Mycopathologia (2007) 163: 21–30
DOI 10.1007/s11046-006-0079-9
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# Ochratoxin A and citrinin nephrotoxicity in New Zealand White rabbits: an ultrastructural assessment*

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Received 10 May 2006; accepted in revised form 30 October 2006

## Abstract

In the present investigation, ochratoxin A (OTA) (0.75 mg/kg feed) and citrinin (CIT) (15 mg/kg feed) were fed alone and in combination to young growing New Zealand White rabbits for 60 days to evaluate renal ultrastructural alterations. The severity and intensity of renal ultrastructural changes varied with the type of the treatment, and predominant and consistent lesions were recorded in the proximal convoluted tubule (PCT) lining cells. The significant changes in mitochondria, the most affected cell organelle in all the treatment groups, included mitochondrial disintegration and distortion, pleomorphism, cluster formation and misshapen appearance such as signet ring, dumbbell, cup and U shapes. Intra-cisternal sequestrations of involuting mitochondria, and thickening of basal layer of PCT epithelial cells with partial detachment, were the characteristic features observed in OTA and combination treatments. CIT treatment revealed crenated nucleus, loss of nucleolus, depletion of cytoplasmic organelles, mitochondrial pleomorphism, nuclear fragmentation, uniform folding of cell membrane and cytoplasmic vacuolations in the PCTs. Focal thickening of the glomerular basement membrane and degeneration of endothelial cells were the prominent alterations in the glomeruli in OTA and combination treatments. Distal convoluted tubules were unaffected in CIT treatment, however, mild to moderate lesions were observed in OTA and combination treated rabbits. It may be concluded that on simultaneous exposure, CIT potentiated the toxic effects of OTA on renal ultrastructure.

**Key words:** citrinin, mitochondria, ochratoxin A, proximal convoluted tubule, rabbits, ultrastructural changes

**Abbreviations:** BM – basement membrane; CIT – citrinin; DCT – distal convoluted tubule; FP – foot processes of podocytes; ICS – intra-cisternal sequestration; IS – interstitial space; Lu – lumen; M – mitochondria; Mv – microvilli; N – nucleus; OTA – ochratoxin A; PCT – proximal convoluted tubule; RER – rough endoplasmic reticulum; **US** – urinary space

## Introduction

Mycotoxin(s) contamination of various food and feed commodities is an important problem not only in terms of human and animal health but can also have serious economic impact causing losses of millions of dollars in terms of production and reproduction. It reduces the nutritional value of food and feeds and is responsible for causing deleterious effects in form of toxicosis in animal and human populations. Among various mycotoxins,

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ochratoxin A (OTA) and citrinin (CIT) are important food born contaminants causing nephrotoxicity. OTA is mainly produced by *Aspergillus ochraceus* (= *A. alutaceus*) [1] and CIT is produced by *Penicillium citrinum* while *P. verrucosum* produces both the toxins [2]. Kidney is the primary target organ for ochratoxin A [3] as well as citrinin [4]. OTA has been shown to induce nephropathy in all the animal species so far tested, including birds and mammals [5] and it also induces renal tumors in rodents. In humans, OTA and CIT have been implicated as causal agents for the development of fatal endemic nephropathy i.e. Balkan endemic nephropathy and an increased incidence of urinary tract tumors [6]. The $LD_{50}$ value of OTA for rabbits was found to be 10 mg/kg body weight [7]. The $LD_{50}$ value of CIT for rabbits is 134 mg/kg body weight [8]. The natural occurrence of low levels of citrinin and ochratoxin A has been reported in maize (18–207 μg/kg and 0.6–1.6 μg/kg) [9] and in fruits (50–70 μg/kg and 260–300 μg/kg, respectively) [10]. OTA and CIT are often encountered simultaneously in the nature [11]. Vrabcheva et al. (2000) [12] reported co-existence of OTA (140 ng/g) and CIT (420 ng/g) in feeds of Bulgarian villages with a history of Balkan endemic nephropathy and the reported citrinin levels were 2–200 times higher than OTA. Tangni and Pussemier (2006) [13] found OTA (17.3–318 ng/g) and CIT (137–344 ng/g) in wheat grains from Belgian grain storages. Under field conditions, citrinin may potentiate the OTA toxicity in various mammalian species. Citrinin can act synergistically with ochratoxin A leading to depressed RNA synthesis in murine kidneys [14]. Since OTA and CIT are nephrotoxic, their simultaneous occurrence as food contaminants might lead to more severe renal damage due to their interaction, additive or synergistic. There is scarce information on sub-cellular changes as a result of interaction of these two mycotoxins. The present investigation was undertaken to assess the renal ultrastructural alterations in rabbits fed a diet containing OTA and CIT, either alone or in combination.

