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Ochratoxin A Induced Hepatorenal Carcinogenesis in Mice (*Mus musculus*)

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Dietary concentration of ochratoxin A (0.25 ml of 50 ppm per animal twice a week) administered to young weanling mice (*Mus musculus*) for 20 weeks caused a wide range of histopathological changes in liver and kidneys leading to formation of hepatoma and renal carcinoma. Combined administration of citrinin and ochratoxin A enhanced the incidence of renal carcinoma in experimental animal, however occurrence of neoplastic lesions in liver by ochratoxin A remained unaffected by simultaneous feeding of citrinin.

Key Words : Ochratoxin A, Citrinin, Carcinogenesis, *Mus musculus*, Hepatorenal Carcinoma

Introduction

Ochratoxin A is potent mycotoxin produced by certain species of fungal genera *Aspergillus* and *Penicillium* (Pitt & Leistner 1991). Its occurrence in cereals, beans, nuts and in meat and dried fish is well documented (Jeswal 1990, Jaio et al. 1994, Scudamore et al. 1995). Ochratoxin A is supposed to be the cause of mycotoxic porcine nephropathy and has also been suggested to be involved in the fatal human disease Balkan endemic nephropathy (Krogh et al. 1977, WHO 1990).

Inspite of comparative early discovery in 1965 (Vander Merwe et al. 1965) it received little attention with regard to its toxicity. Some earlier reports however confirm its nephrotoxic, embryotoxic and immuno-suppressive effects on some mammal, fish and birds (Scott et al. 1977, Harvey et al. 1994, Stormer & Lea 1995). In the present study, hepatorenal carcinogenicity of ochratoxin A was assessed by oral administration of this mycotoxin in lower dose and for longer period. Effect of simultaneous administration of citrinin and ochratoxin A was also observed because in nature both the mycotoxins usually exist as co-contaminants.

Materials and Methods

The experiment were performed with an inbred stock of albino swiss mice (Parent

stock supplied by CDRI, Lucknow). Six weeks old albino mice of either sex (25-28g) were categorised into four group each comprising 20 animals. Group I did not received any toxin and served as control. Group II mice were administered 0.25 ml of 50 ppm ochratoxin A solution per animal twice a week by intubation an hour before their regular meal. Group III were given 0.25 ml of 50 ppm citrinin solution per animal twice in a week. Group IV received both ochratoxin A and citrinin (0.25ml of 50 ppm each) simultaneously. The animals were maintained by providing standard diet (Hindustan Lever) with water *ad libitum.* Each group of animal was kept in separate cages. Appropriate care and hygienic conditions were maintained in order to keep them healthy and free from any infections. Toxin administration was continued for 20 weeks. The doses were adjusted in such a manner that the total amount of ochratoxin A or citrinin supplied to each animal did not exceed the single lethal dose of either ochratoxin A or citrinin or both.

Animals were sacrificed after 24 hr of last treatment by instant cervical dislocation. Liver and kidneys were removed and transferred to separate vials containing Bouin's fixative. After keeping for 24 hr in the fixative, the tissues were finally preserved in 70% ethanol, Paraffin blocks were made and sections were cut at 6μ and stained with haemotoxylin-eosin.

Results and Discussion

Morphologically the kidneys of the toxin treated mice were swollen and turned gray-white to pale yellow-brown in colour. Ochratoxin A ingestion caused a wide range of histopathological changes in kidneys leading to formation of renal carcinoma. The main histological alternations in kidneys were degeneration of glomeruli and degeneration of renal tubules. A well differentiated renal carcinoma was observed in ochratoxin A fed mouse (figures 1, 2).

Histopathological examination of liver of toxin fed mice revealed that ochratoxin A induced several hepatic damages such as

Table 1 *Effect of Ochratoxin A and citrinin administration on induction of hepatorenal carcinoma in mice (**Mus musculus**)*

Treatment groups	No. of effective animals	RENAL DAMAGE				
		No. of showings renal damage	Degeneration of renal tubules	Degeneration of glomeruli	Renal carcinoma	No. of animal hepatic damage
I. Control	20	Nil	Nil	Nil	Nil	Nil
II. Ochratoxin A only	20	12 (60.0)	7 (35.0)	5 (25.0)	2 (10.0)	13 (65.0)
III. Citrinin only	20	11 (55.0)	5 (25.0)	6 (30.0)	Nil	9 (45.0)
IV. Ochratoxin A + Citrinin	20	16 (80.0)	9 (45.0)	7 (35.0)	4 (20.0)	13 (65.0)

