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Effects of Ochratoxin a Contaminated Arabian Coffee Seeds on Liver and Kidney Functions and Structure in Mice: Protective Role of Roasting and Vitamin C

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Abstract: Ochratoxin A (OTA), a mycotoxin mainly produced by *Aspergillus* species, was found to contaminate 43 samples of Arabian coffee seeds collected randomly from different markets in Saudi Arabia. The results showed that the average of contamination ranged between 0.73-34.08 µg kg^{-1}. Roasting the highly contaminated sample for 10 minutes at 100°C significantly reduced toxin contamination by about 46.6%. Coffee drink was prepared from the highly contaminated sample (1.4 mg coffee powder/1 ml saline) in which OTA equaled 2 µg ml^{-1}. 50 ml from this sample was given daily in drinking bottles to 60 adult male mice (weight 15-20 gm) for 4, 6 and 8 weeks. Another group of mice was given a similar amount of uncontaminated roasted coffee drink. Half of the first group was given Vitamin C in a dose of 200 mg kg^{-1}. A control group for each period of the experiment was included. After 8 weeks, biochemical analyses revealed alteration of liver and kidney functions. Uric acid was significantly increased in OTA contaminated group. Serum creatinine, on the other hand, showed no change (serum level<0.5 mg dl^{-1}). Albumin was significantly decreased (11. 38%) while liver enzymes SGOT and SGPT were considerably increased (37.11 and 44.33%) respectively. Histopathological studies supported the above biochemical changes. Minimal changes were noticed after 4 weeks in the form of slight congestion in liver and kidney. At the 6th week, the liver showed marked dilation of central and portal veins, bile duct proliferation and binucleated hepatocytes. A significant finding was the appearance of a large sized nuclei (Megakayrocytes) while some had an acidophilic intra-nuclear inclusions; which were previously reported to be associated with cellular metabolic changes or toxicity. At the 8th week, the hepatocyte vaculation, patchy cellular degeneration and signs of cell apoptosis were observed in some specimens. Kidney tubules showed increased acidophilia and basal striation to face toxicity. Moreover, cellular vaculation and loss of brush border were observed. Some samples showed patches of massive degeneration resembling infarction. Administration of Vitamin C produced marked improvement of biochemical parameters and amelioration of histological changes. Also, carcinogenicity described in literature was not observed. Recent publications proved some chemo protective effects of some coffee constituents such as Kahweol and Cafestol.

Key words: Rostingcoffee% Vitamin C % Mycotoxin % Contaminated coffee % Ocratoxin A % Arabic coffee

INTRODUCTION

OchratoxinA (OTA) is one of the most toxic secondary metabolites produced by a number of *Aspergillus* and *penicillium* species [1, 2]. It is found in a wide range of food stuffs such as cereals and grains, pulses [3] and spices [4]. Coffee seeds are liable to mould contamination especially, if not dried to safe moisture level or if rehydrated during any stage of drying, packing and transportation [5]. Exposure to Ochratoxin A through consumption of contaminated food staff represents a risk of toxicity.

Ochratoxin A was reported to be nephrotoxic [6], heptotoxic, carcinogenic [7], genotoxic [8] and immunotoxic [9] in rats and possibly human. Arabian coffee (*Coffea arabica* L.) is the popular drink in Saudi Arabia. It is widely used as lightly roasted seeds and often marketed as exposed products to air and high humidity, giving a chance for mould or fungal growth and contamination.

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The present study was designed to study the effect of Ochratoxin naturally contaminated Arabian coffee drink on liver, kidney function and structure, as well as the possible protective role of roasting and vitamin (C) administration.

MATERIALS AND METHODS

Materials

Collection of Samples and Mycotoxin Analysis: 43 samples of green coffee beans were collected randomly from different markets in Jeddah, Saudi Arabia during 2006-2007. They were stored at 3-5°C to avoid any toxins formation or microbial contamination before analysis. Ochratoxin A was extracted from each sample by blending Ochratoxin A content of extract which was determined by HPLC after the method of [10]. Standards Ochratoxin A purchased from Sigma Chemical Company (P. O. Box 4508, St. Louis, USA) were used according to AOAC methods [10, 4].

