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Study possibility of some Biomarkers Determind on poisoning of Human by Ochratoxin A

A thesis
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Master in Forensic Evidence Sciences

Submitted by

Wael Saad Hassan

B.Sc. in Biology \ University of Baghdad (2006)

Supervised by

Professor.Dr. Sami Abdel Reda Ali

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا يَذَّكَّرُ إِلَّا أُولُوا الْأَلْبَابِ﴾

صَدَقَ اللهُ العَلِيُّ العَظِيم

سورة البقرة – الآية (٢٦٩)

Dedications

To the one who illuminated the darkness of ignorance.. who brought people out of darkness into the light.. Muhammad the Prophet (may God bless him and his family and grant them peace).. and to his pure and reformed family.. and his chosen companions.. and those who were guided by their guidance to the Day of Judgment.

To the master of reform, my strong master, the trustworthy, al-Husayn ibn Ali (may God's love, prayers and peace be upon him)

To the good and pure martyrs of Iraq

To my mother's strength and patience
My father's courage and dignity
And the love of my brothers and sisters

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After giving thanks and praise to the Creator

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And for all patients and healthy people who contributed by providing samples in order to complete the study

I extend my thanks and appreciation to my respected family who provided moral support for the completion of the study

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List of Abbreviation

NO	Abbreviation	**Meaning**
1	BEN	**Balkan Endemic Nephropathy**
2	PK	**Proteinase K**
3	WHO	**World Health Organization**
4	ALT	**Alanine Aminotransferase**
5	ALP	**Alkaline phosphatase**
6	AST	**Aspartat aminotransferase**
7	BUN	**Blood Urea Nitrogen**
8	CKD	**Chronic Kidney Disease**
9	HPLC	**High performance Liquid Chromatography**
10	SGOT	**Serum glutamic oxaloacetic tranaminase**
11	SGPT	**Serum glutamic pyruvate -tranaminase**
12	EDTA	**Ethylene diamin tetra acetic acid**
13	CBC	**Complete Blood Count**
14	WBC	**White Blood Cells**
15	RBC	**Red Blood Cell**
16	PLT	**Platelets**
17	HGB	**Hemoglobin**
18	HTC	**Hematocrit**
19	EU	**European Union**
20	MCH	**Mean Corpuscular Hemoglobin**
21	MCHC	**Mean Corpuscular Hemoglobin concentration**
22	OTA	**Ochratoxin A**
23	OTB	**Ochratoxin B**
24	(OTα)	**ochratoxin alpha**
25	OTC	**Ochratoxin C**
26	UV	**Ultraviolet**
27	MPN	**Myeloproliferative neoplasms**
28	NTP	**National Toxicology program**
29	TLC	**Thin layer chromatography**
30	AFB1	**Aflatoxin B1**
31	AFG1	**Aflatoxin G1**
32	AFM1	**Aflatoxin M1**
33	ELISA	**Enzyme Linked Immun Sorbent assay**
34	CRI	**Chronic renal insuffciency**

35	UTT	**Urinary tract tumours**
36	PCTs	**Proximal convoluted tubule**
37	hOAT4	**Human organic anion transporter**
38	MLs	**Maximum Levels**
39	DNA	**Deoxyribonucleic acid**
40	RNA	**Ribonucleic Acid**
41	Tyr	**tyrosine**
42	GSH	**glutathione**
43	ROS	**Reactive oxygen species**
44	NRF2	**Nuclear erythroid 2-related factor2**
45	SOD	**Superoxide dismutase**
46	UDL	**Under detection Limited**

Summary

The results should presence of Ochratoxin A in 90% of blood plasma of nephropathy disease patients, while the blood plasma sample of healthy people was 3% with a significant difference. Its percentage among male patients, as the percentage among females is 48.8%, while the percentage among males is 51.1%.

The results also showed a clear superiority in the concentration rates of Ochratoxin A in the blood plasma of patients relative to a healthy person, as its average concentration in female and male patients was (7.015 and 7.071) ng/ml, respectively, while it was in both healthy females and males (0.1, 0.09) ng/mL, respectively.

The results associated with the presence of Ochratoxin A (8-24mg/dl) showed an increase in the level of urea in male patients to 115 mg/dL, while the level in females reached (99.1) mg/dL, while the levels in both healthy females and males were within the(6-21mg/dl) normal level.

The average creatinine level in patients with chronic kidney disease, whether female and male, increased to (4.645, 3.73) ml/dL, respectively, with a significant difference from its levels in a healthy person knowing that the normal level(0.84-1.21mg/dl).

As for albumin levels, it slightly increased in nephropathy than in healthy controls, with an insignificant difference knowing that the normal level(3.5-5.5g/dl)

Also, the level (8–48U/L)of AST enzyme was affected (8-35U/L) in female patients, reaching a ratio of (19.26) international U/L, while its level reached (15.4) international units/liter in healthy patients, with a significant difference at the level of $P<0.05$.

The results indicated that the levels(4.36U/L) of ALT enzyme were within the normal level(17-55U/L) in patients with nephropathy and in a healthy person.

On the other , the ALP enzyme was affected for female patients, reaching a rate of (125.54) IU/L, while for healthy females it 44.86 IU/L, and for male patients, the percentage was close to what it is in female patients knowing that the normal level(40-129U/L)

The tests for physiological blood parameters showed a decrease in the percentage of HCT in male patients, which amounted to (32.68%) with a significant difference from the percentage of a healthy person knowing that the normal level male (42 – 52) and female (37- 47).

And there was a decrease in the level of hemoglobin in male patients compared to its level in healthy controls, with a small significant difference $P<0.05$ knowing that the normal level male (14 -18) and female (12 -16).

The results also indicated a decrease in the number of red blood cells in patients compared to healthy controls with a significant difference of $P<0.001$

The results of physiological blood parameters represented by MCHC, MCH, WBC, and PLT do not showing any significant differences in both males and females from their levels in healthy persons.

1. Introduction:

Fungi, in their toxic species, are among the many sources of food contamination. it is called on the toxin that it produces Mycotoxins, is defined as the secondary metabolites produced by fungi, based on their pharmacological activity it causes many health risks that start from mycoses and may sometimes reach death **(Bennett and Klich, 2003).**

Penicillium, Aspergillus, Claviceps and Fusarium, fungi which produce the mycotoxins as: Ochratoxins; Aflatoxins; T-2/HT-2 Toxins; Deoxynivalenol (DON, Vomitoxin); Ergot Alkaloids; Zearalenone; Cyclopiazonic Acid, Mycophenolic Acid, and Fumonisins, These mycotoxins are responsible for food contamination **(Vivek *et al*., 2014).**

Mycotoxins happen through the growth of molds on some plants such as apple, grains, coffee beans, nuts, and dried fruits, and exposure to mycotoxins occurs by consuming the poisoned food directly, or by eating the meat and derivatives of animals fed on the infected feed. **(World Health Organization, 2018)**. Certain environmental conditions, such as high temperature and humidity, help fungi create these toxins. **(Daou *et al*., 2021).**

Mycotoxins impact human health, as they may cause gangrene, respiratory problems, convulsions, liver cancer, changes in protein metabolism, and reduced immunity, This would affect the economy through the increased health care costs and premature deaths **(Freire and da Rocha, 2016).**

Ochratoxin A is one type of these fungal toxins which is defined as is a mycotoxin produced by ***Aspergillus and Penicillium***, contaminates a wide range of food crops. **(Ruyck *et al.*, 2015).**

Ochratoxins are not one type but include several types which classified into Ochratoxin A (OTA), ochratoxin B (OTB), and Ochratoxin C (OTC), Ochratoxins usually contaminate the cereals **(Reddy and Bhoola, 2010),** Ochratoxin A (OTA) is the most common Ochratoxin in the world **(Ringot *et al.*, 2006).**

The human is exposed to Ochratoxin through eating the contaminated food, and international health organizations have been keen to set the maximum permissible level of OTA in food **(Denli and Perez, 2010).**

Scientific study indicate that OTA has many toxicological impacts like teratogenic, nephrotoxic, immunotoxic, neurotoxic, and hepatotoxic, It also has a role in the causes of Balkan endemic nephropathy, Previous studies have linked some of the harmful effects of OTA, as carcinogenicity; neurotoxicity, it also has many toxicological impacts include teratogenic, nephrotoxic, immunotoxic, neurotoxic, and hepatotoxic **(El Khoury and Atoui, 2010)**

Increasingly being suspected OTA of being the primary cause of Balkan endemic nephropathy **(BEN),** a deadly kidney disease linked to the final stage of urothelial malignancies **(Salwa *et al.*, 2003).**Where It causes kidney fibrosis and degeneration of the renal tubules and leads to low weight, high protein content in urea and anemia **(Belmadani *et al.*, 1998).**

Nephropathy that is endemic in the Balkans and is a kidney disease that occurs in rural communities in the vicinity of the Danube River in Romania and Bulgaria **(Castegnaro *et al.*, 1987).**

This mycoses occurs endemically and affects females more than males **(Chernozemsky *et al.*, 1977)**

after which Ochratoxin A is isolated in large quantities from corn in the United States of America and it was diagnosed as a toxin that affects the urinary system **(Shotwell *et al.*, 1969; Abdul Aziz, 2011).**

OTA has been shown to cause OTA in animals such as birds and porcine nephropathy, but not for adult ruminants **(Paola and Amedeo, 2002)** and this mycotoxin plays a special role in the genesis of mycotoxin porcine nephropathy **(MPN)** a common disease in Scandinavia **(Ciegler, 1972; Marquardt and Prolieh, 1992).**

The illness mostly affects rural areas in Bulgaria, Romania, and Yugoslavia, all of which are located within the Danube Basin. This illness affects about 12% of the population, There have been reports of mortality rates as high as 40%. Affected individuals are virtually primarily between the ages of 30 and 55, as a result of extended exposure to a causative agent. BEN etiology is currently unclear. It may be possible to establish a link between high rainfall (late summer and autumn), which may encourage fungus development and toxigenicity in endemic regions, and the number of persons who died of nephropathy in the following two years. The major causative agent is OTA, which has parallels to the mycotoxin porcine nephropathy caused by this mycotoxin in Scandinavia **(Martin, 2001).**

Aim of study

Investigation of Ochratoxins in blood of patients with nephropathy and knulege the effect of Ochratoxin A and nephropathy and their interference in levels of some biochemical and physiological blood parameters.

The necessities of the research procedures :

there is a lack of studies on this toxin, knowing that it is one of the toxins dangerous to human health and the fact that much of the human food in our country is contaminated with this toxin, and there is no clear method or evidence for the occurrence of poisoning with it. We decided to study this topic.

1.2. Literature Review

1.2.1. Mycotoxin:

Mycotoxin was coined in 1962 in the wake of an unusual veterinary crisis in Britain, in which about 100,000 turkeys perished, and at that time the mysterious turkey disease **(Turkey x)** was linked to a meal of peanuts contaminated with secondary metabolites produced by a fungus. ***Aspergillus flavus***, where scientists predicted the possibility that other secondary metabolites of the fungus could be fatal and deadly, and the period between **(1960 - 1975)** is considered the period of the height of scientists' interest in mycotoxins **(Maggon *et al.*, 1977).**

Mycotoxins are secondary metabolites that are derived from chemicals such as amino acids, terpenes, phenols, peptides, and others. Most mycotoxins are cyclic hydrocarbon compounds, and a few of them may have open chains **(aliphatic) (Abdul Aziz, 2011).**

Mycotoxins represent a real problem because of their low molecular weights that do not stimulate the immune system as well as their resistance to high temperatures as they are not destroyed by the temperature used in cooking food **(Ciegter, *et al.*, 1983).**

Mycotoxins are considered one of the most dangerous fungl toxins, and the reason for this is that bacterial toxins are proteins that can be recognized by the body's immune system, while mycotoxins are toxic chemical compounds of low molecular weight that the immune system cannot recognize and thus no reaction occurs **(Pitt, 1989).**

The reason behind the production of fungi is not fully known, and some theories point to the possibility of helping fungi to compete environmentally in their natural habitat **(Richard, 2012).**

Fungi are present in nature and need many factors to grow and multiply, such as nutrients, heat, and humidity, and during their life cycle, the fungi are able to produce the mycotoxin and are produced in the steady growth phase and can be found in the spores of fungi **(Hussein and Brasle, 2001).**

Fungi produce mycotoxins on field crops during harvest and during storage **(Diaz *et al.*, 2003; Oroian *et al.*, 2009).**

Mycotoxins produced when crops mature under moist conditions such as Trithothin are less important than those that occur under drought stress such as aflatoxins **(Pitt and Hocking, 2006).**

The agricultural crops most affected by the growth of fungi and mycotoxins before and after harvest are field pistachios, corn, cotton seeds, rice, nuts, and grains, as well as animal products such as meat, milk, and eggs that can also be contaminated by feeding **(Rachaputi *et al.*, 2002).**

Often mycotoxins are released into the medium in which the fungi thrive **(Council, 2003).**

The fungi causing the poisoning have been divided into two groups:

1- Storage fungi: They are fungi that multiply during storage, such as ***Aspergillus*** and ***Penicillium***.

2- Field Fungi: They are fungi that are able to reach seeds during plant growth, such as ***Cladosporium spp***. and ***Alternaria spp.***

Mycotoxins lead to many diseases called Mycotoxicosis, such as kidney failure, genetic mutations, weak reproductive system, cancers, and there is no toxin-free area **(Verma, 2004).**

And that which raises the greatest concern is that these toxins lead to liver cancer and esophageal cancer, and the most vulnerable to these diseases are children before the age of five **(Miller and Beardall, 1994; Marasas, 2001).**

People are exposed to mycotoxins in different ways, either indirectly by eating animal-origin foods infected with mycotoxins or directly resulting from eating foods of plant origin contaminated with mycotoxins **(Chassy, 2010).**

Currently, there are more than 300 types of mycotoxins known, and global attention is focused mainly on those that have proven to be carcinogenic or toxic **(Zain, 2011).**

These toxins cost millions of dollars annually as losses worldwide due to their impact on human health, animal health, and agricultural products **(Nida and Ahmad, 2010; Luttfullah and Hussain, 2011).**

Among the most common fungi that produce mycotoxins are some types of fungi such as ***Penicillium, Aspergillus, Fusarium, Alternaria***, which grow on a number of food products **(Barkai-Golan, 2008).**

Mycotoxins differ in the degree of toxicity they cause. For example, Aflatoxin B1 is more toxic than Aflatoxin G1, so the amount of the lethal dose varies from one organism to another within the same species in animals, so the effects of toxins depend on both the dose and the duration of exposure of the organism. **(Peraica *et al.*, 1999).**

Scientists classify mycotoxins on the basis of the organ that is affected by them, so the toxins that affect the liver Hepatotoxins that affect the kidneys, Nephrotoxins and that affect the neruro toxis **(Eaton and Gallagher, 1994).**

Whereas, microbiologists classify mycotoxins based on the fungus that produces these toxins, for example, the toxins produced by the fungus ***Aspergillus*** called ***Aspergillus*** toxins that are produced by the fungus ***Penicillium*** called ***Penicillium*** toxins **(Cole and Cox, 1981).**

As for biologists, they classify mycotoxins according to their effect, which causes cancer called Carcinogens, which cause allergies, and which cause mutations **(Marasas and Nelson, 1986).**

Likewise, chemists have classified mycotoxins into groups, for example, those that contain multiple cations called polyketides, and the cations that contain coumarin are called Coumarin lactones, which are derived from the amino acid-derived **(Council, 2003).**

1.2.2. Ochratoxin A (OTA)

It was discovered in 1965 by the scientist Scott, and its name is due to the first fungus that was isolated from it, which was ***Aspergillus .carbonarius*** **(Ceovic *et al.,* 1991),** It is a crystalline compound whose color ranges from colorless to white and gives Ochratoxin A a greenish luster under ultraviolet rays in an acidic medium and has a blue color in the basic medium **(Budavari *et al.,* 1989).**

It is a mycotoxin, which belongs to ochratoxins, which are a group of mycotoxins that are mainly produced by the following fungi: ***Aspergillus*** in the kinds (***A. alutaceus var. alutaceus; A. steynii and A. westerdijkiae; niger and A. niger A. carbonarius, also Penicillium verrucosum).*** **(Mostrom, 2016).**

Ochratoxin production is affected by several factors, including humidity, temperature, and other factors **(Selouane *et al.,* 2009).**

The optimum humidity values vary for the production of Ochratoxin produced by the fungus ***Aspergillus and the Penicillium*** between (**83-90**) and (**95-99**)%. As for the fungus, ***A.carbonarius***, the moisture value is less than 79% and the optimum value is 99%. (**Pitt and Hocking, 1985)**

The pH values for the production of Ochratoxin A and the growth of fungi range between (**5.5 - 6.5**) **(Rao *et al.,* 2013).**

The optimum temperature for the production of Ochratoxin A is between (**30-35**) C^{c}, And that the fungus ***Penicillium verrucosum***, it is able to grow at a temperature between (**0 - 31**) C^{c}. **(Magan and Aldred, 2005)**

As for the fungus, , ***Aspergillus .carbonarius*** it grows in temperatures that range from 8 to 37C^{c} and the optimum is 30 C^{c} **(Ramos *et al.*, 1998).**

The fungus, ***Aspergillus .carbonarius*** the optimum temperature for its growth ranges between **(32 - 35**) C^{c} and is found in West and Central Africa **(Sweeney and Dobson, 1999)**, And that the optimum temperature for the production of Ochratoxin A toxin is between (**25-30**) C^{c}. **(Marin *et al.*, 1998).**

Also, the presence of microorganisms concurrent with the presence of fungi can affect the growth of fungi and the production of mycotoxins, and many microorganisms have been indicated for their use as agents for controlling biological pests, for example, ***Trichoderma harzianum*** is a fungus capable of producing the enzyme latec and ketanase, which have anti-inflammatory activity. Among them are ***A.niger*** **(Nampoothiri *et al.*, 2004)**

And in a study **(Bae *et al.*, 2004)** showed a clear inhibition of ***A. carbonarius*** growth by ***Bacillus thuringiensis***.

