HISTOPATHOLOGICAL CHANGES IN PIGS EXPOSED TO AFLATOXIN B1 DURING PREGNANCY

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ABSTRACT
The present study was undertaken to find out histopathological changes during experimental induction of aflatoxicosis in sows during pregnancy. In this experiment, 19 sows and 2 boars were used. The sows were divided in to three groups, consisting of control group without toxin in the feed. For treatment groups, aflatoxin was fed at 1 and 3 ppm respectively. The treated sows showed clinical signs of anorexia, jaundice and loss of body weight. The postmortem examination of the treatment groups revealed tannis h dis coloration of liver with insis s ated bile in the gall bladder and atrophied spleen. Histopathological examination of the liver revealed hypertrophy of the bile duct epithelium, dissociation of the liver cords, karyomegaly and extensive fibrosis with appearance of pseudolobulation in certain areas resulting in frank adenoma. Spleen showed depletion of lymphocytes in germinal epithelium area. Kidney showed prominent intertubular hemorrhages and atrophy of the glomeruli. The present study indicated hepatotoxic, nephrotoxic feature of the aflatoxin B1 in pregnant sows.

Key words: Aflatoxin, Hepatotoxicity, Nephrotoxicity, Sow.

INTRODUCTION
In recent years piggery has developed to a great extent and has attained the Industry status. As in any livestock production programmes swine production needs knowledge of management and health coverage. The presence of mycotoxins in food and feed is of great concern for human and animal health. Fungal contamination of agricultural products can occur at any stage from standing crop through harvest and post harvest handling operations until they reach to the consumers. High temperature (27°C) and relatively high humidity (>85%) which normally prevail in tropical countries like India, provide an excellent conditions for mould growth and activity in the stored products. The fungi prefer to grow in dampness with relatively higher temperature, so natural contamination of agricultural products with mycotoxins is comparatively more in underdeveloped and developing countries. This is mostly because of socio economic backwardness, unhygienic, outdated agricultural and storage practices. Approximately 25% of the food supply in the world is contaminated by mycotoxins annually.

The first outbreak of aflatoxicosis in pigs was reported by Loosemore and Markson (1961). Experimental aflatoxicosis of swine has been studied by Harding et al. (1963).

Aflatoxins are immune-suppressors and have different effects on pigs, varying from poor growth rates in weaners and finishers to abortion and agalactia in sows. The first sign of an aflatoxicosis in sows is decreased feed intake and depending on the levels of mycotoxins present, losses can result from deaths, reduced growth rates, poor feed conversion efficiency and carcass condemnations. Levels in excess of 0.5ppm in the diet of lactating sows will reduce piglet growth rates due to aflatoxins in milk (Devegowda et al., 1998). However, studies on the experimental feeding of aflatoxins during pregnancy are not available.
effort has been made here to study Aflatoxicosis in swine’s during pregnancy.

**MATERIALS AND METHODS**

**Production of aflatoxin:** *Aspergillus parasiticus* strain NRRL-2999 obtained from the Institute of Microbial Technology, Chandigarh was used for production of aflatoxin in the laboratory using sterile rice culture material. The method described by Shotwell *et al.* (1966), was adopted for obtaining the toxic material.

Sabouraud’s dextrose agar was inoculated with spores of NRRL-2999 and incubated the petri plates at the room temperature for 7 days.

Fermentation was carried out in Erlenmeyer’s flasks containing 50 g of polished rice, that was soaked with distilled water. These flasks were autoclaved at 15 lbs pressure for 15 minutes and known quantity of spores ($10^6$) were inoculated into the flasks. The flasks were then moistened with 5 to 6 ml of sterile distilled water during the first 2-3 days without causing individual rice to adhere. The flasks were incubated at 28± 1°C for 6-7 days in an incubator. Flasks were shaked constantly to facilitate through mixing of rice grain and uniform growth of mould mycelium. The flasks containing uniform mycelia growth were steamed to destroy spores after 6-7 days, dried and grounded to fine powder using an electric grinder.

**Treatment of feeds and feeding level of aflatoxin:** The pig feed was purchased commercially and was screened for aflatoxin content. The moldy rice powder with known content of aflatoxin was mixed in these feeds to obtain two levels of aflatoxin B1 activity i.e. 1 ppm (1 ug/g of feed) and 3 ppm (3 ug/g of feed). A control feed, free of aflatoxin was also used. All feeds were incorporated with adequate vitamins and minerals.

