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NEPHROTOXICITY INDUCED BY PAN MASALA IN SWISS MICE AND ITS PROTECTION BY ELETTARIA CARDAMOMUM (L.) MATON

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ABSTRACT

This study was designed to investigate the protective effect of cardamom on renal tissue damage caused by pan masala. Experimental animals were divided into 3 groups, control, pan masala treated and cardamom along with pan masala treated. They were exposed till 12 months to observe changes histologically and ultrastructurally. Male Swiss mice were given orally pan masala at a dose of 2% of the feed which caused acute tubular necrosis along with dilation and atrophied glomerulus seen in its light microscopic structures, whereas ultrastructural changes showed pyknotic nucleus, swollen mitochondria and loss of membrane integrity. When cardamom was given at a dose of 0.2% along with pan masala or alone, damages were less showing normal glomerulus with less inflammation, and normal nucleus. It is, therefore, concluded that cardamom may be beneficial in preventing pan masala induced renal tissue damage and shows potential for clinical use.

KEY WORDS

Cardamom, kidney, necrosis, pan masala

INTRODUCTION

The mammalian kidney is a target organ for a wide variety of toxic agents due to its prime function as a blood filter during the excretory process and is also sensitive to drug induced injury[1]. In this experiment, kidney is studied to assess the nephrotoxicity of pan masala and the protective properties of cardamom against it. Pan masala is a dry mixture of tobacco, areca nuts, slaked lime, catechu, and flavouring agents such as menthol, camphor, sugar, rosewater, aniseed, mint, or other spices are sometimes added in different regions. These have been found to promote excessive and harmful use and also lead to dependence[2]. It is sold in small pouches in India, Pakistan, Srilanka, Bangladesh and in migrant populations from these regions in other countries[3]. It has become a very serious health hazard and its increasing trend has given rise to a number of human health problems like cancer, reproductive and developmental defects, cardiovascular problem etc. Various organs have been studied but the data on kidney is scarce. Thus, the present study was designed to evaluate the damages imposed by pan masala on kidney and its protection by cardamom.

MATERIALS AND METHODS

The experiment was cleared by the Ethical committee, Ranchi University, Ranchi, for

conducting research on Swiss mice. Thirty male Swiss albino mice weighing 22± 5g acquired from B. N. Ghosh and Company, CIT Road, Kolkata, were housed in the laboratory under natural condition and allowed water ad-lib. Animals were randomly divided into three groups: control (fed with formulated feed), PMT (fed with formulated diet along with pan masala; 2% of the feed) and PMCT (formulated diet mixed with fixed combination of pan masala and cardamom, 2% and 0.2% of the feed) and were kept for 9 months[4]. Afterwards, mice from the group PMT and PMCT were continued with only cardamom along with the feed for another 3 months to check its ameliorating property.

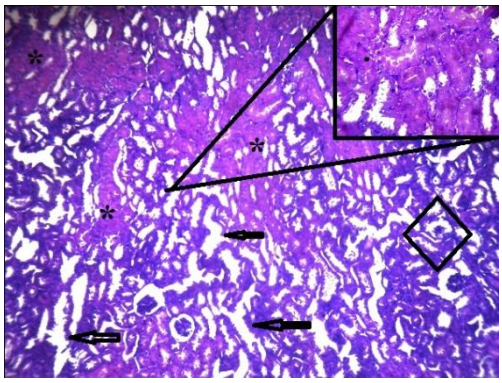
At the end of the experiment, mice from all groups were sacrificed by cervical dislocation under anaesthesia, and kidney was excised. The fragments from harvested tissue were fixed in Bouin's fixative, embedded in paraffin, stained and observed under light microscope.

Another portion was fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer and processed to observe its ultrastructure by Philips CM-10 transmission electron microscope (Netherland) at AIIMS, New Delhi.

RESULTS

Under the light microscope, the kidney of male mice in the control and sham control group showed a typical cortex represented by vascular glomerulus and convoluted tubules, which are lined by cylindrical epithelial cells (figure not shown). However, vascular changes with interstitial edema, severe inflammation and shrunken glomerulus were visible in PMT mice along with tubular dilation (**Fig.1**).

