EFFECTS OF OCHRATOXIN A IN PREGNANT RATS AND THEIR FETUSES

ผลของออคราท๊อกซิน-เอ ต่อหนูท้องและลูกอ่อน

Vorasak Patchimasiri[1] Somchai Pongjanyakul[2] Pongsri Jittanoonta[3]

วรศักดิ์ บัจฉิมะศิริ สมชัย พงศ์จรรยากุล ผ่องศรี จิตตนูนท์

Vipaporn Na Thalang[3] Duangchan Hengswadi[3]

วิภาภรณ์ ณ ถลาง ดวงจันทร์ เฮงสวัสดิ์

[1]Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University Bangkok Metropolis 10900

ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กท. 10900

[2]Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University Bangkok Metropolis 10900

ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กท. 10900

[3]Institute of Food Research and Product Development, Kasetsart University, Bangkok Metropolis 10900

สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร มหาวิทยาลัยเกษตรศาสตร์ กท. 10900

บทคัดย่อ

ออคราท๊อกซิน เอ เป็นสารพิษจากเชื้อราพวก *Aspergillus spp.* และ *Penicillium spp.* สามารถก่อให้เกิดความผิดปกติของตัวอ่อนและความเป็นพิษต่อไตได้ เนื่องจากความเป็นพิษของสารพิษออคราท๊อกซิน เอ ต่อหนูท้องและลูกอ่อนยังมีรายงานน้อยมาก คณะดำเนินการวิจัยจึงทำการศึกษาเกี่ยวกับความเป็นพิษของออคราท๊อกซิน เอ ต่อหนูท้องและลูกอ่อน (สายพันธุ์ วิสตาร์) โดยการฉีดสารพิษนี้เข้าทางใต้ผิวหนังในช่วงแรก (เมื่อตั้งท้องได้ 8–10 วัน) และช่วงหลัง (เมื่อตั้ง

ท้องได้ 15–17 วัน) การตรวจพบเชื้ออสุจิในช่องคลอดให้นับเป็นวันที่ 1 ของการตั้งท้อง และทำการฆ่าเมื่อตั้งท้องได้ 21 วัน จากการศึกษาพบว่า กลุ่มหนูท้องที่ได้รับสารพิษออคราท็อกซิน เอ ขนาด 3 มก./น.น. 1 กก. ในช่วงแรกของการตั้งท้อง พบว่าหนูท้องจำนวน 1 ใน 5 จะตายภายใน 4 วัน หลังจากได้รับสารพิษ เมื่อทำการตรวจผ่าซากหนูท้องที่รอดชีวิตพบว่า แม่หนูท้องมีการแท้ง 100% ขณะที่กลุ่มหนูท้องซึ่งได้รับออคราท็อกซิน เอ ในช่วงหลังของการตั้งท้อง พบว่า 2 ใน 5 ของหนูท้องจะตายภายใน 3 วันหลังจากได้รับสารพิษ โดยมีอัตราการแท้ง 55% ซึ่งสูงกว่ากลุ่มเปรียบเทียบ (20%) และน้ำหนักโดยเฉลี่ยของลูกอ่อน (1.93 ± 0.16 กรัม) น้อยกว่ากลุ่มเปรียบเทียบ (3.62 ± 0.76 กรัม) อย่างมีนัยสำคัญ ($P < 0.01$) นอกจากนี้ยังพบว่าลูกอ่อนเหล่านี้มีความผิดปกติของกระดูกซี่โครง ซึ่งมีลักษณะเป็น wavy ribs แสดงว่าสารพิษออคราท็อกซิน เอ สามารถก่อให้เกิดความผิดปกติต่อลูกอ่อนได้

การเปลี่ยนแปลงทางจุลพยาธิสภาพ ส่วนใหญ่พบที่ ไต ม้าม และธัยมัส วิการที่สำคัญได้แก่ pycnotic nuclei ของ epithelial cells ของ proximal และ distal tubules, necrotic glomeruli ที่ม้ามและธัยมัสพบ lymphoid depletion และ necrosis จากการศึกษาครั้งนี้ สามารถยืนยันได้ว่า ออคราอ๊อกซิน เอ สามารถทำให้เกิดการแท้งอย่างรุนแรง และการแคระแกรนของลูกอ่อน

