Toxic Effects of Citrinin in Animals and Poultry

D.Basheer Ahamad

Department of Veterinary Pathology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tirunelveli, Tamil Nadu, India

Article Received on 20.03.2018 *Article Published on 19.04.2018*

Introduction

Citrinin (CTN) is a secondary product of fungal metabolism, first isolated by Hetherington and Raistrick from a culture of *Penicillium citrinum* Thom (Hetherington and Raistrick, 1931). Meanwhile, several other fungal species within the three genera, Penicillium (*P. expansum*, *P. verrucosum*), Aspergillus (*A. terreus*), and Monascus (*M. ruber*) were also found to produce this mycotoxin (Ciegler *et al*., 1977, Bragulat *et al*., 1977). CTN contaminates maize (Nelson et al., 1985), wheat, rye, barley, oats (Scott et al.,1972), and rice (Tanaka *et al*., 2007). Citrinin was considered as a potential antibiotic (IARC, 1986), but its toxic properties prevented its therapeutic use. Citrinin (CTN), a low molecular weight compound, that melts at 172^{o}C and is soluble in dilute sodium hydroxide, sodium carbonate, methanol, ethanol and other polar solvents (Leatherhead Food Research, 2000) was first isolated as a pure compound from a culture of *Penicillium citrinum* in 1931. Later, yellowish coloured rice imported from Thailand to Japan in 1951 was found to be contaminated with citrinin. Subsequently, it was identified in over a dozen species of *Penicillium* including certain strains of *Penicillium camemberti* (used to produce cheese) and several species of *Aspergillus* (*Aspergillus terreus, Aspergillus niveus*) and *Aspergillus oryzae,* the latter being used to produce sake, miso, and soy sauce (Manabe, 2001).

Citrinin is nephrotoxic and implicated in disease outbreaks in both animals and humans. Acutely lethal doses administered to rabbits, guinea pigs, rats, swine, or mice caused swelling of the kidneys with eventual necrosis (Friss et al., 1969; Jordan *et al*., 1978; Krogh, 1978, Krogh *et al*., 1974). In mice, citrinin is also embryocidal and fetotoxic (Hood *et al*., 1976). In rats, citrinin has similar effects and high doses are teratogenic (Reddy et al., 1982a,b). Citrinin has been implicated in porcine and avian nephropathies and possibly in the fatal renal disease in humans, causing endemic Balkan nephropathy (Friss *et al*., 1969;, Krogh *et al*., 1970 Witlock, et al., 1977). The nephropathy of citrinin in rats was characterized by an enhanced excretion of dilute urine glucosuria, proteinuria, and reduced glomerular filtration rate and renal blood flow (Petkova-Bocharova *et al*., 1991).

The kidney is the major target organ of CTN toxicity, but other target organs such as liver and bone marrow (Gupta *et al*., 1983). CTN could be implicated in porcine nephropathy (Krogh *et al*., 1973). It is frequently found in food and feed in combination with ochratoxin A (OTA), and these two nephrotoxic

mycotoxins are suspected to be involved in the aetiology of a human kidney disease called Balkan endemic nephropathy. In the endemic area in Bulgaria, CTN had higher concentrations in maize and beans intended for human consumption than in the non-endemic area (Petkova-Bocharova *et al*., 1991). CTN is also found to increase the toxicity of OTA either additively (Ahamad et al., 2006) or synergistically (Speijers and Speijers, 2004). The International Agency for Cancer Research (IARC) classified CTN in Group 3 of carcinogens because of the limited evidence of its carcinogenicity to experimental animals and no evidence for humans (IARC, 1986). Citrinin affects the animals in a number of ways i.e. it leads to decreased body weight, immunotoxicity, nephrotoxicity, hepatotoxicity, teratogeniccity. Oxidative stress, apoptosis were found to be the important mechanism for causing these effects

Incidence of Citrinin

Krogh *et al.* (1973) reported ochratoxin A and CTN in cereals with an incidence of 58 and 9 per cent, respectively, and these mycotoxins were also implicated in porcine nephropathy in Denmark. Reiss (1977) considered CTN responsible for "yellowed rice syndrome", an animal mycotoxicosis in Japan. Yoshisawa (1991) observed natural contamination of CTN in corn, rice, rye, wheat, barley, oats and decaying tomato fruits. Abdelhamid (1990) reported the occurrence of various mycotoxins in Egyptian feeds in which 44.2 per cent of feed samples tested were positive for aflatoxin (AF) containing less than 100 ppb, and 15.4 per cent of samples were positive for CTN in the range of 3-70 ppb. An outbreak of pruritis, pyrexia and haemorrhagic syndrome in cattle was attributed to feeding of citrus pulp containing citrinin 30 - 40 ppb (Griffiths and Done, 1991).

In India, Pande *et al.* (1990) reported the occurrence of CTN in feed. Manickam *et al.* (1985) encountered mycotoxicosis in buffaloes due to ingestion of mouldy fodder contaminated with AFB1, CTN, ochratoxin and penicillic acid of which CTN alone was present in around 18 per cent of samples. Sundaram *et al.* (1999) recorded the co-occurrence of CTN and AFB_1 in maize. Out of 229 samples of maize tested, 45 were positive for CTN, of which 23 samples also contained AFB_1. Natural occurrence of citrinin has been reported in various agricultural products like coconut (Kumari and Nusrath, 1987), rice (Reddy *and Reddy.,* 1983;, cereals (Pande *et al.*, 1990), some herbal medicinal plants (0.01 - 0.76mg/ g) (Roy and Kumari, 1991; Chourasia, 1995), groundnut (Subrahmanyam and Rao, 1974; Mehan and McDonald, 1984), sesame (Reddy and Reddy, 1983), bakery bread (Sinha *et al.*, 1994), poultry feeds (Rajeswari and Char, 1991), milk and milk products (Singh *et al.*, 1992), cattle feed (Jeswal and Jeswal, 1990; Ranjan and Sinha, 1991), root drugs (Roy and chourasia, 1990) and dry fruits (Saxena *et al.*, 1988). Ahamad and Vairamuthu (2000) recorded up to 400 ppb of CTN when singly or combination with aflatoxin B_1 (AFB_1); up to 4800 ppb of CTN in combination with ochratoxin A (OTA) up to 320 ppb of CTN in combination with CTN- AFB1- OTA in poultry feed where as CTN alone up to

1800 ppb; CTN with AFB_1 up to 400 ppb, CTN with OTA up to 600 ppb and up to 1200 of CTN when combination of CTN, AFB_1-OTA in feed ingredients such as maize, sorghum, jowar, soya meal, sun flower oil cake and groundnut oil cake.