Likewise, the presence of some microorganisms in the gut of animals **(such as sheep and camels)** has the ability to convert the toxin of Ochratoxin A into Ochratoxin Alpha, which is less toxic **(Malekinejad *et al.*, 2005; Meucci *et al.*, 2010)**

Ochratoxin A production varies by strains of ***A.ochraceoroseus*** according to the different concentrations in which they are grown from yeast and sucrose extracts with a concentration ranging from **(0-4**)% each **(Atalla and Eldlin, 1993).**

Also only it was found that ***Aspergillus*** has the ability to produce Ochratoxin in grains that have a high protein content as well as oilseeds. As for the

Penicillium genus, it has the ability and high ability to produce Ochratoxin toxins in seeds rich in carbohydrates **(Madhyasta *et al.*, 1990).**

Studies have shown that Ochratoxin has multiple toxicities, as it is nephrotoxic**,** genotoxic **(Cosimi *et al.*, 2009),** and immunotoxic **(Khatoon *et al.*, 2013).**

The toxic mechanism of Ochratoxin A is inhibition of protein synthesis **(Sakthivelan and Rao, 2010).**

Induction of oxidative stress by the formation of peroxide lipids and free radicals **(Gillman *et al.*, 1999).**

The kidneys are the target organ for Ochratoxin **(Pfohl - Leszkowicz and Castegnaro, 1999).**

The immunotoxicity of Ochratoxin was observed at a high dose **(Creppy *et al.*, 1991).**

It is known that the Ochratoxin A toxin is produced by several types of fungi, which grow in optimum temperatures and water activity. The following **table(1-1)** showed Ochratoxin-producing fungi, optimal growth conditions, and commodities affected

Table (1-1) Ochratoxin-producing fungi, optimal growth conditions, and commodities affected

Fungi that produce OTA	Water activity	Optimal temperature range (Min-Max) ˚C	Affected food
A. carbonarius	0.82	32-25 (N/A¡40)	Grapes and grape products, including dried vine fruits, table grapes, and wines
A. ochraceus	0.95–0.99	24-31 (8–37)	Smoked and salted dried fish, biltong, dried beans, dried fruit, pepper, nuts, coffee beans, wheat, barley, maize, soya beans, sesame seeds, cereals rice, bran, flour, chickpeas, and , rapeseed.
P. verrucosum	0.80	20 (0–30)	cheese, meat products, and Cereal crops.
A. niger	0.77	35–37 (6–47)	Nuts, strawberries, peaches, melons, figs, citrus, grapes, apples, pears, mangoes, tomatoes, yams garlic, and onions

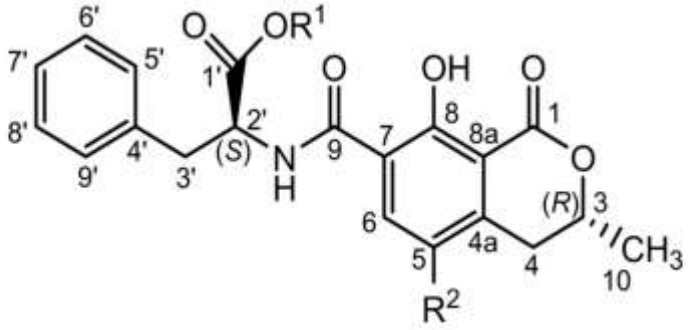
(JECFA, 2001).

Usually, a person is exposed to OTA by eating food contaminated with this toxin, and in possible causes, he may become infected by inhaling air contaminated with this toxin. **(Brera *et al.*, 2007).** Reducing and toasting bread by 20% contributes to reducing exposure to OTA toxin, and studies have not observed that boiling has an effect on the nature of the presence of the toxin. **(O'Brien and Dietrich, 2005).**

1.2.2.1. The chemical structure of Ochratoxin A:

Some types of ***Penicillium*** and ***Aspergillus*** produce ochratoxin A, These fungi grow in certain climatic conditions and colonize on different plants. All foods can be contaminated with ochratoxin A toxin, and none of them is immune to this as long as the climatic conditions necessary for growth are available. It pollutes many food crops such as wheat, barley, and dried fruits, and may contaminate animal products by transferring them to the animal from the contaminated feed with the toxin ochratoxin A**. (Aish, 2004).**

chemically described as **3,4-dihydro-methylisocoumarin deriva (Cole and Cox, 1981),** Ochratoxins are derivatives of an isocoumarin moiety linked to phenylalanine by an amide bond. The most important ochratoxins are ochratoxin A **(OTA),** ochratoxin B **(OTB),** ochratoxin C **(OTC) (Figure1-1)**



Ochratoxin	Substituents	Molecular formula	Molar mass ($g \cdot mol^{-1}$)	CAS number
OTA	$R^1 = H; R^2 = Cl$	$C_{20}H_{18}ClNO_6$	403.8	303-47-9
OTB	$R^1 = H; R^2 = H$	$C_{20}H_{19}NO_6$	369.4	4825-86-9
OTC	$R^1 = C_2H_5; R^2 = Cl$	$C_{22}H_{22}ClNO_6$	431.9	4865-85-4

Figure (1-1)Chemical structure of ochratoxin A, ochratoxin B and ochratoxin C. (Schrenk *et al.*, 2020)

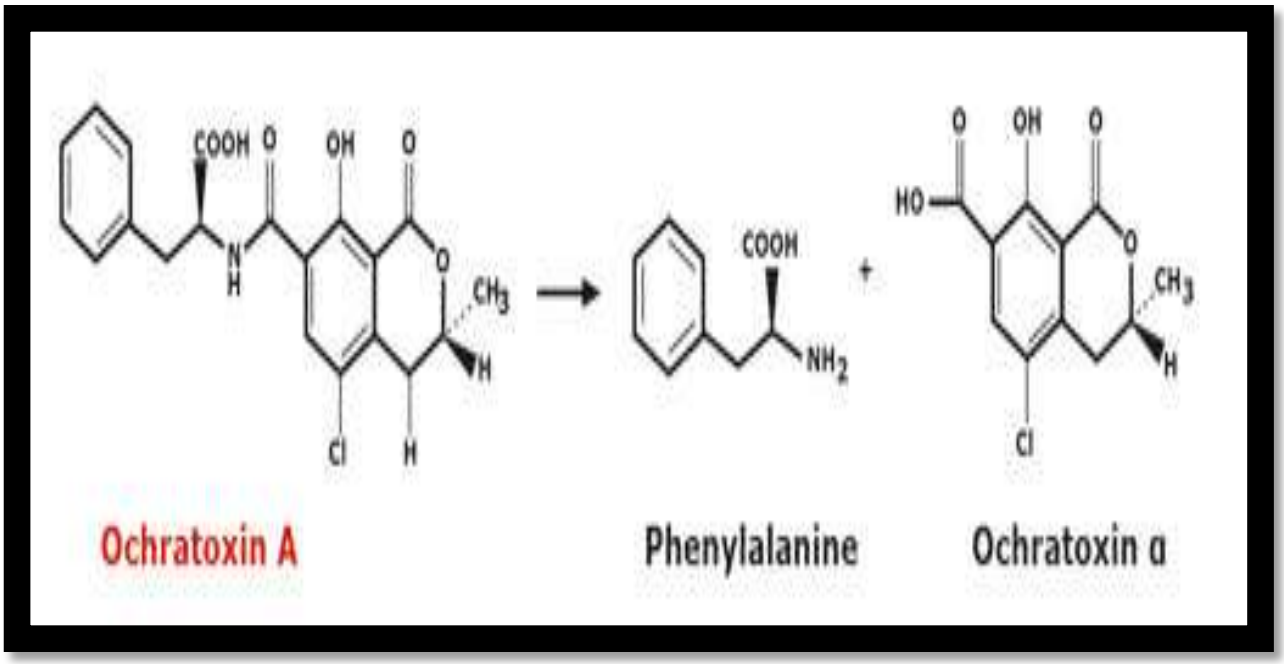


Figure (1-2) Biotransformation of Ochratoxin A (Juodeikiene *et al.*, 2012).

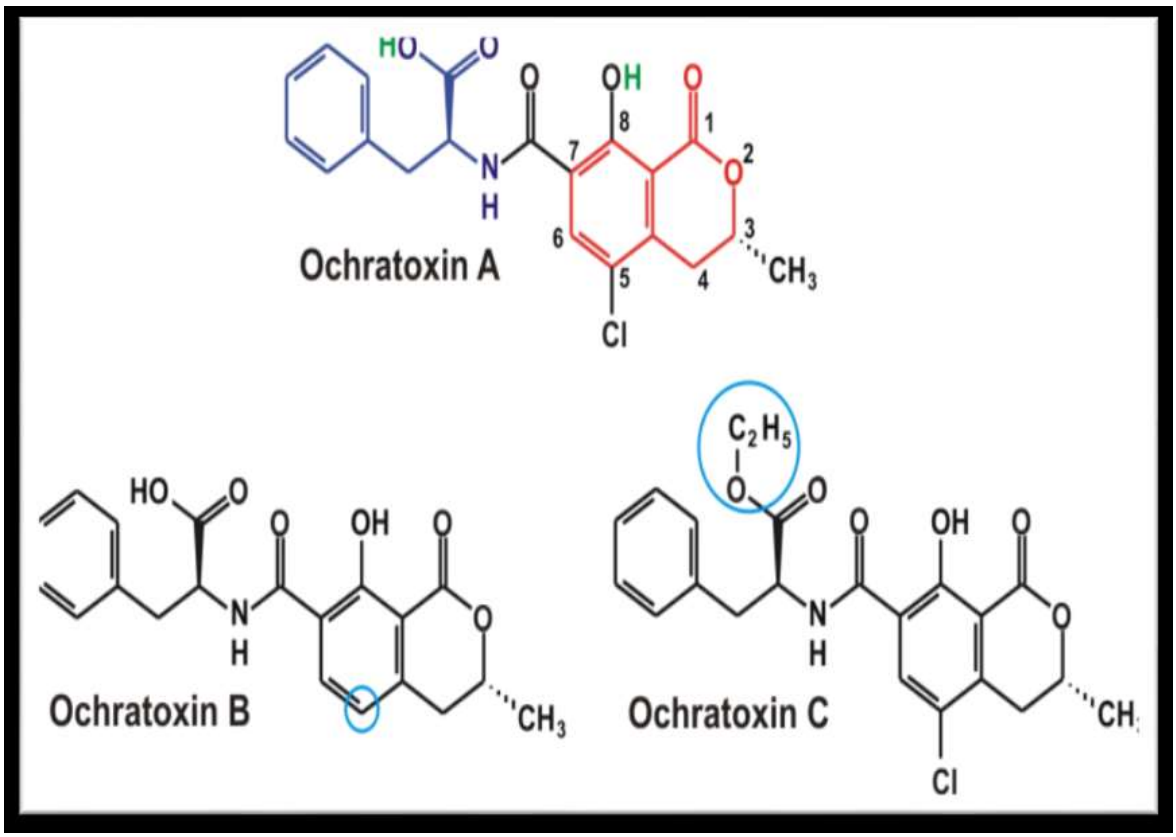


Figure (1-3)The chemical structure of Ochratoxin A, B and C. (Koszegi, and Poór, 2016).

1.2.2.2. Chemical and physical characteristics of ochratoxin A

Crystal white solid material that doesn't smell. bushy fluorescent in ultraviolet rays light. If it is placed in alkaline and acidic solutions, it emits green and blue fluorine, in succession. This is attributed consist two different formats, comprising Open or closed-loop of lactone in a row **(Pohland *et al.*, 1982; john *et al.*, 2012).**

Ochratoxin A dissolves in acidic conditions in polar organic solvents such as alcohol, ketones, and chloroform and is slightly soluble in water and is insoluble in protein ethers and saturated hydrocarbons. In basic media, it is dissolved in aqueous sodium bicarbonate and all basic solutions. Therefore, it is difficult to remove it from food contaminated with it, and Ochratoxin A is weak organic acid due to its toxicity due to the phenolic hydroxyl group in the Dihydroisocoumarin ring **(Müller, 1983; Nwagu and Ire, 2011).**

Under alkaline circumstances, the lactone ring opens, although the process is reversible. Treatment with an excess of sodium hypochlorite totally degrades OTA solutions. **(john *et al.*, 2012).**

Under typical cooking circumstances, OTA is partly degraded **(Müller, 1983),** The water activity of the medium determines the stability of OTA during heating conditions**.(Subirade, 1996; Van der Stegen *et al.*, 2001).**

It melts at 159 °C from benzene-hexane and 169 °C from xylene after recrystallization. Upon recrystallization, (**Van der Merwe *et al.*, 1965),** But after an hour of drying at a temperature of 60 C^c, it melts at 168 - 173 C^c **(Pohland *et al.*, 1982).**

The ultraviolet (UV) spectrum. Extinction coefficients of **37.2 10 $^{-3}$, 0.89 10 $^{-3}$, and 63.3 10^{-3} L.mol–1.cm–1** have been recorded at maximum wavelengths of **214, 282**, and **332 nm**, respectively. **(Cole and Cox, 1981).**

Rotation that is specific. [α]20 D –118° **(c = 1.1 mmol/L in chloroform) (Van der Merwe *et al.*, 1965); [α]21 D –46.8° (c = 2.65 m mol/L in chloroform) (Pohland *et al.*, 1982).** studied **Cagnasso *et al.*, (2019)** the behavior of OTA in water in the pH range 1.0–12.5. The findings show that depending on the pH, four forms interfere, ranging from neutral to trianionic. To address the lack of literature, separate spectroscopic methods were used to determine pKa1,2. The most likely degradation process was then determined using Density Functional Theory (DFT) simulations, The lactone ring begins to hydrolyze in less than an hour, but the breakdown process does not lead to fragmentation until two hours later. This process has not yet been finished after one week. The products of the re-acidification process were also examined. If acidic conditions are quickly restored, OTA breakdown can be reversed, resulting in the formation of a toxic substance once more.

1.2.3. Absorption, Tissue distribution and accumulation, Metabolism and Excretion of OTA

1.2.3.1.Absorption

The OTA is quickly absorbed. The amount of binding to plasma proteins determines the half-life in plasma **(john, *et al.*, 2012).**

The kidney has been shown to be the most highly polluted tissue with OTA in pigs, with levels in the blood being roughly 5-fold higher than in the kidney **(Krogh *et al.*, 1976)**.

OTA and its metabolites are reabsorbed by the kidneys and excreted in the urine, as well as cycled via the enterohepatic system and removed in feces. Microorganisms in the gastrointestinal tract can convert hazardous metabolites to less toxic compounds. Furthermore, ochratoxin A and B can be metabolized by cytochrome P450 enzymes found in a variety of organs **(john *et al.*, 2012).**

In a number of animals, OTA is absorbed in significant amounts from the gastrointestinal tract. (GI) After oral administration, absorption into the systemic circulation ranged from 40% in chickens to 56 percent in rabbits to 66 percent in pigs**. (Galtier, 1977).**

According to studies on OTA absorption, the toxin may be detected promptly in peripheral blood, with the highest concentration in the blood after oral administration of OTA occurring **(Suvi *et al.*, 2020),** The rate of OTA absorption varies greatly between species, and the time to maximal blood concentration after a single oral dose of OTA has been observed to be 0.33ng/ml hours in chickens and 1 hour in rabbits. **(Galtier *et al.*, 1981),** in ruminant

calves, it takes 2–4 hours **(Sreemannarayana *et al.*, 1988),** 4–10 hours in rats **(Suzuki *et al.*, 1977; Galtier, 1977**).