Abstract

Ochratoxin A is a teratogen and a potent nephrotoxin produced by storage fungi in the group of *Aspergillus spp.* and *Penicillium spp.*. Its toxicity on the pregnant rats and their fetuses is rarely reported. On this basis, we present here the data on toxicity of ochratoxin A given subcutaneously to the pregnant rats (Wistar strain) on the early (day 8 through 10 of gestation) and late (day 15 through 17 of gestation) pregnancy. The day on which sperm identified was designated as day 1 of pregnancy. The pregnant rats were killed on day 21 of gestation. At the level of 3 mg/kg of ochratoxin A treated during early pregnancy, one out of five dams died within 4 days

after administration and resulted in acute ochratoxicois characterized by sign of renal failure from necropsy, litters from the s rvival dams were found 100% resorption, whereas the treatment during late pregnancy revealed two out of five pregnant rats died within 3 days after administration, percentage of dead or resorption (55%) was higher than control group (20%) and the mean fetal weight (1.93 ± 0.16 g) were significantly decreased ($P < 0.01$) from control (3.62 ± 0.76 g). In addition, these fetuses showed malformation of the rib : wavy ribs. This indicated that ochratoxin A has the teratogenic effects.

Histopathological changes were mostly observed in kidney, spleen and thymus of both early and late pregnancy treatments. The main lesion revealed pycnotic nuclei of epithelial cells of proximal and distal tubules, necrosis of the glomeruli, lymphoid, depletion and necrosis in spleen and thymus. The studies reported in the present paper confirm that ochratoxin A may cause resorption or dead and fetal growth retardation in the pregnant rat.

Introduction

Ochratoxin A was first isolated from *Aspergillus ochraceus*, but subsequent investigations have revealed that a variety of moulds included in the fungal genera Aspergillus and Penicillium are able to produce ochratoxins. The main producers appear to be *A. ochraceus* and *Penicillium viridicatum*. This subject has been reveiwed by Krogh (1976). In studies of ochratoxin A production by *A. ochraceus*, optimal production occurred between 20°C and 30°C (Schindler & Nesheim, 1970; Bacon *et al.*, 1973). Ochratoxin A is a potent nephrotoxin in several species and has been found as a contaminant in a variety of cereal grain and food as reveiw by Chu (1975). In addition, ochratoxin A also caused pregnant rats to resorb litters (Still *et al.*, 1971). Many investigations

found that ochratoxin A was teratogenic in mice (Hayes *et al.*, 1974), rats (Brown *et al.*, 1976) and hamsters (Hood *et al.*, 1975). However, teratogenic responses and the toxic effects of ochratoxin A in experimental animals was only performed during the period of organo-genesis (day 6 through 12 of gestation). It is not clear whether the effects of prenatal exposure of ochratoxin A during late pregnancy (day 15 through 17 of gestation) were reported elsewhere.

The present study was designed to further assess the teratogenic and toxic effects of ochratoxin A (3 mg/kg) in rats dosed by subcutancously injection during day 8 through 10 and day 15 through 17 of gestation.

Materials and Methods

Virgin white rats, Wistar strain, of average nonpregnant weight 180-200 g. were mated as they came into estrus; the timing of estrus and confirmation of mating being performed by the vaginal smear technique. Day 1 of gestation is verified by observation of sperm in the vagina. The pregnant rats were separated and housed individually in cages and randomly assigned to four groups (Table 1). The pregnant rats (group 2 and group 4) dosed with 3 mg/kg of ochratoxin A dissolved in 0.1 N $NaHCO_3$ subcutaneously as multiple doses on day 8 through 10 and day 15 through 17 of gestation, respectively. Control animals (group 1 and group 3) were given 0.1 N $NaHCO_3$ only on day 8 through 10 and day 15 through 17 of gestation.