**Experimental design:** Group I consisted of seven apparently healthy breedable sows, which were weighing around 35 kg. They were protected against intestinal parasites and were fed with aflatoxin free diet during gestation period of 114 days and served as control.

Group II consisted of seven apparently healthy breedable sows, which were weighing around 35 kg. They were protected against intestinal parasites and were fed with the feed containing 1 ppm of aflatoxin from the day of conception to the end of gestation.

Group III of seven apparently healthy breedable sows, which were weighing around 35 kg. They were protected against intestinal parasites and were fed with the feed containing 3 ppm of aflatoxin from the day of conception to the end of gestation.

All the pigs were placed in respective groups with suitable identification procedures.

**Procedure:** A systematic necropsy was conducted on the pregnant pigs. The organs were examined insitu and gross pathological changes were noted. A representative sample of each organs namely liver, lung, kidney, lung, heart and lymphnodes were collected in 10% formalin for histopathological studies.

**Histopathological studies:** Tissues were processed by paraffin embedding technique. The sections 4 - 5 m thickness and the slides were stained by haematoxylin and Eosin stains, as per the procedure given by Luna (1968) and special staining techniques like Vagieson and Mallory trichome were also done.

**RESULTS AND DISCUSSION**

The pathology of aflatoxicosis in pregnant sows studied by taking 3 groups of sows, where in group II and group III sows were fed with 1 ppm and 3 ppm of aflatoxin in the feed throughout the pregnancy.

**Clinical signs:** The pregnant sows showed characteristic clinical signs of aflatoxicosis in group II and group III. The clinical signs were, anorexia, uneasiness, depression, rough hair coat, diarrhoea, jaundice and less weight gain when compared to control.

**Changes in liver of group-II (90th day):** The liver of pregnant sows during 90th day fed with aflatoxin showed grossly haemorrhages on surface of liver and histological examination, haematoxylin and eosin and tendency for formation of microthrombi in portal vessels were observed. There was mild bile duct hyperplasia with periportal infiltration of mononuclears were observed (Fig. 1 and 2).

These changes were of mild toxicity and similar changes have also been reported by Miller *et*
al. (1981) in experimental aflatoxicosis in swine. So both clinicopathological and histomorphological studies revealed that the aflatoxin caused considerable liver damage. Thus these observations were in agreement with those of Sisk et al. (1968) and Hayes et al. (1978).

**Changes in liver of group-II (100 and 116th day):** The pregnant sows during 100 and 116th day showed grossly congested liver with haemorrhagic spots on the surface. While histological examination showed distorted architecture, dilated sinusoids and haemorrhages in the parenchyma with formation of cystic spaces and disorientation of liver with dissociated liver cells forming gland like structures. These morphological changes of liver of aflatoxin feeding could be categorized as moderate intensity of poisoning of aflatoxicosis as suggested by Miller et al. (1981). Similarly several investigators have reported hematological and serum enzyme alterations induced by aflatoxin consumption in acute and chronic study in young and adult swine (Sisk et al., 1968 ; Miller et al., 1981). The note...
worthy feature was as suggested by Buttler and Wrigglesworth (1966). That prolonged feeding of aflatoxin was acting as a hepatotoxic and carcinogenic. Aflatoxin and their metabolites acting on to cholioles and bileducts resulted in its proliferation and further damaged hepatic cells.

Changes in liver at 3 ppm (90th and 105th day): The liver of pregnant sows during 90th and 105th day fed with aflatoxin grossly showed enlarged liver with petechial haemorrhages on the surface. Histological examination revealed hypertrophy of bile duct epithelium and dissociation of liver cords. In most of hepatic cells, condensed pyknotic nuclei were present. The presence of agglutinated RBS,s aligning to the endothelium of portal veins might have resulted in anorexia. At certain places tendency of liver cells to form alveolar like spaces indicated chronic effects of aflatoxin and its metabolites. Paul et al. (1969) observed that, gross and histopathological lesions of aflatoxicosis in pigs very with the amount of toxin consumed. In the present study the pigs fed with 3 ppm of aflatoxin had most pronounced lesions.

Changes in liver of 116th day (3 ppm): Grossly liver was fibrosed with yellow discoloration and small black spots were observed on entire surface. Histological examination showed, completely destroyed liver architecture with disappearance of lobules. Extensive fibrosis and entwining the group of hepatic cells were seen. The adenomatous pattern was much more perceptible in most of the areas, wherein group of liver cells formed gland like structures and numerous cystic spaces with detached epithelial cells and numerous bizarre shaped cells as well as tumor giant cells were seen. These changes indicated severe chronic toxicity (Fig. 6).