Percentage values are given in parenthesis

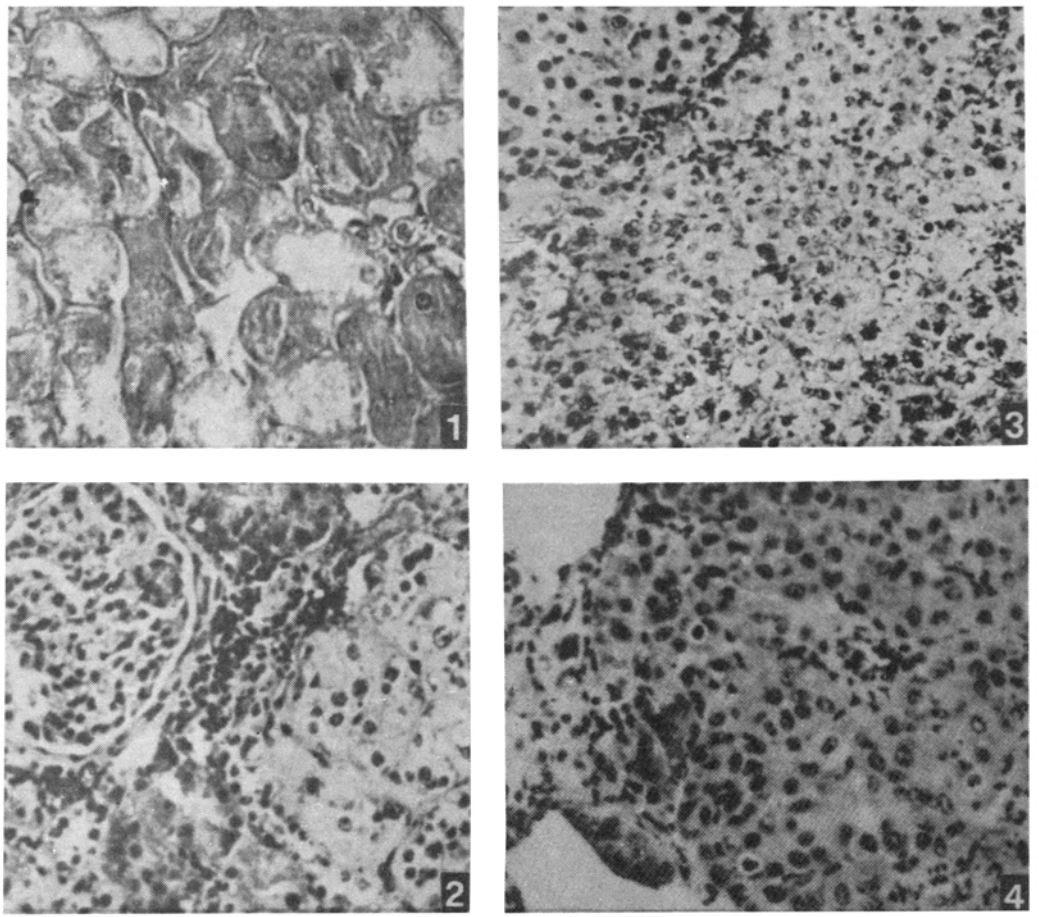

Figure 1-4 : **1**, Transverse section of liver of an ochratoxin A fed mouse showing preneoplastic lesions (X 500); **2**, Transverse section of liver of an ochratoxin A fed mouse showing well differentiated hepatoma (X 500); **3**, Tranverse section of the kidney of a mouse fed ochratoxin A showing degenerating renal tubules (X 360); **4**, Tranverse section of a well developed renal carcinoma in the kidney of a ochratoxin A fed mouse (X 500)

degeneration of hepatocytes and fatty degenerations of liver parenchyma. Preneolastic lesions were frequently observed and hepatoma was detected (figures 3 & 4). Citrinin ingestion also caused several hepatic injury but none of the animal showed

formation of hepatoma. Hepatotoxicity was not enhanced by combined administration of citrinin and ochratoxin A.

The carcinogenic potential of ochratoxin A has not been fully established so far because of the inadequacy of available studies but Kanisawa and Suzuki (1978) reported induction of hepatic and renal carcinoma in 30% of mice fed with ochratoxin A contaminated diet. Subsequently, Kanisawa (1984) reported formation of renal cell tumour in mice fed a diet containing ochratoxin A at 50mg/kg feed for various periods of time. Ochratoxin A induces carcinogenesis by damaging the immune system of an animal, thus under mining its natural defence mechanism against the development of abnormal cells (Jeswal 1995). Induction of renal carcinoma by long term feeding of high dose of citrinin has been reported by Bilgrami and Jeswal (1993).

The present study confirms the hepatorenal carcinogenicity of ochratoxin A. It has been also observed that renal toxicity A is synergistically affected by simultaneous administration of citrinin. The present results suggested the increased risk nephrotoxicosis in man with a mixed contamination of ochratoxin and citrinin in the environment and it is desirable to caution the mass against the hazardous effect of ochratoxin A.

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