of the enzymes which decreased significantly in all the organs (F > F_{crit}) when cardamom was administered along with pan masala (Table 1).

Table 2: Effect of Cardamom on different serum parameters against pan masala induced toxicity in mice after 36 weeks of exposure

Groups	Glucose (mg/dl)	Calcium (mg/dl)	Triglyceride (mg/dl)	Protein (g/dl)	Creatinine (mg%)	GOT (IU/L)	GPT (U/ml)
CNT	224.12±11.31	8.27±0.72	167.69±10.01	61.82±1.67	2.62±0.22	232±9.67	24.40±3.73
PM	401.76±5.99*	26.00±1.13*	344.62±12.18*	141.36±4.89*	10.46±0.83*	449±28.56*	252.2±14.54*
PM+C	315.29±7.15*	18.13±0.68*	281.54±9.14*	97.73±8.36*	3.69±0.22*	320.83±51.13*	139.6±17.2*

*Each value is a mean of 5 determinations ±SD. CNT, Control; PM, pan masala treated group; PM+C, group with co-administration of pan masala and cardamom; *F>F_{crit} compared to control group.*

Biochemical studies indicated significant differences in glucose, calcium, triglyceride, protein, creatinine, GOT and GPT in the serum of mice fed with pan masala containing diet. When cardamom was given along with pan masala, it showed protecting effect on all the parameters. There was a significant rise in the serum glucose, calcium, triglyceride, protein, creatinine, GOT and GPT in mice exposed to pan masala for 36 weeks. Cardamom showed ameliorating effect and the mice fed with cardamom had their serum levels almost near the control values (Table 2).

DISCUSSION

The habit of using pan masala is increasing because of its legality, free availability and relatively cheap cost. However, studies have confirmed that smokeless tobacco is as harmful as smoked tobacco. In fact, chewing tobacco could result in significantly greater deleterious cardiovascular effects due to a larger overall exposure owing to prolonged absorption [12]. Nearly a third of all cancers can be attributed to use of pan masala. The present paper is focussed on the protective property of cardamom against ill effects of pan masala. Cardamom is a highly aromatic and distinctive spice possessing 8% essential oils. Constituents in cardamom include 1,8 cineole, alpha-terpinyl acetate, limonene, and myrcene along with many other volatile terpenoids[13] 1,8 cineole (eucalyptol) is a terpenoid compound that has been shown to possess anti-inflammatory capacity in the rat paw edema model of inflammation[14]. A direct relationship between the degree of protection by cardamom and the level of ACP, ALP and LDH was found.Significant increase in enzymatic parameters demonstrates excessive damage to all the studied organs. Raised serum alkaline phosphatase and acid phosphatase have also been reported in bronchogenic carcinoma and malignant tumors in the lung [15][16][17]. The increase in LDH level may be associated with free radical production from areca nut, catechu and lime, the major ingredients of pan masala[18]. The increased activity can also be correlated with cellular injury, inflammation, tissue damage and progression of fibrosis in chronic interstitial disorders [19][20]. Tissue inflammation or chronic inflammation changes cell immune function causing metabolic dysregulation of lipid metabolism, through pro-inflammatory cytokines leading to hypertriglyceridemia and lipid oxidation[21] [22]. Increased plasma concentration of cytokines leads to altered lipid metabolism[23], altering amino acid utilisation of various tissues involved in lipid metabolism or modifying hypothalamic-pituitory-adrenal axis, increasing plasma concentration of ACTH, cortisol, adrenaline, nor adrenaline and glucagon[21]. All these lead to hepatic lipogenesis, increased synthesis or reduced clearance of triglycerides which induces rapid increase in serum glucose and triglyceride levels. The mechanism by which these cytokines increase serum levels may be due to an increase in the activity of 3-hydroxyl-3methyl glutaryl Co-enzyme A (HMG-CoA Reductase) [24]. Plasma free cortisol flowing through glomeruli cause increase in serum creatinine level and different degree of renal impairment[25]. There was a 4 fold increase in the creatinine level after pan masala exposure depicting kidney damage which can also be attributed to the higher level of ACTH secreted due to chronic inflammation [26]. There was a significant increase in total protein levels possibly due to the increase in globulin fractions and other serum proteins [27]. One of the constituent of pan masla is slaked lime which is reported to induce hypercalcemia [28] similar to our study. Pan masala caused 3 fold increase in serum calcium. However all the serum parameters showed a decline in their value in PM+C treated mice as compared to PM treated mice. A higher level of GOT and GPT was observed in the liver and serum of pan masala treated mice. GPT level in liver increased to 10 times in pan masala treated mice and GOT got doubled. A similar result was observed in the serum values of these transaminases. A rise in GOT and GPT activity is a sensitive indicator of damage to the cytoplasmic and/or mitochondrial membrane due to liver toxicity [29]. These enzymes are involved in amino acid metabolism, therefore an increase in their level indicates tissue damage or toxic effects of liver [30] [31]. Thus the protective property of cardamom was revealed on all the target organs which caused a decrease in all the abnormal elevated parameters in the tissue as well as the serum of pan masala treated mice.

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