Figure 1: Photomicrograph of PMT mice showing severe inflammation (*), also shown in the insat (40x). Dilation of tubules (arrow) and shrunken glomeruli (bounded by square). (9 months; H&E, 10x)



Cortical renal tubules show various degenerative changes with focal tubular necrosis, atrophied and fragmented glomerulus (**Fig. 2**).

After 12 months including last 3 months of cardamom treatment, PMT still showed dilated tubule with slogged of epithelium and also tubules undergoing necrosis and atrophied glomerulus (**Fig.3**). **Fig.4** represents the section of PMCT after 9 months of treatment of pan masala along with cardamom. Normal glomerulus was visible, inflammation was less. Intact exfoliated tubular cells in tubular lumen were seen in the section showing ischemic injury. Signs of amelioration were clearer in PMCT, after cardamom treatment excluding pan masala for last 3 months, with normal glomerulus and tubules in renal cortex. (**Fig. 5**)

Figure 2: Photomicrograph of PMT mice showing necrosis (*) and atrophied glomerulus (large arrow) alongwith fragmented glomerulus (small arrow). (9 months; Mallory's triple stain, 40x)

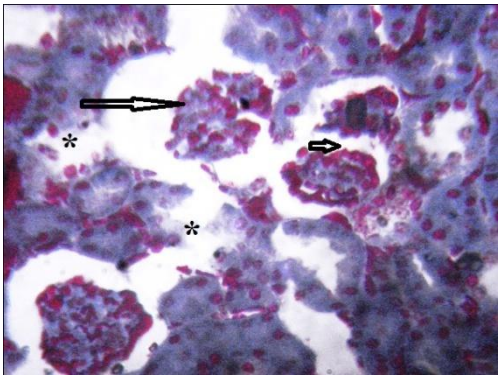


Figure 3: Photomicrograph of PMT mice showing dilated Proximal Convoluted Tubule (T) and atrophied glomerulus (G). (12 months; Mallory's triple stain, 40x)

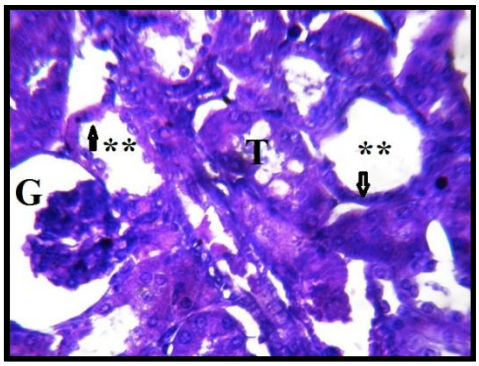


Figure 4: Photomicrograph of PMCT mice showing normal glomerulus (G) along with ischemic injury (*). (9 months; H&E, 40x)

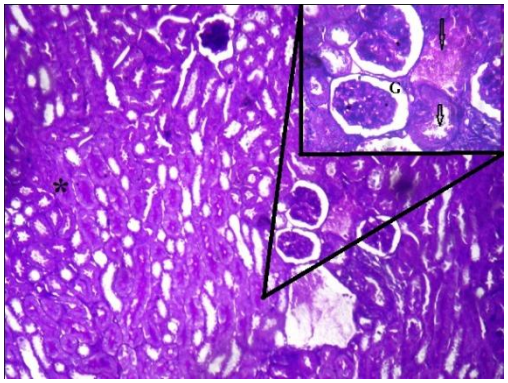
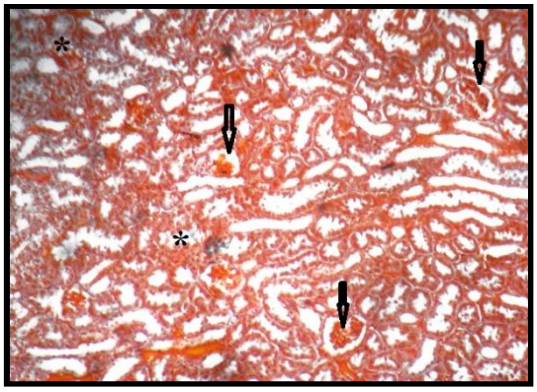