Toxic Effects of Citrinin in Animals

Pigs

A case study by Friis *et al*. (1969) reported the results from pigs administered citrinin in feed at 20, 40 or 100 mg/kg b.w. per day. At the highest dose level, the pigs died in a coma with renal insufficiency and a decrease in appetite was observed in all animals. At 20 and 40 mg/kg b.w. per day growth depression, loss of weight and glucosuria were observed. The data are poorly reported and as such were not used for risk assessment. Sándor *et al*. (1991) described a subacute toxicity study with ochratoxin A and citrinin in swine. Pigs are able to tolerate citrinin concentrations in feed resulting in doses of up to 0.02 mg/kg b.w. per day for a longer period without any signs of toxicity. Szczech *et al*. (1974) reported increased concentrations of glutamic oxalacetic transaminase, isocitric dehydrogenase and lactic dehydrogenase in serum and urine before clinical signs of renal disease were evident in pigs given oral doses of 1.0 and 20.0 mg citrinin/kg b.w. per day. The activities of the enzymes were elevated in urine, whilst no other signs of renal disease were observable, and there was no alteration in blood urea nitrogen. An early elevation in these serum activities is acknowledged to be a sensitive indicator of renal damage. In conclusion, only few studies on adverse effects of citrinin in pigs could be identified. At present, no effect has been reported from pigs given 20 µg/kg b.w. per day (Sándor *et al*., 1991).

Dogs

In an early study, dogs were given citrinin with 5 mg/kg b.w. i.p. survived the entire study, but severe proximal tubule damage was observed during the post-mortem examination. (Kitchen *et al*., 1977a, b, c). Anorexia, emaciation, vomiting and polydipsia/polyuria and consumption of the feed was reported to be „over one month". The dogs had high blood urea nitrogen and creatinine. Two severely affected dogs died, while the others recovered gradually. Renal failure, with renal atrophy, congestion of the glomerula capillary, and diffuse degeneration, necrosis, dystrophic calcification and regeneration of the tubular epithelium were seen @citrinin at 8.3 µg/kg feed (Ahn *et al*., 2007) and severe scrotal dermatitis 150 µg citrinin/kg feed (Little *et al*., 1991).

Toxic Effects of Citrinin in Experimental Animals

Lethal Dose

Acute LD50 of CTN varies with the route of administration, physiological conditions, and animal species Oral LD50 for rats is 50 mg kg-1 b.w. while subcutaneous LD50 is 67 mg kg-1 b.w. (Ambrose and DeEds, 1945). In the Dutch Belted rabbit, oral LD50 is 134 mg kg-1, and in the New Zealand White rabbit it is about 120 mg kg-1 (Hanika *et al*., 1983, Hanika *et al*., 1984). The LD_{50} of CTN for ducks is 57 mg/kg, for a rat 60mg/kg, for chickens 95 mg/kg, and for rabbits 134 mg/kg (Hanika et al., 1983). In a LD50

study with hamsters, citrinin caused slight to mild necrosis of scattered individual lymphocytes in the spleen and the intestinal submucosa (Jordan et al., 1978a). The acute s.c. LD50 for citrinin in rabbits is 20 mg/kg b.w. (Ambrose and DeEds, 1946), with lacrymation and salivation, and mild histopathological changes in the kidneys. At higher s.c. doses (50-75 mg/kg b.w.) given for up to 14 days, citrinin caused marked renal lesions, confirming rabbits as sensitive to citrinin much like guinea pigs (Ambrose and DeEds, 1946). Hanika *et al*. (1983) determined intraperitoneal (i.p.) and oral 72-hr LD50 values of 50 mg/kg b.w. and 134 mg/kg b.w., respectively in rabbits. The LD50 of a single s.c. citrinin dose to rats was 67 mg/kg b.w. (Ambrose and DeEds, 1946).

Clinical Pathology

The CTN causes anaemia along with leucopenia due to lymphopenia,in increase in platelet count, mean corpuscular volume (MCV) in rats (Gupta *et al.,* 1983;Singh *et al.,* 2006) decrease in PCV, haemoglobin (Hb) and total erythrocyte count (TEC) in guinea Pig (Thacker *et al.,* 1977). Anorexia, decreased body weight gain in rats (Lockard *et al.,* 1980; Jordan *et al*., 1978a; Singh *et al.,* 2006); anorexia, dullness, lethargy, loose faeces, polydypsia and dehydration in young growing New Zealand White rabbits (Kumar *et al*., 2007).

Biochemical alterations with decreased BUN and creatinine levels, decrease in total protein, albumin and globulin, increase in activity of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) in rats (Beasley *et al*., 1986; Singh *et al.,* 2006). Increased level of total plasma protein in guinea Pig (Thacker *et al.,* 1977)increased cholesterol esters, increased BUN and serum creatinine in rabbit (Ramadoss and Shanmugasundaram, 1973; Hanika *et al.* 1983). Carlton *et al*. (1974) found in a study on beagle dogs inconsistent changes in leukocyte counts which were probably more related to dehydration of citrinin treated animals than to any direct effect of citrinin (20 and 40 mg/kg b.w. for 2 days administered in gelatine capsules and after that i.p. because of profound emetic effects). Citrinin treated mice (20 mg/kg b.w.; i.p. injections once a week for 6 weeks) shows a decrease in the total count of bone marrow cells (precursors of erythrocytes, leucocytes and megakaryocytes) (Gupta *et al*., 1983).

Pathomorphology

Dietary exposure to citrinin at 250 and 500 mg/kg feed for two weeks was well tolerated by hamsters, with no clinical signs of toxicity, no gross lesions at necropsy and no histopathological changes were observed (Carlton and Szczech, Beagle dogs (Carlton *et al*., 1974) commenced with dry feed containing 50 % of rice moulded by *Penicillium citrinum* and containing 100 mg citrinin/kg. showed poor feed consumption and inappetance. In rats, kidney shows necrosis in kidneys, vacuolar degeneration of renal tubular epithelium, tubular degeneration with presence of proteinaceous casts in the lumen of renal tubules (Lockard *et al.*, 1980, Jordan *et al.,* 1978a, Singh et al., 2007b) The other lesions include

hepatocytic degeneration with engorged sinusoids, karyomegaly in liver, dilated cystic spaces with haemosiderosis in uterus, lymphoid depletion, Haemosiderosis in spleen,villous sloughing with lymphoid depletion in peyer's patches in intestines (Singh *et al.,* 2007b) are seen. In rabbit severe renal tubular degeneration with presence of proteinous casts in tubular lumen. (Kumar, 2005) In hamster, necrosis of renal cortical tubular epithelium, tubular calcification, presence of proteinaceous casts and tubular dilations (Jordan *et al.,* 1978b).

Histopathology

Degenerative changes in renal proximal tubule epithelium were reported in rats given citrinin contaminated feed, but no dose was specified (Friis *et al*., 1969; Krogh *et al*., 1970). In contrast, no clinical or renal histopathological changes occurred in a rat given dietary citrinin for 5 days (~18 mg/kg b.w. per day) (Mantle and McHugh, 1993). Acute lethal doses administered to rabbits, guinea pigs, rats, and swine caused swelling of the kidneys and acute tubular necrosis ((Friis *et al*., 1969; Ambrose and DeEds, 1946). Subchronical oral treatment of rats with water suspension isolated from a strain of *Penicillium viridicatum* Westling caused CTN-induced kidney damage characterised by enlarged kidney, hydropic degeneration, loss of brush border, and pyknotic nuclei in the proximal tubules (Friis *et al*, 1969). Treatment of mice with weekly injections of CTN (20 mg kg-1) for six weeks resulted in a significant decrease in total bone marrow cells, red blood cell precursors, white blood cell precursors, megakaryocytes, decrease in spleen weight, and decrease in the total spleen cell count.

