البحث السابع

Volume 29 Number 3 2001

ISSN. 1110 - 1458

Zagazig University
Faculty Of Vet. Medicine

Zagazig Vet. Journal

Zagazig Veterinary Journal

Zag. Vet. J. (ISSN. 1110-1458) Vol. 29, No. 3, (2001) pp. 1-10

Ochratoxin A In Blood Of Renal Failure Patients And Some Renal Medicaments

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ABSTRACT

In continuing the effort to provide further evidence for the hypothesis that ochratoxin A (OTA) might be involved in the aetiology of renal affections, a survey to determine the occurrence of OTA in renal medicaments and blood of renal failure patients was conducted. 20 samples of 5 types of renal medicaments of herbal origin were analyzed for their OTA content, also 30 serum samples from renal failure patients under dialysis of both sexes , different ages and at different seasons. The samples of renal failure patients were compared to apparently healthy donors. Our results indicated that the examined renal medicaments contain different concentrations of OTA, also , there was a significant differences in OTA content of renal failure patients and healthy donors. A differences in OTA content in renal failure patients in relation to sex and age were observed. A significant difference in OTA content of renal failure patients with changes the season of sample collection were also noticed . From our results we can conclude that the presence of OTA in examined renal medicaments may play a role in renal pathology.

INTRODUCTION

Humans are undoubtedly exposed to ochratoxin A (OTA) through different sources as foods of plant and animal origin, herbs and airborne dust. The presence of OTA in human blood has been suggested as an indicator for indirect assessment of exposure to this nephrotoxic agent. In several countries, therefore, human blood has been collected for the purpose of obtaining more information on the intake of OTA, where in European countries, the frequency of contamination of human sera seems to indicate continuous, widespread exposure of humans to OTA (*1*).

Forty-nine powdered herbal drugs were analyzed for their mold profile and for the potential presence of aflatoxin, sterigmatocystin and OTA. *Aspergillus* and *penicillium* species were predominant, but *Rhizopus, Muor, Cladosporium* and *Aurcobasidium* were found in a few samples. Mycotoxins were not detected in any samples, only one isolated culture was found to be a mycotoxin producer on laboratory media *(2)*.

OTA was found in one of 7 medical plant samples contaminated with *A.flavus* and analyzed for OTA *(3)*.

Ochratoxin A (OTA) is one of the frequent sources of mycotoxin contamination of medical plants in Egypt. It is produced by *Aspergillus and Penicillium* species which considered widely spread mould species. Nine types of medicinal plants were analysed and all of them were contained OTA (30.96-212.68 ng/ml) *(4)*.

OTA was found to be produced by increasing the time of storage of the herbal drugs and in a great quantity than in semi-synthetic media *(5)*.

Many investigators determined OTA in human serum samples collected from South Italy where they found that the mean and median concentrations of OTA in healthy group were 0.53 and 0.44 ng/ml serum, respectively, while the highest mean concentration was found in the group of patients treated from renal failure by dialysis (1.4 ng/ml serum)*(6)* . Similarly, OTA was detected in sera of 368 blood donors with a range of 0.01-5 ng/ml *(7)*. Also, in Sweden, some authors *(8)* examined 39 blood samples from healthy mothers and all blood samples contained OTA in a mean concentration of 167 ng/ml (range 90-940 ng/ml). While in Hungary *(9)*, it was reported that 52 % of randomly collected healthy human blood samples were found to contain OTA (0.2-12.9 ng/ml).

Mysterious deaths of archeologists after opening Egyptian tombs have been suspected, but never proved to be secondary to inhalation of mycotoxins . Some authors *(10)* observed a case of acute renal failure due to inhalation of OTA after working 8 h in a granary closed for several months, a farmer and his wife suffered

respiratory distress, the woman developed non-oliguric acute renal failure and biopsy revealed tubulonecrosis. A strain of *A.ochraceous* producing OTA was isolated from wheat.

