Histological study of the effect of aflatoxin on liver and kidney of female Wistar rats

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Abstract:

Female albino rats were given different oral doses (20 , 40 , and 80 µg/kg) of aflatoxin for 6 weeks. Clear suppression in the body weights was recorded especially in higher doses.The lower doses caused increase in the number of apoptotic hepatocytes in the liver ,while the higher dose (i.e.80 µg/kg)caused necrosis of the hepatocytes accompanied by lymphocytes infiltration . Dose-dependent histological alterations in epithelial cells lining kidney tubules were noted. The main mode of cell death detected in kidney was programmed cell death having the main characteristics of apoptosis such as shrinkage of the cells , condensation and fragmentation of nucleus , hypereosinophilia of cytoplasm and in which no inflammation was seen .

Keywords : aflatoxin, liver, kidney, apoptosis , necrosis

Introduction:

Aflatoxins are one of the most dangerous mycotoxins known, owing to their high toxicity to both animals and human. Aflatoxins B_1, B_2, G_1, and G_2 are mycotoxins that may be produced by three moulds of the *Aspergillus* species: *A. flavus, A. parasiticus* and *A. nomius,* which contaminate plants and plant products. Most of the toxicological data relate to aflatoxin B_1 [1].Ankrah *et al* 1993 [2] exposed mice to AFB1 and AFG1 via their feed (4.8 µg AFG1, 0.8 µg AFB1 (or both) per kg body weight per day and found that AFG1 caused significant accumulation of only neutral fat in the liver, a slight rise in serum triglycerides and intensified hepatorenal inflammation, necrosis and bile duct proliferation. AFB1 caused the accumulation of both neutral fat and fatty acids in the liver, and was cytotoxic to the liver and kidney.Rats and mice differ markedly in sensitivity to AFB1 hepatocarcinogenicity, the former being sensitive and the latter resistant [1].

Aflatoxins are found as contaminants in human and animal food as a result of fungal contamination both pre- and post-harvest, with the rate and degree of contamination being dependent on temperature, humidity, soil and storage conditions.. The range of levels reported for AFB1 was from 0 to 30 µg/kg and for total from 0 to 50 µg/kg [3].

Cell death has found to be occurred in response to aflatoxins [4] and other mycotoxins [5] and there are conflicting observations about the mode of this cell death. Programmed cell death or apoptosis is characterized by a series of typical morphological features, such as shrinkage of the cell, fragmentation into membrane-bound apoptotic bodies and rapid phogocytosis by neighboring cell [6].It is a physiological mode of death and induced in response to various pathologic and physiologic stimuli, such as activation of

cell-surface death receptors, UV radiation, serum withdrawal, and cytotoxic drugs [7 ; 8]. Apoptosis plays an important role in animal development (9), maintenance of tissue homeostasis [7 ; 8] , regulation of the immune system [10] , and the host response to insult [8, 11]. It is morphologically characterized by cell shrinkage, chromatin condensation, membrane blebbing, and formation of apoptotic bodies [11].

The present work deals with the identification of mode of cell death in liver and kidney of female rats in response to exposure to aflatoxin using three different doses.

Material and Methods:

Sixteen female albino rats (weighing 230 – 250 gm) were obtained from the animal house in the college of science and divide into four groups of four animals each.Aflatoxins was prepared in the central veterinary laboratory in Erbil from poultry foodstuffs.Purification of aflatoxin was done using Thomas 1974[12] procedure . Group 1 received distilled water only and served as control.The animals of groups 2 , 3 and 4 received 20µg , 40µg and 80µg /kg body weight / day of aflatoxin respectively . All the animals were received aflatoxin in the mentioned doses in drinking water for six weeks.The animals in all the groups were sacrificed 24 h after the last dose by decapitation , and liver and kidney were immediately removed and fixed in formol saline and processed for paraffin method . The samples were dehydrated , cleared and embedded in paraffin, then 4-6 micrometer sections were stained with haematoxylin and eosin .Other small pieces of both organs have been fixed in 4% glutaraldehyde for plastic method.The later samples were postfixed in osmium tetraoxide , dehydrated, cleared and then embedded in epoxy resin mixture and sectioned by Riechert ultramicrotome into 0.5 micrometer sections which were stained by toluidine blue (4).Apoptotic cells were identified by morphological criteria (cell shrinkage , chromatin condensation and margination) used by Gujral et al 2002[13].