Preparation of Coffee Drink and Vitamin C

a. Coffee drink was prepared from sample proved to contain high percentage of Ochratoxin (34.08 Ug kg^{-1}) was powdered and added (1.4 mg)/1 ml boiled water and about 50 ml was added to drinking bottles [11].
b. Non contaminated coffee drink prepared from samples proved to have no Ochratoxin and prepared as above and added to drinking bottles.
c. Vitamin C (Ascorbic acid) was prepared in a dose of 200 mg kg^{-1} according to [12].

Animals: 30 adult male mice (MFI. Strain), about 2-3 months old and 18-20 in weight, were obtaind from King Fahd Research Center (KFRC) at Kind Abdul Aziz University, Jeddah, transferred to experimental room, then kept for one week for acclimatization (temperature 18-22°C) and light (Fluorescent) and red lights (12:12 h) according to modified method of Al-Hazmi [13]. The animals were divided in to 4 groups. (n=6 animals) as following:

G1: Control group giving normal saline via drinking bottles.
G2: Ochratoxin contaminated coffee drink.
G3: Ochratoxin free coffee drink made from non contaminated samples.
G4: Ochratoxin contaminated coffee drink plus vitamin C (200 mg kg^{-1}).

Table 1: Percentage of Ochratoxin contaminated coffee seeds collected from local markets using HPLC

Sample number	Percentage of OTA contamination (µg kg^{-1})	Sample number	Percentage of OTA contamination (µg kg^{-1})
1	7.32	23	0.00*
2	1.83	24	5.42
3	2.56	25	22.00
4	0.915	26	25.97
5	2.20	27	4.01
6	0.915	28	4.21
7	2.01	29	3.77
8	9.15	30	13.52
9	2.56	31	0.00
10	3.66	32	3.77
11	1.45	33	0.91
12	34.08**	34	1.83
13	2.59	35	2.01
14	1.95	36	2.20
15	0.0	37	3.66
16	0.0	38	4.01
17	0.73*	39	1.94
18	0.73*	40	5.42
19	21.25	41	9.86
20	1.19	42	0.00
21	0.0	43	0.00
22	0.0		

High percentage contamination 34.08**. Least percentage contamination 0.73*. Average percentage contamination 6.046. SD 7.919. ** Sample used as OTA contaminated coffee seeds. * Sample used as uncontaminated coffee seeds

Methods: At intervals 4, 6 and 8 weeks respectively, blood was collected via Jugular veins, centrifuged and plasma was immediately sent for biochemical assay (creatinine, uric acid for kidney functions), albumin and liver enzymes (SGOT, SGPT and alkaline phosphates for liver functions). Tissue samples from kidney and liver were taken from all groups after saline and 10% neutral buffered formalin (NBF) intra-cardiac perfusion to ensure good tissue fixation. Small pieces of each organ (2×2 m) were refixed in 10% NBF. Processing for 5um thick paraffin section was preformed at King Abdul Aziz University Hospital (KAUH) pathology lab and Stained by Haematoxylin and eosin, PAS (periodic acid Schiff). Then the samples were examined by light microscope and photographed using digital camera.

RESULTS AND DISCUSSION

Mycotoxin contamination is one of the most relevant and worrisome problems concerning food and feed safety. It can produce a variety of toxic acute and chronic effects in human [14] and animals [15, 6].

OTA was reported to contaminate a wide range of food stuffs such as cereals and grains [3], dried food [16]and dried figs [1]. Bokhari proved that Arabian coffee

beans collected from Jeddah region were contaminated by Ochratoxin A [17]. The average of OTA ranged between 0.73-34.08 µg kgG^{1}. In the present study, roasting coffee beans according to Saudi way (100°C for 10 min) was found to reduced OTA level to 46.6% (Table 2). However, Van der Stegen proved that roasting produced isomerization and thermal degradation of the toxin leading to about 69 to 96% reduction in OTA contamination. The difference in the percentage of this reduction might be related to the degree of roasting which is light in Saudi coffee and dark in for example American [15].

