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Effect of aflatoxin in the development of pulmonary and systemic aspergillosis in rabbits with *Aspergillus fumigatus* infection : A comparative pathological study

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Rabbits are the most sensitive animals to the aflatoxicosis, the LD_{50} being only 0.3 mg/kg body weight which is comparable to that of ducklings, the lowest among all species (Edds 1973). Meagre reports are available on combined effects of aflatoxicosis and aspergillosis in rabbits (Richard and Thurston 1975). Therefore, the present work was undertaken on comparative pathological study of induced aspergillosis alone and in combination with aflatoxicosis.

Twenty-four young (2-3 months old), healthy New Zealand White rabbits of either sex were maintained on toxin-free experimental diet using soybean-meal in place of groundnut-cake in the feed mixture. The rabbits were randomly divided into 3 groups, comprising 8 animals in each. Group 1 was fed with aflatoxin B_1 prepared and estimated as per Shotwell *et al.* (1966) and Pons *et al.* (1966), and mixed with feed mixture @ 0.35 mg/kg a day till the end of experiment, i.e. 60 days. Group 2 rabbits were maintained on toxin-free diets. At third week group 1 and group 2 were infected with 1 ml of *Aspergillus fumigatus* spores suspension (2.8×10^9 spores/ml) intratracheally. Group 3 served as healthy control. After systematic necropsy of sacrificed (at 15-day-interval) and dead animals, the representative tissue pieces were collected for histopathology and isolation studies as per standard techniques. Formalin fixed tissues were processed routinely to obtain H & E as well as specially (periodic Acid Schiff's, Grocott's and Gridley's method) stained sections.

On necropsy all rabbits of groups 1 and 2, showed emaciation, dehydration and anaemic appearance of carcasses. Trachea was highly congested, oedematous and haemorrhagic with small greyish caseated nodules at the site of inoculation, which were prominent at 20 DPI onwards. Focal consolidation of lungs was invariably noticed in both the groups which later increased in intensity. However, in group 1, lungs were more affected and were found studded with multiple greyish white nodules (Fig.1). The lesions reached to peak of severity between fourth and fifty week of infection. The heart revealed congestion and petechiae on epicardial and endocardial surfaces with greyish white foci which were more severe and prominent in group 1. Mucosae of stomach and intestine are congested, oedematous and denuded in group 1. Hepatomegaly, icteric apperance and patchy congestion with extensive whitish nodular foci on liver surfaces were remarkably noticeable in group 1. While only petechiae with yellowish white streaks were discernible in the liver of group 2 rabbits. The spleen was congested and enlarged in both infected groups. however, group 1 revealed greyish foci earlier at 20 DPI. Group 2 rabbits exhibited marked congestion with greyish white nodules and depressed foci on sub-capsular surfaces of kidneys. These lesions were more severe in toxin-fed-infected group (Fig. 2).

Histopathologically, trachea revealed hyperplastic mucosal lining epithelium, engorged blood vessels, hyperactive mucous glands, oedema and infiltration of inflammatory cells comprising polymorphonuclear and mononuclear cells with granulomatous lesions, which were more prominent in group 1 rabbits (Fig. 3). Sections of lungs in group 2 rabbits, revealed multiple small granulomas characterised by central caseated necrotic area containing fungal elements and surrounded by polymorphonuclear and mononuclear cells; the peripheral are dipicting presence of giant cells at 45 DPI. In group 1, the lesions were more severe with multiple granulomatous foci evincing macrophage and giant cell reaction at 25 DPI, which pronounced at 40 DPI exhibiting presence of radiating fungal elements (asteroid bodies) in addition (Fig. 4). Heart revealed engorged blood vessels with extravasation of RBC and exudation in perimyseal and interstitial spaces leading to distortion of myofibrils with degenerative and necrotic changes. Lesions in stomach / intestine were characterized by engorged blood vessels, serous exudation, infiltration of mononuclear cells and hyperactivity of mucosal and submucosal glands. In liver of Group 1, the initial