To clarify the role of OTA in chronic interstitial nephritis of unknown aetiology *(11)*, food and blood samples from nephropathy patients and controls were collected. The OTA assays showed very different scales of OTA food and blood contamination from 0.1-16.6 µg/kg and 0.1-2.3 ng/ml, respectively in controls and healthy individuals and 0.3-46830 µg/kg for food and 0.7-1136 ng/ml for blood in nephropathic patients.

In 1997, some authors *(12)* found that the incidence and the OTA concentration in blood in hyperendemic village in Croatia was 4.5% (range 2-50 ng/ml) while in control village (where no clinical cases of nephropathy had been found) up to 2.4 % range 2-10 ng/ml. Similarly in Czech Republic, the mean concentration of (0.63 µg OTA/L) blood donors was recorded *(13)*.

Healthy blood donors from the City of Zagreb were checked for the presence of a nephrotoxic mycotoxin OTA, where, the frequency of OTA-positive samples (70.2 ng/ml) of plasma showed seasons variation. In high frequency of positive samples coincided with seasons favoring growth of moulds and production of toxins *(15)*, while in Croatia, the mean concentration of OTA in plasma of general population was 0.39 ng/ml (*16*).

In Italy, OTA was detected in 97% of examined healthy adults with a mean of 0.56 ng / ml ranging between 0.12 and 2.84 ng/ml with exclusion of one sample of 57.2 ng / ml. OTA levels were significantly higher in men than in women and a strong association was found with the season where the values were higher in summer than autumn (*16*). While in Canada, the mean concentration of OTA in healthy volunteers was 0.88 ng/ml with a range of 0.29-2.37 ng/ml (*17*).

OTA was measured in serum samples from healthy individuals and individual suffering from different urinary disorders in Isparta-Turkey by using an analytical method based on the measurement of fluoresence spectra. The mean concentration of OTA in the healthy group was 0.4 ± 0.28 ng/ml and the highest mean concentration was found in the group of patients treated by hemodialysis 2.1 ± 1.2 ng/ml *(18)*.

Similar results were recorded in Tunisia *(19)*, where the authors found that the range of contamination is 0.7–7.8 ng/ml for general population and 12–55 ng/ml for peoples suffering from chronic renal failure. Also, they noticed that 21– 64 % of peoples suffering from nephropathy are OTA positive with a detection limit of 1 ng/ml.

Therefore, the present work aimed to detect the presence of OTA in the renal medicaments from herbal origin and serum of renal failure patients to explore if there is a relationship between contamination of herbs, medicaments and occurrence of renal failure in humans.

MATERIALS AND METHODS

Sampling

a) Medicaments

20 samples of 5 types of medicaments were used for treatment of renal colic, urinary tract infections and renal stones, were randomely collected from different pharmacies. All this medicaments contain the extract of *Cymobopgon proximus* and *Ammi visnaga* (medicinial plants), these medicaments prodeuced by 4 different companies. The medicaments were : Proximol tablets, Proximol effervescent granules, Coliurinal, Sekem renal herbs and Kellagon.

b) Blood samples

30 human blood samples were collected from nephropathy patients under treatment by dialysis (especially those categorised as having a chronic interstitial nephropathy of unknown aetiology); 15 samples male and 15 samples

females ageing from 10 to 70 years. The samples were collected in two seasons : dry season (May–August) and wet season (November-February). Also, six blood samples were collected from apparently healthy volunteers of both sexes.

All samples were undergo a quantitative determination of OTA by using Veratox ® quantitative OTA ELISA Kits, Neogen TM Corporation using automatic ELISA reader which was kindly available from Dept. of Zoology, Fac.of Science, Zagazig University, Benha Branch. The concentration of OTA were determined using standard calibration curve .

Statistical analysis of data were carried out *(20)*.

RESULTS

Results of this investigation are tabulated in Tables (1-4). Table (1) shows the concentration of OTA (ppb) in some renal medicaments, while Table (2) shows the concentration of OTA (ppb) in serum of renal failure patients and apparently health donors. Data in Table (3) represents the values of concentration of OTA (ppb) in serum of renal failure patients in relation to age as well as the percentage of total number of patient, while the data in Table (4) shows the concentration of OTA (ppb) in renal failure patients in relation to seasonal variations.