## Materials and methods

### *Production and analysis of ochratoxin A*

Pure culture of *Aspergillus ochraceus* (NRRL-3174) procured from National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, USA was employed for the OTA production as described by Trenk et al. [15]. Briefly, OTA was produced through the fermentation of sterilised maize at 25°C (±2) by the inoculated fungal culture for 2–3 weeks in dark place with vigorous shaking twice a day to break the clumps. Fermented maize was autoclaved, dried at 80°C and ground to a fine powder. OTA was extracted using chloroform and 10 g diatomaceous earth (Celite-545) to 50 g culture powder and filtered by muslin cloth. Subsequently the crude extract was reduced to $^{1}/_{10}$ volume by vacuum evaporation. Filtrate was passed through chromatography column containing activated diatomaceous earth with aqueous sodium bicarbonate. Initial elution was carried out with n-hexane and followed by benzene: acetic acid (98:2). Ochratoxin A was crystallised by treating thrice with benzene and dried under vacuum. Finally, OTA was estimated using thin layer chromatography and UV-Vis-Spectrophotometer (Genesys$^{TM}$ 10, Thermo Electron Corporation, Pittsford, USA) at 333 nm against the standard toxin procured from Sigma Chemicals Limited, USA [16]. The purity of the toxin produced in the laboratory was found to be approximately 94%.

### *Production and analysis of citrinin*

Citrinin was produced and analysed on sterile maize as described by Jackson and Ciegler (1978) [17]. The culture of *Penicillium citrinum* NRRL 5907, supplied by National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, USA, was used to produce citrinin on partially ground maize. The inoculated maize grain samples were kept at 25°C (±2) for 2–3 weeks and were shaken twice daily for desired growth of mycelial mass. The culture substrate was steamed for 5 minutes, dried at 50°C and ground to a fine powder. The culture was soaked with chloroform, acidified with concentrated HCl and allowed to equilibrate. The suspension was blended, filtered and the chloroform layer was rinsed with water. Then the suspension was extracted with acidified 0.1 M $NaHCO_3$ (conc. HCl, pH 2.5) and condensed to $^{1}/_{10}$ volume. The chloroform crude extract was washed with water and again extracted with 0.1 M $NaHCO_3$. The aqueous layer was acidified to pH 2.5, and toxin was collected by filtration. The citrinin was dissolved in chloroform and estimated

by TLC and Spectrophotometer (Genesys™ 10, Thermo Electron Corporation, Pittsford, USA) at 322 nm against the standard citrinin procured from Sigma Chemicals Limited, USA. The purity of the toxin was found to be approximately 95%.

*Experimental animals*

Sixteen New Zealand White rabbits, 6–8 weeks of age, were procured from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute (IVRI) and housed individually in metal cages. All the experimental procedures were carried out with the prior permission of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC). All animals were house-caged in a temperature- controlled and artificially illuminated room (12 h light/dark cycles) free from any source of chemical contamination. These rabbits were maintained on a standard basal diet supplied by the Feed Processing Unit of the Institute, containing maize (30%), wheat bran (35%), decorticated ground nut cake (25%) and fish meal (10%) with adequate vitamin and mineral supplementation (estimated Crude Protein 16.2% and Metabolizable Energy 2.35 Mcal/kg feed). The mycotoxin free, fresh green fodder (200 g/animal/day) was supplied along with water *ad libitum*. The basal experimental diet was tested prior to start of the experiment in the laboratory for aflatoxin $B_1$, ochratoxin A, citrinin and fumonisin $B_1$; and no detectable levels of these mycotoxins were recorded by thin layer chromatography (lower detection limit 1–5 μg/kg feed).

*Experimental design*

After acclimatization period of one week, the animals were randomly distributed into four groups of four animals each and treated as follows: Group I, diet containing 0.75 mg OTA/ kg feed; Group II, diet containing 15 mg CIT/ kg feed; Group III: diet containing 0.75 mg OTA/kg feed + 15 mg CIT/kg feed; and Group IV, fed standard mycotoxin free basal diet.

*Experimental feed*

The inoculum containing known amounts of OTA and CIT was separately added and thoroughly mixed with the basal diet in proportion so as to arrive at 0.75 mg/kg and 15 mg/kg level of OTA and CIT in the basal experimental feed, respectively. Randomly four aliquots were taken in duplicate from each of the experimental diet to check the homogeneity of toxin concentration for the required levels using thin layer chromatography and spectrophotometric analyses and the resultant variability was observed in the range of ±2–4% for all the experimental diets.