Results and Discussion:

As shown in table 1 , the aflatoxin has caused suppression in the body weights of the rats in the second and third doses in a dose dependent effect while the first dose which represent the lower one caused normal increase in the body weight.There were conflicting results with respect to this point in which some investigators found no significant differences in the body weights or feed efficiency in response to aflatoxin administration [14,15] while recent investigation recorded dose dependent decrease in body weight [16,17,18].

Table 1 : effect of aflatoxin on body weights

Doses / µg/kg	Body weights	
	0 time	After six weeks
0	250 ± 3. 55	305 ± 5. 3
20	248 ± 5. 21	298 ± 6. 34
40	240 ±4. 23	245 ± 4. 9
80	235 ± 2. 01	243 ± 3. 8

Above weights represent mean weight of three animals in each group

± : standard deviation

The liver in the 20 µg/kg dose showed little histological alterations in comparison to control which include appearance of some apoptotic hepatocytes throughout the liver lobules characterized by condensed nuclei and hypereosinophilic cytoplasm .Other recorded changes were hypertrophied nuclei , dilated sinusoids and congested blood vessels with red blood corpuscles (fig.1 & 2).The second and third doses showed dose dependent effects with respect to the number of died hepatocytes. The main mode of cell death in the later doses were programmed cell death and necrosis respectively. As shown in (fig.3 & 4) the main mode of cell death in liver of 40 µg/kg exposed rats is the apoptosis with marginated chromatin and condensed chromatin , while fig.5 shows necrosis of the liver cells accompanied by lymphocytes infiltration in the 80 µg/kg dose. Abdel-Raheim et al 2001[4] mentioned that aflatoxin induced apoptosis in rat hepatocytes. AFB1 was shown to be converted into its epoxide and this derivative produces DNA adducts causing DNA strand breaks and point mutations [19]. Under this pathological condition, the active process of cellular self-destruction, apoptosis, may be occurred. One of manifestations of AFB1–induced toxicity is oxidative stress[20] . Recently, it is accepted that oxidative stress is an apoptosis inducer[21]. Many agents that induce apoptosis are either oxidants or stimulators of cellular oxidative metabolism. AFB1 can lead to direct or indirect caspase-3,an apoptosis marker, activation and consequently to apoptosis in rat liver[4]. The histopathological investigation revealed by Abdel Raheim et al 2001[4] showed that AFB1 induced marked hepatotoxic effects, in the form of degenerative and necrobiotic changes which were manifested by vacuolar degeneration as well as appearance of minute foci of necrosis. The apoptosis was manifested by condensation of nuclear chromatin into defined masses that become marginated against nuclear membrane as well as formation of half moon shaped or round dense nuclear remnants. The cells were shrinkage and the cytoplasm was condensed. All these features were described as a morphological criteria of apoptosis by many investigators [22]. Ochratoxin A which is a mycotoxin caused apoptosis in mice liver with no evidence of cell necrosis at one week of administration while it centrilobular necrosis was evident at two weeks [5].Furthermore, Ueno et al 1995[23] found that Ochratoxin A induced apoptosis at low concentration. However، besides the lipid peroxidation and oxidative DNA damage induced by AFB1, it is mutagenic mycotoxin and could induce DNA damage in genomic DNA at different site [20].Ingestion of this mycotoxin , is know to be capable of inducing acute poisoning, aflatoxicosis, and is believed to be implicated in the development of primary liver cancer [24]. Abdel-Rahiem et al 2001[4] suggested that oxidative damage caused by AFB1 may be one of the underlining mechanisms for AFB1−induced cell injury and DNA damage, which eventually lead to tumorigenesis .Choy (1993)[25] has reviewed the dose-response induction of DNA adducts by AFB1 and its implication to quantitative cancer risk

assessment . Many carcinogenic substances including AFB1 damage DNA. The destructive effects of these agents on the genetic material involve a variety of different mechanisms but often free radicals are involve [4].The present work didn't show any sign of inducing cancer and this may be due to the short term administration of aflatoxin.