The chronic changes exhibited in the liver in the present study were dose dependent. The severity of destruction of liver was increased with the progress of the time.

Changes in spleen (group II): The lymphoid organs like spleen and lymphnode on 90th day showed reduction of organs and histologically minimum lymphoid depletion in the germinal centers as well as periarterial areas. Whereas on 100th day of lymphoid organs like lymphocytes in the germinal centers and periarterial areas were depleted. At 116th day the depletion was aggravated and infact the reticular cell proliferation in spleen and lymphnode was so much, it gave whirl like appreance in the section. At this stage there was considerable reduction in humoral response as well as cell mediated immune response. The replenishing of the depletion of lymphocytes by the proliferated whorl like cells indicated immunosuppression (Fig. 4).

Changes in spleen of 3 ppm: Spleen showed reduction of organ weight when compared to 1 ppm group and also showed the reduction of cortex and medulla and much more proliferation of reticular cells and epitheloid cells in medulla areas. These

FIG. 5: Section of uterus of Group II (1 ppm) pregnant sow showing dilated utrine vessels with agglutinated RBC’s to the endothelium, tortuous uterine glands in the mucosa. H &E 20X

FIG.6: Section of the liver of Group III (3 ppm) pregnant sow showing dissociated liver cords of lobules. Mild bileduct proliferation in the periportal areas and dilated lymphatics. H&E 20X
observations were in agreement with serum antibody level and delayed hypersensitivity reactions, which were considerably reduced.

Thus both humoral and cell mediated immune response were decreased during aflatoxicosis in swine. These observations were essentially similar with those of rabbits and poultry by Nair et al. (1988) in pigs.

**Changes in kidney:** Kidney of group II during 90th, 105th and 116th day showed extensive cortically and medullary haemorrhages and in later stage hypertrophy of tubular epithelium was prominent. Because of cystic dilation of renal tubules with pronounced hypertrophy were observed in group III (Fig. 3 and 4). Paul et al. (1969) found out that aflatoxin not only induced malignant hepatomas in rats but also caused damage to renal glomeruli as well as tubules. Even with the sows this was also true, thus aflatoxin induced proliferative changes in the renal tubules and dilation of distal convoluted tubules of kidney in the aflatoxicosis of pigs. Similar changes were reported by Loosemore and Markson (1961) and Sisk et al. (1968).

**Changes in heart:** Grossly haemorrhages spots were observed on epicardium of sows during 90th, 100th and 116th day. Histologically also dilated blood vessels and myocardial haemorrhages were constant features. At higher doses (3 ppm) stripping of nucleus from muscle fibres was observed, obviously due to the induction of mild toxic changes (Fig 8).

**Changes in lungs:** In all aflatoxin treated animals (1 and 3 ppm), congestion of lungs and haemorrhages of paranchyma were observed. Histologically interalveolar haemorrhages and emphysematous bullae were observed.

**Changes in uterus:** Pregnant sows that were fed with 1 ppm during 90th day showed 14 fetuses and during 100th day, on cutting open of uterus resorpted of fetus was observed by patchy red spots on uterus mucosa. On 116th day also resorption of fetus was observed and implantation sites were marked by reddened areas on the mucosa. When 3 ppm aflatoxin fed sows sacrificed on 100th day the uterine mucosa showed red spot indicating of resorption of uterus and on 116th day also the pregnant sow that revealed red patches on the mucosa membrane was yellowish due to icterus (Fig 5).

In all above cases (1 and 3 ppm) uterine mucosa showed resorption of fetuses. On histological examination uterine mucosa showed, desquamation of lining of endothelium as well as multiple haemorrhages in the submucosa. Whereas, in the case of those sows that delivered fetuses, the histological examination of uterine mucosa and sticking of the RBC’s to the lining endothelium. The findings were as per the observations of Loosemore and Markson (1961) and Sisk et al. (1968).

**CONCLUSION**

To conclude, aflatoxin induced hepatic lesions when fed to pregnant sows at 1 and 3 ppm.
The aflatoxin exposure resulted in hepatic damage in pregnant sows. More importantly aflatoxin at 1 and 3 ppm induced pathological lesions in spleen, lymphnode, kidney, uterus, heart and lungs. Further the cell mediated and humoral immune response were reduced in aflatoxin exposed animals.

REFERENCES