*Electron microscopy*

All the animals from each group were euthanised using overdose of ketamine anesthesia at 60 days post treatment. Kidneys were weighed and small tissue pieces were immediately collected into a petri dish containing chilled 2.5% glutaraldehyde in cacodylate buffer (pH 7.4), trimmed to cubes measuring approximately 1 $mm^3$ and fixed for 6 h at 4°C. The tissues were washed twice (30 min each) with cold 0.2 M phosphate buffer (pH 7.4) and fixed in 1% osmium tetraoxide for 4 h at 4°C. The tissue pieces were then dehydrated in ethyl alcohol, cleared and embedded in epon-araldite resin. Ultra thin sections (600 Å) were cut employing an ultra microtome (Ultracut, Reichert-Jung, Austria), mounted onto copper grids, and stained with uranyl acetate and subsequently with lead citrate [18]. The grids were examined under an electron microscope (Philips M-10, Holland) at the All India Institute of Medical Sciences (AIIMS), New Delhi. Four samples (renal cortex) from each animal of each group were evaluated to judge the severity of lesions. The lesions were classified as mild (+), moderate (+ +), intense (+ + +) and severe (+ + + +) on the basis of changes in renal tubules, glomeruli and interstitium.

## Results

Clinically the rabbits of all the toxin fed groups were comparatively less active than the controls and the signs of toxicity observed were anorexia, dullness, lethargy, loose feces, polydypsia and dehydration. A significant decrease in the body weights of all the treated groups was recorded (1353.75 ± 8.67 g, 1370 ± 7.22 g and 1253.75 ± 10.51 g, respectively in groups I, II and III) when compared with those in the control group (1458.25 ± 6.84 g). The relative

weights of kidneys were comparable between all the toxin fed groups and the control (0.66 ± 0.03%, 0.65 ± 0.03%, 0.67 ± 0.02% and 0.63 ± 0.03% in groups I, II, III and IV, respectively). Relative liver weights in the groups I and II (3.60 ± 0.22% and 3.46 ± 0.14%) when compared with that in the group IV (3.31 ± 0.12%) were insignificantly higher. However, group III (4.12 ± 0.15%) revealed significantly higher relative liver weight as compared to group IV. The relative weights of other vital organs (heart, brain and lungs) did not reveal any significant differences between toxins fed groups and the control group rabbits.

Kidneys of the intoxicated animals revealed mild to moderate paleness on gross examination. Histologically, the renal lesions were more pronounced in OTA and combination treated animals compared to citrinin treated rabbits. The proximal convoluted tubules were consistently affected showing degenerated epithelial cells with occasional karyomegaly; at some places the epithelial cells were desquamated and even shed into the lumen. Distal convoluted tubules were less affected in OTA and combination treated groups and were comparatively normal in citrinin treated group animals. In glomeruli, swelling of parietal epithelial layer was common in OTA and combination treatment groups. The inter-tubular blood vessels were engorged.

*Ultrastructural changes*

In rabbits, the intensity of renal lesions varied between the treatments, whereas within the groups the lesions were similar in nature i.e. mild to moderate in group II, moderate to intense in group I and intense to severe in group III (Table 1).

*Group I (OTA treated)*

Ultrastructural alterations primarily involving proximal convoluted tubular (PCT) epithelium were mainly associated with mitochondria, nucleus and surface microvilli. Degeneration and distortion of mitochondria with complete loss of cristae and formation of empty spaces in the cytoplasm were the consistent findings. PCT epithelial cells were shrunken with condensed cytoplasmic organelles, indistinct nuclear membrane, uneven distribution of nuclear chromatin and disappearance of nucleoli and cytoplasmic vacuolations (Figure 1). Intra-cisternal sequestration of mitochondria undergoing involution and dissolution was observed as a prominent feature (Figure 2). Base-

*Table 1.* The ultrastructural characteristics of renal lesions observed at 60 days post-intoxication of ochratoxin A and citrinin, alone and in combination in rabbits

| †Renal lesions | Group I (OTA) | Group II (CIT) | Group III (OTA + CIT) |
|---|---|---|---|
| *Proximal convoluted tubules* | | | |
| Damaged epithelial brush border | + + + | + + | + + + + |
| Mitochondria | | | |
| Swollen with loss of cristae | + + + | + + | + + + + |
| Distorted or mis-shapen | + + | + | + + + + |
| Intra-cisternal sequestration/involution | + + | – | + + + + |
| Nucleus | | | |
| Loss of nucleoli | + + | + | + + + + |
| Indistinct nuclear membrane | + + + | + + | + + + + |
| Nuclear fragmentation | + + | + | + + + |
| Cytoplasmic vacuolations | + + + | + | + + + + |
| Swollen and degranulated RER | + + | – | + + + |
| Thickened/detached tubular basement membrane | + + + | – | + + + + |
| *Distal convoluted tubules* | | | |
| Mitochondria and cytoplasmic changes | + | – | + + |
| *Glomerulus* | | | |
| Thickening of basement membrane | + + | – | + + + |
| Electron dense deposits in Bowman's capsule | + | – | + + |
| *Interstitium* | | | |
| Widened space due to proteinaceous deposits | + + | + | + + |

†Lesions described as: (–) no lesion, (+) mild, (+ +) moderate, (+ + +) intense; and (+ + + +) severe.

