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Toxicopathological studies on the effects of aflatoxin B_1, ochratoxin A and their interaction in New Zealand White rabbits

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ABSTRACT

New Zealand White rabbits, divided into 4 groups were fed with feed containing aflatoxin B_1 (AFB_1) @ 0.5 ppm (group I), ochratoxin A (OA) @ 1 ppm (group II), AFB_1 and OA @ 0.5 ppm and 1 ppm, respectively (group III) and standard feed (group IV) for 60 days. Mortality and decrease in body weight were highest in the interaction group. Superoxide dismutase, catalase and malondialdehyde levels were increased in all the toxin fed groups with maximum elevation in group III. Significant decrease in the antibody titre to sRBC was observed in groups I and II. Significant reduction of HI and CMI responses was observed in group III. Grossly, liver was the most affected organ in AFB_1 treated animals. Microscopically, vacuolar degeneration of hepatocytes, bile duct epithelial hyperplasia and hypertrophy and peribiliary fibrosis were consistently observed. Ultrastructurally, hepatocytes revealed varying degrees of degeneration, swollen endoplasmic reticulum and pleomorphism of mitochondria. OA produced significant nephrotoxicity with the pale, soft and enlarged kidneys showing discoloured foci over the surface. Microscopically, kidneys revealed degeneration of the proximal convoluted tubules and the testes were atrophic. Ultrastructurally, disruption of the mitochondrial membrane and swelling of the endoplasmic reticulum were observed. In group III, gross and histopathological changes were observed both in liver and kidneys and were of greater severity as compared to those of groups I and II. Ultrastructurally, hepatocytes revealed nuclear distortion, marginated heterochromatin, chromatolysis, electron opaque mitochondria with vacuolations and disarray of cristae and loss of cytoplasmic organelles. The results suggested an additive interaction of AFB_1 and OA in rabbits.

1. Introduction

Mycotoxins are toxic compounds produced by fungi. Animals, as well as human beings, are usually exposed to mycotoxins through their diet (Dvorackova, 1990). The result is acute or sub-acute or chronic mycotoxicosis depending on the factors such as age, sex and species involved (Hasinoff et al., 1990). Among the mycotoxins, aflatoxins are a family of extremely toxic, mutagenic and carcinogenic compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Deiner et al., 1987; Kurtzman et al., 1987). Aflatoxin B_1 (AFB_1) is the most abundant toxic metabolite and widespread contaminant of foods and feed in different parts of the world (Avinash et al., 2004). Of the other mycotoxins, Ochratoxin, commonly produced by two species of fungi, *Penicillium verrucosum* Dierckx and *Aspergillus ochraceus* Wilhelm (Frisvad and Samson, 1991), is potentially as important as the aflatoxins (Bennett and Klich, 2003). The toxicity and clinical signs observed in animals when more than one mycotoxin is present in feed are complex and diverse. The effects observed during multiple mycotoxin exposure can differ greatly from the effects observed in animals exposed to a single mycotoxin (Huff et al., 1988). There has been a general paucity of data on mycotoxin interactions in susceptible species of animals.

Rabbit is among the most sensitive species to the mycotoxin toxicity. Rabbits are highly susceptible to aflatoxicosis having the least LD_{50} of any animal species studied (Newberne and Rogers, 1981). Rabbits are also comparatively more susceptible to ochratoxin A (OA) than mice, rats and guinea pigs (Ponnuchamy, 2000). Because many of the clinical signs and clinico-pathological changes

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of experimental aflatoxicosis in rabbits are similar to those reported in other species of animals, rabbits constitute an appropriate model for studying the mechanisms of the toxic actions in food-producing animals (Clark et al., 1980). Under Indian environmental conditions, occurrence of spontaneous aflatoxicosis and ochratoxicosis, individually and in interaction are quite frequently reported in different species of animals (Panisup et al., 1993; Raina and Singh, 1991; Sharma, 1998). Further, the combined presence of low dose levels of AFB_1 ranging from 0.03 to 2.06 ppm and OA ranging from 0.01 to 1.23 ppm in cereals/feedstuffs in India have been frequently reported (Dwivedi et al., 2004). The alarming feature of mycotoxins is their occurrence in combination and in low dose levels frequently in cereals/feedstuffs which may exert a greater degree of damage (additive or synergistic) in comparison to the individual effects. Systematic experimental studies on the detailed pathological alterations produced by sub-chronic aflatoxicosis and ochratoxicosis, individually and in interaction at the low dose levels are rare, necessitating the present study.