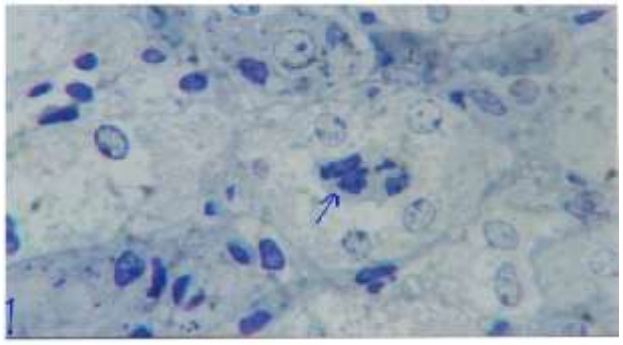

Fig.1Paraffin section of normal rat liver 400X

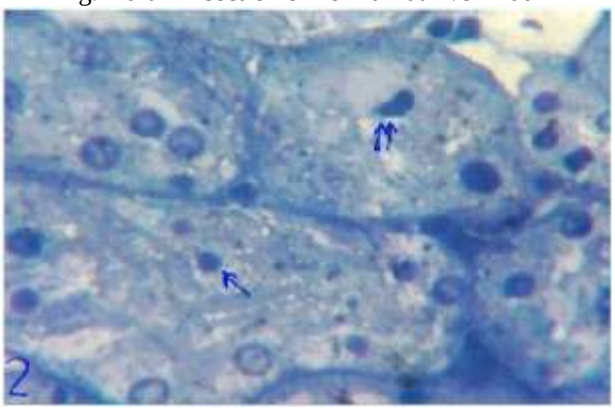

Fig.2 Paraffin section of 20 µg/kg b.w. aflatoxin rat liver ,notioce the congested central vein with blood corpuscles , dilated sinusoids(S), hypertrophied hepatocytes(H) and appearance of apoptotic hepatocytes (↑) 400X

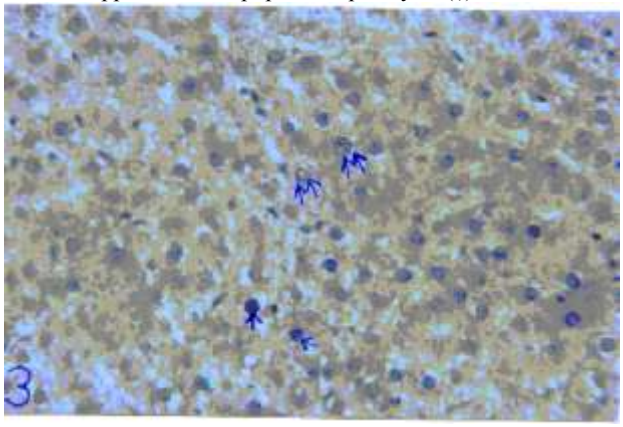

Fig.3 Paraffin section of 40 µg/kg /b.w. exposed rat liver showing numerous apoptotic cells with condensed (↑) and half moon nuclei(↑↑) 400X

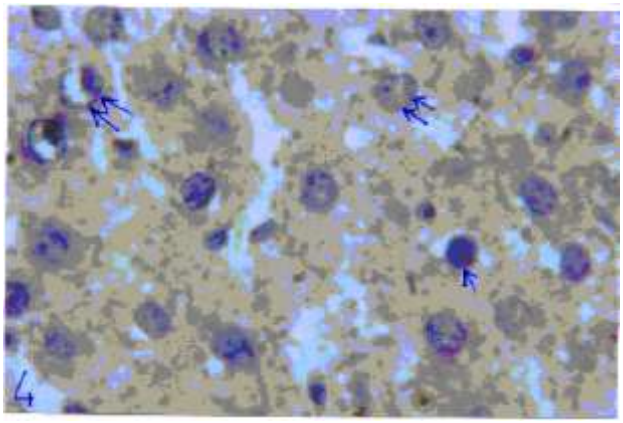

Fig.4 Same section under 1000X.