Animals were fed with regular rat diet and water *ad libitum*. The dams were carefully observed for any behavioral changes following treatments. Maternal weights were recorded weekly during period of pregnancy. On day 21 of gestation, all treated dams were killed with chloroform. The abdomen was explored with midline

Table 1. Summary of trial for pregnant rats treated subcutaneously with 3 mg/kg of Ochratoxin A on day 8 through 10 and day 15 through 17 of gestation.

Group	No. of Dams	Treatment	Days of gestation given	Days of gestation taken
1	5	0.1 N $NaHCO_3$	8–10	21
2	5	3 mg/kg OCA*	8–10	21
3	4	0.1 N $NaHCO_3$	15–17	21
4	5	3 mg/kg OCA	15–17	21

*OCA = Ochratoxin A

incision, then the uterus was removed rapidly, the number of implantation sites, resorbed or dead fetuses, live fetuses, corpora lutea and fetal weights were recorded. Following gross examination, all fetuses were immediately fixed in 95 percent ethanol for staining of skeleton by Alizarin Red S.

Maternal liver, kidney, heart, stomach, thymus and adrenal gland, and fetal liver were fixed in 10% neutral buffered formalin for 48 hours, then sectioned and stained with haematoxylin and eosin routinely.

Results

The results of the ochratoxin A effects in our experiments are summarized in table 2. All the pregnant rats given 0.1 N $NaHCO_3$ subcutaneously on day 8 through 10 or day 15 through 17 of gestation remained healthy at all times and no abnormality in maternal, fetal histology could be demonstrated. All animals dosed with ochratoxin A became unwell and developed staring coats within 24–48 hours with loss of weight for 2–3 days.

Five rats were given 3 mg/kg ochratoxin A on day 8 through 10 of gestation, one was found dead within 3 days after administration and the clinical signs were of sudden onset, anorexia, depression and finally death. The 4 survivors were killed on day 21 of gestation, showed 100% dead or resorptions. Hence the abnormality of the litters could not be determined and the mean body weight gain during pregnancy $(1.58 \pm 0.37$ g/day) was significantly different from those of untreated control rats $(6.02 \pm 0.48$ g/day; $P < 0.01)$. There was, however, histological evidence of toxic injury to liver, kidney, spleen and thymus organs following administration of ochratoxin A on day 8 through 10 of gestation. The main lesions revealed swollen of proximal epithelial cells (Fig. 1), pycnotic nuclei of epithelial cells of proximal and distal tubule (Fig. 2), necrosis of the glomeruli, lymphoid depletion and necrosis in spleen and thymus (Fig. 3).

Two out of five pregnant rats dosed with 3 mg/kg of ochratoxin A on day 15 through 17 of gestation died within 4 days after administration. These animals were more severely affected than the rest of the group in showing acute ochratoxicosis in the kidney. The average fetal weight of the group dosed on day 15 through 17 was only 1.93 ± 0.16 g and the mean body weight gain during pregnancy of the 5 dams were significantly lower than those of control group $(P < 0.01)$. The skeletal anomalites was not found in control fetuses, but wavy ribs occurred in all litters from dams given 3 mg/kg of ochratoxin A during late pregnancy.

Table 2. Toxicity of Ochratoxin A on pregnant rats and their offsprings treated with multiple subcutaneously doses (3 mg/kg) on day 8 through 10 and day 15 through 17 of gestation and sacrificed on day 21 gestation

Treatment	Days of gestation given	No. of Dams	Mean body weight gain during pregnancy (g/day ± SE)	No. of Fetuses	Total Implants	Dead or resorbed (%)	Mean Fetal weight (g ± SE)
0.1 N $NaHCO_3$	8–10	5	6.20 ± 0.48	52	52	–	3.38 ± 0.84
3 mg/kg OCA	8–10	5[a]	1.58 ± 0.37*	20	54	100	–
0.1 N $NaHCO_3$	15–17	4	4.91 ± 0.64	31	39	20.2	3.62 ± 0.76
3 mg/kg OCA	15–17	5[b]	1.70 ± 0.29*	48	54	55.5	1.93 ± 0.16*