Table (1): Ochratoxin A concentration (ppb) in some renal medicaments.

Type of medicaments	Range	Mean ± S.E.
Proximol (tablets)	2.50 – 22.00	12.140 ± 6.068
Proximol (effervescent granules)	7.79 – 36.50	24.072 ± 7.346
Coliurinal (effervescent granules)	2.50 – 21.00	15.450 ± 4.35
Sekem renal herbs	0.00 – 19.69	6.048 ± 4.589
Kellagon (capsules)	22.00 –39.00	30.765 ± 3.687

** Highly significant P<0.01

Table (2): Ochratoxin A concentration (ppb) in serum of renal failure patients and apparently health donors.

Case of Patient	Sex	Range	Mean ± S.E.	Total	
				Range	Mean ±S.E.
Renal failure patients	Male	1.5-105.34	32.08 ± 7.72**	1.5-105.34	38.435 ± 5.152 **
	Female	2.5 –76	44.79 ± 6.42 **		
Healthy donors	Male	0.0 –3.0	1.50 ± 0.866	0.0 –3.0	1.416 ± 0.507
	Female	0.0 –2.5	1.33 ± 0.726		

** Highly significant P<0.01

Table (3): Ochratoxin A concentration (ppb) in serum of renal failure patients in relation t age.

Sex		Age (year)					
		10-20	20-30	30-40	40-50	50-60	60-70
Male	Range	21.88	4.34-24.5	18.5 -105.34	18.5	1.5-64.0	2.5-66.0
	Mean±S.E	--	14.42±10.08	66.28±25.44	--	23.33±20.35	28.64±10.33
Female	Range	66.50	23.0-29.0	51.0-75.0	70	2.5-76.0	7.79-73.0
	Mean±S.E	--	26.0±3.0	64.33±7.06	--	43.17±21.58	32.16± 10.92
Percentage to total No.of patients		6.66 %	13.33 %	20.00 %	6.66 %	20.00 %	33.33 %

Table (4): Ochratoxin A concentration (ppb) in renal failure patients in relation to season variations.

Season	Sex	Range	Mean±S.E.	Total
Dry season (May-August)	Male	22-105.34	55.01 ±10.94**	60.04 ± 6.35**
	Female	28.0 –76.0	65.07±5.86**	
Wet season (November-February)	Male	1.5 –24.5	12.03±3.196	19.54 ± 3.82
	Female	2.50- 51.00	27.04 ±5.83	