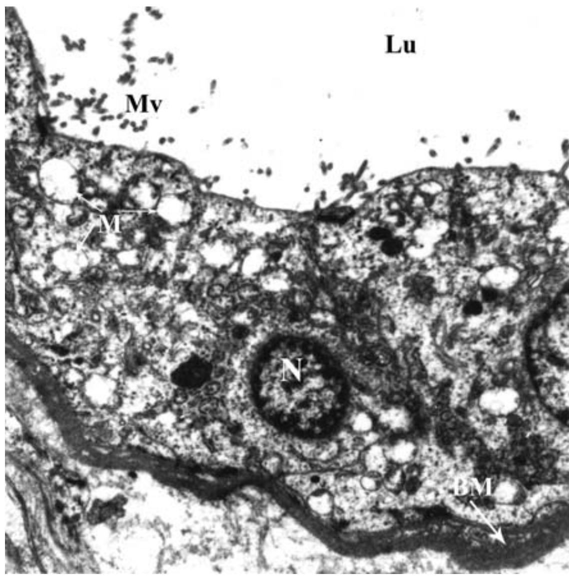


*Figure 1.* Group I: A PCT epithelial cell showing loss of microvilli (Mv), degenerating nucleus (N) with indistinct nuclear membrane, cytoplasmic vacuolation and loss of cytoplasmic organelles including mitochondria (M). Lead citrate & uranyl acetate × 2400.

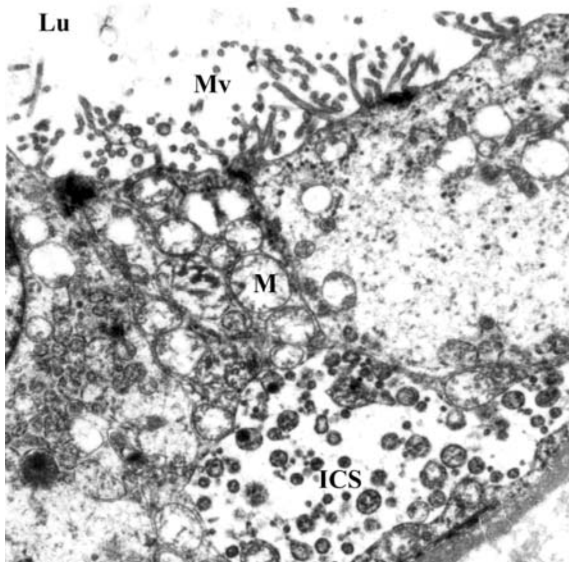


*Figure 2.* Group I: A PCT epithelial cell showing degenerating pleomorphic mitochondria (M) and intra-cisternal sequestration (ICS) of mitochondria (mitochondrial involution and dissolution). Note the area in cytoplasm devoid of organelles. Lead citrate & uranyl acetate × 3000.

ment membrane of the PCT epithelial cells was thickened and folded giving festooned appearance due to detached basal lamina from the reticular lamina (Figure 3). At some places, the interstitial cells showed nuclear fragmentation and cytoplasmic blebing. The interstitial spaces were widened due to accumulation of finely granular proteinaceous material. Glomeruli revealed thickening of glomerular basement membrane (Figure 4). The distal convoluted tubular (DCT) epithelial cells showed mild changes with less severity than those observed in PCT epithelial cells.

*Group II (CIT treated)*

In this group, renal ultrastructural changes were comparatively less severe than in the ochratoxin A treated group. The PCT epithelial cells revealed crenated nuclei, loss of nucleoli, variable depletion of cytoplasmic organelles and peripheral condensation of pleomorphic mitochondria in the cytoplasm and cytoplasmic vacuolations (Figure 5). The basement membranes of PCT epithelial cells and glomeruli were unaffected. Interstitial cells revealed nuclear fragmentation, large intracytoplasmic vacuoles and uniform folding of cell membrane. The DCT epithelial cells did not show any significant alterations and majority of them revealed almost normal appearance.