OCA = ochratoxin A

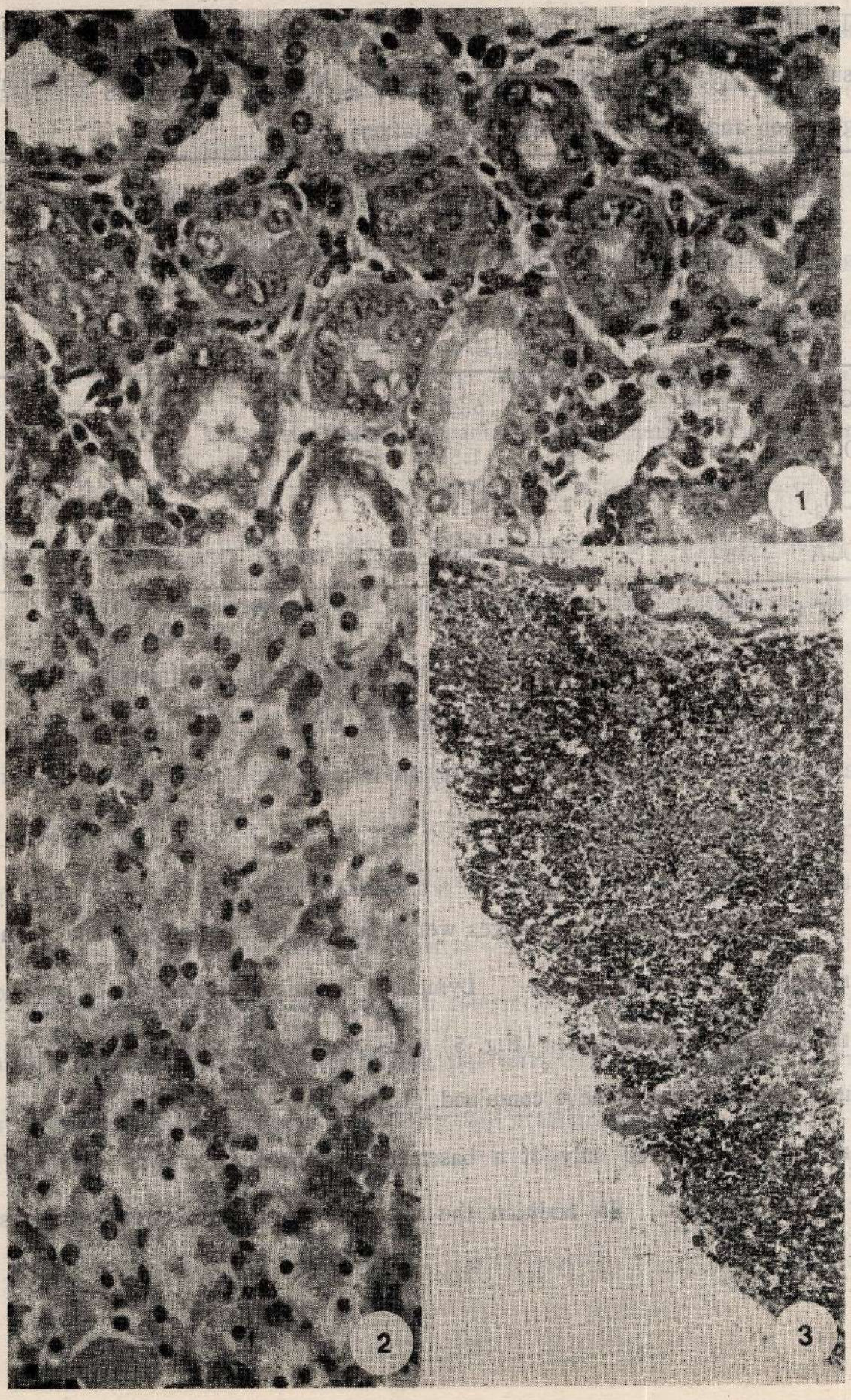
a = one out of five dams died within 3 days after administration

