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The Correlation between Concentration of Aflatoxins and Ochratoxin A and Tumor Patients Cases in Nineveh province

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Abstract

This study was conducted to investigate the correlation between occurrence of Aflatoxins B1 (AFB1), M1 (AFM1), Aflatoxicol, and Ochratoxin A (OA), and tumor patients in Nineveh province. Blood, urine and tissues samples were taken from 33 tumor patients at liver and kidney organs, and 18 samples from healthy volunteer's peoples to estimated the presence and concentration of AFB1, AFM1, Aflatoxicol, and OA contents. It had been found that the AFB1, AFM1, and OA at a high percentage in blood samples from patients group at 42.4, 45.5, and 42.4 % respectively and high range concentrations 4.5 to 13.1, 9.4 to 31.6, and 1.02 to 8.6 ng/100 ml of blood respectively, compared with the above mycotoxins presence in same samples from healthy group. On the other hand there was a highly percentage for occurrence and concentrations of AFB1, AFM1, aflatoxicol, and OA in urine samples from patients group (54.5, 66.7, 60.6 and 42.4 % respectively and the concentrations at 5.2 to 25.1, 10.7 to 36.8, 7.2 to 20.3 and 9.2 to 33.7 ng/100 ml of urine respectively when compared with the same samples from healthy group. Furthermore, found that a high concentration of AFB1, AFM1, and OA in the liver and kidney samples that taken from tumor patients group. The results was confirm the strongly correlation between level occurrence and concentration of myctoxins type and the tumor cases in contributors peoples.

الترابط بين تركيز أنواع سموم الافلاتوكسين والاوكراتوكسين وحالات الإصابة بالأورام لمرضى في محافظة نينوى

كركز محمد ثلج موفق محمود احمد خلف اللويزي

المستخلص

أجريت الدراسة لتوضيح مدى الترابط مابين تواجد كل من أنواع السموم الفطرية (الافلاتوكسين B1 و M1 والاوكراتوكسين في دم وإدرار ٣٣ شخصا مصابين بأورام في كل من الكبد والكلى و١٨ شخص سليم في محافظة نينوى في العراق. أشارت النتائج إلى إن سموم الافلاتوكسين B1 و M1 والاوكراتوكسين تواجدت بنسب مرتفعة معنويا ($p<0.05$) في نماذج الدم للأشخاص المصابين بالأورام إذ كانت بنسب ٤٢,٤ ، ٤٥,٥ و ٤٢,٤ % على التوالي وكذلك في مدى تركيز من هذه السموم مابين ٤,٥-١٣,١، ٩,٤-٣١,٦ و ١,٠٢-٨,٦ نانوغرام/١٠٠ مل من عينات الدم على التوالي، عند المقارنة مع تواجد السموم المشار إليها في عينات الدم المأخوذة من الأشخاص الأصحاء. كذلك أشارت النتائج إلى ارتفاع معنوي في نسبة تواجد وتركيز كل من سموم الافلاتوكسين B1 و M1

والافلاتوكسيكول والاوكراتوكسين في نماذج الإدرار من الأشخاص المصابين بالأورام التي كانت ٥٤,٥، ٦٦,٧، ٦٠,٦ و ٤٢,٤ % على التوالي ومدى تركيز مابين ٢,٥-١,٢٥، ١٠,٧-٨,٣٦، ٢,٧-٣,٢٠ و ٢,٩-٧,٣٣ نانوغرام/مل من الإدرار على التوالي عند المقارنة مع نفس النماذج في عينات إدرار مجموعة الأشخاص الأصحاء. فضلا عن ذلك فقد وجد إن تركيز سموم الافلاتوكسين B1 و M1 والاوكراتوكسين قد تواجدت بتراكيز مرتفعة في كل من عينات الكبد والكلى للأشخاص المصابين بالأورام فيهما. تأكد من النتائج الربط القوي بين تواجد أنواع السموم الفطرية قيد الدراسة وحالات التورم في كل من الكبد والكلى في الأشخاص المصابين.