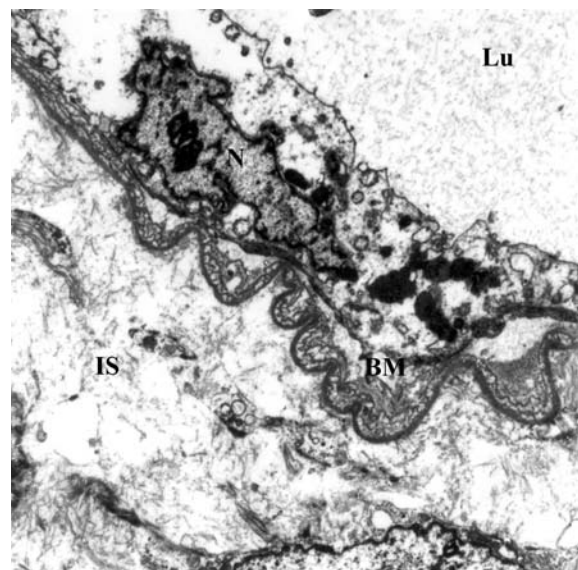


*Figure 3.* Group I: A degenerating PCT epithelial cell showing loss of microvilli (Mv) and cytoplasmic organelles and degenerating nucleus (N). Reticular lamina is multilayered and folded giving a festooned appearance. Lead citrate & uranyl acetate × 1500.

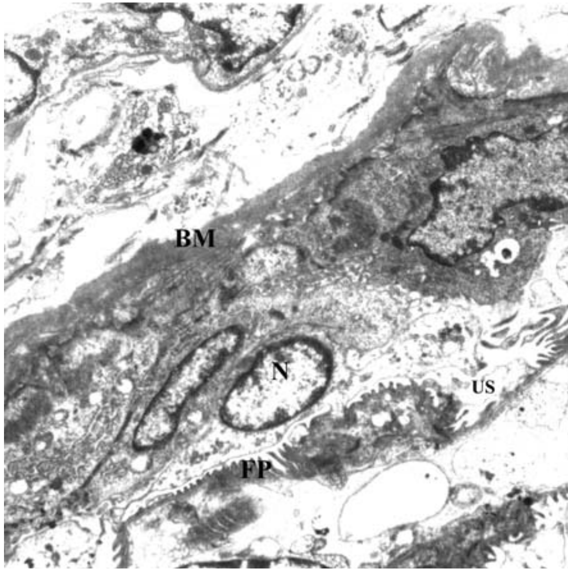


*Figure 4*. Group I: Glomerulus showing focal thickening of glomerular basement membrane (BM). Lead citrate & uranyl acetate × 1900.

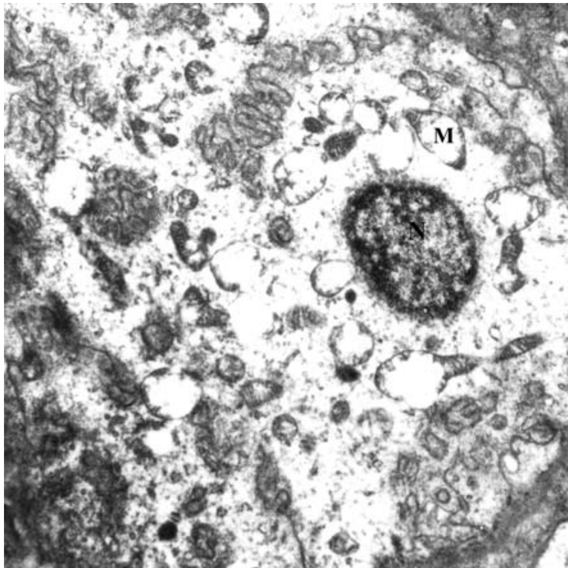


*Figure 5*. Group II: A PCT epithelial cell undergoing necrosis showing loss of nucleolus and cytoplasmic organelles. Note the presence of pleomorphic degenerating mitochondria (M). Lead citrate & uranyl acetate × 3000.

*Group III (Combination treatment)*

The ultrastructural changes were most severe and prominent in the combination group than those observed in the individual toxin treated groups. PCT epithelium revealed complete loss of brush border and/or thickening and blunting of microvilli (Figure 6). Intra-cisternal sequestration of mitochondria undergoing involution and dissolution, a characteristic feature, was consistently observed in the cytoplasm of PCT epithelial cells (Figure 7). Mitochondrial pleomorphism, cluster formation and mis-shapen appearance such as signet ring, dumbbell, cup and U shapes were consistently observed. Dilated and degranulated rough endoplasmic reticulum (RER) was frequently noticed (Figure 8). Epithelial cells of Bowman's capsule showed large sized electron dense deposits with excessively thickened basal layer (Figure 9). Some of the PCT cells and interstitial cells revealed cytoplasmic condensation, nuclear fragmentation and bleb formation (Figure 10). The DCT epithelial cells revealed changes of moderate severity compared with those observed in the PCT epithelial cells.