Significant biochemical changes in kidney and liver function tests were observed in mice after 8 weeks of experiment (Table 3). Serum uric acid showed significant increase (36.36%) while creatinine levels showed no change (serum level<0.5 mg dlG^{1}). Albumin was significantly decreased (11.38%) while liver enzymes SGOT and SGPT were significantly increased (37.11 and 44.33%) respectively.

Hoehler and Marquardt [18] found that OTA increased plasma concentration of uric acid ($p<0.001$) when OTA was added to the diet of chicks. Gekle and Silbernagle [19] found that OTA in rats caused a reduction of glomerular filtration rate if given in a dose (0.5 mg kgG^{1} for 6 days) which resulted from different arteriolar resistances and a reduction of renal blood flow.

Previous studies reported an alteration of kidney and liver function tests upon exposure to mycotoxins. El-Sawi *et al*. [20] reported an alteration of uric acid, creatinine and liver enzymes in mice exposed to Verrucarian J mycotoxins. An increase in creatinine and blood urea were reported by Edrington *et al.* [21] in lambs and by Bucci *et al*. [22] in rats exposed to fumonisins mycotoxins.

Histological study of both liver and kidney may explain the above biochemical changes compared to control. Drinking OTA contaminated coffee altered liver parenchyma, showed marked individual sensitivity (Plate I, Fig. 1) and also showed the normal structure of liver in control mice. The cells are arranged in cords separated by hepatic sinusoids their nuclei are rounded. The cytoplasm showed basophilic areas (site of protein synthesis). Portal area contained bile duct and portal vessels.

Uncontaminated Coffee drink produced dilation of both bile canliculi and hepatic sinusoids. The cytoplasm is more acidophilic that may point to an increase of mitochondria and smooth endoplasmic reticulum needed for detoxification processes (Plate I, Fig. 2).

Table 2: Effect of roasting Arabia Coffee seeds (100°C for 10 mins) in reducing perecentatge of Ochratoxin (OTA) contamination using HPLC

Sample numbber	OTA (µg kgG^{1}) Before roasting	After roasting	Reduction percentage (%)
1	44.00	17.40	60.5
2	21.90	15.20	30.6
3	154.00	71.20	53.7
4	46.60	27.33	41.4
Average	66.60	32.80	46.4 ($p<0.01$)

Table 3: Effect of Arabian coffee drink (1.4 mg kgG^{1}) and Ochratoxin contaminated coffee (2 mg kgG^{1}) on some biochemical measurement in rat blood after 8 weeks of administration

Animal groups	ALB (IU/L)	SGOT (IU/L)	SGPT (IU/L)	CREA. (mg dlG^{1})	Uric Acid (mg dlG^{1})
Control (G1)					
Mean±S.E	325±34.6	194±21.08	57±3.46	p<0.50	2.64±0.1
Uncontaminated coff. (G2)					
Mean ±S.E	298±46.2	199±34.1	60±2.6		2.78±0.14
Change (%)	8.31%	2.58%	3.26%		5.3%
Significant	P>0.05	P>0.05	P<0.05	P<0.5	p>0.05
Contaminated coff. (G3)					
Mean±S.E	288±23.3	280.34.1	64±1.7		3.6±0.15
Change (%)	11.38%	44.33%	12.28%		36.36%
Significant	P<0.05	P<0.05	P<0.05	p<0.5	P<0.05
Contaminated coff. + Vit. C (G4)					
Mean±S.E	310±34.5	187±13.9	52±2.89		3.01±0.20
Change (%)	-4.62%	-3.61%	-8.77%		14.02
Significant	p>0.05	p>0.05	p>0.05	P<0.5	P<0.05

