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HISTOPATHOLOGICAL DAMAGES IN RATS BY AFLATOXIN CONTAMINATED FEED[1]

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ABSTRACT

Feeding of albino rats with aflatoxin-contaminated feed (at the rate of 40-60 ppb per day per animal) for 30 weeks showed histopathological damages in skin, liver and kidney. Loss of hairs, presence of "horn-pearls" and non-differentiated cellular mass in the dermis were observed in the skin. The liver contained large cells, either of degenerating nature or with distinct mitotic configurations. Mass of non-differentiated hyperchromatic cells were found in the meduallary region of the kidney.

INTRODUCTION

Mycotoxin induced histopathological changes in animals were reported for the first time about two decades back (Newberne *et al.*, 1964a, Butler, 1974). Since then, the volume of literature is gradually growing (IARC, 1976; Wyllie and Morehouse, 1978). However, all such works suffer with two major drawbacks : (i) the amount of toxin administered was unusually high, and (ii) the mode of toxin administration was through intubation and intramuscular injection. We are fully aware that under natural conditions human and cattle populations never get such high doses. Moreover, the toxins do not enter normally through these routes. In the present work, the normal course of toxin-administration, i.e., oral route was preferred in a concentration ranging from 40-60 ppb. This dose is substantially low than that consumed by cattle and men, because cases of toxin being present in concentrations as high as 1000 ppb have been reported (Amla *et al.*, 1970, 1974; Bhat *et al.*, 1978; Daradhiyar, 1980; Sinha, 1980).

MATERIALS AND METHODS

Normal feed of 6-8 weeks old, albino swiss rats were supplemented with mould-infested bread containing crude aflatoxins (B_1, B_2, G_1 and G_2) in concentration ranging from 40 to 60 ppb per day per animal. All the animals were pathogen free, and kept in separate cages at room temperature. Care was taken to ensure that the diets were properly supplemented with protein, vitamins and minerals. Six male animals served as control, while the other six got the treatment. One lot of the animals was sacrificed after 15 weeks, and the other after 30 weeks of the treatment. Organs for histologic studies were promptly removed and fixed in Bouin's fixative.

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Paraffin blocks were cut at 6 μm, and sections were stained with haemotoxylin-eosin.