Utrastructural Pathology

Kumar et al. (2007) investigated the utrastructural lesions in kidney of citrinin-treated animals focused on proximal convoluted tubules (PCT) and interstitial cells, while epithelial cells of distal convoluted tubules had almost normal appearance. In epithelial cells of the PCT citrinin caused loss of nucleoli, depletion of cytoplasmic organelles, peripheral condensation of pleomorphic mitochondria and cytoplasmic vacuolation. Degenerative and necrotic changes were mild to moderate. The basement membrane of PCT epithelial cells and glomerulai were unaffected. Mitochondrial swelling and misshapen appearance are consistent with the theory that the lesions of this organelle are crucial in the mechanism of citrinin toxicity.

Toxic Effects of Citrinin in Poultry

Lethal Dose

Mehdi *et al*. (1983) reported LD50 values of 56 and 57 mg/kg b.w. for turkey poults and ducklings, respectively

Clinical Pathology

Clinical Signs

Citrinin fed to mature laying hens at concentrations of 0, 50 or 250 mg/kg diet for three weeks had no effect on body weight, feed consumption, egg production, egg weight, or quality of eggshell. Moderate diarrhoea, which subsided once the birds returned to their normal diet, was observed after approximately three weeks at the highest dose level (Ames *et al*., 1976).The important clinical signs

includes stunted growth, decreased body weight gain, watery droppings in poultry, (Campbell *et al*., 1981, Mehdi *et al*., 1984; Uma and Vikram Reddy, 1995; Ahamad, and Vairamuthu,2001; Ahamad *et al*., 2009).

Haemato-Biochemical Changes

In haematological study**,** increased PCV, reported no significant change in PCV, Hb and TEC in poultry (Campbell *et al,*. 1981;Uma and Vikram Reddy, 1995; Manning *et al.*, 1985; Ahamad et al., 2006). Citrinin was fed to broiler chickens at concentrations of 300 mg /kg feed showed lower body weight and an increased water consumption. At the last day of the study, serum protein, albumin and globulin were significantly higher in the birds fed citrinin than in controls (Manning et al., 1985). but no significant alteration was recorded in chicken when fed 150 and 300 ppm of CTN (Ahamad, and Vairamuthu,2001; Ahamad *et al*., 2006; Ahamad, 2000).

Gross Pathology

Ames *et al.* (1976) observed enlargement of liver and kidneys in broilers fed with 62.5, 125, 250 and 500 ppm CTN for 3 weeks. Roberts and Mora (1978) fed 130 and 260 ppm of CTN in broilers and noticed haemorrhagic jejunum, mottled liver and enlarged kidneys. When broilers were fed with 62 per cent of mouldy ration for 6 weeks, there was atrophy of spleen, bursa of Fabricius, thymus and testes. Heart was oedematous, congested and necrotic. The gall bladder was distended and bone marrow was pale. Beasley *et al.* (1980) reported swollen kidneys and discoloured gizzards in broiler chicks fed with *P. lanosum* contaminated feed. Uma and Vikram Reddy (1995) noticed enlargement, congestion and mottling of kidneys; enlargement, congestion and fragility of liver; petechiae in fascia and thigh muscles and dilatation of heart in broiler chicken fed 125 and 250 ppm CTN for 6 weeks.

CTN treated with 150 and 300 ppm revealed moderate to severe enlargement, moderate congestion and petechiae in kidneys. Bursa of fabricius revealed enlargement and congestion during 1st and 2nd wk and atrophy during 3rd wk of sacrifice. The liver was swollen with paleness and moderate yellowish discolouration with distended gallbladder. (Ahamad and Vairamuthu, 2001; Ahamad *et al*., 2009).The intestine revealed mild to moderate mucosal congestion with mucous exudation . The caecum showed severe dilatation. Multifocal peticheal haemorrhages were recorded in epicardium and endocardium of the heart and thigh muscles. (Ahamad and Vairamuthu, 2001; Ahamad *et al*., 2009).

Histopathology

In chicken, hyperplastic glomeruli, atrophy of tubules, hydropic degeneration of epithelial cells of the proximal and collecting tubules, glomerular atrophy and hyalinisation, coagulative necrosis and hydropic degeneration of tubular epithelial cells of kidney (Roberts and Mora 1978, Mehdi *et al.* 1983; Ahamad and Vairamuthu, 2001; Ahamad et al., 2009)

Toxic effects are not described in broiler chicks fed a diet containing 65 mg citrinin/kg feed (Carlton, 1980). Up to 260 mg citrinin/kg feed to broiler chicks

diarrhoea was observed at the two highest concentrations. At necropsy these chicks had haemorrhages in the jejunum as well as enlarged livers and kidneys. All dietary levels resulted in lymphocyte and eosinophil infiltrations of the liver, kidneys and pancreas. Anaplastic areas of the kidney and pancreas, is observed at the highest concentration of 260 mg/kg citrinin in chickens (Roberts and Mora, 1978). Citrinin was fed to broiler chickens at concentrations of 300 mg /kg feed showed proximal tubular intra-nuclear membrane-bound inclusions, misshaped mitochondria, as well as an increase in size and number of peroxisomes and secondary lysosomes(Manning *et al*., 1985). At autopsy the kidneys were observed to be swollen, pale and friable" with the tubular epithelial cells noted to be necrotic. Kidneys of CTN group birds revealed severe toxic tubular nephrosis chareccterised with mild to moderate vascular changes with congestion and inter tubular haemorrhages, moderate hypertrophy of tubular epithelium with narrowing of proximal convoluted tubules, moderate to severe hydropic degeneration with multifocal necrosis of proximal convoluted tubular epithelium, multifocal mononuclear cell (MNC) infiltration with a few heterophils and diffuse mild to moderate intertubular fibroblast proliferation and cellular debris in collecting ducts were observed. The PCT revealed detachment of tubular lining epithelium from basal layer. The lumen contained desquamated epithelial cells. These similar findings were reported by Roberts and Mora (1978) when broiler chicks were fed with 33 to 260 ppm CTN for 6 weeks, 95 mg/kg BW by crop gavage or mixed with diet (Mehdi *et al.,* 1981) , 125 and 250 ppm of CTN in feed for 6 wks (Uma and Reddy,1995) and 150 and 300 ppm fed in broiler chicks for 4 wks Severe degeneration with detachment of lining epithelium of proximal convoluted tubules might contribute to watery droppings in poultry. (Ahamad and Vairamuthu, 2001; Ahamad *et al*., 2009).

In liver, CTN treated chicks revealed moderate toxic hepatitis charecterised by diffuse mild sinusoidal dilatation, engorgement of blood vessels with vacuolar degeneration with multifocal necrosis of hepatocytes with infiltration of lymphocyte and heterophils, moderate to severe diffuse Kuffer cell hypertrophy, bile duct hyperplasia, formation of acinar pattern of hepatocytes were observed. ,These similar findings were recorded by earlier workers in broiler chicks when fed with 130 and 260 ppm CTN in feed for 6 wks and 47.5 to 500 ppm CTN in multiple dose , 90.100.and 110 mg CTN/kg BW in single oral dose (Mehdi *et al.,* 1983); 125 and 250 ppm CTN in feed for 6 wks in broiler chicken. (Uma and Reddy,1995) and 150 ppm fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001). In addition to above lesions, there was mild to moderate periductular and perivenous fibrosis and capsular thickening of liver was also observed. These changes might be due to CTN which pass through central vein and bile duct for such a long period for detoxification or excretion which acts as irritant which leads to proliferation of fibroblast around perivenous and periductular areas (Ahamad *et al*., 2009)

In bursa of fabricius, mild congestion and generalized mild lymphoid

depletion, mild to moderate lymphocytolysis was marked in cortex and medulla of follicles degeneration and necrosis of follicular epithelium with a few mitotic figures, glandular or cystic transformation of follicles and moderate to severe interfollicular fibrosis were reported by when the broiler chickens were fed with 47.5 to 500 mg CTN /kg BW in multiple dose (Mehdi *et al,* 1983); CTN.150 and 300 ppm fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001;Ahamad et al ., 2009). These findings might incriminate the hypothesis of immunotoxic effect of CTN.