2. Materials and methods

2.1. Production and analysis of mycotoxins

Pure cultures of *A. flavus* (NRRL-5518) and *A. ochraceus* (NRRL-3174) were procured from National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, USA. OA was produced by fermentation of maize as per the method of Trenk et al. (1971) and AFB_1 was produced using low salt synthetic liquid medium as per the method of Reddy et al. (1971) with slight modifications. For purification of AFB_1 and OA, procedures of AOAC (1995) were followed and both these mycotoxins were estimated by using thin layer chromatography (TLC) and spectrophotometry.

2.2. Experimental feed

The inoculum containing known amounts of AFB_1 and OA were separately added and thoroughly mixed with the basal ration (tested negative for the presence of any possible residual mycotoxins) in proportion so as to arrive at the respective dose levels. Aliquots were taken from the mixed diet and further quantified for the concentration of both the mycotoxins by thin layer chromatography (TLC) and spectrophotometry to ensure proper mixing.

2.3. Experimental animals

32 male New Zealand White rabbits were procured from the Laboratory Animal Resource (LAR) Section of the Indian Veterinary Research Institute, Izatnagar, India. All animals were individually house-caged (stainless steel cages) in a temperature controlled and artificially illuminated room (12:12 h light/dark cycles). All the rabbits were provided with water *ad libitum*. The experiment was conducted after the due approval from the Institutional Animal Ethics Committee (IAEC) constituted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The rabbits were acclimatized for a period of 1 week before the commencement of the trial.

Details	Group I	Group II	Group III	Group IV
1. Age of rabbits	2–3 months	2–3 months	2–3 months	2–3 months
2. No. of rabbits	8	8	8	8
3. Duration of the experiment	60 days	60 days	60 days	60 days
4. Dosage	0.5 ppm AFB_1	1 ppm OA	0.5 ppm AFB_1 + 1 ppm OA	Toxin free feed
5.Route of administration	Mixed with feed	Mixed with feed	Mixed with feed	–
6. Sacrifice intervals	30th and 60th days of the trial			
7. No. of rabbits sacrificed at each interval	4	4	4	4

2.4. Experimental design

2.5. Clinical signs

Animals of all the groups were closely observed twice daily throughout the experimental period for clinical signs. Body weights of all the animals were recorded at fortnightly intervals.

2.6. Estimation of enzyme antioxidants

Following antioxidants were estimated in the tissue homogenates prepared from liver and kidneys.

2.6.1. Superoxide dismutase (SOD)

SOD activity was determined in the liver and kidney homogenates as per the procedure described by Madesh and Balasubramanian (1998). A colorimetric assay involving superoxide dependent reduction of the tetrazolium dye, MTT (3-(4,5-dimethyl thiazol-2-yl)2,5-diphenyl tetrazolium bromide) to its formazon by SOD was measured at 570 nm.

2.6.2. Catalase

Catalase activity was measured in the homogenates by the method of Maehly and Chance (1954). The utilization of H_2O_2 by catalase was measured spectrophotometrically as decrease in optical density at 254 nm.

2.6.3. Lipid peroxidation

Lipid peroxidation in tissue homogenates was studied by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al. (1979). The results were expressed as nM of malondialdehyde (MDA) formed per gram of tissue per 30 min from the homogenates.

2.7. Immunological studies

2.7.1. Humoral immune response

Haemagglutination (HA) test was carried out by micro titration techniques according to the procedure described by Beard (1980). The rabbits were sensitized with sheep red blood cells (sRBC) by intraperitoneal injection of 0.25 ml of sRBC suspended in PBS @ 1.25×10^6 cells/animal. After initial sensitization with sRBC, serum was collected on 45th day of the trial. Briefly, HA test was performed in U shaped micropersplex plate. Two-fold serial dilution of serum was prepared in PBS keeping the final volume of 0.05 ml in each well except in control well, which contained PBS alone. 0.05 ml of 0.5% sRBC suspension was added to all the wells. A known negative control was also included. The HA pattern (a diffuse sheet of agglutinating RBCs covering the bottom of the well) was read