** Highly significant P<0.01

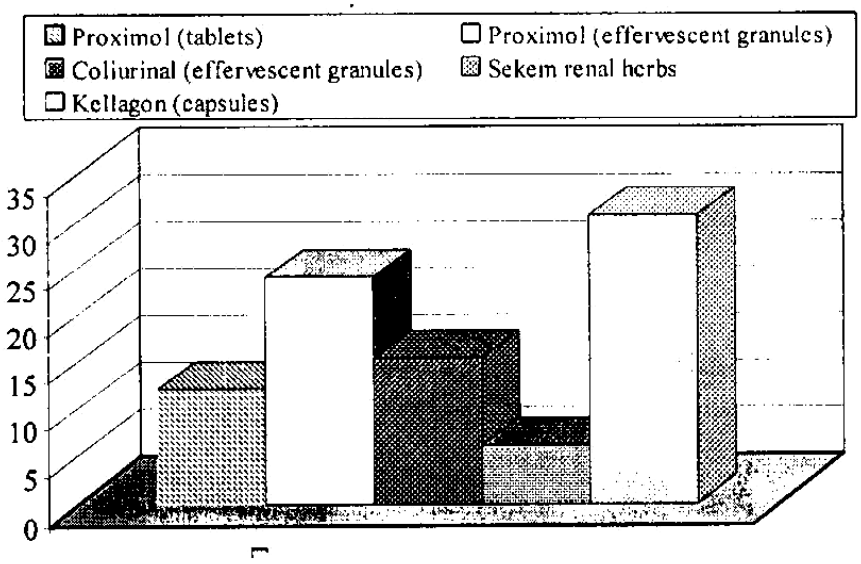

Fig.(1) : Concentration of OTA (ppb) in some renal medicaments

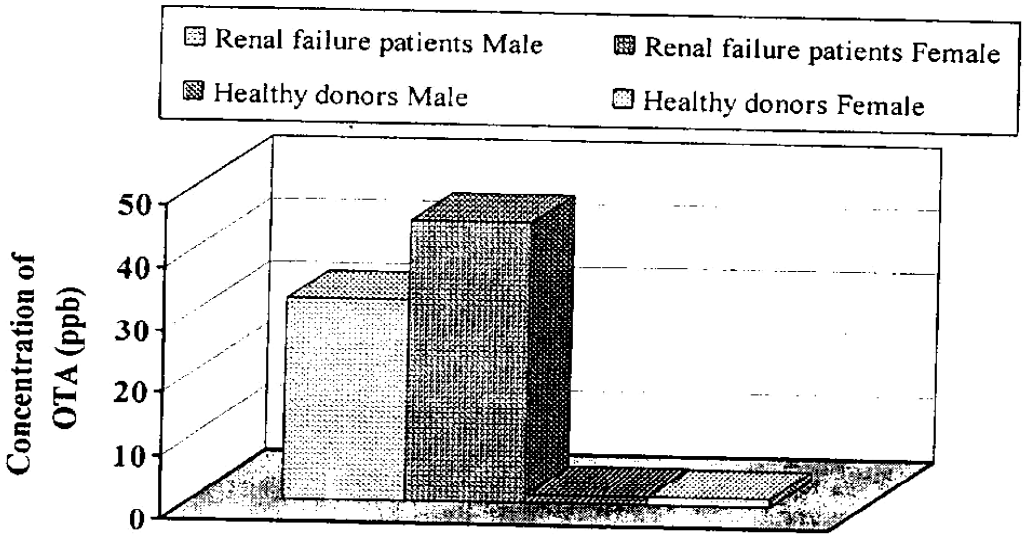

Fig.(2a) Individual concentration of OTA (ppb) in serum of renal failure patients and apparently healthy donors

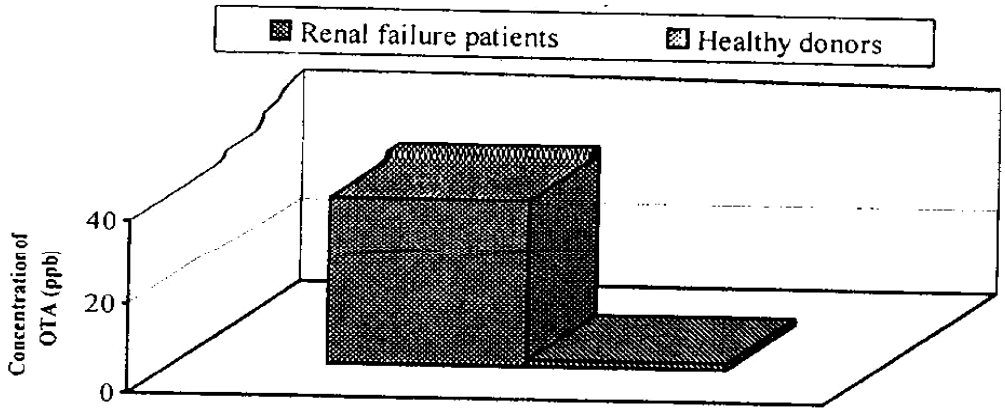

Fig.(2b) Total concentration of OTA (ppb) in serum of renal failure patients and apparently healthy donors