In spleen**,** CTN group birds showed mild to moderate diffuse lymphoid depletion with reticular cell hyperplasia in broiler chicks for 4 wks @150 and 300 ppm CTN fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001;Ahamad *et al* ., 2009Mehdi *et al*. (1981) in broiler chicks when fed with single oral dose of 90,100 and 110 mg CTN /kg BW and). In thymus , mild lymphoid depletion was observed from from 2^{nd} to 4^{th} wks of sacrifice . Similar findings were reported by Mehdi *et al.* (1981) studied in another experiment in broiler chicks when fed with single oral dose of 47.5,71,100,250 and 500 mg CTN /kg BW and 150 and 300 ppm fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001;Ahamad *et al* ., 2009).In heart, Loss of cross-striations, enucleation, sarcolysis and hyalinization of fibres with mononuclear cell infiltration Mild congestion and multifocal haemorrhages mild to moderate myocardial degeneration with multifocal mononuclear cell infiltration in intermyofibrils were also recorded in broiler chickens when fed with 125 and 250 ppm CTN in feed for 6 wks (Roberts and Mora,1978; Uma and Reddy, 1995) and 150 and 300 ppm fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001;Ahamad et al ., 2009). Pancreas showed mild to moderate congestion and focal haemorrhages, mild diffuse vacuolar changes,Perivascular cellular infiltration of lymphocytes, eosinophils, reduced zymogen granules and multifocal necrosis of pancreatic acinar epithelium with MNC infiltration (Roberts and Mora, 1978; Maryamma *et al.*, 1990; Ahamad and Vairamuthu, 2001; Ahamad *et al*., 2009). **Lungs** of CTN fed group birds showed mild congestion and haemorrhages, multifocal MNC and heterophilic infiltrationwith mild bronchiectasis in broiler chickens when fed with 125 and 250 ppm CTN in feed for 6 wks (Uma and Reddy, 1995) and 150 and 300 ppm fed in broiler chicks for 4 wks. (Ahamad and Vairamuthu, 2001; Ahamad *et al* ., 2009). Intestine of CTN treated chicks showed mild congestion in mucosl and submucosal layer of intestine increased goblet cell activity, focal necrosis of enterocytes and mild desquamation of intestinal mucosa, catarrhal changes with infiltration of mononuclear cells in the lamina propria, mucosa and hyperplasia of the glandular epithelium in small intestine in broiler chickens when fed with 125 and 250 ppm CTN in feed for 6 wks (Uma and Reddy (1995) ; 150 and 300 ppm fed in broiler chicks for 4 wks (Ahamad and Vairamuthu, 2001; Ahamad *et al*., 2009).The gross and histopathological changes in CTN fed birds were severe toxic nephrosis in kidneys, severe atrophy of bursa of fabricius, moderate toxic

hepatitis, moderate acute myocarditis, moderate acute pancreatitis, mild lymphoid depletion in spleen and thymus and catarrhal enteritis are observed. Since, the proximal convoluted tubules of kidneys were severely damaged, there might be the possibilities of hindrance in resorption of large quantities of sodium and chloride, water , required concentration of glucose , amino acid, calcium, phosphates uric acids, proteins and potassium and leads to watery droppings and mild depression in CTN fed chicks. The immunotoxic effects could also be observed severely in bursa of fabricius (Ahamad and Vairamuthu, 2001; Ahamad *et al* ., 2009).

Apoptosis and Oxidative Stress

There are scarce reports on apoptosis caused by citrinin. Multiple effects on mitochondrial function in renal proximal tubules (RPT) and renal cortical mitochondria which might have contributed to the development of cell death in rat renal proximal tubule in rats treated with 250 mg of CTN. Aloe *et al*. (1991). Number of apoptotic cells in dam (kidney, liver, spleen) as well as foetuses (kidney and liver) on analysed by FACS When citrinin was fed @ 10ppm in pregnant wistar rats (Singh, 2005). Martin *et al.* (1986) reported that CTN induced single and double strand breaks in the DNA of intact *E. coli* and this activity was prevented by NADPH, catalase and superoxide dismutase, suggesting the involvement of free radicals in the mechanism of citrinin-induced damage. The cytochrome c release from mitochondria to cytoplasm. The presence of antioxidants in cultures did not effectively suppress CTN-induced cytotoxicity and caspase-3 activity. These findings suggest that CTN induces apoptosis in HL-60 cells by stimulating cytochrome c release followed by activation of multiple caspases, but oxidative stress may not play a role in the apoptotic process by CTN in HL-60 (Yu *et al.,* 2006).

Immunotoxicity

In thymus mild to marked necrosis of lymphocytes occurred mostly in the cortex, in contrast to the cloacal bursa where the necrosis and lymphoid depletion was more prominent in the medulla in 7-day old ducklings treated orally with a single dose (30, 40 and 50 mg/kg b.w.) of citrinin and this study confirmed the cytotoxicity of citrinin to splenic lymphocytes in vitro (Mehdi *et al*., 1983). Splenic cells from male CD1 mice, receiving 0.0,0 .12,0 .60 and 3 mg citrinin / kg i/p daily for 2-4 weeks, when cultured with or without mitogens, phytohaemagglutinin (PHA), pokeweed mitogens (PWM), and lipopolysaccharide exhibited splenic lymphocyte proliferation. However, delayed type hypersensitivity, measured as footpad swelling in response to sRBC, was not affected (Reddy *et al*., 1988). Similarly, De Souza *et al*. (1999) also observed stimulation of lymphocyte proliferation when lymphocytes from spleen and thymus of sensitised white mice were cultured in presence of 4 different concentrations of CTN (0, 5, 10 and 20 mg/kg). Lymphoid depletion and necrosis in both cortex and medulla in the bursa of Fabricius and pronounced lymphoid necrosis in the medulla and cortex, lymphoid atrophy in thymic cortex

were noticed in chicks fed with single oral dose of 47.5, 71, 100, 250 and 500 mg CTN per kg bw (Mehdi and Carlton, 1981). Campbell *et al.* (1981), however, did not observe any effect on humoral and cell mediated immunity when day- old broiler chickens were fed a starter mash with 0, 125, 250 or 500 ppm CTN for 3 weeks. Significant decrease in both cell mediated and humoral immune response was seen when citrinin was fed @ 10ppm/ kg feed to pregnant rats and the effect beibg additive with endosulfan fed @ 1 mg/kg body weight (Singh, 2005).