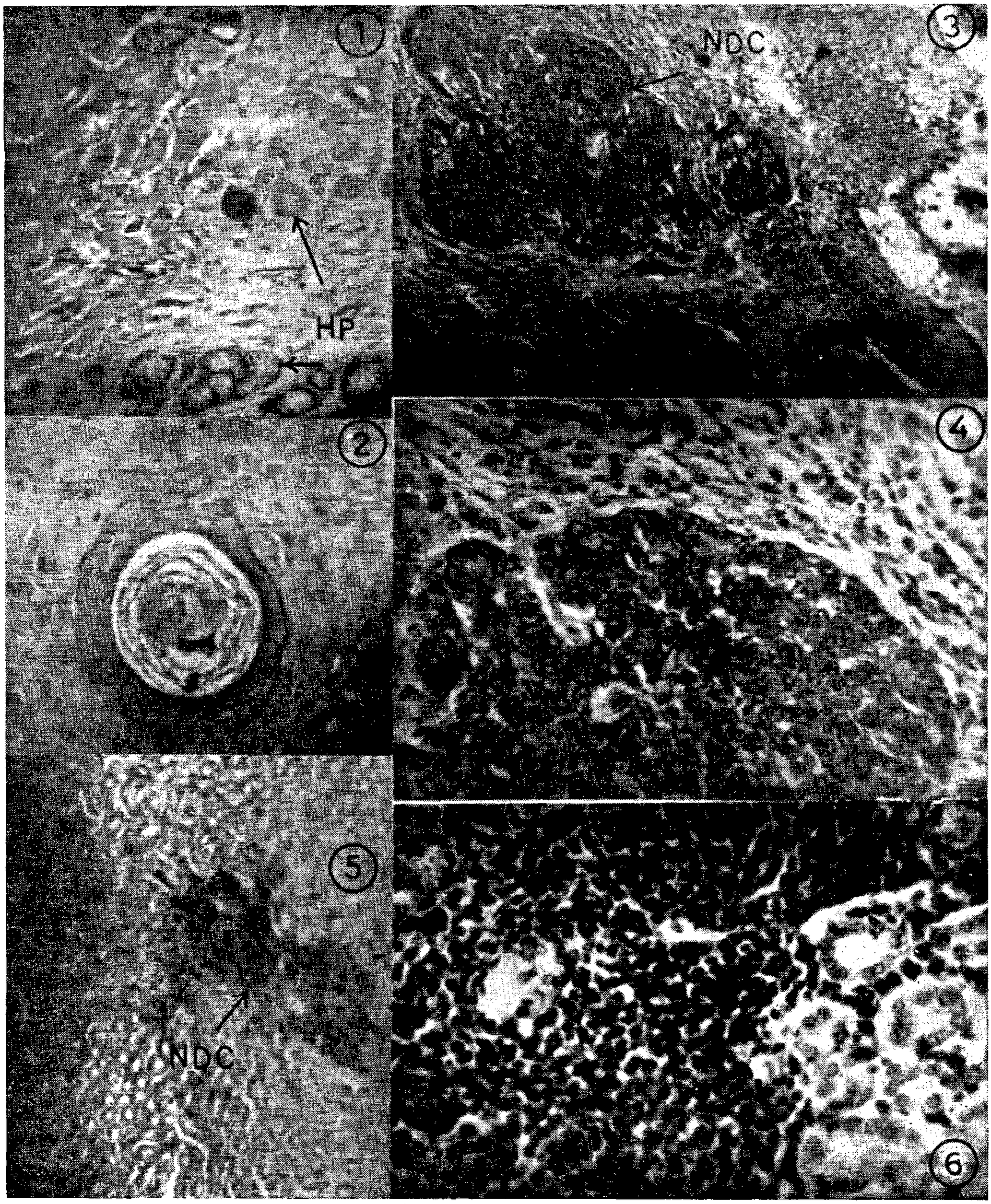
RESULTS AND DISCUSSION

One of the early symptoms that appeared in the treated variant of the animals was the loss of hairs, more distinctly on the posterodorsal surface of the body. After 12 weeks of the feeding, minute red papillae of pin-head size, numbering 3 to 10 per square centimeter were observed. Biopsy of such skin after 15 weeks revealed the presence of deep infiltration of non-differentiated embryonic neoplastic cell-masses which were smaller in size and got deeply stained (Fig. 1). Presence in dermis of these cell masses, is in all probability, akin to squamous cell carcinoma in human beings (Lever and Lever, 1983). Thick-walled capsular structures, identical to fully keratanized epidermal cells forming "horn-pearl", were also noted at different stages of their development. Such pearls are very characteristic of early squamous cell carcinoma Grade-I (Fig. 2). Most of the hair follicels were devoid of hairs. The sebaceous and sweat glands exhibited atrophic changes. The connective tissue showed focal oedematous separation. At still advanced stage, many of the "Pearls" and the cell-masses, perhaps, aggregated totether to give rise to a sufficiently large tumour in the lumbo-sacral region after 30th week. Histological preparations of such tumour could reveal the presence of layers-after layers of squamous cells having non-differentiated small-sized cells in between. Large, vacuolated cells showing degenerative changes were also found in abundance (Figs. 3 & 4), and are suggestive to a case of secondary carcinoma. The glandular elements, present in abundance, suggest it to be of apocrine nature. The malignancy was frequent. Whether this carcinoma was of sebaceous or pseudoriferous glands, is difficult to explain at this stage. It is also possible that the neoplastic cells came from liver or from some other organs to the skin.