Figure 5: Photomicrograph of PMCT mice showing normal glomerulus (arrow) and necrotic tubules (*) in one part of the section showed. (12 months; Crossman stain, 10x)



Electron microscopy of injured tubules from PMT mice revealed swollen brush border microvilli and thinning of basal membrane in the proximal tubules while the control group showed array of vertically oriented mitochondria and extensive infolding of the basolateral plasma membrane (**Fig. 6 and Fig.7**)

Figure 6: An electron micrograph of control kidney's proximal convoluted tubule. mitochondria (M), nucleus (N), basal membrane (BL). (9 months; 1100x, scale bar-2µm).

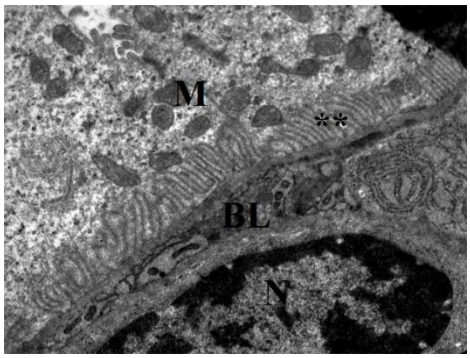
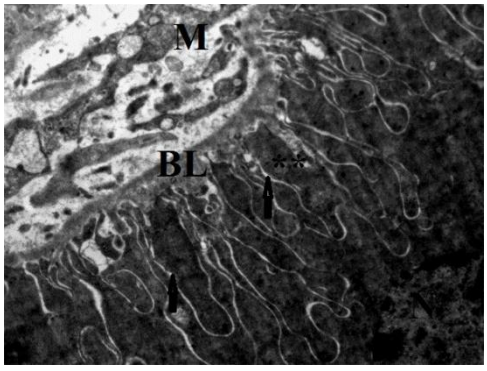


Figure 7: An electron micrograph of PMT kidney. There was a increase in the space between the interdigitations (arrow) and the basal membrane. Brush borders were swollen (**). (9 months; 1100x, scale bar-2µm).



Tubular necrosis was visible with the presence of pyknotic nucleus, swollen mitochondria, alterations in lysosomes and loss of other cytoplasmic organelles (**Fig.8**). Degenerative changes in mitochondria, including swelling and loss of cristae are often visible (**Fig.9**). PMCT mice showed signs of amelioration with the presence of almost normal brush border, normal nucleus, and mitochondria in the proximal convoluted tubule (**Fig.10**).

Figure 8: An electron micrograph of PMT kidney. Note pyknotic nucleus (arrow), lysosomal vacuoles (*), and swollen mitochondria (M). (9 months; 1100x, scale bar-2µm).

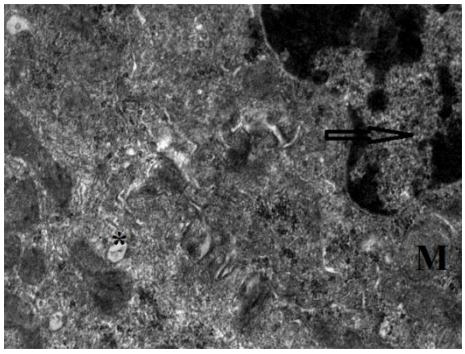


Figure 9: An electron micrograph of PMT mice showing reduced invisible cristae (**) and, in some cases, exhibit lost membrane integrity (*). (9 months; 1100x, scale bar-2µm).