Plate I

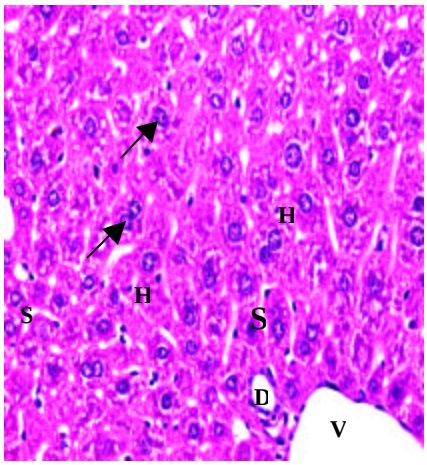

Fig. 1 Control

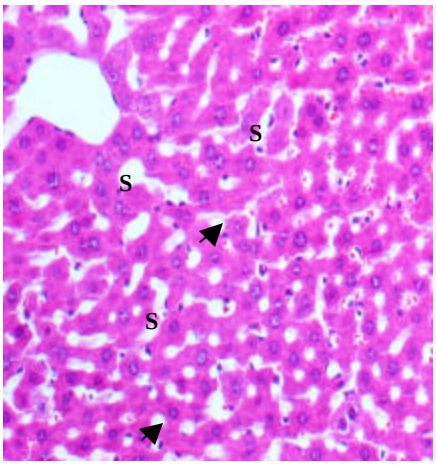

Fig. 2 Uncontaminated coffee

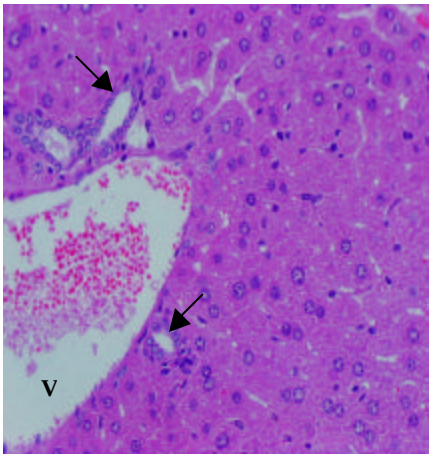

Fig. 3 OTA contaminated coffee (6 weeks)

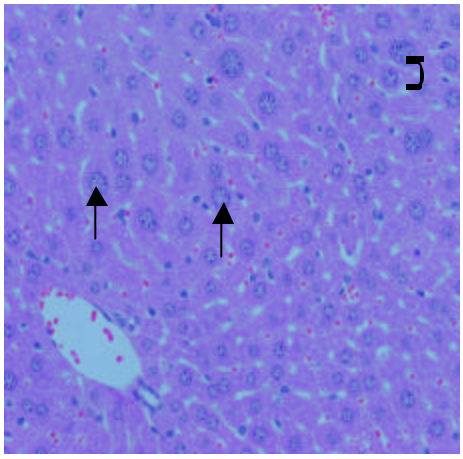

Fig. 4 OTA contaminated coffee (6 weeks)

Plate I

Fig. 1: Light micrograph of mice control liver showing one of the portal areas containing a branch of portal vein (V) and a bile duct (D). Hepatocytes cell cords (H) are separated by regular sinusoids (S). The cytoplasm of the cells are acidophilic with basophilic areas represent protein synthesizing regions (r E R) . The nuclei are rounded and vesicular (↑). (H & E X 400)

Fig. 2: Light photomicrograph of mice drinking uncontaminated coffee, notice the dilated bile canaliculi (↑) and blood sinusoids (S). The cytoplasm is highly acidophilic representing an increase in SER for detoxification (H & E X 400)

Fig. 3: Photomicrograph of mice liver after 6 weeks of drinking OTA contaminated coffee showing dilation of central vein (V) and proliferation of bile ducts (D) (↑). (H & E X 400).

Fig. 4: Light micrograph of mice liver after 6 weeks of drinking OTA contaminated coffee showing large sized nuclei (Megakaryocytes) (↑). Some contain acidophilic intra-nuclear inclusions (⇑). (H & E X 400)

Plate II

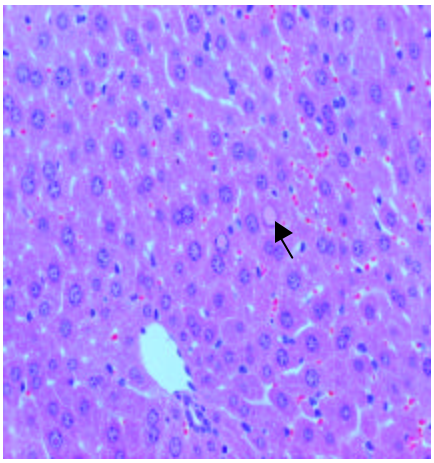

Fig. 1 OTA contaminated coffee (6 weeks)