Genotoxicity

Genotoxicity of CTN has not been unequivocally established because various test systems gave both positive and negative results. CTN-induced oxidative stress did not affect DNA (Liu *et al*.,2003). In contrast to negative results, various cell cultures exposed to CTN showed a significant increase in micronucleus (MN) frequency (Šegvić Klarić *et al*., 2007). This increase was also noticed in HEPG2 cells, human lymphocytes, and Chinese hamster V79 cells, but CTN concentrations showing genotoxicity differed between cell cultures (Knasmüller *et al*., 2004; Dömnez-Altuntas *et al*., 2007; Pfeiffer *et al*., 1998)

Mutagenicity

CTN was not mutagenic to *S. typhymurium* (Knasmüller et al., 2004; Sabater-Vilar *et al*., 1999). However, when a primary hepatocyte culture was added, strain TA98 showed a significant dose-dependent mutagenic response, and strain TA-100 a slight positive response. These results indicate that CTN requires a complex cellular biotransformation to become mutagenic.CTN have shown clastogenic activity *in vitro* and *in vivo*, including a variety of chromosomal aberrations, save for sister chromatid exchange (SCE). In a study of Chinese hamster ovary cells and HEK293, CTN did not produce any significant difference in either SCE frequency or DNA gaps and breaks (Liu *et al*., 2003). CTN-induced SCEs in Chinese hamster V79-E cells in the presence of S9-mix (Thust and Kneist, 1979). CTN was found to be aneugenic because it caused concentration-dependent mitotic arrest, regardless of incubation time. This effect was reversible after the removal of CTN (Thust and Kneist , 1979). has found CTN induces chromosome abnormalities and breaks in bone marrow cells in young weanling mice (Jeswal, 1996) and in adult mice included breaks, centric fusions, rings, and gaps (Bouslimi *et al*., 2008).

Developmental and Reproductive Toxicity

Citrinin is an embryocidal and foetotoxic agent (Hood *et al*., 1976). In rats which received a single s.c. dose of 35 mg citrinin/kg b.w. on days 3-15 of gestation, no skeletal malformations of the foetuses were observed. However, enlarged kidneys, internal hydrocephalus and cleft palates were found. As in this experiment 30-50% of the pregnant dams died and the resorption rate of foetuses in the treated group was higher than in controls, it needs to be considered that maternal toxicity has influenced the outcome of this study. (Reddy *et al*., 1988). Citrinin was administered in the feed at a concentration of 10 mg/kg feed

(equivalent to approximately 1 mg/kg b.w.) at GD (6 - 20 days post coitum), foetal resorption rate was increased and 6.8 % of the examined foetuses showed severe malformations, including internal hydrocephalus and notched and contracted kidneys (Singh *et al*., 2007b). About 10 % of all foetuses were retarded with incomplete ossification of the skull bones. Histological investigations of the foetal kidneys showed tubular degeneration, medullar tubular necrosis and interstitial fibrosation (Singh *et al*., 2008). The embryotoxic potential of citrinin citrinin tested 1 - 10 µg, injected subgerminally or intra-amniotically at different days of the egg incubation period) alone and together with ochratoxin A. The most pronounced alterations in the early embryonic development were observed following an exposure on day three, including embryonic death (up to 60 %), and embryos showed morphological alteration on their heads (exencephaly, microophthalmia and cleft beak) (Vesela *et al*., 1983). These data are in line with earlier studies by Ciegler *et al*. (1977) describing also exencephaly, exopthalmia, crossed beaks and occasional crooked necks. CTN (30 mg/kg) were subcutaneously injected in pregnant Sprague-Dawley rats, foetal resorptions and the foetal body weights were not significantly different except on day 8 of gestation following CTN treatment. (Mayura *et al*.,1984).Recent *in vitro* studies with mouse embryonic cells confirmed the previous *in vivo* data as blastocysts treated with citrinin showed apoptosis and significant decreased implantation rates (Chan and Shiao, 2007). An increased rate of apoptosis was observed in mouse embryoblasts following ex vivo treatment with citrinin at a concentration of 30 µg/ml (Chan, 2007). In further in vitro experiments, Chan (2008) observed a significant reduction in the rate of oocyte maturation, fertilization and embryonic development. Qingqing *et al*. (2010) investigated the effects of citrinin on the reproductive organs of male mice. Sperm counts were decreased and the number of abnormal spermatozoa was increased. Histological examination revealed an increased diameter of the testicular seminiferus tubule. Serum testosterone concentrations were decreased and a significantly lower pregnancy rate was observed when females were mated with the citrinin-exposed males and no embryos occurred in the females mated with males given the highest dose of 6.25 mg/kg b.w. *In vitro* and *in vivo* studies provided clear evidence for reproductive toxicity and, teratogenic and embryotoxic effects of citrinin. The doses tested in the *in vivo* experiments exerted, however, clear signs of maternal toxicity, including nephrotoxicity, indicating that these effects might be secondary to maternal toxicity.

Carcinogenicity

IARC (1986) concluded that there was limited evidence for the carcinogenicity of citrinin to experimental animals and that no evaluation could be made of the carcinogenicity of citrinin to humans. Citrinin is classified in group 3 (not classifiable as to its carcinogenicity to humans). In an 80-week chronic toxicity study, all treated rats showed, starting from week 40, focal hyperplasia of the tubular epithelium and small renal adenomas were observed (Arai and

Hibino, 1983). No evidence for carcinogenicity was found in a model fish species (Oryzias latipes) which was exposed to citrinin for 24 weeks (Hatanaka et al., 1982). In an earlier study, Shinohara *et al*. (1976) considered whether dietary citrinin could act as a tumour promoter in male Sprague-Dawley rats. Arai and Hibino (1983) reported that the long term feeding, with a high dietary exposure to citrinin (initially ~70 mg/kg b.w. per day) showed high incidences of adenomas in rats.

Conclusions

It is concluded that citrinin is a nephrotoxic mycotoxin. Studies on immunotoxicity of citrinin do not allow a conclusive evaluation. Citrinin and aflatoxin B1 has additive effect where as CTN and Ochratoxin A has synergistic effects. *In vitro* and *in vivo* studies provided clear evidence for reproductive toxicity and, teratogenic and embryotoxic effects of citrinin. The doses tested in the *in vivo* experiments exerted clear signs of maternal toxicity, including nephrotoxicity, indicating that the teratogenic effects might be secondary to maternal toxicity. Citrinin is not mutagenic, but induces micronuclei, aneuploidy, and chromosomal aberrations in vitro in mammalian cells. *In vivo* it induced chromosome abnormalities and hypodiploidy in the bone marrow of mice. This carcinogenicity effect of citrinin in rats, showing high incidences of adenomas in the kidneys.