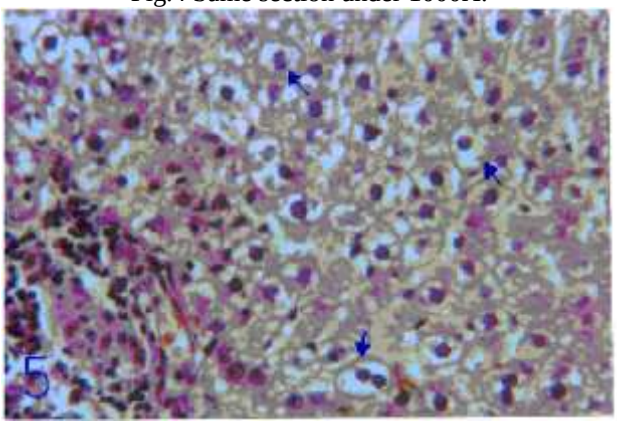

Fig 5 Paraffin section of 80 µg/kg /b.w. exposed rat liver showing numerous necrotic cells (↑) 400X

The hepatocytes have active proliferative response towards toxin insult [26] and this explains the appearance of mitotic figures especially in the 40 µg/kg dose(fig.6).

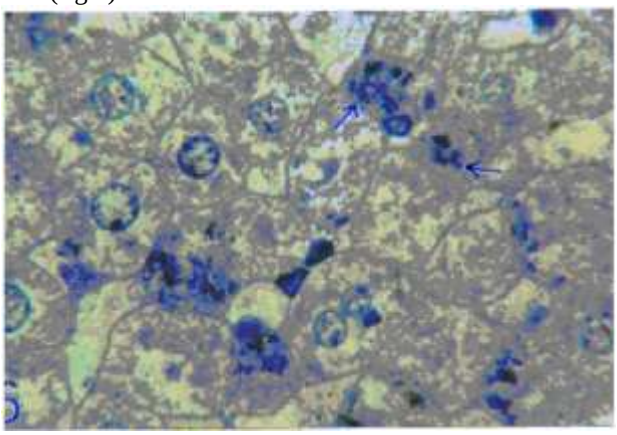

Fig.6:Plastic section 40 µg/kg /b.w. exposed rat liver showing mitotic figures
(↑) ,Toludiune blue.1000X

Parallel to liver , kidney tubules showed signs of degeneration in a dose dependent response.At low dose (i.e.20 µg/kg) no clear effect was seen except appearance of few apoptotic cell in the lumen of kidney tubules and little degeneration of some tubules in comparison with control (fig7 , 8), while at higher doses (i.e.40 µg/kg and 80 µg/kg) clear degeneration of epithelial cells were detected (fig. 9,10 respectively) .The mode of cell death was programmed cell death or apoptosis with condensed or fragmented nuclei (fig.11, 12,13, 14).In 80 µg/kg dose , some necrotic cells have been appeared in the lining of

the tubules (fig.14). There is no need for phagocyting died cells by neighboring macrophages as occurred in other tissues [6] ,because the died cells can detached from the basement membrane into the lumen of the tubules (fig 11).The later phenomenon has been recorded in kidney tubules of an ischaemic acute renal failure in rats [27].In the 80 µg/kg dose , dilated tubules were seen in the medulla (fig.15 & 16).