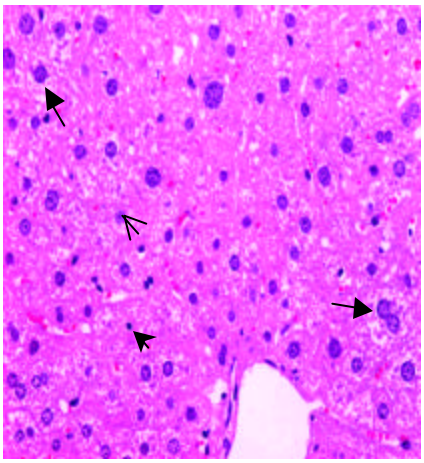

Fig. 2 OTA contaminated coffee (8 weeks)

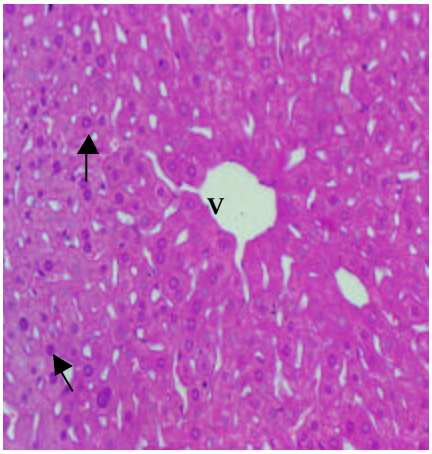

Fig. 3 OTA contaminated coffee (8 weeks)

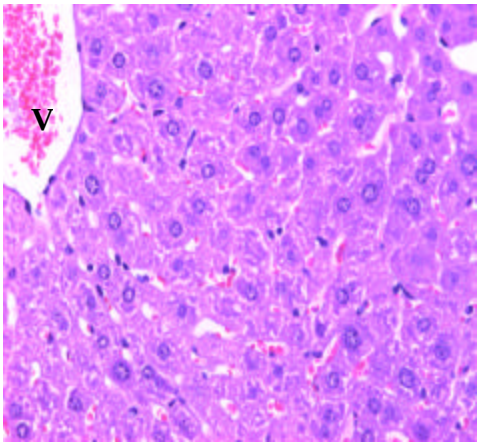

Fig. 4 OTA contaminated coffee with vitamin C

Plate ?

Fig. 1: Light micrograph of mice liver after 6 weeks of OTA contaminated coffee drink showing nuclei with large acidophilic intra-nuclear inclusions (↑). (H & E X 400)

Fig. 2: Light micrograph of mice liver after 8 weeks of drinking O T A contaminated coffee showing hepatocyte vaculation. The nuclei are small (1) , some underwent karolysis (↑). (H & E X 400)

Fig. 3: Part of mice liver after 8 weeks of O T A contaminated coffee drink showing signs of apoptosis in the form of acidophilic cytoplasm and small dark nuclei (↑). (H & E X 400)

Fig. 4: Light micrograph of mice liver receiving vitamin C. with O T A contaminated coffee drink showing nearly normal parenchyma. (H & E X 400)