References

Ahamad, D. B., Vairamuthu, S., Balasubramaniam, G.A., Muralimanohar, B and Balachandran,C. (2006). Individual and combined effects of citrinin and aflatoxin B1 in broiler chicken: A clinicopathological study *Indian Journal of Veterinary Pathology, 30(1):32-35.*

Ahamad, D. B. and Vairamuthu S. (2000). Occurrence of citrinin mycotoxin in compounded feed and feed ingredients in and around Namakkal area (Tamil Nadu) *Indian Journal of Environment and Toxicology, 10 (2):84-86.*

Ahamad, D. B. and Vairamuthu, S. (2001). Individual and combined effects of citrinin and aflatoxin B1 in broiler chicken : A pathomorphological study *Indian Journal of Veterinary Pathology 25(1&2)* :32-34

Ahamad, D. B., Vairamuthu, S., Titus George, Balachandran, C.V., Muralimanohar, B and Balasubramaniam, G.A., (2009). Pathological features of citrinin toxicosis in broiler chicks. *Indian Veterinary Journal, 86(10):1014-1016*

Aloe, M.D., Wyatt, R.D. and Schnellmann, R.G. (1991). Mitochondrial dysfunction is an early event in ochratoxin A but not oosporin toxicity to rat renal proximal tubules. *Toxicol. Appl. Pharmacol*., 107: 73-75.

Ambrose AM, DeEds F. (1945).Acute and subacute toxicity of pure citrinin.

Proc Soc Exp Biol Med;59:289-291.

Ambrose AM, DeEds F. (1946). Some toxicological and pharmacological properties of citrinin. *J Pharmacol Exp Ther*;88:173-186.

Arai M and Hibino T, (1983). Tumorigenicity of citrinin in male F344 rats. *Cancer Letters*, 17, 281-287.

Beasley, V. (1999). Estrogenic and anti-androgenicToxicants. *International Veterinary Information Service,* Ithaca NY; A2623.0899.

Bernhoft A, Keblys M, Morrison E, Larsen HJ and Flaoyen A, (2004). Combined effects of selected Penicillium mycotoxins on in vitro proliferation of porcine lymphocytes. *Mycopathologia*, 158, 441-450.

Bouslimi A, Bouaziz C, Ayed-Boussema I, Hassen W and Bacha H, 2008. Individual and combined effects of ochratoxin A and citrinin on viability and DNA fragmentation in cultured Vero cells and on chromosome aberrations in mice bone marrow cells. *Toxicology*, 251, 1-7.

Bragulat MR, Martínez E, Castellá G, Cabañes FJ. (2008). Ochratoxin A and citrinin producing species of the genus Penicillium from feedstuffs. *Int J Food Microbiol*;126:43-48.

Campbell, M.L., Doerr, J.A. and Wyatt, R.D. (1981). Immune status in broiler chickens during citrinin toxicosis. *Poult. Sci.,* **60:** 1634.

Carlton WW and Szczech GM (1978). Citrinin. In: Mycotoxicoses in Laboratory Animals. Volume 2. Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An encyclopaedic Handbook. Eds Wyllie TD and Morehouse LG. Marcel Dekker, New York, 371 pp.

Carlton WW, Sansing G, Szczech GM and Tuite J. (1974). Citrinin mycotoxicosis in beagle dogs. Food and Cosmetics Ttoxicology, 12, 479-490.

Chan WH and Shiao NH. (2007). Effect of citrinin on mouse embryonic development in vitro and in vivo. *Reproductive Toxicology*, 24, 120-125.

Chan WH. (2007). Citrinin induces apoptosis via a mitochondria-dependent pathway and inhibition of survival signals in embryonic stem cells, and causes developmental injury in blastocysts. *Biochemistry Journal*, 404, 317-326.

Chourasia, H.K. (1995). Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. *Mycological Research*, 99: 697 - 703.

Ciegler A, Vesonder RF and Jackson LK (1977). Production and biological

activity of patulin and citrinin from *Penicillium expansum*. *Applied and Environmental Microbiology*, 33, 1004-1006.

Desouza, M.M.S., Freire, R.B. and Carvalho, C. (1999). Evaluation of the immunomodulatory action of citrinin by stimulation of lymphocyte blastogenesis. *Revista Brasileria de Medicina Veterinaria*, 21(2): 69-71.

Dömnez-Altuntas H, Dumlupinar G, Imamoglu N, Hamurcu Z, Liman BC (2007) Effects of the mycotoxin citrinin on micronucleus formation in a cytokinesis-block genotoxicity assay in cultured human lymphocytes. *J Appl Toxicol* 27:337-41.

Friis P, Hasselager E and Krogh P (1969). Isolation of citrinin and oxalic acid from *Penicillium viridicatum* Westling and their nephrotoxicity in rats and pigs. *Acta Pathologica et Microbiologica Scandinavica*, 77, 559-560.

Griffiths, I.B. and Done, S.H. (1991). Citrinin as a possible cause of the pruritis, pyrexia, haemorrhagic syndrome in cattle. *Vet Rec.,* 129(6):113-117.

Gupta M, Sasmal D, Bandyopadhyay S, Bagchi G, Chatterjee T and Dey S. (1983). Hematological changes produced in mice by ochratoxin A and citrinin. *Toxicology,* 26, 55-62.

Hanika C, Carlton WW and Tuite J, (1983). Citrinin mycotoxicosis in the rabbit. *Food and Chemical Toxicology*, 21, 487-493.

Hanika C, Carlton WW, Boon GD, Tuite J. (1984). Citrinin mycotoxicosis in the rabbit: clinicopathological alterations. *Food Chem Toxicol*;22:999-1008.

Hetherington AC, Raistrick H. *(1931).* Studies on biochemistry of microorganisms. Part XIV. On the production and chemical constitution of a new yellow coloring matter, citrinin produced from glucose by *Penicillium citrinum* Thom. Phil Trans Roy Soc London, *Ser B;220:269-96*

Hood RD, Hayes AW and Scammell JG. (1976). Effects of prenatal administration of citrinin and viriditoxin to mice. *Food and Cosmetics Toxicology*, 14, 175-178.

IARC (International Agency for Research on Cancer), (1986). Citrinin. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, volume 40. 67.

Jeswal P. (1996). Citrinin-induced chromosomal abnormalities in the bone marrow cells of Mus musculus. *Cytobios* 86:2933.

Jeswal, Punam and Jeswal, P. (1990). Mycotoxin contamination in cattle feeds from Bihar. *National*

Academy Science Letters, **13:** 431 - 433.

Jordan WH and Carlton WW, (1978a) Citrinin mycotoxicosis in the Syrian hamster. *Food and Cosmetics Toxicology*, 16, 355-363.

Jordan WH, Carlton WW and Sansing GA, (1978b). Citrinin mycotoxicosis in the rat. 1. Toxicology and pathology. *Food and Cosmetics Toxicology*, 16, 431-439.

Jordan, W. H., W. W. Carlton, and G. A. Sansing. (1977). Citrinin mycotoxicosis in the mouse. *Food Chem. Toxicol*. 15:29-34.

Jordan, W. H., W. W. Carlton, and G. A. Sansing. (1978). Citrinin mycotoxicosis in the Syrian hamster. *Food Chem. Toxicol.* 16:355-363.

Kitchen DN, Carlton WW and Hinsman EJ. (1977c). Ochratoxin A and citrinin induced nephrosis in Beagle dogs. III. Terminal renal ultrastructural alterations. *Veterinary Pathology*, 14, 392-406.

Kitchen DN, Carlton WW and Tuite J. (1977a). Ochratoxin A and citrinin induced nephrosis in Beagle dogs. I. Clinical and clinicopathological features. *Veterinary Pathology*, 14, 154-172.

Kitchen DN, Carlton WW and Tuite J, (1977b). Ochratoxin A and citrinin induced nephrosis in Beagle dogs. II. Pathology. *Veterinary Pathology*, 14, 261-272.