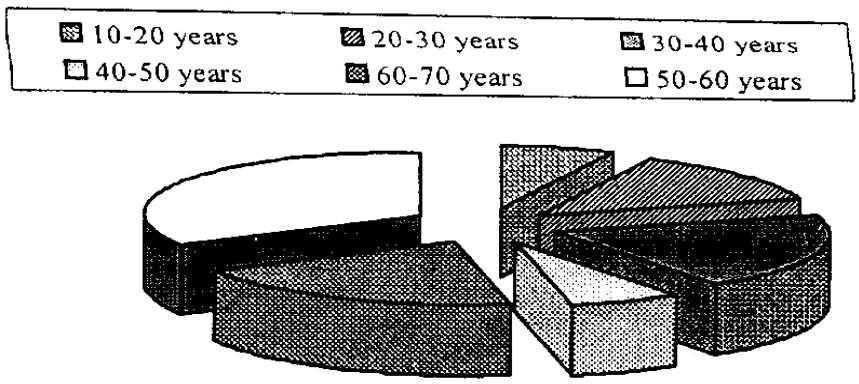

Fig.(3a) Percentage to total number of patients

Fig.(3b): OTA concentration (ppb) in serum renal failure pateints in relation to age

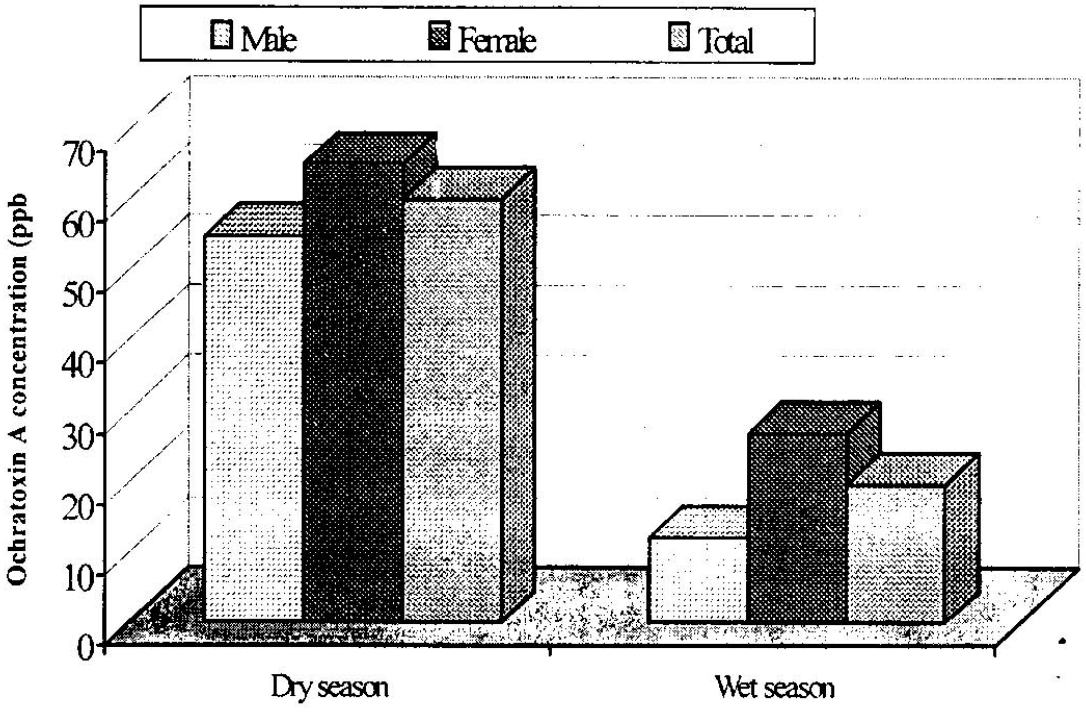

Fig.(4) OTA concentration (ppb) in renal failure patients in relation to seasonal variations

DISCUSSION

OTA is a mycotoxin produced by ubiquitous *Aspergillus* and *pencillium*. It was found allover the world in feed and human foods and blood as well as in animal blood and tissues. The most threatening effect of OTA are its nephrotoxic and carcinogencity and its increasingly involvement in the Balkan endemic nephropathy (BEN), a human chronic interstitial nephropathy which is most of time associated to urinary tract tumor ***(21)***.