According to several research, the stomach is the primary source of absorption in the GI tract for most animals. is the, since OTA is uncharged and hence more lipid-soluble under acidic circumstances. **(Roth *et al.*, 1988).**

The authors of research in which radiolabelled OTA was administered orally to mice proposed this location of absorption as the major route of uptake. However, the findings of this study are ambiguous, and they may be read as indicating that OTA moves quickly from the stomach to the small intestine, with absorption occurring in the intestine rather than the stomach **(Fuchs *et al.*, 1988).**

While other studies using animals with ligated intestinal loops suggest that the main site of absorption in the digestive tract is the small intestine, where the most absorption took place in the proximal jejunum **(Kumagai and Aibara, 1982).**

The biliary excretion of OTA has been demonstrated, and examination of intestinal contents and serum following single doses of OTA to rats indicated additional peaks in OTA concentration profiles over time. These findings show that OTA is circulated via the enterohepatic system **(Fuchs *et al.*, 1988; Roth *et al.*, 1988).**

1.2.3.2.Tissue distribution and accumulation

OTA that has been absorbed attaches to blood proteins like albumin and travels throughout the body **(Hagelberg *et al.*, 1989).**

Because OTA binds strongly to human serum albumin and shares a binding site with other known anionic substances such as warfarin, naproxen, and phenylbutazone, there is a risk of OTA–drug interactions **(Il'ichev *et al.*, 2002).**

The kidney is reported to have the greatest tissue concentrations in pigs and chickens, followed by the liver, muscle, and fat in that order, Or muscle first, followed by the liver, then fat **(Madsen *et al.*, 1982; Harwig *et al.*, 1983; Mortensen *et al.*, 1983; Jorgensen and Petersen, 2002).**

After subcutaneous injection of a single dosage of radiolabeled OTA, just 0.1 percent of the dose was detected in the fetus of pregnant rats, with maximal OTA levels reaching 2–3 days after treatment **(Ballinger *et al.*, 1986).**

Following the administration of large dosages of OTA to chickens at a rate of 10 mg/kg feed, OTA has been found in eggs **(Juszkiewicz *et al.*, 1982).** unless at lower doses of 1 mg/kg body weight **(Piskorska-Pliszczynska and Juszkiewicz, 1990).**

OTA reduces egg production in hens in a dose-dependent manner, perhaps resulting in fewer eggs from exposed chickens entering the food chain. Increased egg-shell staining has also been linked to hens' use of OTA, leading in decreased egg salability **(Page *et al.*, 1980).**

OTA has been found to pass the placenta, causing the fetus to be exposed to contamination with OTA **(Fukui *et al.*, 1987).** In a limited study on a pregnant pig, this was proven, The blood levels in the mother of a pregnant pig fed naturally contaminated feed was 0.2 ng/ml, whereas the concentration in the progeny varied from 0.075–0.12 ng/ml at delivery **(Barnikol and Thalmann,**

1988). Subsequent studies about OTA in the offspring did not reveal the same result **(Patterson *et al.*, 1976; Mortensen *et al.*, 1983).**

It has been proven that OTA moves into the milk of some animal species such as rabbits, rats, and humans to milk **(Ribelin *et al.*, 1978),** OTA is carried over into the milk of dairy sheep to the tune of 1% **(Niderkorn *et al.*, 2006).** In ruminants, however, transfer to milk is limited. **(Ribelin *et al.*, 1978),** Although certain exceptions may arise if cows are consuming significant amounts of OTA, it may be quickly hydrolyzed by the rumen bacteria and pH to a less harmful metabolite, the ochratoxin α, at low or moderate doses of OTA **(Breitholtz-Emanuelsson *et al.*, 1993; Skaug, 1999; González-Osnaya *et al.*, 2008).**

OTA transfer to milk was shown to be effective in research **(Hallen *et al.*, 1998),** with estimated milk: plasma concentration ratio of 0.6 in female rats exposed to OTA. Similarly, the milk: blood concentration ratio was 0.4 at 24 hours and 0.7 at 72 hours in lactating rats given a single dose of OTA, showing that transfer to milk is very fast **(Breitholtz-Emanuelsson *et al.*, 1993).**

OTA has been found in the blood and kidneys of rats and rabbits' offspring in perinatal investigations **(Hallen *et al.*, 1998; Ferrufino-Guardia *et al.*, 2000).**

Micco's *et al.*, (1991 and 1995), two human researches reveal that OTA is transmitted to human milk as well. In the first research, OTA concentrations in breast milk samples obtained from nursing women ranged from 1.7 to 6.6 ng/ml in nine out of fifty samples. In the second research, OTA contamination was found in 22 of 111 breast milk samples, ranging from 0.1 to 12 ng/ml.

However, because neither research revealed the mothers' OTA exposure, an estimate of OTA transfer efficiency to breast milk could not be calculated **(Aish, 2004).**

In a study of samples from 40 women, **(Breitholtz-Emanuelsson *et al.*, 1993)** found that the concentration of OTA in human milk was around 10-fold lower than that in human blood **(concentrations varied from Milk 10–40 ng/L and blood 90–940 ng/L in milk and blood, respectively).**

1.2.3.3. Metabolism of Ochratoxin A

Understanding the biotransformation and metabolism of mycotoxins in the human body, animals, plants and microorganisms is very important for food safety assessment and control of food and feed contamination with these toxins **(Qinghuawu *et al.*, 2011).**

Thus, clarifying the stages of Ochratoxin A metabolism in animals and humans is very useful for determining food safety. Ochratoxin A can be metabolized in plants, which causes health problems in the digestive system of animals and humans who eat these plants **(Mally *et al.*, 2004).**

Ochratoxin A (OTA) is metabolized in a variety of ways in rodents, especially mice. Ochratoxin A (OTA) is metabolized in the kidneys, liver, and intestines. The important excretion pathways are in urine and feces. The main metabolites of OTA in rodents are: OTA, 4(S) - OH - OTA, OH - OA- 10 - 4(R), OTB, 4-OH-OTB, OTHQ, OP – OTA. Ochratoxin alpha is one of the most important non-toxic metabolites that can be synthesized by the enzyme carboxy peptidase. 4(R)-OH-OTA is the major hydroxy metabolite in mice, while 10-OH-OTA is

the major hydroxy metabolite in rabbits and is catalyzed by cyp450**. (Størmer, *et al.*, 1981).**

In ruminants that are more resistant to mycotoxins including Ochratoxin A than in non-ruminant animals, Ochratoxin A is mainly degraded by bacteria and microorganisms to Ochratoxin A alpha and phenylanine **(Hult *et al.*, 1976).**

Ochratoxin A can be converted by stage I or II enzymes and most metabolites show little or no intestinal toxicity. Ochratoxin A is broken down to Ochratoxin Alpha by bacterial enzymes **(Madhyastha *et al.*, 1990).**

Ochratoxin A can be hydrolyzed by opening the lactone ring in alkaline conditions, which leads to the formation of a highly toxic compound called lactone - opened OTA. As for the 4-OH-OTA 4-hydroxyl Ochratoxin A compound, it is a product of microsomal oxidation of the toxin with low toxicity **(Størmer *et al.*, 1980).** Another less toxic compound is Ochratoxin A 10-hydroxyl **(10-OH-OTA) (El-Adlouni *et al.*, 2006)**.

Some studies indicate that after dechlorination, Ochratoxin A is converted to Ochratoxin B with less genotoxicity **(Faucet-Murquis *et al.*, 2006; Hadjeba *et al.*, 2012).**

Other metabolites derived from Ochratoxin A have been observed in the tissues, blood and urine of animals and humans, namely hex/pen glucuronide - OTA and glutathione OT - GSH **(Tozlovanu *et al.*, 2012).**

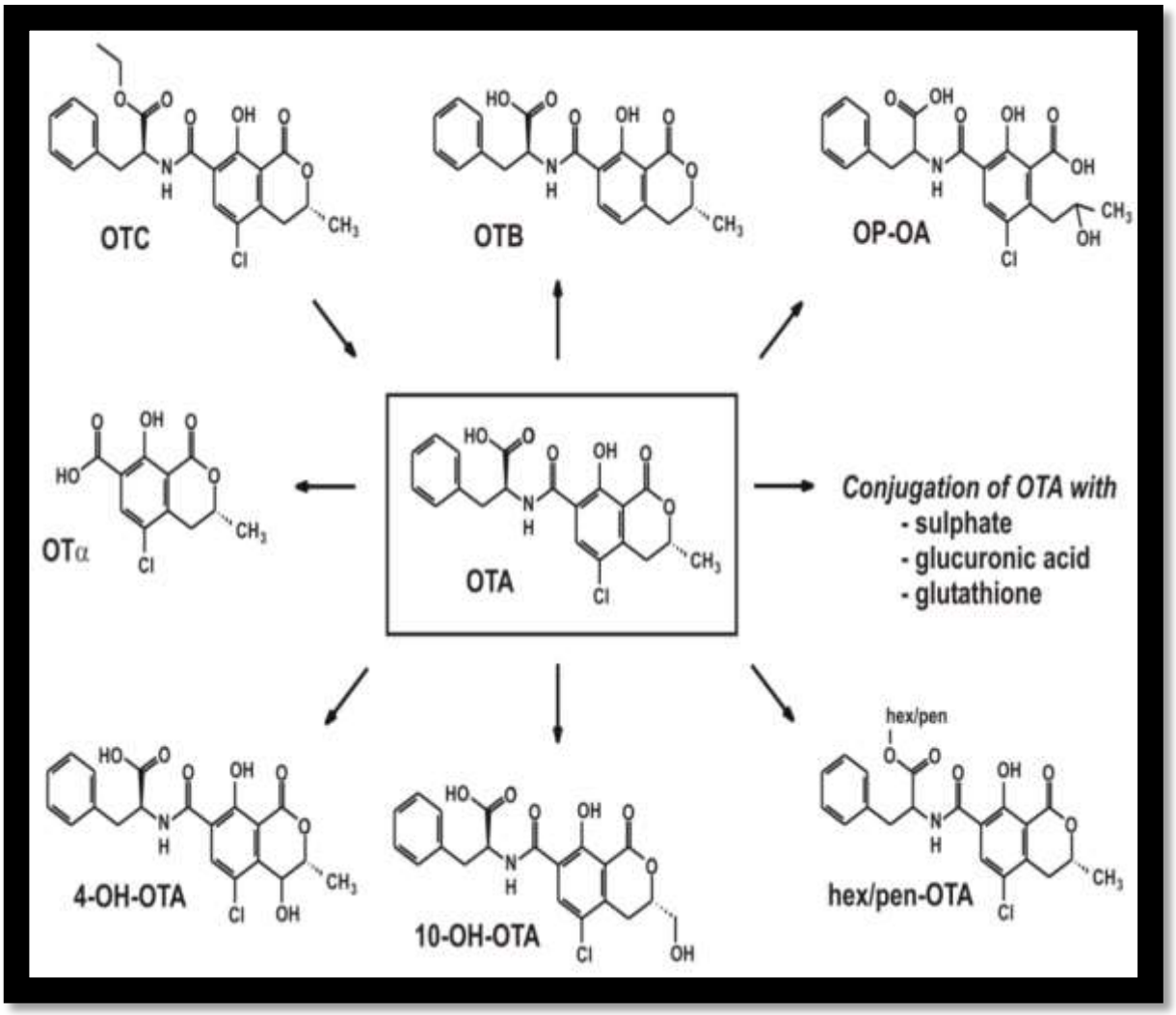


Figure (1-4): The stages of metabolism of Ochratoxin A (Koszegi and Poór , 2016)

1.2.3.4. Excretion:

OTA is slowly being eliminated in the majority of animals. The major routes through which OTA is removed from the blood through the bile duct into the feces or via glomerular filtration into the urine **(Aish, 2004).**

Following oral administration of OTA, OchratoxinA, and the 4R-OH-Ochratoxin A epimer was found in the urine and feces of rats. In the feces, OTA

and Ochratoxin accounted for 12 percent and 9 percent of the original dosage, respectively, whereas ochratoxin and OTA accounted for 25–27 percent and 6 percent of the dose in the urine**(Storen *et al.*, 1982).**

Hydrolysis of ochratoxin conjugates by intestinal bacteria is crucial in hepatoenteric recycling in some animals. Fecal excretion can account for more than 50% of the dosage administered in goats and calves, while less than 10% of the dose was excreted as Ochratoxin-A **(OTα)** in sheep **(Nip and Chu, 1979; Sreemannarayana *et al.*, 1988).**

there may be significant variations across species in the percentage and rate of OTA clearance by the bile duct and kidney **(Aish, 2004),** because Effect some factors affecting the amount of OTA removed from the bloodstream as enterohepatic circulation and serum protein binding influence. OTA is excreted in the bile and reabsorbed from the intestine by enterohepatic circulation**.** In laying hens, biliary excretion of OTA is a primary excretory pathway for orally given OTA**.** Two studies have looked at the mechanism of OTA clearance. OTA transport is conducted on the apical side of the proximal tubule by human organic anion transporter 4 **(hOAT4).** and the basolateral side of the proximal tubule by the hOAT1 and 3 transporters**(Armorini *et al.*, 2015).**

1.2.4.Ochratoxin A in Foods and Feeds

In The opinion of the Scientific Panel on contaminants In The Food chain on a request from the commission related to Ochratoxin A In food, clarified that: OTA contaminates many foodstuffs such as grains, grain products, pulses, coffee, beer, grape juice, dry grape fruit, wine, cocoa products, nuts and spices, Contamination of animal feed with OTA may result in the presence of residues in blood serum and edible offal, OTA contamination of meat, milk and eggs is achieved, but in a small percentage, not the same as in agricultural crops, which is a large percentage. Additional EU Maximum Levels for Ochratoxin A on the Horizon maximum levels (MLs) have been in place for OTA in foodstuffs such as cereals, dried vine fruit, coffee and some spices for several years. Recently, high levels of OTA were found in some other foods. In response to these findings, the Commission has proposed setting MLs for these commodities, by evaluating some occurrence data for these foods. The following MLs have been proposed **(Gerda, 2017).**

We will clarify these levels according to what is stated in European union regulations in the following table**.**

Table (1-2) European union regulations for Ochratoxin A in food maximum levels (µg/Kg)

	Food	OTA
1	Soluble coffee, dried vine fruit (currants, raisins and sultanas). liquorice placed on the market for the final consumer. herbs and herbal teas.	10 µg/kg
2	Unprocessed cereals, roasted coffee beans and ground roasted coffee, excluding soluble coffee	15 µg/kg
3	sunflower and pumpkin seeds, pistachios, hazelnuts or all tree nuts	5 µg/kg
4	cocoa powder, Wine, fruit wine, all beverage from grape.	2 µg/kg
5	Baby foods for infants and young children.	0.5

(Gerda, 2017; Schrenk *et al.*, 2020).

The highest percentage of ochratoxin A food poisoning was reported in cereal grains, while the lowest percentage was in other foodstuffs such as chocolate, spices, and coffee. It may also toxinanimal products such as meat, dairy, and tissues.

1.2.5. Effects Of Ochratoxin A

1.2.5.1.Biochemical Effects Of OTA

Because of its structural closeness to the important amino acid phenylalanine, OTA has a biological impact. The main impact appears to be protein synthesis inhibition. However, RNA and DNA synthesis inhibition has also been linked to its mode of action. Because of its Phe moiety, OTA can bind to the active site of Phe-tRNA synthetase and block amino acid acylation and peptide elongation competitively. Phe is missing or substituted with other amino acids, such as tyrosine **(Tyr),** in analogues of OTA that either do not inhibit amino acid tRNA synthetase or inhibit the amino acid-specific tRNA synthetase **(Aish *et al.*, 2004).**

OTA, on the other hand, is a weak inhibitor of Phe-tRNA, with binding affinity orders of magnitude lower than Phe, ranging from 300-fold lower in yeast **(Km = 1.3 mmol/L for OTA compared to 3.3 mol/L for Phe**) **(Creppy *et al.*, 1983)** to 20-fold lower in rat liver cells Km = 0.28 mmol/L for OTA compared to 6 mol/L for The inhibition of the enzyme by OTA can be easily reversed by adding Phe **(Zanic-Grubisic *et al.*, 2000).**

The mortality of a single intraperitoneal injection of OTA was totally avoided by administering phenylalanine at the same time**.** However, the lower relative effectiveness of OTA compared to Phe may be compensated by the higher concentrations of OTA in cells, since an in vitro research found that OTA influx into cells is efficient, with OTA concentrations 200–300-fold higher within cells compared to the culture media **(Creppy *et al.*, 1983).**

Other enzymes that employ Phe as a substrate can also interact with OTA. In vitro studies revealed that OTA inhibits phenylalanine hydroxylase, an enzyme that catalyzes the irreversible hydroxylation of Phe to Tyr, a critical step in Phe catabolism. However, the interaction with this enzyme is rather weak, with apparent inhibition constants of Ki = 12 M for liver and Ki = 13 M for kidney phenylalanine hydroxylase reported, similar to the interactions with Phe-tRNA synthetase OTA has been hypothesized to cause lipid peroxidation and the production of superoxide and hydrogen peroxide radicals, which is unrelated to its structural resemblance to Phe. Experiments have demonstrated that following oral administration of OTA, ethane exhalation, a measure of lipid peroxidation, rose seven-fold in rats, supporting this impact on radical generation **(Zanic-Grubisic *et al.*, 2000).**

Furthermore, **Meki and Hussein (2001)** found that when OTA **(250 g/kg body weight)** was given orally four times a day for four weeks, levels of malondialdehyde **(MDA),** a lipid peroxidation product, were substantially higher in blood, liver, and kidney tissues than in control rats. Gautier ***et al.,*** (2001b) also found that giving rats OTA **(1 mg/kg body weight)** raised the amount of haem oxygenase-1, an oxidative stress-responsive protein, in the kidney five-fold, but MDA levels in plasma, kidney, and liver remained constant.