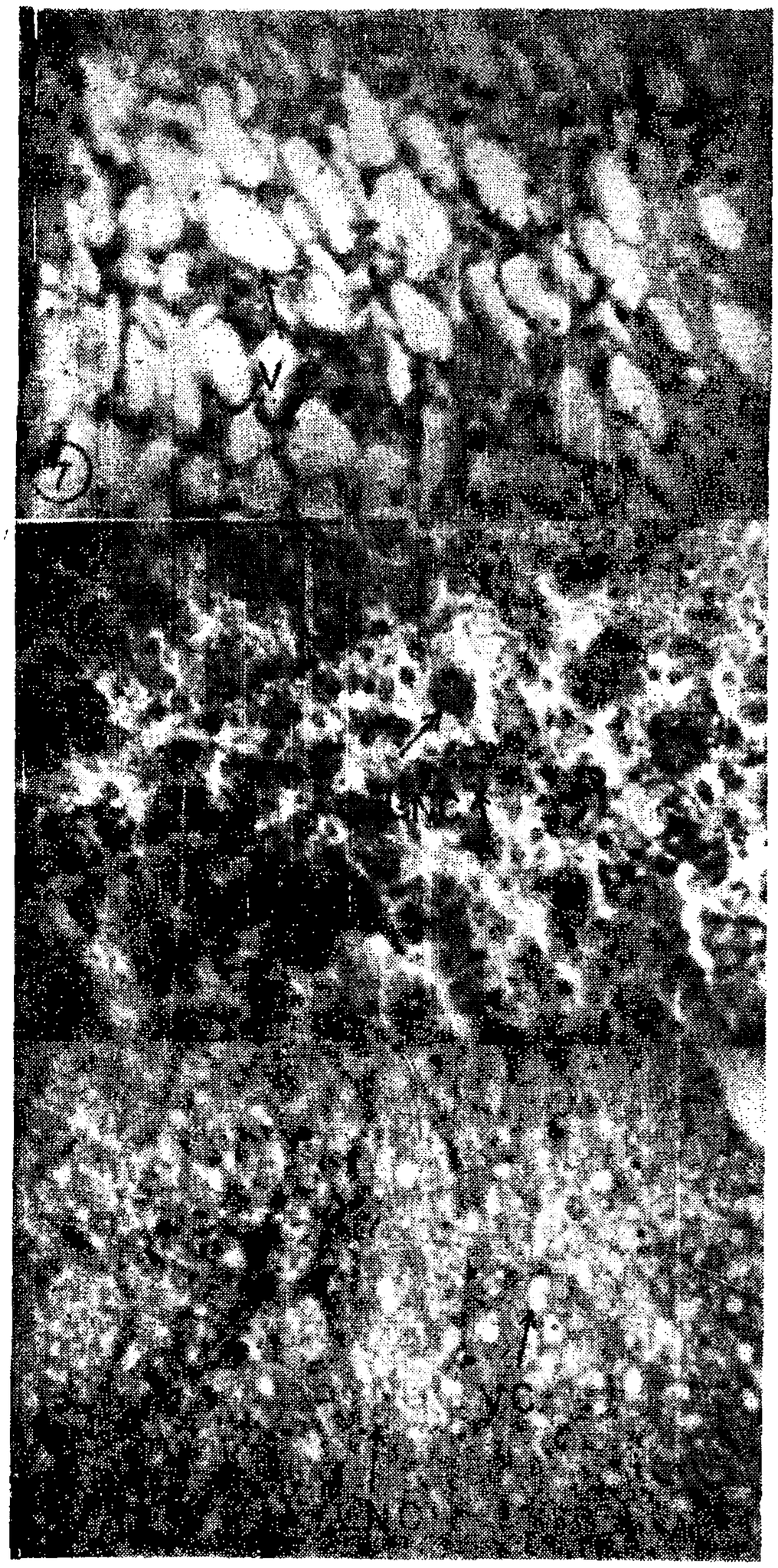
Kidney is also likely to be affected by these toxins because it is through this organ that the (toxins) metabolic wastes are eliminated from the body (Newberne *et al.*, 1968; Butler, 1974; Singh and Chauhan 1983). One of the histopathological damage was the presence of fairly large areas of neoplastic elements comprising masses of nondifferentiated cells with hyperchromatic nuclei. These elements were found in the medullary region of the kidney (Fig. 5 & 6).

Liver is one such organ, where the disastrous effects of aflatoxins have been reported most (Newberne *et al.*, 1964b, 1968; Wogan, 1966; Wogan and Newberne 1967; Krishnamachari *et al.*, 1975a, b, c). Two distinct categories of damages were foundin liver : (a) toxic degeneration of the liver parenchyma and (b) carcinoma. The toxic degeneration was evident by the presence of vacuolated giant-sized cells (VC) giving a foamy appearance (Fig. 7). The latter category (carcinoma) of the change was evidenced by the cells having larger nuclei with mitotic configurations of chromosomes (LNC) (Fig. 8). Both categories of damages were found occurring side-by-side even within one focus of thousand times magnification (Fig. 9). At other places, either only the degenerating cells or the metaplastic ones were appreciably present.

No apparent damage was found in the gastrointestinal tract. It is a bit difficult to conceive that the cells of this system, which come in direct contact with the toxin, would more or less remain unharmed. Perhaps, the damaged cells of epithelium got quickly replaced by the healthy, newly produced daughter cells (these cells



Figs. 1-6. Fig. 1. "Horn-pearls" (HP) in the dermis of the skin (× 32). Fig. 2. A magnified view of the Horn-pearl (×30). Fig. 3. A cluster of non-differentiated cells (NDC) in the tumour (×32). in Fig. 4. A magnified view of Fig. 3 (×80). Fig. 5. Non-differentiated cell mass (NDC) in the medulla of the kidney (×32) Fig. 6. A magnified view of the cell mass of the Fig. 5. (×80).



Figs.7—9. Fig. 7. Vacuolated degenerating cells (VC) of liver (×100). Fig. 8. Large liver cells with nuclei having mitotic configurations (LNC) (×100). Fig. 9. T. S. of liver abounding in vacuolated (VC) and large nucleated (LNC) cells(×100).

belong to renewing cell population, where division and differentiation continues for the whole life), and thus could hide the damage and even compensate for it.

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