Introduction-

Mycotoxins are chemically diversified low molecular weight compounds produced as secondary metabolites of filamentous fungi such as *Aspergillus* ,*Penicillium* and *Fusarium* and others genera over a variety of foodstuffs (Moss,1996). Mycotoxicoses are characterized as feed-related, nontransferable, and non infections diseases (Bennett and Klich, 2003). Moreover, the severity of mycotoxins depends on the type and concentration of mycotoxins, the duration of exposure, gender, age, and health status of animal or human being (Chowdhury *et al.*, 2005). Among the different types of mycotoxins aflatoxins have received greater attention than other mycotoxins because of their established carcinogenic effect in various animals and their acute toxicological effects in humans (IARC, 1993). Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are major Aflatoxins produced by fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus* and the rare *A. nomius*. These fungi are ubiquitous, and under favorable conditions can grow on a wide variety of agriculture commodities. AFB1 is the most toxic and carcinogenic and is most abundantly produced by fungi. Though the liver is considered the primary target of AFB1 toxicity (IARC, 1987). The International Cancer Research Institute defines aflatoxin as a class 1 carcinogen (Henry et al. 1999). Appositive correlation has been established between estimated AF intake determined from the level of aflatoxin of either market food samples or cooked food samples and the incidence of liver cancer in a number of studies in African and Asian countries (Van Rensburg, et al. 1985 and Gorelick, et al. 1993 and Cullen and Newberne, 1994). With respect to ochratoxin A (OA) is a widely distributed mycotoxin produced mainly by *Aspergillus ochraceus* and *Penicillium verrucosum* under diverse environmental conditions (Delacruz and Bach, 1990 and Chu, 2002). Ochratoxin A has been detected in a variety of nutrients and in the majority of human blood samples (Scott, et al. 1998). The main target organ from OA is the kidney because tubular concentrations of free OA are several-fold higher than plasma concentrations (Bahnemam, et al. 1997). Epidemiological studies have shown a positive correlaton between human nephropathies-especially certain forms of interstifial nephritis- and either dietary OA exposure of plasma concentration of OA in the nanomolar range (Godin, et al. 1997 and Wafa, et al. 1998). The objective of current research was to investigate the association between the presence of AFB1, AFM1, aflatoxicol and OA in blood, urine and tissues of organs and the liver and kidney tumor cases of some patients' in Nineveh province.

Materials and Methods

Sample collections: Thirty three samples from each of bloods, urine, liver and kidney tissues were collected in screw vials from 33 patients' whose have liver and kidney tumors, and 18 samples

of blood and urine from healthy group peoples in Nineveh province.

Sample preparations: Blood samples were centrifuge at 3000 rpm for 10 mints. to obtained the plasma. Urine samples were collected in 50 ml volume sterile screw plastic containers. Tissues of liver and kidney samples were collected in 10 ml volume sterile screw plastic containers. All types of samples were stored at -20 ° c until used in mycotoxins assay.

Ochratoxin A assay: Tow milliliters from plasma and urine samples were taken and added to its 2% from 1M of acetic acid (vol/vol) at pH 4.5, after prepared the chromatographic column according to AOAC, (1984), the toxin was eluted by used 1% of HCl in methanol (vol/vol). then was evaporated the elute to dryness by used the rotary evaporated system (Germany), and redissolved residual in 1 ml of methanol in small screw glass containers and stored at 4 ° c until used in mycotoxins analysis. The liver and kidney tissues samples were homogenized well after mixed with 60% of acetonitrile and extracted the toxin by eluted with hexan from the chromatographic column, the eluted then dryness by used the rotary evaporator and redissolved with 1 ml of methanol in small glass screw containers and stored at 4 ° c until used in mycotoxins analysis. Methanol samples from plasma, urine and liver and kidney tissues were injected in the pump of HPLC system (LC-10-Shimadzu-Japan) which used pump 4015 and fluorescence detector at 340 and 465 nm, flow rate 0.5 ml/ mint. 50 µl for the samples were injected according the procedure that mention in Monaci *et al.*, (2005). All unknowns toxin was compared with the standard solution of ochratoxin A which provided by Sigma Company (USA).

Aflatoxins assay: The extraction of AFB1 and AFM1 from plasma and urine samples according to Clara *et al.*, (2002), were obtained by used the chloroform and then with hexan for eluted it's from chromatographic column which prepared according AOAC, (1984), and then evaporated by used rotary evaporator system (Germany), until dryness. While the extraction of above toxins from liver and kidney tissues by homogenized its and adding 2 ml of citric acid after mixing well. From the chromatographic column was eluted the toxins with the chloroform and then evaporated the eluted to dryness by used the rotary evaporator. The extraction of aflatoxicol toxin from urine samples was obtained by washing the chromatographic column with chloroform and then evaporated the elute by used the rotary evaporator. All extraction samples were redissolved with 1 ml of chloroform and storage in small screw glass container at 4° c until used to analysis. AFB1, AFM1, and Aflatoxicol were determined in extracts of plasma, urine, and liver and kidney tissues, after injected in HPLC system (LC-10-Shimadzu-Japan) which used pump 4015 and fluorescence detector at 366 and 418 nm, flow rate 0.8 ml/ mint. 20 µl for the samples were injected according the procedure that mention in Clara *et al.*, (2002). All unknowns toxin was compared with the standard solution of AFB1, AFM1, and aflatoxicol which provided by Sigma Company (USA).