It's unknown how OTA may cause lipid peroxidation to happen. OTA, on the other hand, is thought to form a complex with flavoprotein-bound iron **(Fe3+)** to promote Fe2+ reduction and subsequent oxygen binding. This might result in the production of oxygen radicals in the long run **(Omar *et al.*, 1990).**

However, decreasing the amounts of chemicals or enzymes that eliminate reactive oxygen species might be an alternate method. When OTA-treated rats

were compared to controls, glutathione **(GSH),** glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase, and glutathione-S-transferase levels were substantially lower in the liver and/or kidney **(Meki and Hussein, 2001).**

1.2.5.2. Toxic Effects of Ochratoxin A

After the OTA toxin enters the animal or human body by swallowing the OTA toxin directly or indirectly or inhaling it, then the OTA toxin is absorbed by the digestive system, and it binds with the blood protein **(Ringot *et al.*, 2006),** The blood contains proteins, the most important of which are globulins and albumin, and the OTA toxin binds with albumin. albumin or serum albumin and It is often referred to as albuminuria is a protein produced in the liver. And there is blood plasma, which makes up 50% of human plasma Maintains the osmotic pressure of the blood compartment **(Nicholson and Wolmarans, 2000).**

Exposure to any type of mycotoxin causes harm to humans and animals, and as for Ochratoxin toxins, The presence of OTA in the human and animal body causes several effects, OTA is the most harmful to the body, and has many effects such as Toxicity; Renal Toxicity; Neurotoxicity; Immunotoxicity; Reproductive toxicity and Carcinogenicity. The presence of OTA toxin in the human body causes several toxic effects and has been defined Toxicity as concatenation of events When the body is exposed to the influence of a toxic chemical, that evolves with metabolism and food distribution, Then it interacts with various cell molecules such as protein and DNA, leading to different toxicological results **(Singh, *et al.*, 2018).**

Nephrotoxicity

Following the development of oxidative stress in renal cells, OTA appears to have a strong nephrotoxic impact. The kidney is the most common location of OTA toxicity, with the middle (S2) and terminal (S3) portions of the proximal convoluted tubules being the most affected **(Jung *et al.*, 1989).**

Nephrotoxicity is defining as rapid deterioration in the kidney function due to toxic effect of medications and chemicals**.(Ma *et al.*, 2019).**

Because of the high binding of this toxin to albumin, the first demonstration mechanism of OTA nephrotoxicity indicated that tubular secretion is the primary mode of OTA excretion. Its clearance via glomerular filtration is minimal, and greater levels in proximal tubular cells might be due to the reabsorption of tubular toxins. In the latter, OTA raised the concentration of reactive oxygen species **(ROS),** which resulted in an increase in the production of 8-oxoguanine **(Tao *et al.*, 2018).**

After inhibiting the erythroid oxidative stress response pathway 2-like 2 **(Nrf2)** and its Keap-1 inhibitor, glutathione production, oxidized glutathione recycling, and oxidoreductase activity are all reduced, making cells and tissues more susceptible to oxidative stress **(Limonciel *et al.*, 2014).**

in **Loboda *et al.*, (2017)** found that OTA causes nephrotoxicity in porcine tubular epithelial cells after Nrf2 inhibition, with increased expression of profibrotic, proinflammatory, and proapoptotic factors and decreased levels of claudin-2 and vascular endothelial growth factor, as well as decreased claudin-2 levels and vascular endothelial growth factor.

Furthermore, OTA inhibits regucalcin, a gene involved in calcium homeostasis. By increasing cytosolic calcium, disruption of Ca 2 homeostasis plays a key role in renal cell death **(Klaric *et al.*, 2012).**

In vivo research in laboratory animals revealed that OTA caused histological kidney damage in pigs, as well as reduced T-cell viability, decreased IL-2 concentrations, and increased TNF concentrations. Treatment with OTA did not cause lipid peroxidation or an increase in 8-oxo-7,8-dihydro-20-deoxyguanosine **(8-OHdG)** in the kidneys in male F344 rats, but it did cause a significant rise in trimethylamine N-oxide excretion in the urine **(Gan *et al.*, 2017).**

Low doses of OTA and CIT, alone or in combination, caused apoptosis and lipid peroxidation in rabbit kidneys, which appears to play a key role in nephrotoxicity characterized by nuclear fragmentation and cytoplasmic flow **(Kumar *et al., 2014).***

In birds, OTA causes microscopic, degenerative, necrotic, and inflammatory changes in the kidney, and the ultrastructure revealed remarkable and consistent changes in proximal convoluted tubules **(PCT),** with severe mitochondria and reticulum endoplasmic lesions **(Patial *et al.*, 2013).**

In pig kidneys, the effects of PCV2 infection on OTA-induced nephrotoxicity were studied. The toxin-induced nephrotoxicity was exacerbated by PCV2 infection, as evidenced by p38 phosphorylation and autophagy as evidenced by the expression of Atg5, LC3 II, and p62 proteins. Induction of p38 phosphorylation, annexing V/PI binding, caspase-3 activation, and autophagy in PK15 cells were all seen in the in vitro research **(Gan *et al.*, 2018)**

In vitro nephrotoxicity studies in PK15 cells and primary porcine splenocytes revealed that dosages of 2.0e8.0 and 0.5e4.0 mg/mL for 24 hours caused cytotoxicity and apoptosis through p38 and ERK activation and phosphorylation **(Gan *et al.*, 2017)**

When LLC-PK1 cells were treated with OTA, the activity of superoxide dismutase **(SOD)** did not alter at the mRNA level, but its enzymatic activity dropped. OTA reduced the levels and activity of GST and gamma-glutamylcysteinyl synthetase mRNA, as well as transactivation of activator protein 1 (**AP- 1**) and Nrf2-associated factor 2, which drive GST gene transcription **(Boesch *et al.*, 2008; Boesch *et al.*, 2009)**

Several studies indicate that OTA may be a cause of nephropathy in the Balkan countries **(BEN),** This disease is known as endemic nephropathy in the Balkans, and it is a renal disease that occurs in rural communities in the vicinity of the Danube River in Romania and Bulgaria **(Castegnaro *et al.*, 1987).**

Females are more impacted than males in this disease **(Chernozemsky *et al.*, 1977)**. While the start of the illness may go unnoticed, the decrease in kidney function becomes apparent as the condition progresses. The main symptom is severe nephropathy, which is frequently accompanied by urinary tract malignancies. Only symptomatic treatment, including hemodialysis, is available for this. BEN develops progressively up to death over the course of 5 to 10 years **(Martin, 2001; Stoyanov, *et al.*, 2021).**

the World Health Organization (**WHO**) reviewed critically the available data and provided the following description of the disease Insidious start of progressive and very slowly progressing renal failure. It generally occurs without a nephrotic syndrome or hypertension. Anemia is present, as is

moderate proteinuria and a little urine deposit. The kidney concentration power is reduced in all cases and is out of proportion to the degree of glomerular filtration restriction; non-inflammatory interstitial nephropathy with heavy tubular epithelial damage and only late and secondary destruction of the glomeruli, eventually leading to extreme renal contraction... The last stage is distinguished by fibrosis, particularly in the outer zone of the cortex, where tubules are completely absent and numerous fibrotic glomeruli are present. Chronic and/or acute inflammation is not common at this time **(Fuchs and Peraica 2005)**

1.2.6. Methods for detecting mycotoxins:

The methods for detecting mycotoxins are of two main types, which are analytical methods and immunological methods **(Aycicek *et al.*, 2005).**

1.2.6.1. Analytical methods:

To determine mycotoxins in food and feed, there are several different analytical methods, including high-performance liquid chromatography **(HPLC)** and thin layer chromatography **(TLC) (Azizollahi *et al.*, 2013),** and chromatography is the most widely used method for the analysis Mycotoxins **(Shephard, 2016)**

1.2.6.1.1. Thin-layer chromatography (TLC)

It is the first method that has been used in the process of identifying mycotoxins and is still used to the present time, and this technique is used in many laboratories as a rapid visual examination of some mycotoxins such as **(AFB1, AFG1, OTA, AFM1),** which allows a qualitative classification either if it is combined with a density It allows a semi-quantitative division **(Li *et al.*, 2017),** and since mycotoxins are polar compounds, they are often separated on silica plates using a group of mixtures of organic solvents that represent the mobile phase, and the detection is done by placing the chromatographic slide inside a suitable tank for organic solvents. Such as acetone, acetic acid, chloroform and ethermethanol **(Ismail, 2014),** then these solvents move up to the top on the solvent slide by capillary action and when it reaches a certain limit, the slide is taken out of the tank and then ultraviolet rays are used to detect these toxins, which appear in the form of colored spots **(Aycicek *et al.*, 2005**),

and the RF **(flow coefficient)** can be calculated through the fluorescence intensity of the sample and its volume measurement depending on the distance traveled by the solvent **(Ismail, 2014).**

One of the disadvantages of this technique is its inaccuracy, and the toxins that are less than 1 mcg/kg cannot be detected by this technique **(Papp *et al.*, 2002),** and one of the advantages of this technique is that it is fast, inexpensive, simple and easy to use **(Sotanaphun *et al.*, 2009)**

1.2.6.1.2. High-performance liquid chromatography (HPLC)

It is an important qualitative and quantitative technique used in various fields of research and industries and to estimate pharmaceutical and biological samples **(Malviya, 2010),** and this technique is currently used to identify and detect mycotoxins **(Wacoo, 2014).** The mobile phase consisted of solvent A (methanol/water, 80:20, v/v) **(Liu *et al.*, 2006)**

The sample to be analyzed is placed at the top of the column and distributed between two phases **(fixed and mobile).** The components of the samples are separated depending on the degrees of solubility of the compound in the mobile phase and the degrees of adsorption of the sample compounds and molecules of the stationary phase**.** Calculation of the time period by calculating the detention time of the compound compared to the retention time of the appearance of curves for the standard material**,** and the area and height of the curve of the compound are measured and plotted on a graph **(Mousa *et al*., 2013).**

The sample in this technique needs to be cleaned before being injected into the separating column, and it is complicated and expensive **(Ismail, 2014).** As for its advantages, it is considered a technique that is characterized by accuracy, speed, and sensitivity to very low concentrations **(Herzallah, 2009).**

1.2.6.2. Immunological methods:

ELISA (Enzyme Linked Immun Sorbent assay)

It is a diagnostic technique that is widely used in medicine and industries to monitor and is also used in medical and biological research **(Gan and Patel, 2013)**, and this technique can be carried out in a direct and indirect way and has been widely used in the detection of mycotoxins, especially the direct method **(Chu, 1996),** and that the direct method consists of an immunoassay plate coated with antibodies, while the indirect method is the conjugated toxin protein that is used to coat the plate for immunoassay **(Aycicek *et al.*, 2005).**

The principle of ELISA is based on the interaction of antibodies with the base material of mycotoxins, where the toxin competes with the enzyme accompanying the toxins in the standard kit. As for the indirect method, the conjugated enzyme is linked and the base material is converted into a chromatic fluorescence to be measured using this technique **(Gheorghe *et al.*, 2008)**. and is measured at a wavelength of 450 nm and the higher the concentration of mycotoxins, the lower the absorption **(Ibrahim, 2016)**

This technique is characterized by rapid antigen detection, and the standard kit for this technique is available, easy to use, low in cost and with high accuracy **(Nguyen *et al.*, 2019).**

1.2.7. Level OTA in blood serum

In human blood levels in Tunisia has demonstrated a likely rate of human contamination 82% of positive sample in the general population (3.5 ± 6.8 ng/ml) at the detection limit of (0.1ng/ml) and 100% in cases of whatever the etiology these percentages are lower when the detection limit is set to (1ng/ml). These results led us to search for a possible association of the presence of OTA in human blood with cases of nephropathy as has been found in the Balkan peninsula . The first study was performed in the Sahel region in Tunisia surrounding the city of Monastir .it showed that a certain number of people having chronic interstitial nephropathy of unknown etiology were OTA positive **(Achour *et al.*, 1993).**

In Italy Breitholtz-Emanuelsson *et al.*, (**1994**) found significantly higher OTA concentration in blood of patients with renal stones or cysts. Get 1.4 ng\ml while it was in chronic renal insufficiency 0.60 ng\ml but in the blood of healthy persons was 0.53 ng\ml.

Otherhand the OTA concentration in blood of dialysed patients was 1.97 ng\ml but in blood of healthy persons was 0.71 ng\ml (**Jimenez *et al.* 1998).**

In the blood of patients with bladder cancer **Ozcelik *et al.*, (2001)** found significantly higher concentration of OTA 2.1 ng\ml than in control 0.4 ng\ml. while **Grosso *et al.*, (2003)** did not find this in a group of patients with urinary tract tumours of different localization.

In France, in two patients with chronic renal failure, OTA found in the blood at very high concentration get 205 ng\ml and 367 ng\ml in blood (Fillastre, 1997).

Wafa *et al.,* (1998) showed that the maximum OTA level in healthy controls was0.91 ng\ml and 10.15 ng\ml in patients with nephropathic syndromes in Egypt.

Malir *et al.,* (2001) illustrated that OTA concentration in blood were higher in patients with chronic renal insufficiency treated with dialysis than in healthy individuals indicting that OTA accumulation due to renal failure cannot be eliminated with dialysis. This is confirmed by the lack of the difference in OTA concentration when measured before and after dialysis in patients with chronic renal insufficiency.

Fuchs and peraica (2005) showed that Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin that is thought to be implicated in the aetiology of endemic nephropathy in the Balkans (BEN). The frequency of this human deadly illness in Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Serbia, and Monte Negro is linked to a relatively high incidence of usually uncommon renal pelvis and ureter urothelial tumors. Despite the fact that OTA was identified more frequently and/or in greater concentration in the food and blood of residents in BEN-affected areas than in other areas, the role of OTA in the development of BEN remains unknown. Dialysis patients with chronic renal failure had a higher blood factor.

Table (1-3) OTA in blood of patients with other kidney diseases

County	Positive\analyzed (%)	Mean (ng\ml)	Range (ng\ml)
Czech Republic			
H	**1514\1620 (93)**	**0.25**	**0.1-13.7**
D	**-\99**	**0.8****	**0.05-0.40**
CRI	**-\103**	**0.4****	**0.05-3.10**
Italy			
H	**-\65**	**0.53**	**0.12-2.0**
D	**-\28**	**1.4***	**0.18-14.0**
CRI	**-\13**	**0.60**	**0.20-1.40**
Spain			
H	**40\75 (53)**	**0.71**	**0.52-4.0**
D	**58\72 (78)**	**1.97****	**0.52-11.7**
Tunisia			
P	**62\62 (100)**	**0.53**	**0.12-8.06**
CRI	**23\26 (88)**	**0.99**	**0.11-5.80**
UTT	**15\21 (71)**	**0.26**	**0.14-0.74**
Turkey			
H	**-\40**	**0.4**	**0.19-1.43**
D	**-\35**	**2.1*****	**0.6-5.5**
UTT	**-\15**	**1.08****	**0.4-25**

*** H, healthy volunteers or blood donors; CRI, chronic renal insufficiency; D, dialysed patients; P, patients hospitalized for other than kidney diseases; UTT, urinary tract tumours; *p<0.01; **p<0.001; ***p<0.0001.**

(Breitholtz-Emanuelsson *et al.*, 1994; Jimenez *et al.* 1998; Malir *et al.*, 2001; Ozcelik *et al.*, 2001; Grosso *et al.*, 2003; Fuchs and Peraica, 2005).