A future long term exposure to aflatoxin is recommended for detecting more toxicological information about the effect of this mycotoxin on these two important and other organs.

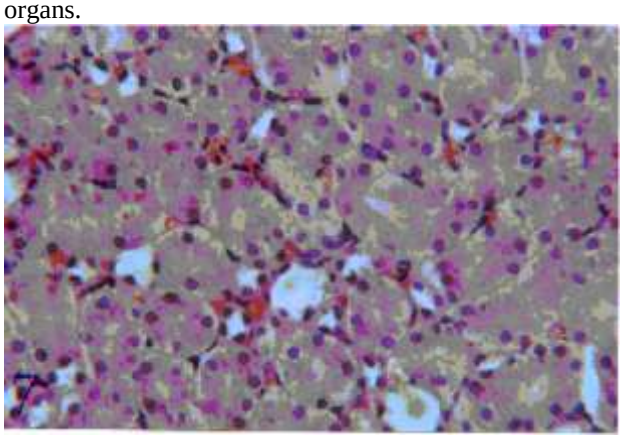

Fig.7:Kidney tubules in control animal 400X

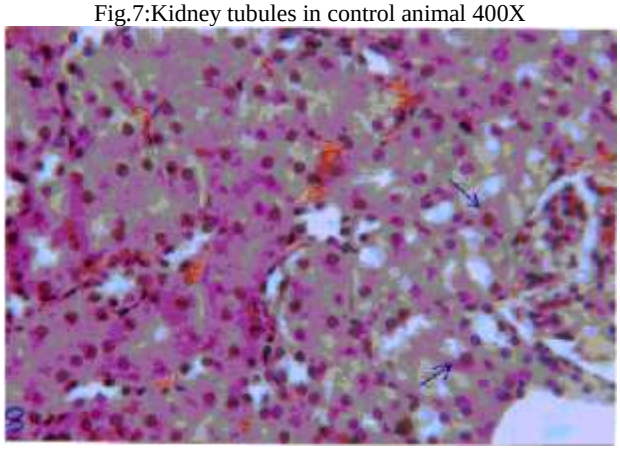

Fig.8 : Kidney tubules in 20 µg/kg /b.w. exposed rat showing few apoptotic cells
with little degeneration of the tubules (↑).400X

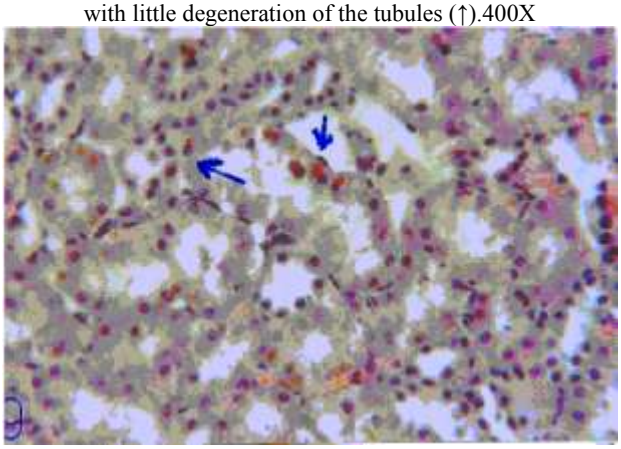

Fig.9 Degenerated kidney tubules in the cortex of 40 µg/kg /b.w. exposed rat, 400X

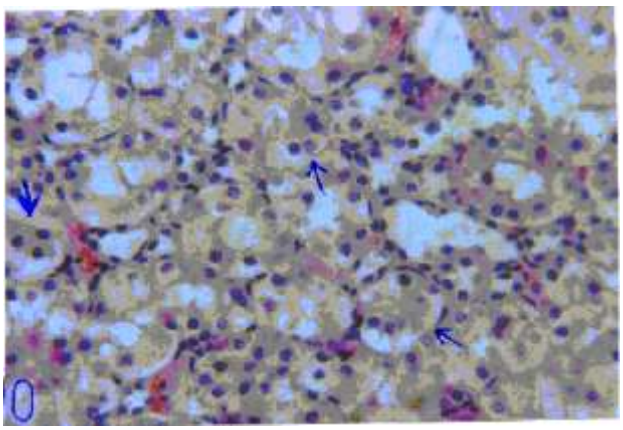

Fig 10 Degenerated kidney tubules in the cortex of 80 µg/kg /b.w. exposed rat, 400X