*Group IV (Control)*

PCT epithelial cells revealed the presence of closely packed, uniform microvilli, well formed nuclei and normal shape, size and number of cytoplasmic organelles (Figure 11) including densely packed mitochondria with distinct cristae arranged in the form of tubular rays arising from their membrane,

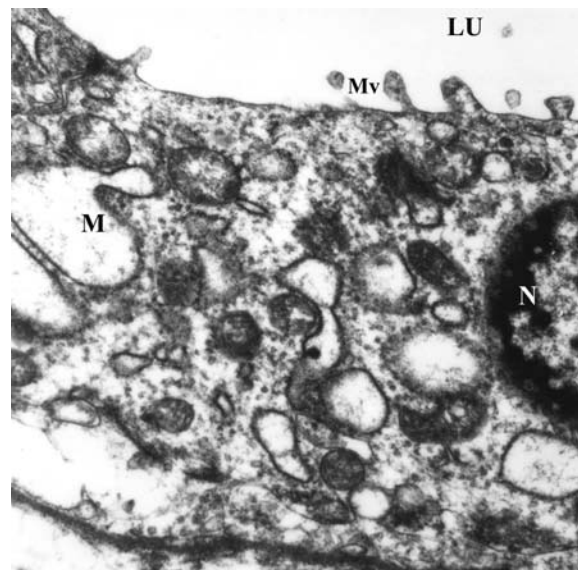


*Figure 6*. Group III: A PCT epithelial cell showing blunting and loss of microvilli (Mv). Note pleomorphic mitochondria (M) with loss of cristae. Lead citrate & uranyl acetate × 4800.

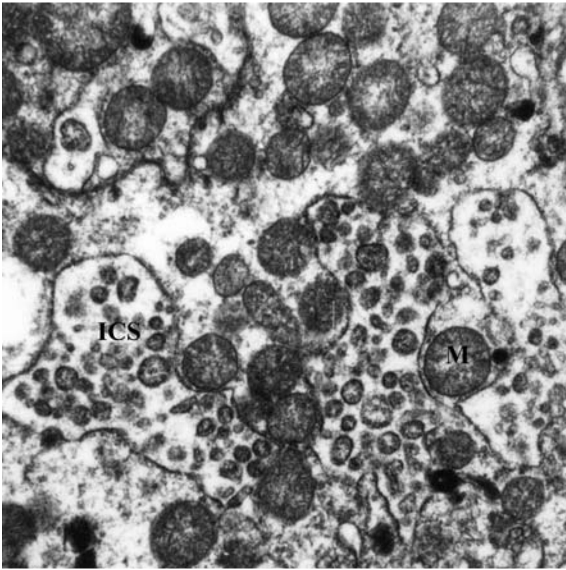


*Figure 7*. Group III: A PCT epithelial cell showing intra-cisternal sequestration (ICS) of mitochondria (mitochondrial involution and dissolution). Lead citrate & uranyl acetate × 4800.

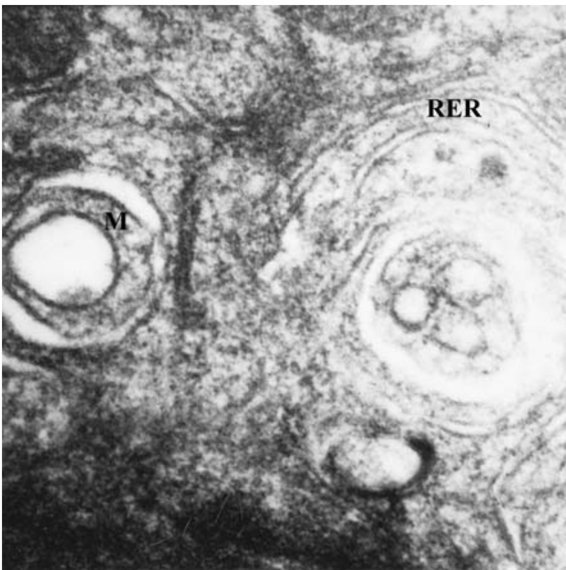


*Figure 8*. Group III: A PCT epithelial cell showing degranulated RER and signet ring form of a mitochondrion. Note a cluster of degenerated mitochondria (M). Lead citrate & uranyl acetate × 19000.

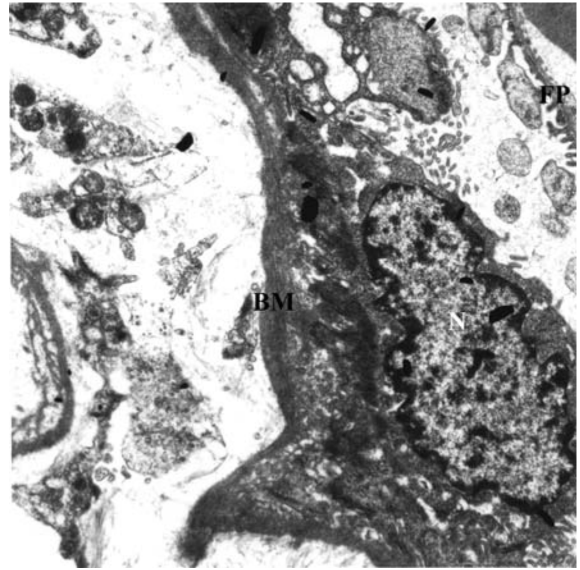


*Figure 9*. Group III: An epithelial cell of Bowman's capsule showing electron dense deposits in the excessively thickened basal layer. Lead citrate & uranyl acetate × 2400.