Results and Discussion

The occurrence of mycotoxins in blood samples from patients and healthy groups were illustrated in Fig. 1. Data show the occurrence of AFB1, AFM1, and OA at 42.4, 45.5 and 42.4% respectively in blood samples from patients group compared with 33.3, 38.9, and 37.6% respectively in blood samples taken from healthy group.

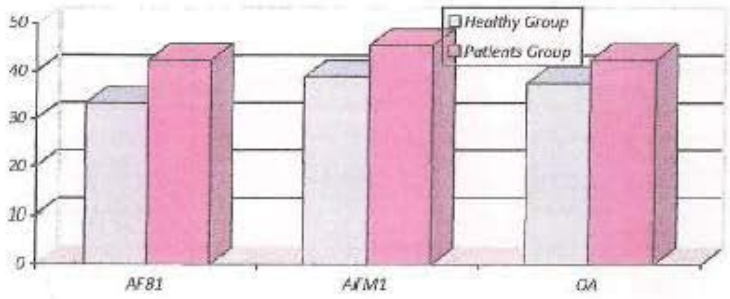

Fig.(1):-The percentage occurrence of AFB1, AFM1, and OA in blood samples.

The range concentration of above mycotoxins in blood samples were investigated in table 1. The data founds the range concentrated of AFB1, AFM1, and OA was at 4.5 to 13.1, 9.4 to 31.6, and 1.02 to 8.6 ng/100 ml respectively in blood samples of paticnts group when compared with 2.2 to 7.1, 4.0 to 8.6, and 2.1 to 4.2 ng/100 ml respectively in blood samples of healthy group.

Table (1):- The concentration of AFB1, AFM1, and OA in blood samples.

Groups	AFB1	AFM1	OA
	ng/100ml		
Healthy group	2.2-7.1	4.0-8.6	2.1-4.2
Patients group	4.5-13.1	9.4-31.6	1.02-8.6

Data in Fig 2. was also demonstrated the percentage of AFB1, AFM1, aflatoxicol, and OA at 54.5, 66.7, 60.6, and 42.4 % from the total urine samples of patients group compared with 33.3, 44.4, 44.4, and 33.3 % respectively for urine samples of healthy persons group.

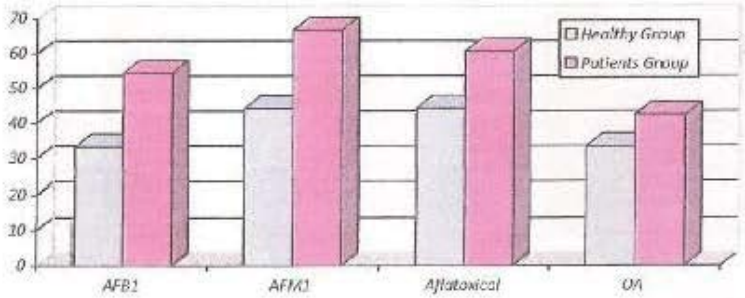

Fig.(2):-The percentage of AFB1, AFM1, Aflatoxicol, and OA in patients and healthy urine samples.

While the range concentrations for AFB1, AFM1, Aflatoxicol, and OA were at 5.2 to 25.1, 10.7 to 36.8, 7.4 to 20.3, and 9.2 to 33.7 ng/100 ml urine respectively for the sample of patients group compared with 2.6 to 12.5, 4.3 to 17.2, 3.5 to 11.4, and 4.9 to 18.5 ng/100 ml urine respectively for the samples of healthy persons group.

Table(2):-The concentration of AFB1, AFM1, Aflatoxicol, and OA in patients and healthy urine samples.

Groups	AFB1	AFM1	Aflatoxicol	OA
	ng/100ml			
Healthy group	2.6-12.5	4.3-17.2	3.5-11.4	4.9-18.5
Patients group	5.2-25.1	10.7-36.8	7.4-20.3	9.2-33.7

Results from the data show the strongly relationship between the occurrence of types of mycotoxins (AFB1), (AFM1), aflatoxicol, and (OA) in blood and urine samples and the tumor cases of peoples. The highly levels of concentrations from mycotoxins in blood, and urine samples indicated that the mycotoxins get the patents from the foods contaminate with it directly or cross-over and effects on human health and finally produce these tumor cases. The number of tumor cases in liver and kidney founds as 17 and 10 cases respectively and the number of liver tissue samples which found to contaminated with AFB1, AFM1 and OA were 13, 14, and 5 cases means as 65, 70, and 25% respectively, while the number of kidney tissue samples were founds as contaminated with the same above toxins were as 4, 6, and 8 cases which means 30.77, 46.15, and 61.54% respectively. The concentrations of AFB1, AFM1 and OA toxins in liver

samples were founds at range between 1.27-3.60, 0.92-4.98, and 0.2-1.72 ng/gm respectively and in kidney were as between 0.71-2.26, 1.23-2.92,and 1.3-5.35 ng/gm respectively. The comparing a high concentrations of AFB1, AFM1, Aflatoxicol, and OA in blood , urine and liver and kidney tissues samples of tumor patients group with healthy group were found and confirmed the strongly roles of these mycotoxins as factors which causes the tumor cases for the patients (Gorelick, et al. 1993 and Cullen and Newberne, 1994).