The concentration of OTA in some renal medicaments of herbal origin were shown in Table (1) and graphically illustrated in Fig.(1) where the highest mean of concentration were found in Kellagon capsules (30.765± 3.687) which contain the extracts of *Ammi visnaga* and *Cymbopogon proximus*, while the lowest mean of concentrations were found in Sekem renal herbs which contain the extract of *Ammi visnaga* fruits , *Cymbopogon proximus* leaves and *Ambrosia* leaves. Some authors ***(4)*** reported that *Cymbopogon proximous* have 33,31 and 40 colony of A. ochraceous and a mean concentration of OTA 46.57±1.07; 45.35±10.79 and 49.81±0.39 in 3 different localities of Kalubia Governorate, Egypt. Also, *Ambrosia martima* contain colonies of *A.ochraceous* which produce OTA in high concentrations (52.80±13.99 ; 30.96±7.17 and 68.58±12.59). This may explain why this medicaments (which used for treatment of renal troubles) contain the nephrotoxic mycotoxin OTA. How can we imagine that we treat renal patients (renal infections, renal colic and renal stones) with medicaments which have a nephrotoxin which may exacerbate (increase) his sickness!!

Data in Table (2) and Fig. (2 a and b) showed the concentration of OTA in serum of renal failure patients under dialysis and apparently healthy donors, where it can notice that there is a high significant difference between concentration of OTA in serum of renal failure patients and healthy donors (36.435±5.152 and 1.416±0.507, respectively). This is in agreement with the previous reports of many investigators (***6,11,12,18, 19***). A higher level of OTA in dialysis group compared to control could be explained by the reduced glomerular filtration rate of the patients (***18***). Also, from this table it could observe a highly significant differences in male renal failure patients and male healthy donors (32.08±7.72 and 1.5 ±0.866, respectively), and similarly between female renal failure patients and female healthy donors (44.79 ± 6.42 and 1.33 ± 0.726,respectively). The concentration of OTA in serum of female renal failure patients was higher than in male ones (44.79 ±6.42 and 32.08 ±7.72, respectively), this is in contrast with the results reported by another authors (***16, 17***) as they found that there is no association between sex and concentration of OTA in serum of renal failure patients.

Concerning the relationship between the concentration of OTA in serum of renal failure patients and patient's age, data were tabulated in Table (3) and Fig. (3), where it can notice that the higher percentage of patients was present in the age between 60-70 years, followed by patients aged 50-60 and 30-40 years, then patients aged 20-30 and the lowest percentage was located in the age between 10-20 and 40-50 years. Regarding the concentration of OTA in patient's serum, data of Table (3) and Fig.(3b) showed clearly that the highest concentration was present in the age period between 30-40 years in male patients (66.28 ± 25.44), while in female patients, the higher concentration was in patients aged 16 years (66.5%) followed by patients with age belonged to 30-40 years (64.33 ± 7.06). Previous reports ***(22)*** noticed that higher concentrations of OTA were located in the stratum of 30-40 years (males and females) and in the age group over 60 years (females) which agreed with our results in case of male but partially agreed in case of female patients. In case of female patients, our results were in agreement with another author ***(9)*** who observed that the sensitivity to mycotoxin OTA is known to be inversely related to age. No relationship between age

and concentration of OTA in patients serum were recorded (***16, 17***).

The relationship between concentration of OTA in patient's serum and season of sample collection were presented in Table (4) and Fig.(4), where we can notice a highly significant differences between dry (May - August) and wet (November-February) seasons. Dry season have a higher concentration of OTA (60.04 ±6.35) than in wet season (19.54 ±3.82). This was previously reported (***23,8, 16***). High concentrations of OTA in patient's serum coincided with seasons favouring growth of moulds and production of toxins (***14***).

CONCLUSION AND RECOMMENDATIONS

OTA is a widespread mycotoxin, when ingested as a food contaminant, it is very persistent in human beings with a blood half-life of 35 days after a single oral dosage. OTA is neither stored nor deposited in the body, but body distribution may impose serious damage to kidneys (***24***). From our results we can concluded that using contaminated medicinal herbs with OTA in drug manufacturing may produce a contaminated medicaments with OTA. This contaminated medicaments which used for treatment of renal affections may itself produce renal affections due to their OTA content. Also, from our data, OTA may have a role in the human urinary pathology, The medical plant materials, if stored improperly allowing mould growth, so it should be analyzed for mould and mycotoxin prior to use. It would appear that OTA presents a true potential hazard for humans as its occurrence is widespread especially if taken as a medicaments.