b = two out of five dams died within 4 days after administration

*significantly different ($P < 0.01$) from control

SE = Standard error

The histopathological changes were found in thymus, spleen and kidney in dams treated during late pregnancy. Lymphoid depletion and necrosis of lymphocytes in the thymus (Fig. 4) and spleen (Fig. 5) were observed. Many proximal and distal convoluted tubules in the kidneys contained necrotic tubular epithelial cells and some proximal tubules consisted only of a basement membrane that was devoid of renal tubular epithelial cells. In addition the necrosis of glomeruli were also observed (Fig. 6).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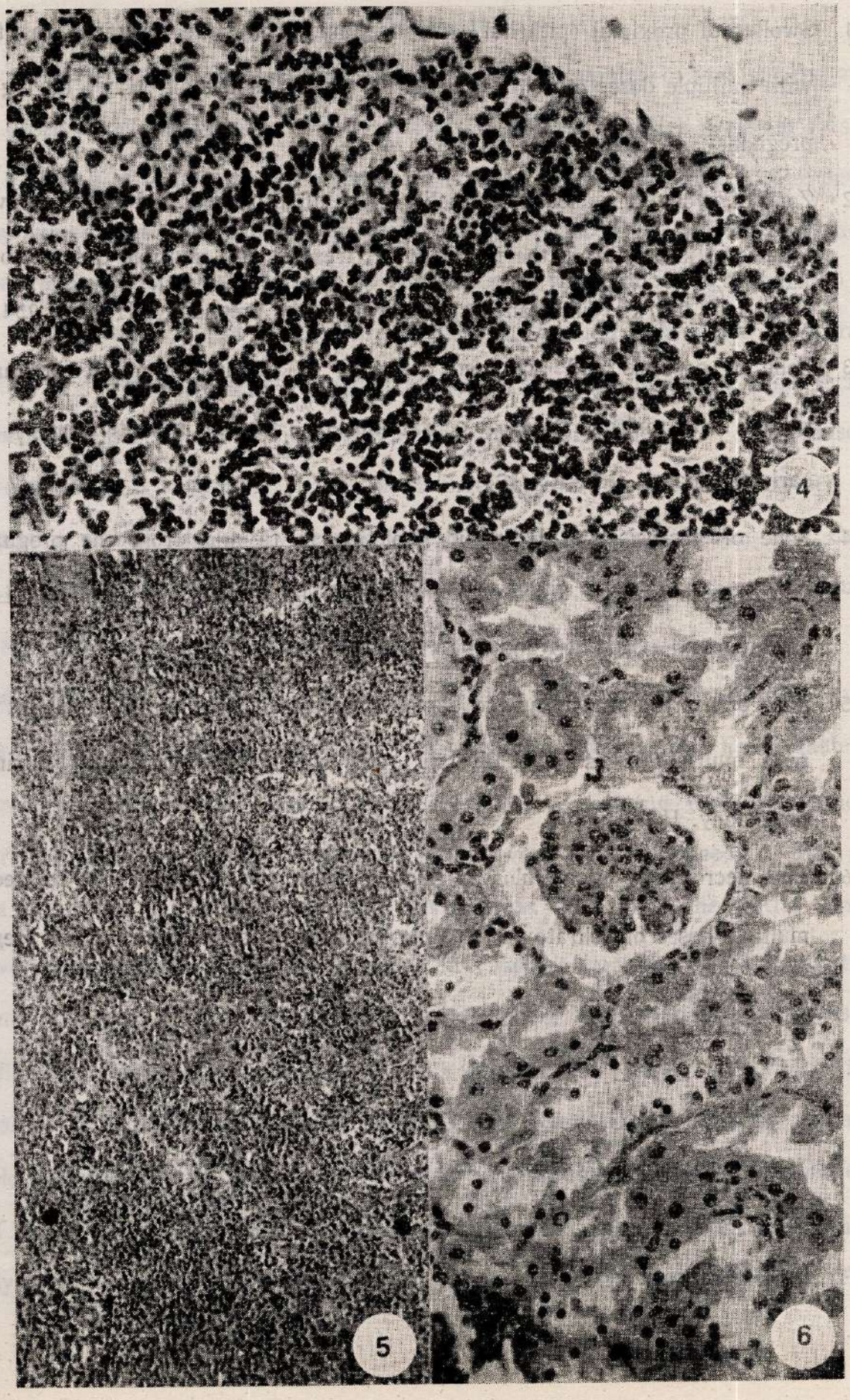
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Fig. 1 Swollen of proximal epithelial cells in kidney from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during early pregnancy. H & E × 594