Plate III

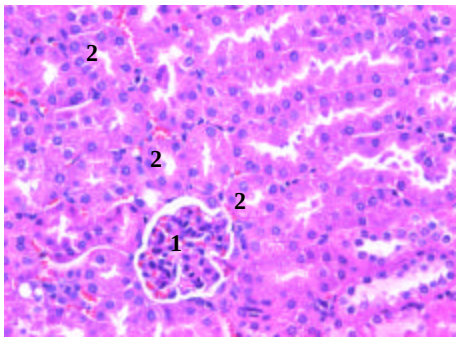

Fig. 1 Control

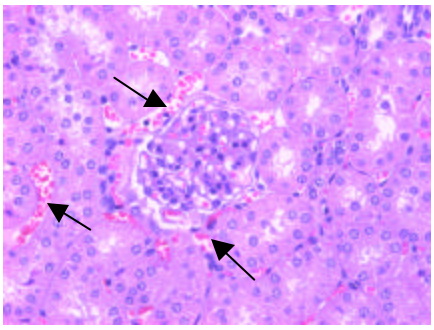

Fig. 2 G2

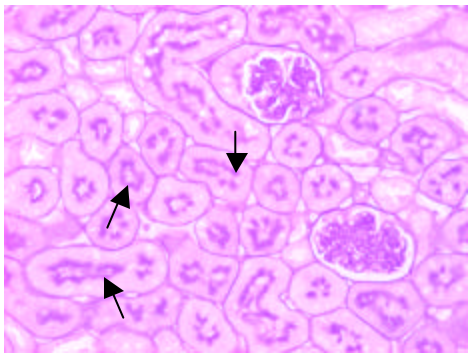

Fig. 3 Control

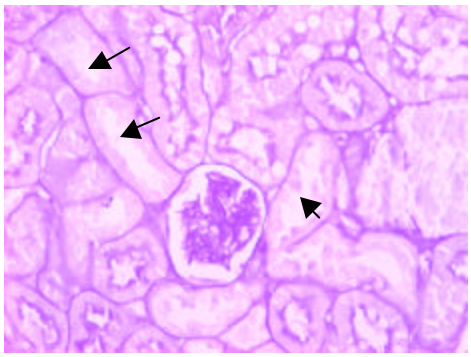

Fig. 4 G3 (8 weeks)

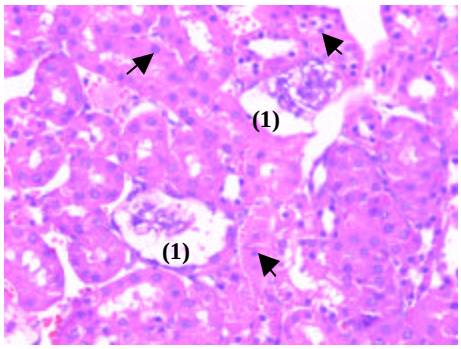

Fig. 5 G3 (6 weeks)

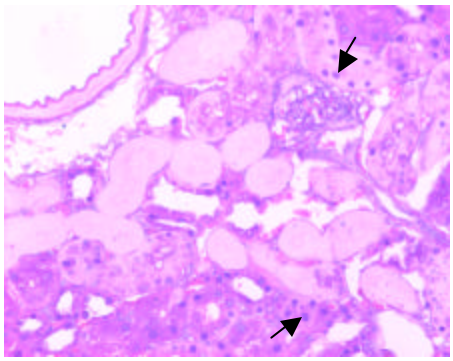

Fig. 6 G3 (8 weeks)

Plate III

Fig. 1: Light photomicrograph of control mice kidney showing a renal corpuscle (Bowman capsule and glomerules) (1) and various kidney tubules (2). (H & E X 400)

Fig. 2: Light photomicrograph of mice kidney after 6 weeks of drinking O T A uncontaminated coffee showing vascular congestion (↑) and tubular enlargement (v). (H & E X 400)

Fig. 3: Light micrograph of control kidney stained by PAS showing well defined brush border of tubular cells (↑). (H & E X 400)

Fig. 4: Light micrograph of mice kidney after 6 weeks of O T A contaminated coffee drink showing loss of brush border of most renal tubules (↑). (H & E X 400)

Fig. 5: Part of mice kidney after 6 weeks of O T A intoxication showing focal atrophy of glomeruli (1) and signs of apoptosis in the form of dark small nuclei (↑) in tubular cells. (H & E X 400)

Fig. 6: Part of mice kidney after 8 weeks of O T A intoxication showing sever focal hyaline degeneration of kidney tubules (structure less and stained acidophilic) (v). The rest of tubules showing dark small nuclei (↑) and acidophilic cytoplasm which may be signs of apoptosis. (H & E X 400)

Minimal changes were observed after 4 weeks of drinking OTA contaminated coffee, in the form of slight dilation and congestion of hepatic vessels and increase in hepatocyte acidophilia. These changes pointed to a response of hepatocytes to OTA toxicity with possible increase in smooth endoplasmic reticulum and mitochondria, the organelles known to be associated with detoxification processes and results in cytoplasmic acidophilia.