2.1.Methodology

2.1.1. Study design

The present study included a case-control study from November 2020 even February 2021. Samples were selected from the patients attending the medical City–Kidney transplant Center **Ghazy AL–Hariri Hospital for Surgical Specialities\ Baghdad.** The sociodemographic aspects of the patients were collected through the self–reported technique (**student Questionnaire**) including age, genetic history of family, stages of nephropathy, and having any current chronic diseases such as nephropathy. They were also exposed to a medical examination for signs and symptoms of nephropathy by the subspecialized doctor based on the World Health Organization (**WHO**) criteria.

For relationship purposes, patients were divided into certain etiology of nephropathy. patient groups were compared to a group who do not have a disease (**apparently healthy**) as a control subject.

Table (2-1) laboratory equipment and origin

No	used laboratory equipment	Manufacturer and Origin
1	Centerifuge	Hettich/EBAZO,Germany
2	Incubator	GallemKamp,Englan
3	Autoclave	GallemKamp,England
4	Water Distiller	Dahin,Korea
5	Refrigerator	Concord,Lebnano
6	Electric shaker	Bioneer,England
7	C4000,Architect	Abbtt, Made in Japan
8	HPLC	SYKAM,Made in Germany
9	Heamatology auto analyzer CBC	NIHON KOHDEN, Made in Tokyo Japan

Table (2-2) Materials and Instrument used in the study

NO	APParatus	Manufactures
1	Test tube	China Mhco,China
2	Test tub rack	China Mhco,China
3	Stand	China Mhco,China
4	Beaker	Volac,England
5	Volumetric Flask	Volac,England
6	Micropipette (100_1000uL)	HUMAN Humapette,Germany
7	Micropipette (10_100ul)	HUMAN Humapette,Germany
8	Tube Plain Without Additives	AFCO-DISPO,Made in Jordan
9	EDTA tube	Plastilab,china
10	Gel tube	Plastilab,china
11	Syriges (10)ml	Provi,Made in PRC
12	Medical cotton	Medicicare Hygiene Limited,Lebanon

13	Tip Micropipette (10_100ul)	AFCO-DISPO,Made in Jordan
14	Tip Micropipette (100_1000uL)	AFCO-DISPO,Made in Jordan
15	Latex Examination Gloves powdered	RZ,Mad in Malaysia
16	Ethanol 70%	Scharlau-Spain
17	Distilled Water for HPLC	Scharlau-Spain
18	Acetonitrile	Supelco,Made in USA
19	Phosphate buffer pH7.4(PBS)	BDH,ENGLAND
20	Sonic bath	FALC,INSTRUMENTS,Made in Italy
21	Sodium hydroxid	BDH,ENGLAND
22	Formic acid	BDH,ENGLAND
23	Filter ,0.2TF	Whatman ,Mede in Germany
24	Eppendrof (1.5ml tube)	Vitrex, Meae in China

Table (2-3) Kits and chemicals

NO	APParatus	Manufactures
1	Chloroform(stabilized With amylene) extra pure Minimum assay (GC)99.0%	THOMAS BAKER, Maed in INDIA
2	Proteinase K Kit	Intron Biotechnology-Korea,Geneaid
3	Urea Kit	Architect,Abbott, Japan
4	Creatinine Kit	Architect,Abbott, Japan
5	Albumin KIT	Architect,Abbott, Japan
6	Alanine Aminotransferase Kit (ALT) Kit	Architect,Abbott, Japan
7	Alkaline phosphatase Kit	Architect,Abbott, Japan
8	Aspartate transaminase(AST)Kit	Architect,Abbott, Japan
9	Standard OchratoxinA	Sigma- USA

2.1.2. Study groups

A total of 200 persons were, 100 **(50male and50female)** of them **nephropathic** patients,100 **(50male and 50 female)** Healthy people do not suffer from nephropathy. The study protocol was approved by the ethical research committee, College of Applied Medical Sciences, University of Karbala and Baghdad Health Directorate.

2.1.3. Patient Criteria

Patients were selected from **Ghazy AL–Hariri Hospital for Surgical Specialities\ Baghdad** in Iraq. The study consists of 100 Patients with nephropathy who were selected from the Kidney Transplant Center in the Baghdad governorate. A questionnaire was applied in order to identify important sociodemographic characteristics, general health status, family history of chronic diseases, and dietary habits.

2.1.4. Exclusion Criteria

Generally, patients who reported congenital diseases in kidney, urinary tract infections, medication consumption at the time of the study were not included. for the uncertain etiology nephropathy group, the history of diabetes and\or hypertension was not included also.

2.1.5. Control Criteria

Control group of an apparently healthy 100 (**50 male and 50 female**) were chosen from well-known volunteers participants. Blood samples were

drawn from the volunteers, participants had no history of renal diseases. The percentage of female and male adult individuals was about the patient's frame. The age of the participants was also convergent in the whole study group. Demographic information of participants was also collected through the self–reported technique (**student questionnaire**).

2.1.6. Approval of ethical

The protocol of the study was approved by the Ethical Committee of The College of Applied Medical Sciences in Karbala and the Baghdad Health Department's tune in the Medical City of Baghdad. Samples were obtained after consent from patients or the patients' relatives.

2.2. Methods

The blood parameters analysis was conducted in the medical city educational laboratories complex, Also, the extraction and detection of the poison was carried out in the laboratories of the department of environment and water of the Ministry of Science and Technology.

2.2.1.Blood Collection And Storage

For all participants, 5 ml of blood was taken from a vein via a sterile syringe, 4 ml into a gel tube and 1 ml into an EDTA tube and then transferred by container to the central laboratory. Samples were settled for 15 min and then centrifuged for 15 min at 3000 rpm to separate the serum**.**

The serum was then transported by micropipette to the sterile container (**Eppendrof1.5ml tube**)for storage which was conducted by divided each serum into two parts and then stored at – 20C^{c} until analyses. stored serum samples then used to measure the following:

1.Renal Function Test (**s.creatinine, s.urea, s.albumin**)

2. Liver Function Test (**ALT**),(**AST**),(**Alkaline phosphatase**)

3. Qualitative and Quantitative detection of serum Ochratoxin A

Blood tube EDTA(**1ml**) It is used for the purpose of analyzing the blood Picture (**CBC**).

2.2.2. Investigation of OTA in blood serum of chronic kidney disease of uncer etiology

This experiment acalculated to **AL-Musoui (2015)** this study was conducted as follows:

Before starting work all the glasses were sterilized in autoclave device for 15 minute at 120 c^{o} each day, also all work surfaces were cleaned by Isopropyl alcohol 70.

2.2.2.1. Proteinase K solution

Proteinase K solution was prepared According to the instructions of the Korean Junaid Company. It was prepared by adding 1.1ml pure sterile distilled water to 22mg of proteinase K powder (**M.wt 28900**).

2.2.2.2.Standard OTA preparation for HPLC

1mg has been taken from the Ochratoxin standard placed in a coical flask with a capacity of 10ml and acetonitrile added to it to the point. The concentration has become (**100 ppm**) and by using the dilution law, the injected concentration was prepared.

2.2.2.3. Extraction of Ochratoxin A in serum samples

Serum samples in the size of 2ml were taken in a sterile test tube and added to each one of them 50uL from proteinase K solution and leave to react for 10 minutes. After that the mixture was exposed to Centrifugation for 15 minute at 3000rpm, then from each sample the filtrate was taken and the precipitate was neglected.

Chloroform of (**1ml**) was added to each filtrate (**twice its size**) and shake vigorously in the electric shaker device, where it formed two layers (**chloroform layer and serum layer**), chloroform layer was separated by separating funnel and put in sterile other glass tube and let to evaporate.

2.2.2.4. Evaluation of Ochratoxin A level in blood Serum of human conducted according to Skarkova *et al.*, (2013)

HPLC model SYKAMN (**Germany**) was used to analyze add detection of Ochratoxin . The mobile phase was an isocratic acetonitrile :D.W:formic acid (**50:47:3**)at flow rate at 1.0mL/min, column was C18-ODS (**25 cm* 4.6mm**) and the detector Florescent (**Ex=365 nm , Em =445 nm).**

2.2.2.5. Preparation of sample Ochratoxin A for HPLC

Ochratoxin was extracted from samples (**20ml**) by homogenization with 20 mL acetonitrile: H_2O (**6:4,v=v**) for 2 minutes. The extract was filtered and 4mL of the filtrate was diluted with 25mL phosphate buffer saline pH 7.4

(**PBS**). The samples were degassed in a sonic bath for 30 minutes then the pH was adjusted to 7.2 using 2 M sodium hydroxide 5 mL of acetonitrile is added to the sample and then stored until the analysis is performed.

The concentration of Ochratoxin A was calculated by adopting a curve on the absorption values of standard poison concentrations, The concentration was calculated according to the retention time of detention estimated using the following equation:

$$\textbf{substance concentration} = \frac{Standard\ substance\ concentration * model\ space}{Standard\ material\ area}$$

$$ppb = \frac{diluion\ factor}{\frac{volume}{weight}}$$

2.2.3.Method for Quantitation Serum Urea

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using CLSI protocol NCCLS EP9-A.

Serum and urine results from the Urea Nitrogen assay on the AERO SET System were compared with those from a commercially available urease methodology.

Serum and urine results from the Urea Nitrogen assay on an ARCHITECT cSystem were compared with the Urea Nitrogen assay on the AERO SET System.

Principles of the procedure

The Urea Nitrogen assay is a modification of a totally enzymatic procedure first described by Talke and Schubert (1965). The test is performed as a Kinetic assay in which the initial rate of the reaction is linear for a limited period of Tim. Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction .catalyzed by glutamate dehydrogenase(**GLD**) converts ammonia and a-Ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (**NADH**) to nicotinamide adenine dinucleotide (**NAD**). Two moles of NADH are oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample.

2.2.4. Method for Quantitation Serum Creatinine Level

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using CLSI protocol NCCLS EP9-A2 with passing –Bablok regression. Serum and urine results from the Creatinine assay on an AEROSET System and were compared with those from a commercially available alkaline picrate methodology. Serum and urine results from the Creatinine assay on the ARCHITECT c System were compared with those from the creatinine assay on the AEROSET System.

Principles of the procedure

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample. Methodology:

Kinetic Alkaline Picrate For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

2.2.5. Method for quantitation serum Albumin

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using CLSI protocol NCCLS EP9-A.

Serum results from the albumin BCG assay on the AEROSET System were compared with those from a commercially available Bromocresol green methodology.

Serum results from the Albumin BCG assay on the AEROSET System

Principles of the procedure

The Albumin BCG procedure is based on the binding of bromocresol green specifically with albumin to produce a colored complex. The absorbance of the complex at 628 nm is directly proportional to the albumin concentration in the sample. Methodology: Bromocresol green For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

2.2.6. Method for quantitation serum ALT

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using CLSI protocol NCCLS EP9-A. Serum results from the ALT assay on the AEROSET System were compared with those from a commercially available NADH oxidation methodology .

Serum results from the ALT assay on an ARCHITECT c System were compared with the ALT assay on the AEROSET System.

Principles of the procedure (ALT)

present in the sample catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase (LD) is reduced to L-lactate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Methodology: NADH (without P-5'-P)

2.2.7. Method for quantitation serum AST

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using protocol CLSI protocol NCCLS EP9-A.

Serum results from the ALT assay on the AEROSET System were compared with those from a commercially available NADH oxidation methodology.

Serum results from the AST assay on an ARCHITECT c System were compared with the AST assay on the AEROSET System

Principles of the procedure (AST)

Present in the sample catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate, forming oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and malate dehydrogenase (**MDH**) is reduced to L-malate. In this reaction, NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD.

2.2.8. Method for quantitation serum Alkaline phosphatase

The analysis was carried out according to the instructions of the Japanese company Abbott.

Correlation studies were performed using protocol CLSI protocol NCCLS EP9-A

Serum results from the Alkaline phosphatase assay on the AEROSET System were compared with those from a commercially available ***p-nitro phenyl*** phosphate hydrolysis methodology Serum results from the Alkaline Phosphatase assay on an ARCHITECT cSystem were compared with those from the Alkaline Phosphatase assay on the AEROSET System.

Principles of the procedure Alkaline Phosphatase

The test was concluded According to **Otto *et al.*, (1946).** Several substrates have been used to measure alkaline phosphatase. activity such as glycerophosphate,1 phenyl phosphate1, and ***p-nitro phenyl*** phosphate.2 Bowers and McComb3 improved the method. optimized this method to include a chelated metal-ion buffer of zinc, magnesium, and HEDTA. This Alkaline Phosphatase procedure is a modification of this method. Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless ***p-nitro phenyl*** phosphate (**pNPP**) to give p-nitro phenyl and inorganic phosphate. At the pH of the assay (**alkaline**), the ***p-nitro phenol*** is in yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Methodology: Para-nitro phenyl Phosphate

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

2.2.9. Measurement of physiological of blood parameters

The analysis was carried out in the laboratories of the Kidney Transplant Center, Martyr Ghazi Hariri Hospital, Medical City, using the hematology device auto analyzer (**NIHON KOHDEN**). And including calculated RBC, MCH, MCHC, HCT, HGB.

3.1. Investigation of OTA in blood serum of chronic kidney disease of uncertain etiology (nephropathy)

The results showed 90 out of 100 samples which is rated **(90%)** of blood samples taken from patients infected with chronic kidney disease of uncertain etiology which contained OTA have a significant difference of blood samples contaminated with OTA **(3 only 3%)** that are taken from a healthy person **(Table 3-1)**

This study also illustrated the number of blood samples collected from female and male patients affected with the OTA was 44 **(48.8%)** and 46 **(51.1%)** respectively with no significant difference between them**. (Table 3-2)**

The results of this study were agreement with many studies. AL-Musoui **(2015)** found that (23.07%) of patients' blood specimens had OTA in their blood reaching to 10%. He showed that males are highly infected with OTA by (87.5%) while females were (70%). **Micco *et al*., (**1995) illustrated that 22 out of 111 breast milk samples were contaminated with OTA in range 0.1-12 ng/ml.

Breithoitz- Emanuelsson *et al*., (1993) found that concentration of OTA in human milk was approximately lower than in human blood.

Table (3-1) percentage of OTA in nephropathy serum patients.

Case	NO. of persons with OTA	NO. of persons without OTA
Patients(CKD)	90(90%)	10(10%)
Control healthy persons	3(3%)	97(97%)

x^2calcuate=152

x^2table=3.84

Our study accepted the alternative hypothesis (Ha) and rejected the null hypothesis (HO). In other words, the presence of OTA is related with nephropathy.

(Table 3-2) The percentage of OTA in nephropathy Basedon gender.

Gender	NO.of patients with OTA	Percentage%
Females	44	48.8
Males	46	51.1

x^2calculate=0.4

x^2=table(at 0.05)3.84

The results revealed that the level of OTA in blood serum rise in female patients with nephropathy to 7.015 ng/ml with a significant difference **(p<0.001)** from the level of OTA in blood serum in the control group. As well as, the level of OTA in the blood serum of male patients with nephropathy and with OTA reached to **7.071ng/ml** with a significant difference **(p<0.001)** from the level of OTA in blood serum in the control group reached to 0.09 ng/ml (**Table 3-3**)

The result of this study was agreement with **Achour *et al*.,. (1993)** who found that OTA in blood serum of 82 persons with (3.5 - 6.8) ng/ml concentration between (3.5 ± 6.8) ng/ml and detection of OTA in blood of 100 other persons (concentration 1 ng/ml)

results have allowed establish the presence of OTA in human plasma in both populations. The incidence of positive values for OTA in blood was over 50% with respect to the levels found. The averages where similar to those reported in the countries except in San Vicente de Tagua – Tagua where the women's group presented values higher than other reports (Grosso *et al.*, 2003). The OTA concentration in the patient's blood with different kidney disease and healthy subjects was compared in several countries in all studies the concentration of OTA in the patient's blood with chronic renal insufficiency treated with dialysis was significantly higher than in the blood of control subjects. (Ozcelik *et al.*, 2001)

The increased OTA **concentration** was not found in cases of other acute renal insufficiency that had developed on the basis of other diseases without pre-existing nephropathy (Malir *et al.*, 2001)

Breitholtz – Emanuels *et al.,* (1994) found significantly higher **concentration** of OTA than in control.