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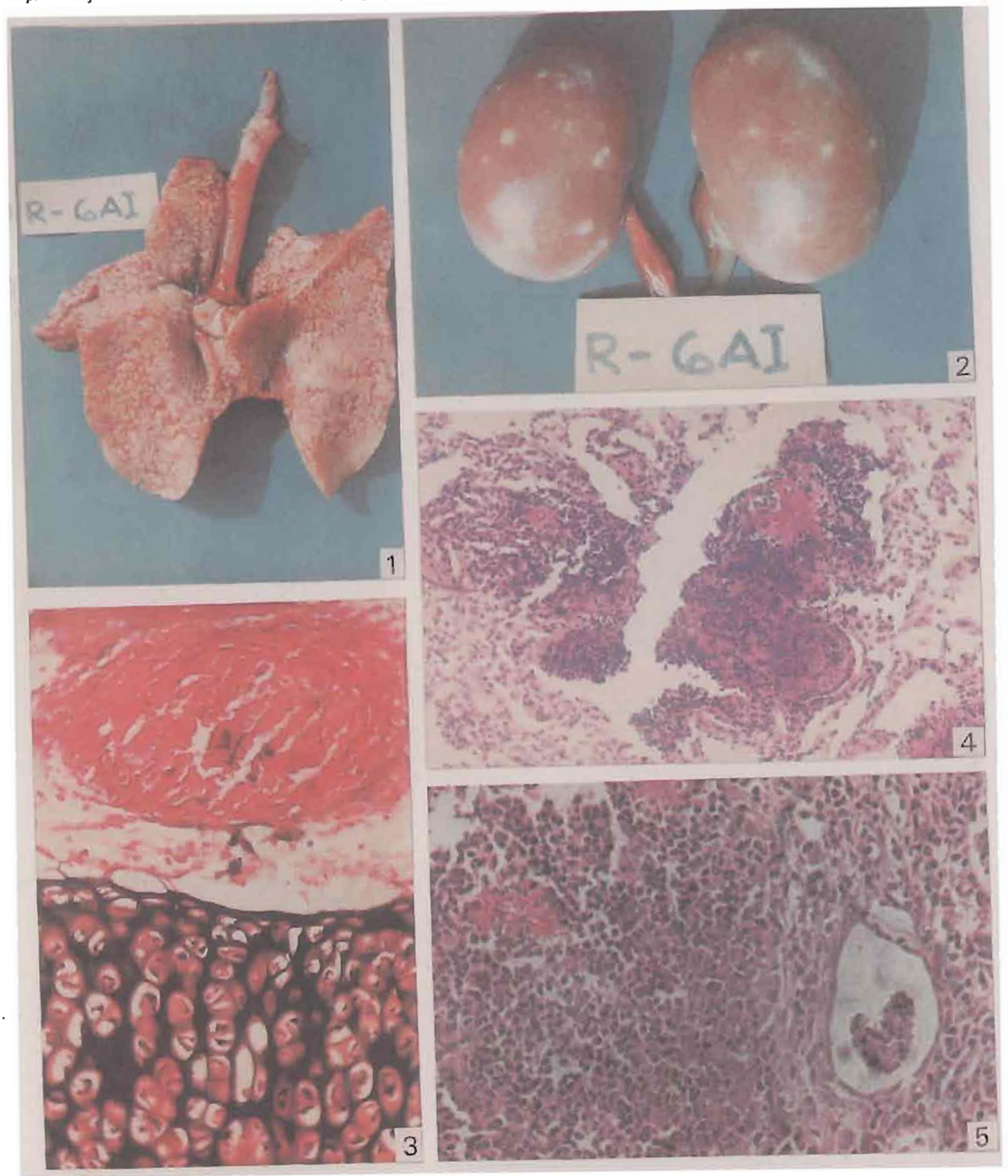

Figs 1–5. 1. Lungs (aflatoxin-fed-infected Group 1) : Studed with multiple raised greyish-white nodules imparting granular appearance. 2. Kidneys (Group 1) : Greyish-white patches and nodules embedded in subcapsular surface. 3. Trachea (Group 1) : Mycotic granuloma showing hyphae of *A. fumigatus* in the centre (Grocott's × 100). 4. Lung (Group 1) : Asteriod fungal bodies of *A. fumigatus* in aspergillotic grannuloma depicting extensive macrophage reaction (H & E 0 × 160). 5. Kidney (Group 1) : Mycotic granuloma evincing mixed cellular reation around the hyphae of *A. fumigatus*. (H& E × 150).