After 6 weeks, vascular dilation and bile duct proliferation were observed (Plate I, Fig. 3). Similar results were reported by Rajendran *et al*. [23] in cattle and by Aydin *et al*. [6] in rat. Binucleated hepatocytes or cells with large sized nuclei (Karyomegaly) were frequent (Plate I, Fig. 4). Nuclei with acidophilic intra-nuclear inclusions were also seen. The inclusions were of various sizes, some were so large to occupy the whole nucleus (Plate II, Fig. 1). Karyomegaly was described by Sponendlin and co-workers in rat liver and kidney when injected with 0.8 µg OTA for 90 days and was explained by accumulation of genetic material, a sign that preceded cancerous transformation [24, 25]. Intra-nuclear inclusions observed in the present results have been described within nuclei associated with aging [26], virus infection, alteration in cellular metabolism and exposure to toxic drugs [27, 28]. The high incidence of such inclusions in association with toxic pollution requires further study on its nature and mechanism of formation.

The present study showed that cumulative effects of the toxin produced more parenchymatous toxicity in both liver and kidney. Individual variation in the severity of changes was also observed due to variation in genosenstivity [29].

After 8 weeks of OTA contaminated coffee drink liver changes were more severe. Vacuolar degeneration of heptocytes and nuclear changes were observed (Plate II, Fig. 2). Apoptotic changes in the form of increased cytoplasmic acidophilia and dark small nuclei were observed in some hepatic lobules (Plate II, Fig. 3). Apoptosis after OTA was also reported by Atroshi *et al*. [30] in the liver of mice. He attributed the changes to oxidative damage of DNA or reduced antioxidant enzymes by the liver. Plate III showed the histological structure of kidney in both control and experimental groups (Fig. 1-6).

Compared to control kidney (Fig. 1 and 5), vascular congestion and slight increase in tubular and nuclear sizes were observed in animals drinking coffee (uncontaminated) (Fig. 2). After 6 weeks, focal atrophy of glomeruli, loss of tubular cell brush borders (Fig. 6) and apoptotic change were observed in some tubules (Fig. 3). More affected samples (8 weeks) showed patches of massive hyaline degeneration. The tubules were structure-less and homogenously stained with eosin (Fig. 4). Hepato-nephrotoxic effects of OTA were reported by many workers [6]. Renal tumors were reported by Bendele *et al*. [31].

Based on the current literature evidence, the mechanisms involved in OTA toxicity may be related to oxidative stress and enhanced lipid peroxidation [7]. Marked alteration in antioxidant enzyme were observed in both human and animals exposed to mycotoxins [32]. Vitamin C is well known for its antioxidant activity [33]. It was found in the present study to produce significant improvement of biochemical parameters and histological changes in both liver and kidney (Table 3). Hoelar and Marquardt [18] found that OTA toxicity can be partially counteracted by vitamin E not by vitamin C. Atroshi *et al*. [30] proved that vitamin C in combination with other antioxidants such as vitamin E, Zn, Mg and selenium, can exert some inhibition of apoptosis caused by OTA in mice liver. Signs of neoplastic changes described by other authors [31, 34] were found in the present study to be minimal except of infrequent appearance of karyomegally and nuclear inclusions in hepatocytes. This may be due to possible chemopreventive components of coffee such as Kahoweol and Cafestol, which were proved by many authors to have anti-mutagenic and anti-carcinogenic properties. Cavin *et al*. [35] suggested that Cafestol and Kahweol components of coffee have a chemoprotective activity against aflatoxin B induced gentoxicity in both rats and humans. Huber *et al*. [36] found that coffee components such as Kahweol and Cafestol (K/C) have a protective effect on mutagenic damage caused by aflatoxin B in rat and human and associated with a lower rate of colon tumors. The mechanism is through enhancement of hepatic levels of anti-oxidant enzymes, glutathione and glutathione S-transferase (GST). Further studies on the effect of coffee and their components in reduction of mycotoxicity in general and OTA in particular will be needed.

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