REFERENCES

1) ***Hald,B. (1991):*** Ocratoxin A in human blood in European Countries. IARC Sci. Publ.,(115):159-164.

2) ***Hitokoto, H.; Morozumi, S.; Wauke, T.; Sakai, S. and Kurata, H.(1978) :*** Fungal contamination and mycotoxin detection of powdered herbal drugs. Appl. Environ. Microbiol. 36(2): 252-256 .

3) ***Halt, M. (1998) :*** Moulds and mycotoxins in herb tea and medicinal plants . Eur. J. Epidemiol. 14 (3) 269-274 .

4) ***Bakry, H.H.; Abou salem, M.E. and Elham A. El-Shewy (1999):*** Gamma radiation as a control of mycotoxins and their producing fungi in medicinal plants. Beni-Suef Vet. Med. J. 9 (3b) : 599-620.

5) ***Efuntoye, MO.(1999):*** Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. Mycopathologia 147(1) : 43-48 .

6) ***Breitholtz-Emanuelsson, A.; Minervini, F.; Hult, K. and Visconti, A. (1994) :*** Ochratoxin A in human serum samples collected in southern Italy from healthy individuals and individuals suffering from different kidney disorders .Nat. Toxins , 2(6) : 366-370 .

7) ***Zimmerli, B. and Dick, R. (1995):*** Determination of Ochratoxin A at the ppt level in human blood , serum , milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: methodology and Swiss data. J. Chromatogr. B. Biomed. Appl. 666 (1) : 85-99 .

8) ***Breitholtz- Emanuelsson, A.; Olsen, M.; Oskarsson, A.; Palminger, I. and Hult, K. (1993):*** Ochratoxin A in cow's milk and in human milk with corresponding human blood samples . J. AOAC. Int. 76 (4) : 842-846 .

9) ***Kovacs, F.; Sandor, G.; Vany, A.; Domany, S. and Zomborszky-Kovacs, M. (1995) :*** Detection of Ochratoxin A in human blood and clostrum. Acta. Vet. Hung. 43(4) : 393-400 .

10) Di Paolo, N.; Guarnieri, A.; Garosi, G.; Sacchi, G.; Mangiarotti, AM. And Di Paolo, M. (1994): Inhaled mycotoxins lead to acute renal failure. Nephrol. Dial. Transplant 9 (4) : 116-120 .

11) Maaroufi, K.; Achour, A.; Betbeder, AM.; Hammami, M.; Ellouz, F.; Creppy, EE. and Bacha, H. (1995 b): Foodstuffs and human blood contamination by the mycotoxin ochratoxin A: Correlation with chronic interstitial nephropathy in Tunisia. Arch. Toxicol. 69 (8) : 552-558 .

12) Radic, B.; Fuchs,R.; Peraica,M. and Lucic, A. (1997) : Ochratoxin A in human sera in the area with endemic nephropathy in Croatia. Toxicol. Lett. 91 (2) : 105-109 .

13) Ruprich, J. and Ostry, V. (1993 b): Health risk assessment of the mycotoxin ochratoxin A to humans: Czech Republic-Brno-1991/92. Cent. Eur. J. Public. Health 1(2) : 86-93 .

14) Domijan, AM.; Peracia, M.; Fuchs, R.; Lucic, A.; Balija, M.; Bosanac,I. and Grgicevic, D. (1999): Ochratoxin a in blood of healthy population in Zagreb. Arch. Hig. Rada. Toksikol. 50 (3):263-271.

15) Peraica, M.; Domijan, AM.; Fuchs, R.; Lucic, A. and Radic, B. (1999) : The occurrence of ochratoxin A in blood in general population of Croatia . Toxicol. Lett. 110 (1-2) : 105-112 .