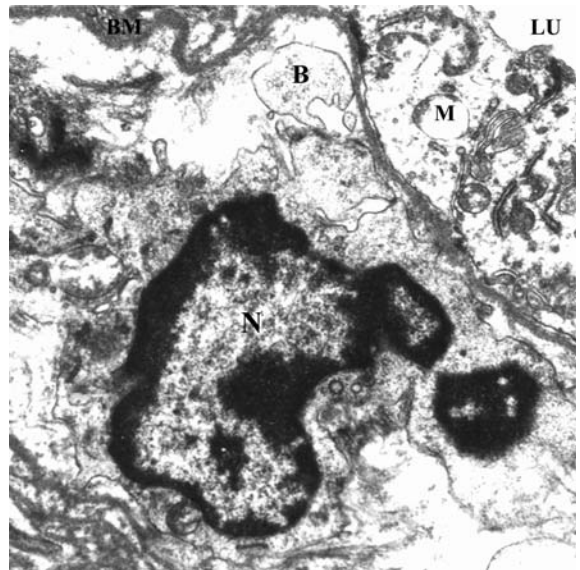


*Figure 10*. Group III: An interstitial cell undergoing apoptosis showing chromatin condensation and margination; nuclear (N) fragmentation with formation of cytoplasmic blebs (B). Lead citrate & uranyl acetate × 3800.

smooth endoplasmic reticulum, rough endoplasmic reticulum, lysosomes etc. The glomerulus contained glomerular tuft with mass of mesangial cells and capillary loops having normal lining endothelial cells with foot processes and glomerular basement membrane.

## Discussion

In the present study, low dietary levels of OTA (0.75 mg/kg feed) and CIT (15 mg/kg feed), alone and in combination (0.75 mg OTA /kg feed plus 15 mg CIT/kg feed) caused significant renal

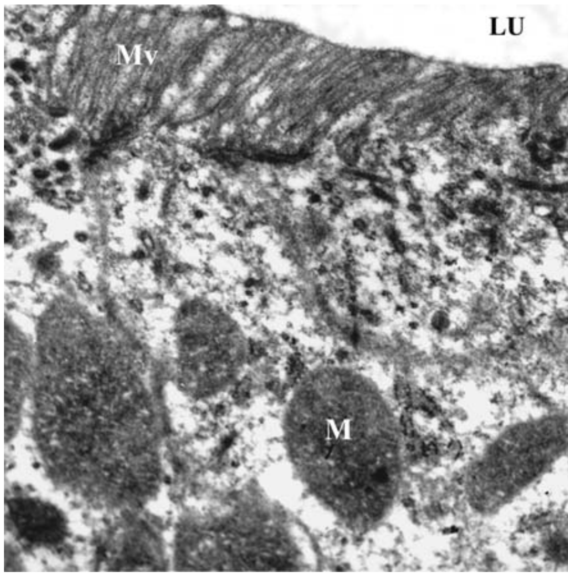


*Figure 11*. Group IV: A PCT epithelium showing abundant, long slender microvilli (Mv) on the surface and normal morphology of mitochondria (M) in the cytoplasm. Lead citrate & uranyl acetate × 3300.

ultrastructural changes in the rabbits. All the intoxicated groups revealed similar types of lesions, the difference being only in the severity and intensity of the lesions. Prominent and consistent lesions were recorded in PCTs in all the treated groups. The degenerative and necrotic changes were relatively intense in the OTA treated animals, severe in the combination group and mild to moderate in the CIT treated rabbits. In the PCT epithelial cells, mitochondria were found to be most sensitive to both OTA and CIT induced injury. The mitochondrial alterations observed in the present study were similar to those described in chicks, pigs and rats [19–21]. Mitochondrial swelling and misshapen appearance might be due to the accumulation of intracellular water as a result of toxic stress. OTA inhibits mitochondrial oxidative phosphorylation by acting as a competitive inhibitor of carrier proteins in the inner mitochondrial membrane [22]. Beside mitochondrial changes, extensive loss of brush border, atrophy of microvilli, cytoplasmic vacuolations with loss of organelles, degenerating nucleus with indistinct nuclear membrane, and loss of nucleolus were the other findings observed consistently suggesting extensive damage to the epithelial cells of the PCT. Nuclear changes noticed in the toxin treated groups might be due to free radical formation as a result of toxic injury to cells [23]. Oxidative damage including lipid peroxidation might be one of the mechanisms of cellular damage in the toxicity due to OTA [24].