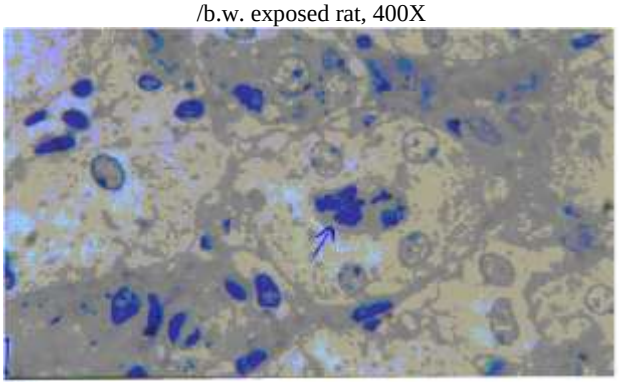

Fig 11:Plastic section through kidney tubules of Degenerated kidney tubules in the cortex of 40 µg/kg /b.w. exposed rat ,Notice the fragmented nucleus of apoptotic cell detached from the basement membrane of the tubule (↑) 1000X

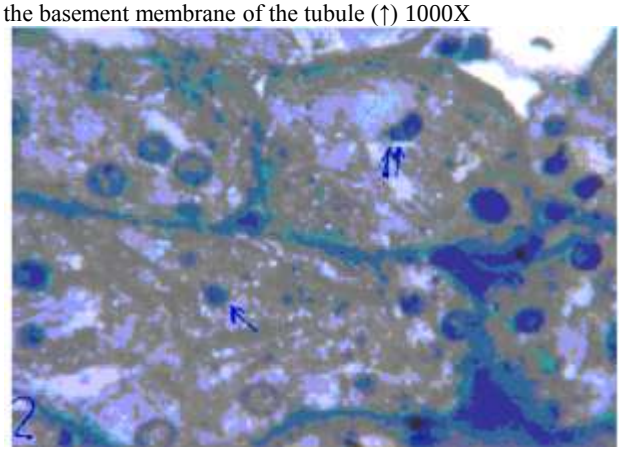

Fig 12Plastic section through kidney tubules of Degenerated kidney tubules inthe cortex of 80 µg/kg /b.w. exposed rat ,Notice the half moon nucleus ofapoptotic cell (↑↑) 1000X

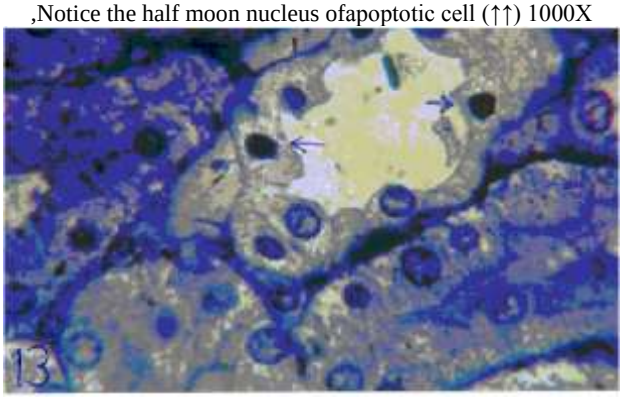

Fig13 Plastic section through kidney tubules of Degenerated kidney tubules inthe cortex of 60 µg/kg /b.w. exposed rat ,Notice apoptotic cells (↑) 1000X