3.2. The conceutdation of OTA Basedon nephropathy gender.

Table (3-3) Effect of nephropathy and Gender in the concentration of OTA ng/ml **blood Serum**

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female	0.100 ± 0.000	7.015 ± 0.623	**0.0001 ****
Male	0.09 ± 0.028	7.071 ± 0.644	**0.0001 ****
Total	**0.095 ± 0.0208**	**7.043 ± 0.630**	**0.0001 ****

*** means** significant **differences (P <0.05)** **** means high** significant **differences (P <0.001)**

3.3. Effects of Nephropathy and Gender in some physiological and Biochemical human Blood serum Parameters

3.3.1. Urea

Urea concentration in blood serum of female patients with nephropathy and borne OTA group was 99.1 mg\dl, while the concentration in blood serum of control group **(healthy)** was 26.72 mg\dl with a high significant difference from the levels of patient's blood serum. Also, the level of urea in the blood serum of male patients with nephropathy was 115 mg\dl with a high significant difference from the levels 27.76 mg\dl of urea in blood serum of control group. **(Table 3.4)**

This result is agreement with the results of **Sharma *et al.*, (2011)** who found out that the creatinine and urea concentration change inversely. They are valuable in determining the degree of renal impairment because they correlate with variations in glomerular filtration rate. Blood urea levels increased when renal function declines to about 25-50 percent normally, which is a very sensitive indication of renal failure **(Sharma *et al.*, 2011)**

Urea levels in the blood were considerably higher, indicating serious kidney damage. The findings are compatible with histopathological sections of the kidney. Biochemically, blood levels of urea are considerably greater, whereas protein levels are significantly lower than in the control group, indicating that kidney function is impaired **(Lippoldl *et al.*, 1992).** Increased urea levels in the blood are a significant indication of kidney impairment and toxicity **(Mir and Dwivedi, 2010).**

Table (3.4) .The level of Urea mg/dL basedon nephropathy and gender .

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(6-21mg/dl)	26.72 ± 10.581	99.1 ± 47.690	**0.0001 ****
Male N.V(8-24mg/dl)	27.76 ± 9.312	115 ± 56.779	**0.0001 ****
Total	**27.24 ± 9.930**	**107.33 ± 52.774**	**0.0001 ****

*** means** significant **differences (P <0.05) ** means high significant differences (P <0.001)**

3.3.2. Creatinine

The results showed that creatinine levels in the serum of female patients were 3.73 mg\dl but in females serum of control **(healthy)** was 0.571 mg\dl with a high significant difference between them.whil, in the blood serum of male patients the creatinine level was 4.645 mg\dl while the concentration in males serum control group was 0.526 mg\dl with high significant at $p < 0.001$ between them **(Table 3-5)**

This results is consistent with the fact that creatinine levels in the blood were considerably elevated, indicating serious renal damage. The results also are congruent with kidney histological sections and biochemically, where serum creatinine level is considerably higher. Protein level is significantly lower than in the control group, indicating kidney and liver function impairment **(Stoev *et al.*, 2012)**

Experiments reveal that the concentration of OTA is greater in the kidneys, **(Persi *et al.*, 2014)**. Increased creatinine level in the blood is a significant indication of kidney impairment and toxicity **(Mir and Dwivedi, 2010)**

Table (3-5). level of Creatinine (mg/dL) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(0.7-1.2)	0.571 ± 0.240	3.73 ± 1.795	**0.0001 ****
Male N.V(0.7-1.2)	0.526 ± 0.232	4.645 ± 2.940	**0.0001 ****
Total	**0.548 ± 0.236**	**4.187 ± 2.466**	**0.0001 ****

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.3.3. level of albumin (g/dL) blood serum Basedon nephropathy and gender

The results illustrated that the concentration of albumin in the blood serum of female patients with nephropathy was 4.436 g/dl, while in blood serum of control group was 3.869 g/dl without significant differences between them no significant.

Also, the similar results that appeared in the blood serum of males with nephropathy and borne OTA addition of control treatment were with no significant difference. **(Table 3-6).**

This result agrees with **Ringot *et al*., (2006)** they found that OTA is strong albumin binding its elimination.

The OTA-human serum albumin complex lacks stability. This finding fully explains why humans have by far the longest OTA elimination half-life **(Studer-Rohr *et al*., 2000).** OTA major function is to prevent protein synthesis **(AL-Jumiley, 2014)**

One of the indications of individuals with renal illness is a low amount of albumin in the blood, which is caused by malnutrition. Patients are provided with nutrients and lost components are restored to raise the amount of albumin in the blood, which improves their condition **(Mitch, 2002)**

Table(3-6). level of albumin (g/dL) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(3.97-4.94)	3.868 ± 0.467	4.436 ± 3.922	**0.3117**
Male N.V(3.97-4.94)	4.024 ± 0.684	4.318 ± 1.380	**0.1802**
Total	**3.946 ± 0.588**	**4.377 ± 2.926**	**0.1503**

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.3.4. level of Aspartat aminotransferase (U/L) blood serum Basedon nephropathy and gender

The results showed AST level in blood serum of patients reached to 19.26U/L with significant differences of level the same enzyme in blood serum of control group (15.4U/L)

The same enzyme decreased in the blood serum of male patients and those affected with OTA reached to18.8 U/L (**Table 3-7**).

The outcome is consistent with in terms of biochemistry. Blood AST levels are, whereas protein levels are much lower than in the control group, indicating impaired kidney function **(Zhang *et al*., 2016)**

AST is a plasma enzyme that is typically found inside the cells of the liver. The presence of this substance in the blood plasma implies tissue damage or organ malfunction **(Wells *et al*., 1986)** that inhibiting AST activity in the liver resulted in OTA poisoning.

Abdel-Tawab *et al*., (2001) and **Rej *et al*., (1973)** they are found that there was the enzyme AST in the blood which was shown to be significantly lower in individuals with chronic renal disease, according to the study. On the contrary to a healthy control group, a low enzyme level can be caused by a variety of factors. The usual amount of AST activity is confirmed. Several studies have been conducted, where a potential vitamin B6 deficiency is thought to be the cause (pyridoxal). Phosphate is a cofactor for enzymes.

Table (3-7) level of Aspartat aminotransferase (U/L) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(5-34)	15.4 ± 8.359	19.26 ± 10.495	**0.0446 ***
Male N.V(5-34)	15.26 ± 7.520	18.8 ± 10.815	**0.0603**
Total	**15.33 ± 7.911**	**19.03 ± 10.605**	**0.0057 ***

*** means** significant **differences (P <0.05) ** means high** significant **differences (P <0.001)**

3.3.5. level of blood serum level of Alanine Aminotransferase (U/L) blood serum Basedon nephropathy and gender

The results indicated that ALT was in the blood serum of healthy females control subjects, at 7.86, U/L but ALT was in female patients with nephropathy and OTA carriers. They have reached to 9.98 U/L with no significant difference between them.

In the healthy males control group, ALT reached to 9.0 4 U/L, but the enzyme ALT increased to 11.4 U/L in male patients with nephropathy and OTA carriers. There was a little difference in the group of male patients with nephropathy who were also OTA carriers. A toxin do not appear to have a difference value larger than (**$p < 0.05$**), in which this finding is consistent with biochemically.

ALT levels are considerably greater in the serum, whereas protein levels are significantly lower than in the control group, indicating that kidney function is impaired **(Stoev *et al*., 2012)**

The ALT plasma enzyme is typically found in the liver cells. Its presence in high concentrations in blood plasma implies tissue damage or organ failure **(Wells *et al*., 1986)**

Endogenous cortisol elevation caused by OTA poisoning was observed to rise over the time course of suppression of liver enzyme activity or production of ALT in the liver and muscle **(Abo-Hegab, 1985)**

According to the study, the enzyme ALT in the blood was shown to be significantly lower in individuals with chronic renal disease. On the contrary of a healthy control group, a low enzyme level can be caused by a variety of factors. The usual amount of ALT activity is confirmed by ALT activity. Several

studies have been conducted. A potential vitamin B6 deficiency is thought to be the cause **(**pyridoxal**).** Phosphate is a cofactor for enzymes **(Rej *et al.*, 1973)**

Table (3-8). level of blood serum level of ALT(U/L) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(0-55)	7.86 ± 2.740	9.98 ± 9.921	**0.1485**
Male N.V(0-55)	9.04 ± 3.885	11.42 ± 11.744	**0.1768**
Total	**8.45 ± 3.397**	**10.7 ± 10.839**	**0.0490 ***

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.3.6. level of Alkaline phosphatase (U/L) blood serum Basedon nephropathy and gender

This study proved that ALP enzyme in the blood serum of the healthy females control group was 44.86 U/L, while the enzyme increased among the female patients with nephropathy, and carriers of OTA toxin, reached to 125.54 U/L with significant difference from control group (**Table 3-9**)

Also, the level enzyme was normed in the blood serum of the healthy males control group, while the level ALP enzyme increased in the group of male patients with nephropathy and toxin carriers OTA showed a statistically significant difference of high value less than (**P<0.001**). This result agrees with **Zhang *et al*., (2012)** who found that serum of ALP increased in blood serum of patients with nephropathy as result of impairment .

Alkaline phosphatase is derived from more than one tissue including bone, where one of the bone metabolites, abnormalities such as chronic kidney disease causes increased osteogenic differentiation which leads to increased transit of many bound protein compounds such as alkaline phosphatase. Alkaline phosphatase leads to a significant increase in its level from the enzyme ALP in the blood **(Torres, 2002; Rej *et al*., 1973)** The study showed that there was a significant increase in the activity of ALP enzyme in the blood among patients with chronic kidney disease in comparison to the healthy control group.

Table (3-9) level of ALP (U/L) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(40-150)	44.86 ± 26.140	125.54 ± 71.491	**0.0001 ****
Male N.V(40-150)	48.48 ± 29.591	135.62 ± 71.339	**0.0001 ****
Total	**46.67 ± 27.837**	**130.58 ± 71.234**	**0.0001 ****

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4. Effect of nephropathy and gender in some blood physiological for human

3.4.1. White Blood Cells

The number of WBC in the blood serum is normal in the healthy females control group, while the number is in the patient's blood. Borne OTA which was 9.148×10^3/ UL was without significant difference between them. It obtained the same results number in males patient and borne OTA group addition the males control, it was 8.37×10^3 /UL and 9.008×10^3 /UL of patient group respective without significant difference at p <0.00 1.(**Table 3-10)**

This result agrees with Gupta *et al.,* (**1983**) they are found that OTA effect white blood cells and differential count of white blood cells causing an increase in the numbers of white blood cells. Also, Paul *et al.,* (**1979**) reported that Mycotoxins cause an increase in the number of white blood cells.

Table(3-10) level of blood WBC × 10^3/UL serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(4.8-10.8)	8.346 ± 2.640	9.148 ± 3.546	**0.2026**
Male N.V(4.8-10.8)	8.37 ± 2.248	9.008 ± 4.535	**0.3750**
Total	**8.358 ± 2.437**	**9.078 ± 4.050**	**0.1293**

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.2. Platelets

The value of PLT was **284.544×10^3/ UL** in blood serum of healthy female control group, while the value of PLT increased in the female patients with nephropathy and carriers of OTA reached to $317.98X10^3$ \ UL as shown in table (3-11). There was no significance greater than the value of (**p< 0.05**). In addition, the plat to (288×10^3\UL) in the group of male patients with nephropathy and carrying OTA toxin showing a significant difference and a value greater than (**P<0.05).** This result agrees with many studies that reported to anemia is a common complication of nephropathy can contribute to platelet dysfunction and aggravate the bleeding tendency **(Miale, 1972).** Moreover**,** other studies found out that OTA effect in clotting time and, decrease in the PLT level **(Bandyopodhyay *et al*., 1979).**

Table(3-11) level of PLT $\times 10^3$ /UL blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(130-400)	284.544 ± 95.404	317.98 ± 99.207	**0.0890**
Male N.V(130-400)	286.61 ± 91.425	288.14 ± 115.463	**0.9411**
Total	**285.577 ± 92.968**	**303.06 ± 108.142**	**0.2217**

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.3. Hematocrit

The results showed that HCT decreased in blood serum of female and male patient borne OTA was 34.82% while the percentage in blood serum of control group reached to 36.7% without no significant differences between them.

Furthermore, similar results obtained in percentages of HCT for male patients with nephropathy where this percentage was 40.03% in male's blood serum of the control group with significant differences. (**Table 3.12**).

This result agrees with Paul *et al., (***1979**) they were showed that OTA impact clotting time hemoglobin level, total red blood cell and white blood cell counts and the total number of red blood cells were both significantly reduced this mycotoxin.

Kazmi *et al,* (**2001**) demonstrated that HCT decreases up to 30% in blood serum of patients with nephropathy.

The reduction in HCT might be due to the removal from circulation as a results of OTA-induced blood extravasation **(Jordan *et al*., 1977)**

Table(3-12). level of HCT 100% blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(37.0-47.0)	36.76 ± 4.182	34.82 ± 7.121	**0.0999**
Male N.V(42.0-52.0)	40.03 ± 6.258	32.68 ± 10.04	**0.0001 ****
Total	**38.39 ± 5.544**	**33.75 ± 8.728**	**0.0001 ****

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.4. Hemglobin g/dL

The results showed that value of HGB was 11.98 g/dL in blood serum of healthy group, while it was 11.8274 g/dL in blood serum of females with nephropathy and with OTA. HGB was 11.44 g/dL in male's blood serum with decrease nephropathy and with OTA while the amount of HGB in blood serum of control group was 12.84/ **g/dL (Table 3-13**).

This result agrees with Miale (1972) who proved that OTA caused a decrease in the hemoglobin level. Also, another study found out that OTA caused significant decrease in the hemoglobin level (**Huff *et al*., 1979**).

High prevalence of nephropathy and decline in Hb concentration with even modest reductions are in kidney function **(Hsu *et al*., 2002).**

Jordan *et al*., (**1977**) showed that OTA decreased HGB level may be related to the elimination of circulation as a result of OTA induced extravasation of the blood.

Babitt and Lin (**2012**) found that anemia of nephropathy has been linked to iron losses. Patients with nephropathy who tend to lose iron have impaired dietary absorption of iron from gastrointestinal tract and have functional iron deficiency

Table(3-13). level of HGB g/dL blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(12.0-16.0)	11.98 ± 1.287	11.8274 ± 1.808	**0.6279**
Male N.V(14.0-18.0)	12.84 ± 1.992	11.443 ± 2.758	**0.0046 ***
Total	**12.413 ± 1.724**	**11.635 ± 2.328**	**0.0079 ***

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.5. Red Blood Cell

The value of RBC was 4.435 $x10^6$ cell\mm^2 in serum of the healthy females control group, while the value of RBC normal in the group of female patients with nephropathy and with OTA with significant differences at $p<0.050$. On the other hand, the value of RBC in blood serum of males group and affected with OTA reached to 4.072 $\times10^6$ while it was 4.875 x10/UL in blood serum of control group with significant differences. **(Table 3-14)**

This result agrees with Gupta *et al*., (1983) who found this mycotoxin caused a significant decrease in the total count of red blood cells.

Joske *et al.*, (1956) they found that nephropathy considers major factors in the progression of anemia occurrence.

The decrease in the number of red blood cells causes the body to be unable to absorb iron and vitamins from food, which leads to the depletion of iron stores in the body **(AL-Jumily, 2014).** The decrease in the RBC may be related to the elimination of circulation as a result of OTA induced extravasation of the blood. **(Jordan *et al.*, 1977)**

Table(3-14). level of RBC $\times 10^6$/uL blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(4.20-5.40)	4.435 ± 0.843	4.041 ± 0.831	**0.0206 ***
Male N.V(4.70-6.10)	4.875 ± 0.897	4.072 ± 0.902	**0.0001 ****
Total	**4.655 ± 0.894**	**4.056 ± 0.863**	**0.0001 ****

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.6. Mean Corpuscular Hemoglobin concentration

The results of MCHCg/dl levels in female blood serum infected with nephropathy and with OTA was 31.764 g/dl. Also, the level of MCHC in blood serum for control group was 32.658 g/dl with no significant differences between them.

Similar results occurred in male blood serum of patient and control without significant differences between them **(Table 3.15**)

This result agrees with this finding that corresponds with the fact that the MCHC was reduced by 25% and 19%, respectively, when compared to the control values (**P< 0.05**). This indicates that anemia caused by a high dose of OTA has erythrocytes that are smaller than usual and lower hemoglobin content than normal. **(Wintrobe *et al*., 1974)**

The total volume of erythrocyte count cells and hemoglobin concentration in circulating erythrocytes did not change significantly with graded levels of dietary OTA, whereas the mobilized cell volume and mean hemoglobin concentration decreased significantly (**P< 0.05**) with the highest concentration of OTA. **(Huff *et al.*, 1979)**

In anemia diagnosis, the estimated blood McHC is very important **(Coles, 1986)**

Table (3-15). level of level of MCHC g/dL blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(31.0-34.7)	32.658 ± 2.601	31.764 ± 5.340	**0.2898**
Male N.V(31.0-39.0)	32.317 ± 2.159	31.96 ± 3.760	**0.5618**
Total	**32.487 ± 2.384**	**31.862 ± 4.596**	**0.2281**

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

3.4.7. Mean Corpuscular Hemoglobin

MCHpg level in the blood serum of female patients with nephropathy and affected with OTA was 30.06 pg and the same parameter of control group was 27.113pg without significant differences. Moreover, MCHpg in the blood serum of male patients with nephropathy and borne OTA was 28.986 pg, while in the blood serum of control group was 26.52 pg no significant differences. (**Table 3-16**)

This result agrees with **Coles (1986)** who found that MCH have particularly shown that changes in MCHpg possibly attributable to a defensive reaction against OTA toxicity through stimulation of erythropoiesis or may be associated with decreased erythrocyte count hemoglobin and HTC.