Knasmüller S, Cavin C, Chakraborty A, Darroudi F, Majer BJ, Huber WW, Ehrlich VA. (2004). Structurally related mycotoxins ochratoxin A, ochratoxin B, and citrinin differ in their genotoxic activities and in their mode of action in humanderived liver (HepG2) cells: implications for risk assessment. *Nutr Cancer* 50:190-7.

Krogh P, Hald B, Pedersen EJ. (1973). Occurrence of ochratoxin A and citrinin in cereals associated with mycotoxic porcine nephropathy. *Acta Pathol Microbiol Scand B* 81:68995.

Krogh P, Hasselager E and Friis P. (1970). Studies on fungal nephrotoxicity. 2. Isolation of two nephrotoxic compounds from *Penicillium viridicatum* Westling: citrinin and oxalic acid. *Acta Pathologica et Microbiologica Scandinavica*, 78, 401-413.

Krogh, P. (1978). Mycotoxic nephropathy in swine, pp. 236-256. In T. D. Wylle and L. G. Morehouse (eds.), Mycotoxic fungi, mycotoxins and mycotoxicoses, Vol. 2. Marcel Dekker, New York.

Krogh, P., Hald, B. and Pederson, E.J. (1973). Occurrence of ochratoxin A and citrinin in cereals associated with mycotoxic porcine nephropathy. *Acta Pathol*

Microbiol Scand [B] Microbiol Immuno., 81(6): 689-695.

Krogh, P., N. H. Axelsen, F. Elling, N. G. Hanses, B. Hald, J. H. Jensen, A. E. Larsen, A. Dadsen, H. P. Mortensen, T. Moller, O. K. Peterson, V. Burnkov, M. Rostguard, and O. Aulund. (1974). Experimental porcine nephropathy: Changes of renal function and structure induced by ochratoxin A-contaminated feed. *Acta Pathol. Microbiol. Scand. Sect A. supp.* 246:1-21.

Kumar M, Dwivedi P, Sharma AK, Singh ND and Patil RD. (2007). Ochratoxin A and citrinin nephrotoxicity in New Zealand White rabbits: an ultrastructural assessment. *Mycopathologia*, 163, 21-30.

Kumar M, Dwivedi P, Sharma AK, Telang AG, Patil RD and Singh ND. (2008). Immunotoxicity of ochratoxin A and citrinin in New Zealand White rabbits. *World Rabbit Science*, 16, 7-12.

Kumari, C. K. and Nusrath, M. (1987). Natural occurrence of citrinin and ochratoxin A in coconut products. *National academy of sciences, India, science letters*, 10: 303 - 305.

Leatherhead food research association, Randalls road, leatherhead, surrey KT22 7RY, England (2000).

Little CJL, McNeil PE and Robb J. (1991). Hepatopathy and dermatitis in a dog associated with the ingestion of mycotoxins. *Journal of Small Animal Practice*, 32, 23-26.

Liu BH, Yu FY, Wu TS, Li SY, Su MC, Wang MC, Shih SM. (2003)Evaluation of genotoxic risk on oxidative DNA damage in mammalian cells exposed to mycotoxins, patulin and citrinin. *Toxicol Appl Pharmacol*;191:255-63.

Lockard, V.G., Phillips, R.D., Hayes, A.W., Berndt, W.O. and Neal, R.M. (1980). Citrinin nephrotoxicity in rats: a light and electron microscopic study. *Exp. Molecul. Path.*, 32: 226-240.

Manabe, M. (2001). Fermented foods and mycotoxins. *Mycotoxins,* 51: 25-28.

Manickam, A., Palaniswami, A. and Neelkandan, S. (1985). Mycotoxicosis in buffaloes. *Ind. Vet. J.,* 62(8): 711-714.

Manning RO, Brown TP, Wyatt RD and Fletcher OJ. (1985). The individual and combined effects of citrinin and ochratoxin-A in broiler chicks. Avian Diseases, 29, 986-997.

Mantle PG and McHugh KM. (1993). Nephrotoxic fungi in foods from nephropathy households in Bulgaria. *Mycological Research*, 97, 205-212.

Mayura, K., Parker, R., Berndt, W.O. and Phillips, T.D. (1984). Effect of simultaneous prenatal exposure to ochratoxin A and citrinin in the rat. *J. Toxicol. Environ. Hlth.,* **13(4-6):** 553-561.

Mehan, V. K. and McDonald, D. (1984). Mycotoxin producing fungi in groundnuts. Potential for mycotoxin contamination. *Oleagineux,* **39:** 25 - 29.

Mehdi NA, Carlton WW and Tuite J, (1983). Acute toxicity of citrinin in turkeys and ducklings. Avian Pathology, 12, 221-233. Mehdi NA, Carlton WW and Tuite J, 1984. Mycotoxicoses produced in ducklings and turkeys by dietary and multiple doses of citrinin. *Avian Pathology*, 13, 37-50.

Mehdi NA, Carlton WW and Tuite J. (1984). Mycotoxicoses produced in ducklings and turkeys by dietary and multiple doses of citrinin. *Avian Pathology*, 13, 37-50.

Mehdi NAQ, Carlton WW, Tuite J. (1983)Acute toxicity of citrinin in turkeys and ducklings. *Avian Pathol*;12:221-33.

Nelson TS, Kirby LK, Beasley JN, Johnson ZB, Ciegler A.)1985). The effect of drying method and storage time on citrinin activity in corn. *Poult Sci*;64:464-8.

Pande, N., Saxena, J., Pande, H. and Pande, N. (1990). Natural occurrence of mycotoxins in some cereals. *Mycoses,* **33:** 126 - 128.

Petkova-Bocharova T, Castegnaro M, Michelon J, Maru V. (1991). Ochratoxin A and other mycotoxins in cereals from an area of Balkan endemic nephropathy and urinary tract tumours in Bulgaria. In: Castegnaro M, Pleština R, Dirheimer G, Chernozemsky IN, Bartsch H, editors. Mycotoxins, endemic nephropaty and urinary tract tumours. Lyon: IARC;. p. 83-7.

Pfeiffer E, Gross K, Metzler M. (1998). Aneuploidogenic and clastogenic potential of the mycotoxins citrinin and patulin. *Carcinogenesis* 19:1313-8.

Qingqing H, Linbo Y, Yunqian G and Shuqiang L (2010). . Toxic effects of citrinin on the male reproductive system in mice. *Experimental and Toxicologic Pathology*

Rajeswari, K. R. and Char, N, L. (1991). Mycotoxins in poultry feeds. *Poult. Adv.*, **24:** 65 – 68.

Ramadoss, C.S. and Shanmugasundaram, E.R.B. (1973). Effect of citrinin on liver metabolism in rabbits. *Ind. J. Biochem. Biophys.,* **10:** 296-297.

Ranjan, K. S. and Sinha, A. K. (1991). Occurrence of mycotoxigenic fungi and mycotoxins in animal feed from Bihar, India. *J. Fd. Sci. Agri.,* **56:** 39 - 47.

Reddy RV, Taylor MJ and Sharma RP, 1988. Evaluation of citrinin toxicity on the immune functions of mice. *Journal of Food Protection*, 51, 32-36.