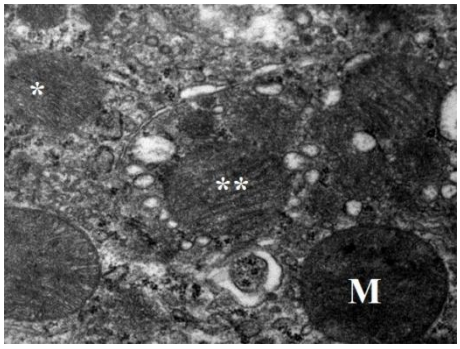
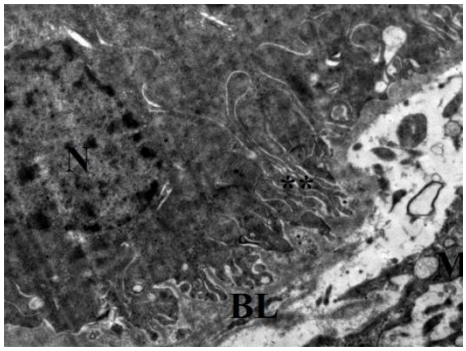


Figure 10: An electron micrograph of PMCT mice showing amelioration. Basal membrane (BL), nucleus (N), mitochondria (M) (9 months; 1100x, scale bar-2µm).



DISCUSSION

The results of this work indicate that pan masala is directly nephrotoxic to mouse glomeruli and tubular kidney cells in vivo. Human victims can also develop severe renal damages with clinical signs and symptoms such as acute renal failure, usually due to acute tubular necrosis by consuming pan masala. The habit of using it is increasing because of the cheapness, bright pouches, easy availability, sweet taste and forceful misleading advertisements. Chewing tobacco could result in significantly greater deleterious cardiovascular effects due to a larger overall exposure owing to prolonged absorption [5].

Enhanced toxicity in proximal tubular cells in PMT occurs due to the extensive cellular uptake of pan masala by endocytosis and other transport pathways[6, 7, 8]. It also occurs via basolateral delivery of endogenous and exogenous organic ions (anions and cations) by peritubular capillaries[6, 7]. Transport of toxicants into cells, followed by movement through the intracellular space via various regulated carrier proteins, and subsequent exit from the cells via apical transport proteins enhances toxicity in proximal tubular cells. Loss of function mutations in and competition for apical secretory transporters[9], which reduces toxin efflux from cell into urine, may promote accumulation of toxic substances within proximal tubular cell and cause cellular injury via apoptosis or necrosis. This extensive trafficking increases renal tubular exposure and risk for elevated concentration of pan masala. Another cause is the tissue inflammation due to pan masala exposure as observed in the PMT group which changes cell immune function, increased plasma concentration of cytokines, ACTH, cortisol, adrenaline, nor adrenaline and glucagon. Plasma free cortisol flows through glomeruli cause increase in serum creatinine level and different degree of renal impairment[10,11,12].

There are several toxic metals in pan masala such as lead and cadmium. They carry serious health risks as they can accumulate in the body and in the food chain and also pass through kidney during filteration process[13]. The kidney also possesses CYP450 enzymes that participate in drug metabolism[14,15]. Mutation of this gene by toxic pan masala could increase nephrotoxic risk as well[6]. Significant renal exposure to potential nephrotoxins, such as pan masala, occurs due to the high rate of this toxin delivery to the kidney, a result of the high blood flow to the kidney, which approaches 25% of cardiac output. Many renal cells, particularly those in the loop of Henle, exist in a relatively hypoxic environment due to the high metabolic rates required to actively transport many solutes via Na^+–K^+ -ATPase driven transport. This excess cellular workload and hypoxic environment promotes increased sensitivity to injury when exposed to pan masala, a potential nephrotoxic substance[16, 17].

Many herbs are proved to decrease high blood pressure and improve kidney functions, including cardamom[18]. It prevents DNA damage, improves liver function, kidney functions, regulates pH balance and acts as a detoxifying agent. Cardamom diet showed also pronounced improvement of renal function[19]. 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 [20]. 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[21].Thus, it can be concluded that the properties of cardamom should be utilized in the medical fields to protect

the human health from the hazardous impact of pan masala.

CONFLICT OF INTEREST

There is no conflict of interest in the research.

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