16) Palli, D.; Miraglia, M.; Saieva, C.; Masala, G.; Cava, E.; Colatosti, M.; Corsi, AM.; Russo, A. and Brera, C. (1999) : Serum levels of ochratoxin a in healthy adults in Tuscany : correlation with individual characteristics and between repeat measurements. Cancer Epidemiol. Biomarkers Prev. 8(3) : 265-269 .

17) Scott, PM.; Kanhere, SR.; Lau, BP.; Lewis, DA.; Hayward,S.; Ryan,JJ. And Kuiper-Goodman, T. (1998) : Survey of Canadian human blood plasma for ochratoxin A. Food Addit. Contam. 15(5) : 555-562 .

18) Ozcelik, N.; Koscedilar, A.; and Soysal, D. (2001): Ochratoxin A in human serum samples collected in Isparta-Turkey from healthy individuals and individuals suffering from different urinary disorders . Toxicol. Lett. 121 (1): 9-13 .

19) Maaroufi, K.; Achour, A.; Hammami, M.; el May, M.; Betbeder, AM. ; Ellouz, F.; Creppy, EE. and Bacha, H. (1995a): Ochratoxin A in human blood in relation to nephropathy in Tunisia . Hum. Exp. Toxicol. 14 (7) : 609-614 .

20) Snedecor, G. W. and Cochran, W.G. (1967) : Statistical Methods , 6th Ed., pp. 2580280. The Iowa state Univ. press, Ames , I A .

21) Creppy, EE.; Baudrimont, I. and Betbeder, AM. (1995) : Prevention of nephrotoxicity of ochratoxin A , a food contaminant. Toxicol. Lett. 82 (83) : 869-877 .

22) Ruprich, J. and Ostry, V. (1993 a) : Study of human exposure to ochratoxin A and assessment of possible sources . Cent. Eur. J. Public. Health 1(1) : 46-48 .

23) Golinski, P.; Grabarkiewicz-Szczensna, J.; Chelkowski, J.; Hult, K. and Kostecki, M. (1991) : Possible sources of ochratoxin A in human blood in Poland . IARC Sci. Publ. (115) : 153-158 .

24) Petzinger, E. and Zigler, K. (2000) : Ochratoxin A from a toxicological perspective. J. Vet. Pharmacol. Ther. 23 (2) : 91-98 .

الملخص العربـــــى

أوكراتوكسين أ فى دم مرضى الفشل الكلوى وبعض علاجات الكلى

حاتم حسين بكرى و إلهام عبد المنعم الشيوى

كلية الطب البيطرى جامعة الزقازيق فرع بنها قسم الطب الشرعى و السموم

استمراراً للجهد المبذول لتقديم أدلة أكثر لنظرية إعتبار الأوكراتوكسين أ كأحد مسببات إصابـــات الكلى ، أُجرى مسح لتحديد وجوده فى علاجات الكلى و دم مرضى الفشل الكلوى حيث تم فيه تحليل ٢٠ عينة من ٥ أنواع مختلفة من علاجات الكلى ذات الأصل العشبى و ٣٠ عينة دم من مرضـــى الفشـل الكلوى تحت العلاج بالغسيل الكلوى . وقد قورنت عينات مرضى الفشل الكلوى من متبرعين أصحـــاء ظاهرياً .

وقد أوضحت النتائج احتواء علاجات الكلى على تركيزات مختلفة من الأوكراتوكسين أ ، كذلـــك فروق ذات قيمة معنوية واضحة فى تركيز الأوكراتوكسين أ فى دم مرضى الفشل الكلـــوى عنـــه فـــى المتبرعين الأصحاء . كما وُجدت اختلافات فى تركيز الأوكراتوكسين أ تبعـــاً للمراحـــل العمريـــة و الإختلافات الموسمية و كذلك بين الجنسين .

و من هذه الدراسة يمكن استخلاص أن تواجد الأوكراتوكسين أ فى عينات علاجات الكلى التـــى فحصها من الممكن أن يلعب دوراً فى حدوث أمراض الكلى .