In the present study, focal thickening of glomerular basement membrane and degenerated endothelial cells were the other changes frequently noticed in the OTA and combination groups. These findings were in consensus with the earlier reports during the experimental ochratoxicosis in pigs, rats and poultry. The nuclear fragmentation with cytoplasmic bleb formation (apoptosis) observed in the present study was a characteristic feature recorded in OTA and combination groups. Although, OTA induced apoptosis has been earlier reported *in vivo* in rat kidney and *in vitro* in human kidney epithelial cells [25] but not in case of rabbit.

In CIT group, the PCT epithelial cells revealed crenated nucleus, loss of nucleolus, depletion of cytoplasmic organelles, mitochondrial damage with pleomorphism and their condensation at the periphery. These findings were in agreement with earlier findings in rabbits, dogs and poultry [26–28]. Citrinin has been reported to cause inhibition of cellular respiration and decreased dehydrogenase activity in the rat kidney [29]. Mitochondrial condensation might lead to respiratory stimulation or altered membrane permeability and altered mitochondrial configuration. CIT also induces apoptosis in HL-60 cells by stimulating cytochrome-c release from mitochondria followed by activation of multiple caspases [30]. However, nuclear fragmentation and uniform folding of cell membrane in the interstitial cells as observed in the present study have not been reported earlier.

In combination group, complete loss of brush border, thickening and blunting of microvilli, swollen, dilated and degranulated rough endoplasmic reticulum and mitochondrial pleomorphism with loss of cristae were consistent changes. These findings were largely in agreement with earlier reports in dogs and poultry. Nuclear degeneration with loss of nucleolus and nuclear fragmentation and cytoplasmic blebs formation (apoptosis) were also the characteristic features. CIT has been reported to cause antagonistic effect at lower concentration [2.5 and 5 μmol/l] and additive effect at higher concentration [7.5 and 15 μmol/l] with OTA for activation of caspase 3 enzyme in

IHKE cells [31]. In addition to glomerular basement membrane, thickened basal layer of Bowman's epithelial cells was also noticed. The characteristic thickening of basal layer of the PCT epithelial cells with partial detachment and intracisternal sequestration of involuting mitochondria were the additional new findings recorded in the combination group. The thickening of basement membrane might be responsible for tubular malfunction, as it is known to act as a barrier for the macromolecular transport system in kidney [32]. The reason for this could be longer duration and dose differences in our study as compared to earlier reports. Since there is no report pertaining to combined effect of both the mycotoxins in rabbits, these lesions could be attributed solely to OTA, as no such lesions were induced by citrinin alone. Mitochondrial pleomorphism and mis-shapen appearance including signet ring, dumbbell, cup and U shapes were the characteristic features in the combination group. The ring shaped, mis-shapen and tortuous mitochondria have been reported earlier in experimental ochratoxicosis in chicken and rats. Absence of such severe alterations in mitochondria in the individual treatment groups in the present study suggested the additive interaction effect of these two mycotoxins. Though the involution and dissolution of mitochondria might be encountered occasionally in normal tissues, but the greater frequency of its occurrence might be attributed to cellular injury induced by these mycotoxins. From the present study it is evident that mitochondrial damage thus appears to play a vital role in the mechanism of nephrotoxicity.

OTA was found to be more nephrotoxic than citrinin in rabbits as also reported earlier in *in vitro* studies on LLC-PK1 renal cells [33]. OTA and CIT might have acted as substrates for the proximal tubular anionic transport system [34]; this finding corroborates the severe changes observed in the PCT epithelial cells in the present study. OTA and CIT have been observed to act synergistically on pig renal tubules for transport of tetraethylammonium and paraminohippurate [35].The synergistic interaction of these toxins related to end points of nephrotoxicity has also been suggested [36]. Contrary to these reports of synergistic interaction of both the mycotoxins, the lesions in our study were strongly suggestive of additive interaction as described in Table 1. OTA either alone or in combination with CIT was found to affect glomeruli and DCTs whereas CIT alone failed to induce any alterations either in glomeruli or in DCTs, further supporting our contention of more severe nephrotoxicity due to OTA than CIT.

On the basis of comparative evaluation of renal ultrastructural changes induced by OTA and CIT, alone and in combination in the present study it may be concluded that the simultaneous exposure of CIT (with OTA) even at sub-clinical dietary levels potentiated the OTA induced nephrotoxicity at ultrastructural level, strongly suggesting their additive interaction in rabbits.

## Acknowledgements

The authors would like to acknowledge the help extended by Dr. A. G. Telang (CADRAD) to carry out the mycotoxin analytical work. This study was financially supported in part by the NATP-CGP (ICAR) Project No. II/221.

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