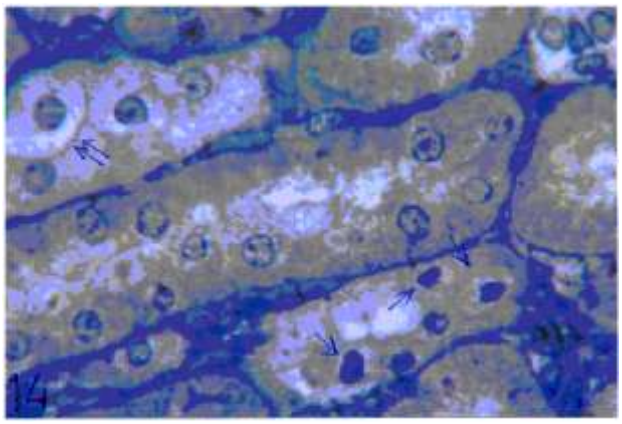

Fig 14: Plastic section through kidney tubules of Degenerated kidney tubules inthe cortex of 80 µg/kg /b.w. exposed rat ,Notice apoptotic (↑) andnecrotic(↑↑)cells 1000X

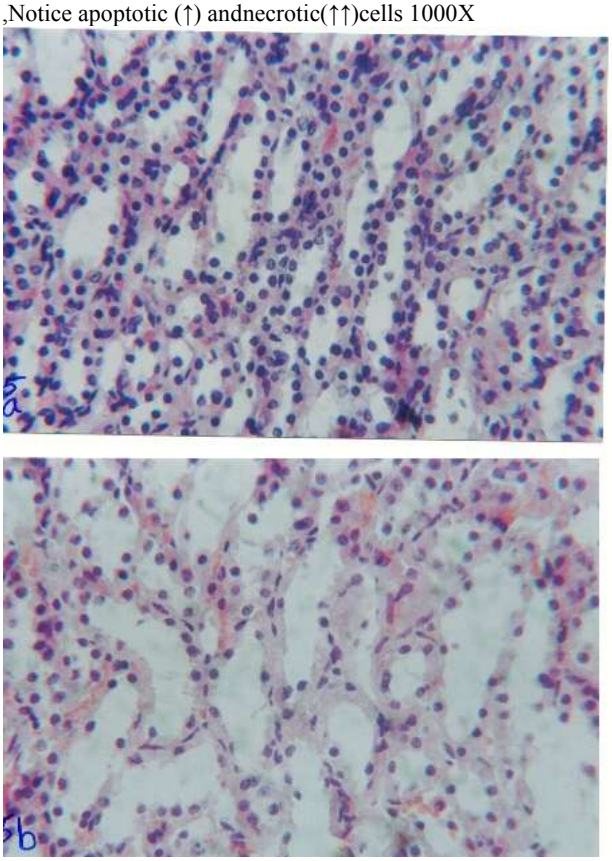

Fig.15:Tubular dilation in the medulla :a)In 40 µg/kg /b.w , b)In 80 µg/kg /b.w400X

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دراسة نسيجية لتأثير الافلاتوكسين على كبد وكلية إناث الجرذان البيض

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الملخص:

تم إعطاء إناث الجرذان البيض جرعات عن طريق الفم (٤٠، ٢٠، ٨٠ ملغم /كغم من وزن الجسم) من مادة الافلاتوكسن لمدة ستة اسابيع متتالية. لوحظ كبت واضح لوزن الجسم ولاسيما في الجرع العالية. وقد سببت الجرع الواطئة زيادة في أعداد الخلايا الكبدية الميتة غير أن الجرع العالية ولاسيما ٨٠ ملغم /كغم من وزن الجسم سببت تنخر الخلايا مصحوباً بترشحات لمفاوية. ولوحظت تغييرات نسيجية وحسب الجرع في الخلايا الطلائية المبطنة للنبيبات الكلوية.ان طريقة الموت الرئيسية للخلايا هي الموت الخلوي المبرمج والمتصفة بانكماش الخلايا وتكثف النواة أو تقطعها وفرط الايوسين للسايتوبلازم مع عدم حدوث التهاب.