Table (3-16). level of level of MCH (Pg) blood serum Basedon nephropathy and gender

Gender	Control (Mean ± SD)	Patients (Mean ± SD)	P value
Female N.V(27.0-31.0)	27.113 ± 5.810	30.064 ± 9.125	**0.0566**
Male N.V(27.0-31.0)	26.52 ± 5.239	28.986 ± 10.319	**0.1351**
Total	**26.816 ± 5.512**	**29.525 ± 9.706**	**0.0161 ***

* means significant differences (P <0.05) ** means high significant differences (P <0.001)

Conclusions

1- The presence of ochratoxin A is in the blood of most people who suffer from nephropathy and at the same time this toxin has been found in a small number of healthy people

2- This study proved the presence of high concentrations of ochratoxin A in the blood plasma of people suffering from disease, and this may be due to the ability of the toxin to cause tissue damage in the kidney tubules, which leads to poor efficiency of the kidney function in excreting toxic substances.

3- The presence of ochratoxin A in the blood plasma is an important vital indicator to infer the extent of contamination of foodstuffs consumed in local markets in the country.

4- The presence of ochratoxin A in the blood plasma and at any concentration is a dangerous indicator of human health, as this toxin is cumulative and has many vital goals in the human body.

Recommendations:

1. According to the results of this study AST, ALT, ALP urea, and creatinine, increased levels in blood serum of human are considered as Biomarkers for poisoning by Ochratoxin A.

2. Activating the supervisory role in border ports and airports and following a tight mechanism in the procedures for entering foodstuffs, such as conducting comprehensive and high-accuracy checks on foods that are imported from outside the country, and preventing the entry of any food commodity contaminated with toxic fungi or their toxins

3. Working to create appropriate storage conditions for the various foodstuffs in the country to prevent the occurrence of fungal infections during transport and storage operations

4. Conducting extensive survey studies in the country to determine the levels of contamination with mycotoxins, including Ochratoxin A, and then determining the permissible levels of toxins in food. Iraq is one of the countries that did not specify the percentages of toxins allowed in various foodstuffs.

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Appendix

Results of patient samples for females and males

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
1	55	Male	89	2.3	4.4	40	7.2	130	12.6	4.1	30.7	31.5	9	6	70	UDL
2	45	Male	104	3.9	4.5	29	8.3	185	8.8	3.74	23.5	30.3	13	6	340	8.5
3	53	Male	266	6.2	3.0	24	7.8	193	8.0	3.1	26.0	29.9	3	6	125	5.6
4	62	Male	97	3.5	3.9	38	10.8	292	12.6	4.6	30.1	30.3	14	6	92	7.2
5	45	FEMALE	120	3.3	3.2	3.2	7.3	237	12.6	4.2	30.0	33.1	18	6	93	6.0
6	48	Male	175	6.6	3.4	32	14.6	323	10.6	3.96	26.7	33.1	13	12	150	6.3
7	52	Male	122	5.0	4.1	22	12.1	250	7.3	3.6	28	30.7	14	6	36	6.9
8	58	Male	120	4.4	4.5	37	8.2	234	12.33	4.20	31.1	32.1	18	8	109	6.9
9	61	Male	206	8.5	3.8	20	6.2	197	6.9	2.31	29.9	33.2	31	82	125	6.5
10	50	FEMALE	138	6.5	3.3	28.1	6.9	223	8.5	3.3	28.1	30.2	13	6	150	7.0
11	60	FEMALE	115	3.1	3.9	38	6.1	229	11.6	372	29.6	32.3	67	26	363	7.2
12	46	FEMALE	19	2.6	3.1	32	11.6	492	11.6	4.4	26	30.1	17	6	151	7.9
13	40	Male	111	9.4	3.6	32	6.1	403	10.6	4.23	30.1	33.8	22	7	150	7.2
14	45	Male	131	4.2	4.3	39	12.5	315	10.9	3.73	2.92	31.1	9	6	130	7.7
15	57	FEMALE	121	3.9	3.6	41	8.6	268	13.1	4.10	31.9	31.9	23	8	78	6.4
16	45	Male	132	6.5	4.1	3.4	8.0	282	12.3	4.5	28.3	31.1	21	10	44	6.8
17	53	FEMALE	101	2.6	30.5	35	8.0	282	12.6	4.76	28.0	30.9	14	6	82	7.9
18	52	FEMALE	120	6.7	4.1	37	10.9	329	13.33	3.06	26.0	31.1	9	6	72	7.5
19	52	Male	70	5.1	3.3	35	7.7	225	11.6	4.1	28.2	33.1	13	6	70	6.9
20	60	FEMALE	109	3.8	3.3	33	7.7	280	9.7	3.97	26.4	28.6	18	6	162	7.1
21	50	Male	140	3.9	4.4	40	11.4	383	13.3	5.1	32.1	35.1	23	19	101	6.5
22	48	FEMALE	110	2.8	2.9	28	6.7	244	9.3	2.78	90.1	31	10	6	162	7.2
23	54	Male	167	3.2	5.4	26	10.1	414	8.6	3.1	26	30.1	29	13	137	6.6
24	65	Male	103	3.2	4.1	36	6.1	222	12.0	4.1	26.3	32	6	6	44	6.9
25	54	FEMALE	103	3.3	1.4	33	6.5	228	12.6	4.49	30.3	31.9	17	6	101	5.6
26	45	FEMALE	146	10.7	4.9	37	12.9	323	12.33	4.6	26.2	33.3	17	6	37	6.7
27	51	Male	106	9.3	5.0	21	10.0	206	7.0	3.1	26.3	30.5	17	9	277	6.0
28	65	FEMALE	202	6.2	4.0	33	8.8	151	12.3	4.6	30.0	30.9	12	6	151	6.3
29	45	Male	69	1.0	3.1	21	15.6	261	7.0	3.74	23.5	30.3	20	11	189	7.7
30	53	FEMALE	49	4.5	5.9	36	6.1	217	11.1	3.29	30.4	34.8	16	6	253	6.2
31	55	Male	171	10.5	3.7	38	6.9	212	12.6	4.3	27.1	31.2	9	6	85	6.5
32	52	FEMALE	116	4.4	4.2	38	9.9	251	11.6	3.3	28.3	31.1	16	6	98	6.5
33	45	FEMALE	118	3.4	4.0	40	9.9	382	13.3	4.5	30.1	35.3	43	31	99	7.1
34	58	Male	89	2.8	3.4	24	4.3	220	8.0	3.7	25.1	31.9	10	6	50	7.2
35	45	Male	55	3.4	3.6	37	6.9	230	12.3	4.76	25.8	33.2	16	33	120	UDL
36	54	Male	50	2.7	3.3	43	9.4	341	12.5	4.27	29.2	29.6	27	27	88	UDL
37	42	FEMALE	30	0.9	4.0	42	13.9	373	15.0	4.0	28.2	30.1	16	11	55	7.9
38	59	FEMALE	98	4.4	4.2	37	6.2	228	11.3	3.3	28.1	30.2	19	7	127	7.2
39	45	Male	110	3.7	3.8	41	14.6	444	11.6	3.9	29.2	31.1	13	7	165	7.9
40	52	Male	19	2.8	3.4	49	8.0	282	17.33	5.1	29.1	32.1	19	6	120	6.4
41	59	Male	112	2.3	3.6	37	11.8	442	12.3	4.2	29	33.2	15	7	180	6.8
42	59	Male	28	3.2	4.7	25	3.6	188	7.9	2.78	28.4	31	17	20	208	8.5
43	47	FEMALE	113	4.7	6.0	30	7.7	248	10.0	3.32	30.1	32.4	20	10	132	6.9
44	60	Male	113	3.3	3.6	39	6.8	313	13.2	4.9	26.9	33.8	9	12	90	UDL

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
45	51	FEMALE	119	3.5	1.2	37	12.3	433	11.33	3.5	28.9	31.2	23	12	155	7.5
46	40	FEMALE	42	5.8	3.1	37	11.5	366	12.3	4.6	27	33.1	20	11	152	6.8
47	48	FEMALE	31	2.8	4.5	35	15.6	353	11.6	3.8	28.6	31.4	9	6	143	7.0
48	64	Male	101	1.1	9.2	37	5.3	314	12.3	4.0	26.0	30.1	24	14	128	7.2
49	55	Male	77	2.6	6.2	43	8.1	251	14.4	4.0	31.2	30.3	29	14	160	7.9
50	46	FEMALE	113	3.7	4.2	30	6.3	341	10.0	3.1	28.3	30.1	23	12	151	6.4
51	55	FEMALE	71	2.3	3.5	33	8.8	155	12.5	3.2	39.1	37.8	21	9	56	UDL
52	48	Male	96	6.6	4.2	4.9	6.2	355	16.33	5.91	24.4	29.6	23	19	154	6.3
53	45	Male	49	2.4	3.9	37	8.2	229	12.4	4.44	31.3	30.2	29	14	102	6.4
54	55	FEMALE	49	1.2	4.8	37	8.1	398	12.3	4.8	26.3	30.3	5	7	140	7.9
55	50	FEMALE	70	2.1	6.3	35	7.7	358	12.2	3.7	32.9	34.8	52	10	76	UDL
56	50	FEMALE	128	2.1	3.7	40	7.7	248	10.0	3.32	30.1	32.4	24	6	54	6.6
57	40	FEMALE	110	4.7	3.2	25	6.3	412	13.1	3.6	36.3	52.4	30	9	103	UDL
58	49	FEMALE	120	3.4	4.7	47	15	482	15.1	5.5	27.7	31.7	16	11	138	6.5
59	55	Male	101	2.1	4.3	29	7.9	376	9.6	3.74	23.5	30.3	16	6	88	6.3
60	51	FEMALE	92	0.4	3.9	47	17.6	606	15.6	5.5	31.3	32.8	20	11	76	7.0
61	52	Male	302	6.1	4.2	38	9.7	313	12.6	4.4	30.1	33.5	12	6	55	7.1
62	57	Male	98	3.5	4.1	34	6.9	342	14.0	3.2	28.1	30.3	17	6	155	8.0
63	51	FEMALE	57	3.3	1.5	32	6.2	380	10.6	3.64	28.0	30.9	19	8	99	6.6
64	60	FEMALE	120	4.6	7.9	39	8.2	313	13.0	4.09	27	31.3	20	6	42	6.6
65	57	FEMALE	70	3.6	3.6	36	7.9	283	12.0	4.3	30.9	32.4	22	10	130	7.3
66	52	FEMALE	95	3.5	3.9	35	8.8	453	11.6	3.72	29.7	32.3	15	6	135	7.9
67	65	Male	113	3.4	2.7	22	5.9	269	7.33	3.1	26.9	31.3	10	6	108	7.9
68	45	Male	120	2.9	7.5	50	8.3	332	17.6	7.3	28.9	31.3	9	6	152	7.7
69	63	Male	282	8.0	7.3	30	16.3	378	10.0	5.4	26.1	30.1	32	6	128	7.7
70	51	FEMALE	315	5.6	4.3	28	5.7	155	8.8	3.3	26.7	30.4	13	6	260	8.0
71	57	FEMALE	47	1.5	3.1	33	7.1	295	11.8	4.8	24.5	35.7	9	10	75	UDL
72	55	Male	102	4.6	3.7	50	16.7	235	15.1	5.2	29.1	30.2	25	10	153	7.2
73	51	FEMALE	67	3.4	4.2	37	8.2	212	12.6	4.5	28.1	34.5	17	30	55	UDL
74	85	FEMALE	107	3.9	3.8	43	18.1	157	14.3	4.6	30.1	32	9	6	77	6.4
75	67	Male	51	1.2	4.2	37	7.8	153	12.33	3.5	26.6	30.1	21	6	394	7.5
76	47	Male	92	17.4	5.2	28	9.9	853	9.3	3.1	26.0	29.1	21	11	155	7.5
77	45	FEMALE	108	5.2	3.4	28	15.2	553	9.33	6.7	19.7	28.4	9	6	32	7.9
78	50	FEMALE	102	5.5	3.7	31	7.1	355	10.1	4.1	26.5	3.4	16	6	162	6.6
79	50	FEMALE	57	3.6	3.8	26	5.6	264	8.7	3.7	28.3	32.7	23	8	153	6.5
80	38	FEMALE	41	2.4	4.3	41	6.4	381	12.6	4.3	30.2	31.1	21	6	127	6.7
81	53	FEMALE	27	0.7	4.7	42	8.0	296	14.0	4.7	32.1	32.5	26	67	393	6.9
82	50	Male	48	3.1	3.3	21	6.1	217	11.7	3.2	36.5	55.1	10	9	78	UDL
83	54	Male	170	4.4	4.9	45	5.5	165	15.0	4.9	31.1	37.1	13	6	150	7.6
84	68	Male	125	5.0	4.2	33	6.4	206	11.0	4.25	30.3	33.1	17	6	150	6.9
85	60	FEMALE	97	2.4	3.9	35	9.7	237	11.6	4.2	27.1	30.1	20	9	130	6.5
86	45	FEMALE	110	6.2	4.5	42	6.0	321	14.0	4.9	28.2	33.3	21	6	122	6.5
87	53	FEMALE	113	3.4	4.1	41	6.6	308	13.6	5.1	27.2	33.2	11	6	35	6.5
88	63	Male	84	1.9	4.5	34	8.7	354	14.4	4.5	28.1	31.3	23	12	179	7.2
89	52	Male	119	9.4	3.6	32	6.1	403	10.6	3.73	93.8	29.2	22	7	150	7.1

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
90	45	Male	98	3.1	8.9	37	13.8	179	12.3	3.8	25.9	30.3	75	15	255	7.3
91	47	FEMALE	72	4.2	3.1	42	5.8	343	13.0	4.9	29.6	31.3	15	6	70	7.7
92	65	Male	101	3.3	1.9	43	3.6	333	14.33	4.56	29.2	31.7	17	9	117	6.0
93	50	FEMALE	125	1.5	3.8	36	9.2	373	12.0	3.3	26.1	32.2	16	6	160	7.4
94	40	Male	111.0	7.3	4.1	25.0	3.6	188.0	8.4	2.8	28.4	31.0	8	6	76	6.6
95	48	FEMALE	120	3.1	3.3	25	6.7	347	8.3	2.4	30.8	31.9	23	10	150	7.3
96	57	Male	94	4.8	3.7	20	30.9	260	6.6	2.78	28.4	31.1	29	20	211	7.2
97	64	Male	151	3.7	3.5	31	7.2	153	10.33	5.3	32	36.3	22	8	162	7.1
98	51	FEMALE	112	4.7	3.3	25	20.3	411	8.1	2.23	29.0	28.1	13	6	149	8.0
99	45	FEMALE	120	4.4	4.0	40	8.0	325	13.33	4.30	29.1	33.3	17	6	111	8.1
100	64	Male	110	3.5	4.6	45	6.3	362	14.0	3.3	27.9	30.1	26	6	26	7