Reddy, A. S. and Reddy, S. M. (1983). Elaboration of mycotoxin by fungi associated with til (Sesamum indicum L.). *Current science*, India, 52: 613 - 614.

Reddy, R. V., A. W. Hayes, and W. O. Berndt. (1982b). Disposition and metabolism of l4C citrinin in pregnant rats. *Toxicology* 25:161-174.

Reddy, R. V., K. Mayura, A. W. Hayes, and W. O. Berndt. (1982a). Embryocidal, teratogenic and fetotoxic effects of citrinin in rats. *Toxicology*, 25:151-160.

Reddy, R.V., Taylor, M.J. and Sharma, R.P. (1988). Evaluation of citrinin toxicity on the immune functions of mice. *J. Fd. Protection,* 51(1): 32-36.

Roberts WT and Mora EC, 1978. Toxicity of *Penicillium citrinum* AUA-532 contaminated corn and citrinin in broiler chicks. *Poultry Science*, 57, 1221-1226.

Roy, A. K. and Chourasia, H. K. (1990). Mycotoxin incidence in root drugs. *Int. J. of Crude Drug Research,* 28: 157 - 160.

Roy, A. K. and Kumari, V. (1991). Aflatoxin and citrinin in seeds of some medicinal plants under storage. *Int. J. Pharmacog.,* 29: 62 – 65.

Sabater-Vilar M, Maas RFM, Fink-Gremmels J. (1999). Mutagenicity of commercial Monascus fermentation products and the role of citrinin contamination. *Mutat Res*;444:7-16.

Sándor G, Busch A, Watzke H, Reek J and Ványi A. (1991). Subacute toxicity testing of ochratoxin-A and citrinin in swine. *Acta Veterinaria Hungarica*, 39, 149-160.

Sansing GA, Lillehoj EB, Detroy RW and Miller MA. (1976). Synergistic toxic effects of citrinin, ochratoxin A and penicillic acid in mice. *Toxicon,* 14, 213-220.

Saxena, J., Pande, H. and Pande, N. (1988). Mycotoxin producing potentials of the fungi isolated from dry fruits. *Proceedings of the Indian National Science Academy, B - Biological Sciences*, 54: 195 - 197.

Scott PM, van Walbeek W, Kennedy B, Anyeti B. (1972). Mycotoxins (ochratoxin A, citrinin and sterigmatocystin) and toxigenic fungi in grains and other agricultural products. *J Agric Food Chem*. 20:1103-9.

Šegvić Klarić M, Želježić D, Domijan A-M, Peraica M, Pepeljnjak S. (2007). Citotoxicity, genotoxicity and apoptosis induced by

ochratoxin A and citrinin in porcine kidney PK15 cells: Effects of single and combined mycotoxins. *Toxicol Lett* 172(Suppl. 1):S56.

Shinohara Y, Arai M, Hirao K, Sugihara S, Nakanishi K, Tsunoda H and Ito N, (1976). Combination effect of citrinin and other chemicals on rat kidney tumourigenesis. Gann, 67, 147-155.

Singh ND, Sharma AK, Dwivedi P, Patil RD and Kumar M. (2007a). Citrinin and endosulfan induced maternal toxicity in pregnant Wistar rats: pathomorphological study. Journal of *Applied Toxicology*, 27, 589-601.

Singh ND, Sharma AK, Dwivedi P, Patil RD and Kumar M, (2007b). Citrinin and endosulfan induced teratogenic effects in Wistar rats. *Journal of Applied Toxicology*, 27, 143-151.

Singh ND, Sharma AK, Dwivedi P, Patil RD and Kumar M. (2008). Experimentally induced citrinin and endosulfan toxicity in pregnant Wistar rats: histopathological alterations in liver and kidneys of fetuses. *Journal of Applied Toxicology*, 28, 901-907.

Singh ND, Sharma AK, Dwivedi P, Patil RD, Kumar M and Ahamad DB. (2006). Toxicity of endosulfan and citrinin alone and in combination in pregnant rats: clinico-haematological and serum biochemical alterations. *Indian Journal of Veterinary Pathology*, 30, 27-31.

Singh, N.D. (2005). *Pathology of Induced Toxicity of Citrinin and Endosulfan Alone and in Combination in Pregnant Rats*. MVSc thesis, Indian Veterinary Research Institute, Izatnagar.

Singh, R. S., Sharma, M. K. and Chander, H. (1992). Incidence of Penicillium toxins in milk and milk products. *Ind. J. Anim. Sci.,* **62:** 285 - 286.

Sinha. K., Gajendra, P. and Prasad, G. (1994). Monitoring and identification in marketed bakery bread during monsoon. *Indian Phytopathol.,* **47:** 164 – 167.

Speijers GJA, Speijers MHM. (2004).Combined toxic effects of mycotoxins. *Toxicol Lett* 153:91-8.

Subrahmanyam, P. and Rao, A. S. (1974). Occurrence of Aflatoxin and Citrinin in groundnut (Arachis hypogaea L.) at harvest in relation to pod condition and kernel moisture content. *Current sci.*, **43:** 707 - 710.

Sundaram, T.K., Chandrasekaran, D., Natarajan, A. and Mohan, B. (1999). Multimycotoxins: an overview of their occurrence in feed ingredients and feeds in Namakkal paper presented in 18th Annual conference of the society of toxicology held at Veterinary

college and Research Institute, Namakkal, pp. 23-24.

Szczech GM, Carlton WW and Lund JE (1974). Determination of enzyme concentrations in urine for diagnosis of renal damage. *Journal of the American Animal Hospital Association*, 10, 171-174.

Tanaka K, Sago Y, Zheng Y, Nakagawa H, Kushiro M. (2007). Mycotoxins in rice. *Int J Food Microbiol* 119:59-66.

Thacker HL, Carlton WW and Sansing GA. (1977). Citrinin mycotoxicosis in the guinea-pig. *Food and Cosmetics Toxicol.*, 15, 553-561

Thust R, Kneist S. (1979). Activity of citrinin metabolized by rat and human microsome fractions in clastogenicity and SCE assay on Chinese hamster V79-E cells. *Mutat Res* 67:321-30.

Uma, M. and Vikram Reddy, M. (1995). Citrinin toxicity in broiler chicks : Haemato-biochemical and pathological studies. *Ind. J. Vet. Path.,* **19(1):** 11-14.

Vesela D, Vesely D and Jelinek R. (1983). Toxic effects of achratoxin A and citrinin, alone and in combination, on chicken embryos. *Applied and Environmental Microbiology*, 45, 91-93

Witlock, D. R., R. D. Wyatt, and M. D. Ruff. (1977). Morphological changes in the avian intestine induced by citrinin and lack of effect of aflatoxin and T-2 toxin as seen with scanning electron microscopy. *Toxicon* 15:41-44

Yoshisawa, T. (1991). Natural occurrence of mycotoxins in small grain cereals (Wheat, barley, rye, oats, sorghum, millet and rice) : Smith, J.E. and Henderson, R.S. (Eds) Mycotoxins and Animal Foods, CRC Press, Boca Ration pp. 301-324.

Yu FY, Liao YC, Chang CH, Liu BH. (2006). Citrinin induces apoptosis in HL-60 cells via activation of the mitochondrial pathway. *Toxicol Lett* 161:143-51.