Table(3):- The concentration of AFB1, AFM1, and OA in liver and kidney of tumor patients

Type of tumor organs	No. of tumor organs	Type of mycotoxins in organs	No. and % of tumor organs contaminated with mycotoxins		Concentration of mycotoxins in tumor organs
			No.	%	ng/gm
Liver	20	AFB1	13	65	1.27-3.60
		AFM1	14	70	0.92-4.98
		OA	5	25	0.2-1.72
Kidney	13	AFB1	4	30.77	0.71-2.26
		AFM1	6	46.15	1.23-2.92
		OA	8	61.54	1.3-5.35

Furthermore, the levels of percentage and concentrations of above mycotoxins in blood and urine samples from healthy peoples were also might be indicate as important risks for all peoples living in Nineveh province and other cities in Iraq since these levels were causes as multi disease which returned to a negative effects on immune response at a less probability. The source of these mycotoxins were exactly by the consumed of foods contaminated with these mycotoxins resulting passed and accumulated to the internal organs that tend to obtain the concentration level which causes these cases in such patients, However, the healthy peoples might be subjected to an increased contaminated foods with such toxins that lead to increase their toxins levels and may caused the tumor organs.

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Effect of phenolic compounds Extractions of Green tea (*Camellia sinesis L*) On the glutathione –S- Transferase Alloxan Experimental Induced-Diabetic Rabbit.

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Abstract

Diabetes Mellitus is considered as a member of oxidative stress syndrome. It is associated with an imbalance between types of free radicals and scavenger's system. This study showed the effect of the polyphenol compounds extracted from green tea (Camellia Sinesis L.) on alloxan induced diabetic rabbits to determine their role in treatment of diabetes mellitus, their effect on enzymatic antioxidant and to know the histological effects on the diabetic kidney. The rabbits group was divided into four groups, each group consist of 7 rabbits. The first group was healthy rabbits (normal group) as compared With second group .The second group diabetic without treatment as control group with the last three groups. The third group diabetic treated with extract of green tea 100mg/kg of body weight as a single daily dose. The fourth group diabetic treated with extract of green tea 200mg/Kg of body weight as a single daily dose. After treatment for twenty week blood and tissue samples were taken for analysis and the result were as follow : There was a significant decrease in GSTS. When polyphenol extract of green tea was used with dose rate 100mg /kg of body weight (fourth group) gave a significant increase ($p<0.05$) in all parameters as compared with diabetic group (without treatment). The present results revealed that alloxan was effectively induced diabetes by partial destruction of b-cells of pancreas which lead to elevation of blood glucose level. As a consequence of hyperglycemia the abnormal effect was obvious in certain tissues in the body which attributed to the effect of diabetes .Histological investigations shows that all the lesions in the kidney that result from diabetes such as hypertrophy, degeneration and hylanosis of the glomeruli.

تأثير مركبات الفينولك المستخلصة من بذور العنب على أنزيم كلوتاثيون ترانزفيريز- ايس في الارانب المستحدثة مرض السكري بمادة الالوكسان

بثينة عبـد الحميد

المستخلص

يعد مرض السكري من العناصر المتلازمة لضغط المؤكسدات ذات الصلة بالتوازن بين أنواع الأوكسجين الفعال ونظام الكاسحات.استهدف هذا البحث دراسة تاثير المركبات الفينولية المستخلصة من الشاي الاخضر camellia sinensis L. في الارانب المحدث بها السكري بواسطة الالوكسان لمعرفة علاج داء السكروتأثيرهما على الأنزيم الكلوتاثايون ولمعرفة التأثيرات النسيجية التي تطرأعلى الكلية.شملت هذه الدراسة الدراسة على ثمانية وعشرون من الأرانب وبعمر (٦-١٠) شهرا وقسمت إلى مجاميع.كل مجموعة تضم ٧ من الأرانب.المجموعة الأولى: مجموعه أرانب أصحاء(مجموعه سيطرة)للمجموعة الثانية . المجموعه الثانيه :أحدث بها سكري وبقيت بدون علاج (مجموعه سيطر)للمجاميع الثلاث الأخيرة .المجموعه الثالثه و الرابعة أحدث بها سكري وعولجت بخلاصة الشاي الأخضر و بتركيز ١٠٠ ملغم/كغم و٢٠٠ ملغم/كغم على التوالي من وزن