Fig. 2 Zone of pycnotic nuclei of epithelisl cells of proximal and distal convoluted tubules in kidney from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during early pregnancy. H & E × 954.

Fig. 3 Zone of necrosis and lymphoid depletion in thymus from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during early pregnancy. H & E × 104.

Fig. 4 Zone of necrosis and lymphoid depletion in thymus from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during late pregnancy. H & E × 400.

Fig. 5 Zone of necrosis and lymphoid depletion in spleen from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during late pregnancy. H & E × 100.

Fig. 6 The necrosis of glomerulus in kidney from the pregnant rat treated with multiple doses of ochratoxin A (3 mg/kg, subcutaneously) during late pregnancy H & E × 454.

Discussion

Before this experiment was performed, we attempted to find out the appropriate doses of ochratoxin A. The results of our studies in which pregnant rats were given 5 mg/kg of ochratoxin A on day 8 through 10 of gestation died within one day after administration. There were signs of acute ochratoxicosis characterised by the sign of renal failure. Hence, a dose level of ochratoixin A was reduced to 3 mg/kg.

As tested in this study confirm that multiple administration of ochratoxin A (3 mg/kg) during either early pregnancy or late pregnacy induced a high incidence of resorption and decreased in body weight gain during pregnancy. The reason for the weight loss is unknown. It appeared that dehydration due to decreased water consumption was at least partly responsible for the weight loss (Thacker and Carlton, 1977). Hence, the effects of ochratoxin A given to the pregnant rats were similar to those reported by many investigators (Brown *et al.*, 1976; Hayes *et al.*, 1974). They concluded that multiple exposures of rats to doses of ochratoxin A larger than 1 mg/kg results in ochratoxicosis and the loss of litters approaches 100%. Unfortunately the 100% resorbed fetuses were observed in the treated group during early pregnancy, so the teratogenic effects and the mean fetal weight were not determined in this group. It was appeared that ochratoxin A killed the embryos soon after the initial exposure. However, the significant difference in mean fetal weight from the pregnant rats treated with multiple doses of 3 mg/kg of ochratoxin A during late pregnancy was observed. This finding revealed that ochratoxin A had a definite direct effect on growth in the fetuses. The mechanism through which growth retardation is nuknown, but several possible explanations are considered. Ochratoxin A may had a direct effect on fetuses, (Hood *et al.*, 1975). More and Galtier (1974) proposed that ochratoxin A impaired glycolysis in maternal liver. Hayes *et al.* (1974) reported that ochratoxin A was

teratogenic when given to mice at 5 mg/kg (i .p) on one of gestation days 7 through 17. They found a variety of skeletal anomalies, mostly in ribs and vertebrae. In the present study in rat, ochratoxin A given during late pregnancy (day 15 through 17 of gestation) also caused a skeletal anomalies, mostly wavy ribs. It is suggested that further studies will be necessary to establish the teratogenic effects of ochratoxin A during late pregnancy.

The toxic lesions were found primarily in kidney, thymus and spleen of both ochratoxin A treated groups. Necrosis of renal tubular epithelium mainly involved the convoluted segments of the proximal and distal tubules. Necrosis of lymphoid cells were also found in thymus and spleen. These toxic lesions seen in the ochratoxin A treated rats were similar to those described in the guinea pig (Thacker and Carlton, 1977) and rats (Munro *et al.*, 1974)

The results of the present study indicate that ochratoxin A, when fed to the pregnant rat during early pregnancy produces the embryotoxic effect more than those of the ochratoxin A treated group during late pregnancy.

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