Results of healthy samples for females and males

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
1	50	MALE	19	0.1	4.4	40	9.4	249	13.0	4.76	30.1	33.0	10	6	47	UDL
2	40	MALE	45	0.5	4.5	40	6.9	350	13.4	4.30	31.3	33.5	12	6	43	UDL
3	43	MALE	39	0.4	3.9	31	7.4	227	10.4	4.76	21.9	33.5	3	6	7	UDL
4	51	FEMALE	22	0.8	4.3	39	10.7	451	13.0	4.44	29.8	33.4	14	8	45	UDL
5	33	MALE	39	0.6	3.5	37	11.5	355	12.3	4.9	27.0	33.3	10	7	33	UDL
6	33	FEMALE	24	0.6	4.0	35	5.9	479	11.7	3.5	33.1	33.2	12	6	21	UDL
7	40	FEMALE	30	0.7	4.1	38	7.6	355	12.6	3.80	29.3	32.1	15	7	30	UDL
8	32	MALE	40	0.9	3.0	39	14.2	545	13	5.1	3.0	33.3	11	6	17	UDL
9	33	MALE	36	0.9	4.4	46	7.7	357	15.3	5.9	26.3	34.2	9	6	50	UDL
10	30	FEMALE	45	0.4	4.5	35	11.5	309	11.6	4.3	27.1	33.7	13	6	16	UDL
11	40	FEMALE	24	0.7	4.4	34	5.6	318	12.1	4.5	29.1	35.3	10	7	20	UDL
12	43	MALE	42	0.5	4.4	33	7.6	393	11.1	4.3	26.1	33.3	17	19	68	UDL
13	47	MALE	45	0.10	3.9	31	14.9	212	10.3	3.76	27.4	33.6	9	6	30	UDL
14	42	FEMALE	15	0.7	3.2	40	7.4	187	13.3	3.30	28.1	31.9	25	10	44	UDL
15	40	FEMALE	27	0.6	3.4	36	5.8	277	12.0	4.11	29.2	33.3	28	10	38	UDL
16	41	FEMALE	20	0.1	3.3	34	10.1	175	11.4	4.3	27.1	43.1	15	6	60	UDL
17	50	MALE	40	0.4	3.3	39	4.8	232	13.7	5.5	24.9	35.1	14	15	52	0.07
18	30	FEMALE	44	0.3	4.1	39	7.7	230	13.0	4.8	30.8	33.1	14	7	42	UDL
19	46	FEMALE	36	0.9	3.8	37	10.7	334	12.4	5.2	24.2	34.5	10	9	20	UDL
20	35	FEMALE	14	0.4	3.8	40	7.9	220	13.3	4.5	29.1	33.5	11	6	86	UDL
21	30	MALE	21	0.8	3.1	39	7.8	203	13.1	5.10	24.1	33.3	10	6	41	UDL
22	54	MALE	35	0.7	3.5	42	7.6	330	14.1	5.5	26.0	33.3	14	6	96	UDL
23	30	MALE	28	0.8	3.2	44	7.4	335	14.6	4.5	32.4	33.1	29	17	94	UDL
24	44	FEMALE	23	0.4	3.6	36	13.8	348	12.1	5.60	21.5	33.3	15	7	20	UDL
25	36	FEMALE	18	0.3	3.8	33	7.1	262	11.0	5.5	20.0	33.6	18	7	50	UDL
26	48	FEMALE	18	0.8	3.5	38	7.5	245	12.3	4.1	30.0	32.3	16	6	75	0.1
27	45	MALE	33	0.9	3.4	47	4.7	175	14.6	5.0	31.9	33.8	17	6	76	UDL
28	44	MALE	29	0.8	3.1	44	9.4	293	14.6	4.95	29.1	33.18	10	7	52	UDL
29	47	MALE	18	0.5	4.1	38.5	8.9	341	12.3	4.57	27.3	28.1	9	6	20	UDL
30	32	FEMALE	31	0.9	4.9	36	8.3	212	12.0	3.9	3.90	33.1	56	16	104	UDL
31	63	FEMALE	40	0.6	3.9	38	6.9	294	12.6	4.12	30.1	33.1	9	7	30	UDL
32	63	FEMALE	45	0.9	3.4	33	6,0	391	11.0	3.17	34.7	33.3	10	6	17	UDL
33	40	FEMALE	16	0.6	4.1	31	4.9	336	10.3	3.17	32.5	32.0	24	6	87	UDL
34	47	MALE	13	0.6	4.6	54	9.4	387	17.6	5.70	32.3	33.4	11	6	32	UDL
35	45	MALE	30	0.3	3.2	45	7.1	198	12	5.12	23.3	26.6	24	14	102	0.11
36	30	FEMALE	20	0.3	3.5	37	5.9	348	12.0	3.33	36.3	32.2	10	8	85	UDL
37	50	FEMALE	45	0.9	3.1	32	4.3	187	10.6	3.3	35.1	32.2	9	7	11	UDL
38	36	MALE	22	0.6	4.6	45	7.1	260	15.0	5.3	28.0	33.3	10	9	20	UDL
39	35	FEMALE	27	0.7	3.5	40	7.0	260	13.0	4.75	28.3	32.5	11	6	15	UDL
40	32	FEMALE	33	0.6	4.3	39	9.3	350	13.0	4.30	30.0	41.0	22	6	62	UDL
41	41	FEMALE	32	0.7	3.4	30	7.4	164	10.0	3.21	31.1	33.1	20	9	55	UDL
42	59	FEMALE	25	0.6	4.2	41	6.4	302	13.0	4.3	30.0	31.2	13	7	44	UDL
43	30	FEMALE	30	0.8	4.1	40	8.4	253	12.0	4.91	24.4	29.6	15	7	89	UDL

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
44	39	FEMALE	25	0.7	4.1	40	6.2	251	13.0	4.3	39.0	32.0	10	6	44	UDL
45	49	FEMALE	39	0.8	3.6	30	5.2	261	10.0	3.7	27.1	33.3	9	6	21	UDL
46	30	MALE	27	0.9	4.3	33	4.7	215	11.1	3.7	30.0	32.1	10	6	13	UDL
47	31	FEMALE	21	0.7	3.5	39	8.6	234	13.0	4.5	28.8	33.3	12	8	25	UDL
48	40	FEMALE	45	0.8	3.4	31	12.3	105	12.3	4.05	30.4	33.4	9	7	31	UDL
49	30	FEMALE	16	0.5	3.3	42	10.0	282	14.0	5.70	24.1	33.3	11	9	35	UDL
50	39	FEMALE	45	0.9	4.2	21	5.9	228	7.0	3.1	22.1	33.3	10	7	12	UDL
51	36	FEMALE	16	0,7	3.6	40	6.3	316	13.0	4.20	30.2	32.5	10	7	33	UDL
52	47	FEMALE	25	0.7	3.8	37	9.6	394	12.3	4.5	27.3	33.1	19	6	60	UDL
53	32	MALE	33	0.7	3.5	45	13.8	335	15.0	5.30	28.1	33.3	10	6	44	UDL
54	45	MALE	45	0.8	4.6	28	6.4	212	9.3	3.20	29.0	33.5	13	6	50	UDL
55	42	FEMALE	16	0.5	3.3	34	6.8	507	11.1	3.52	31.25	32.1	20	6	78	UDL
56	35	MALE	20	0.5	4.1	39	7.3	218	13.1	4.93	26.1	33.1	6	7	31	UDL
57	30	MALE	17	0.5	4.2	43	6.5	354	11.0	3.17	34.0	32.1	11	6	54	UDL
58	42	MALE	45	0.8	4.3	28	9.7	12.5	9.0	3.81	23.1	32.0	16	11	45	UDL
59	50	FEMALE	28	0.5	4.3	40	8.2	253	12.0	4.91	24.4	29.6	10	6	30	UDL
60	30	MALE	21	0.3	7.2	25	11.3	310	7.5	4.4	18.6	29.5	9	7	10	UDL
61	46	MALE	23	0.6	4.5	32	7.5	238	10.3	3.20	32.1	31.9	10	9	20	UDL
62	40	FEMALE	25	0.3	4.4	41	9.3	255	13.7	5.3	25.3	33.3	9	8	16	UDL
63	82	FEMALE	17	0.2	3.4	33	7.3	221	11.6	3.4	34.2	34.3	12	7	23	UDL
64	35	MALE	19	0.5	3.3	40	8.5	322	13.3	4.1	31.7	33.2	13	14	25	UDL
65	50	FEMALE	45	0.9	4.6	38	9.8	528	10.6	5.78	18.4	27.6	11	6	71	UDL
66	52	MALE	27	0.6	4.1	48	6.9	209	15.3	5.47	28.0	31.4	10	7	20	UDL
67	42	MALE	30	0.4	4.8	35	8.5	266	10.9	3.73	29.2	31.1	13	5	10	UDL
68	49	MALE	33	0.8	3.5	40	7.3	237	13.3	4.7	28.2	33.2	11	12	92	UDL
69	50	MALE	25	0.6	4.3	47	7.5	330	15.1	4.90	30.8	31.9	12	9	17	UDL
70	39	FEMALE	26	0.3	3.3	38	9.9	341	12.1	4.57	26.4	31.6	8	10	30	UDL
71	35	FEMALE	32	0.7	4.5	41	7.2	252	12.2	4.72	26.3	30.1	9	11	33	UDL
72	40	MALE	17	0.2	3.2	42	7.5	330	11.2	7.24	15.5	26.61	19	10	88	UDL
73	50	MALE	40	0.8	4.3	38	9.8	520	10.6	5.76	18.4	27.6	19	12	70	UDL
74	52	FEMALE	15	0.3	4.1	37	17	303	12.0	4.57	26.3	31.7	13	7	20	UDL
75	43	MALE	23	0.5	4.3	48	9.7	232	14.7	5.67	25.9	38.4	10	8	32	UDL
76	50	MALE	35	0.7	4.6	40	6.8	395	13.6	5.90	23.5	33.3	13	9	30	UDL
77	44	MALE	27	0.3	4.2	33	4.3	238	11.6	4.20	27.6	34.9	25	11	94	UDL
78	42	MALE	30	0.6	4.4	33	7.6	391	11.0	4.3	25.5	33.3	18	9	11	UDL
79	40	MALE	23	0.3	3.6	44	6.8	262	14.4	5.2	28.7	32.7	12	7	34	UDL
80	48	FEMALE	20	0.4	4.1	40	16.2	14.2	12.7	4.54	28.0	31.3	19	8	94	UDL
81	41	FEMALE	39	0.7	4.5	36	7.7	223	12.1	4.3	28.13	33.3	15	10	50	UDL
82	48	FEMALE	28	0.5	3.7	40	6.4	220	13.0	4.90	26.5	32.1	10	7	52	UDL
83	40	MALE	22	0.3	4.3	45	12.7	445	14.9	4.98	29.9	33.0	29	21	97	UDL
84	31	FEMALE	10	0.2	4.1	41	7.7	172	12.6	4.79	26.3	30.7	16	6	14	UDL
85	44	MALE	19	0.3	4.3	44	7.8	260	13.4	5.5	24.3	30.4	10	8	30	UDL
86	32	MALE	15	0.1	4.2	36	7.3	325	10.9	4.95	22.0	30.1	22	7	13	UDL
87	53	MALE	27	0.6	3.3	47	10.7	232	13.7	5.67	24.1	28.9	14	15	57	UDL

No	Age	Gender	s.urea	Crea	ALB	HTC	WBC	PLT	HGB	RBC	MCH	MCHC	AST	ALT	ALP	OTA
88	38	FEMALE	9	0.1	4.5	42	10.7	330	11.2	7.24	15.5	26.6	34	11	55	UDL
89	49	MALE	30	0.5	4.1	49	8.7	230	14.4	6.80	21.1	29.1	11	9	30	UDL
90	30	MALE	18	0.3	3.7	35	8.5	266	11.6	3.73	31.0	33.1	35	17	78	UDL
91	46	MALE	23	0.6	4.4	40	9.1	325	13.2	5.11	25.8	32.9	34	7	90	UDL
92	54	FEMALE	30	0.8	3.1	30	9.6	328	10.1	5.6	18.0	33.11	10	8	20	UDL
93	37	FEMALE	21	0.3	4.5	42	9.8	337	13.1	4.49	24.2	31.0	16	6	69	UDL
94	46	FEMALE	33	0.8	4.1	33	5.8	365	9.2	4.73	19.5	27.41	24	12	64	UDL
95	33	MALE	10	0.1	4.0	50	8.2	164	15.8	6.86	26.3	31.4	23	10	97	UDL
96	49	MALE	24	0.5	4.2	44	9.7	196	14.0	4.91	26.5	31.6	20	7	80	UDL
97	30	FEMALE	6	0.1	3.2	41	7.4	220	13.8	5.93	23.2	33.3	29	21	97	UDL
98	50	MALE	29	0.7	4.4	37	7.7	255	12.4	3.57	34.7	33.1	34	13	98	UDL
99	40	MALE	15	0.3	3.2	41	7.7	314	13.7	4.80	28.5	33.1	16	7	57	UDL
100	35	MALE	22	0.2	3.7	38	8.2	250	12.7	5.0	25.4	33.2	26	6	27	UDL

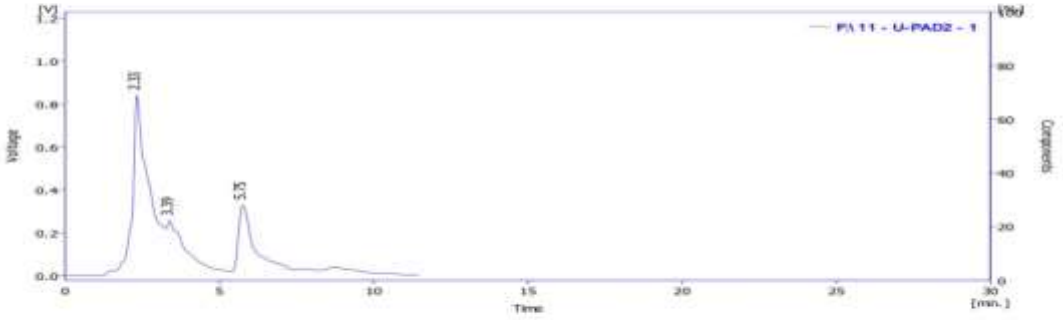
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 11
Sample : 11
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\11 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	12036.389	579.762	71.1	69.9	0.28	
2	3.388	1277.235	59.815	7.5	7.2	0.18	
3	5.748	3627.019	189.531	21.4	22.9	0.30	
	Total	16940.562	829.107	100.0	100.0		

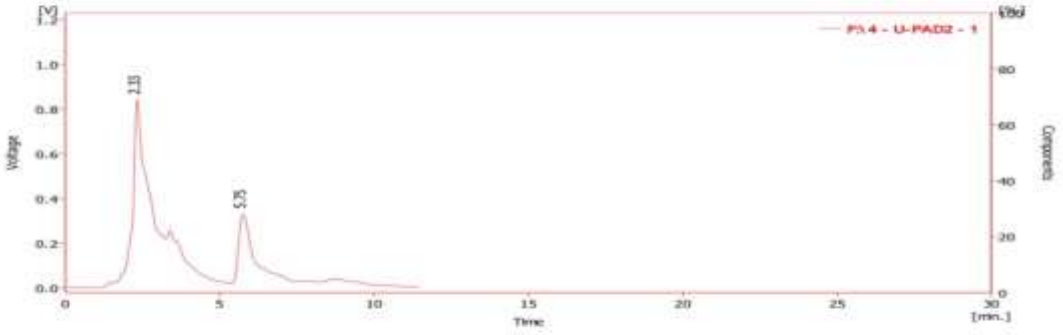
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 4
Sample : 4
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 30.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\4 - U-PAD2 - 1)

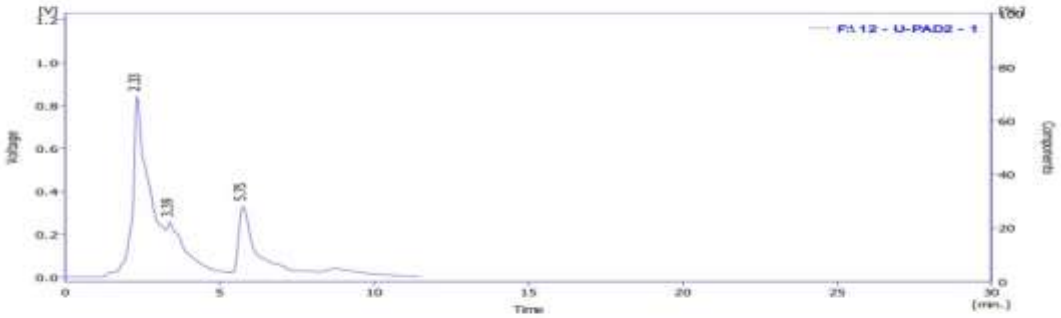
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	8709.170	561.083	70.6	74.8	0.26	
2	5.748	3619.853	189.465	29.4	25.2	0.30	
	Total	12329.023	750.548	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 12	Amount	: 0
Sample	: 12	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\12 - U-PAD2 - 1)

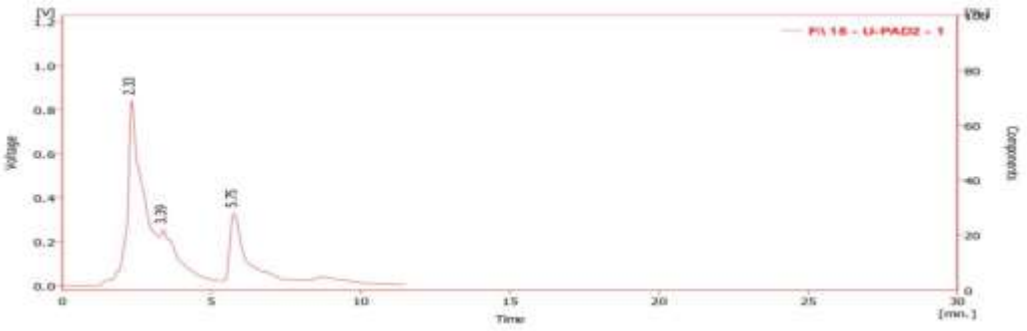
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	9520.360	566.882	66.7	68.1	0.27	
2	3.388	863.284	51.502	6.1	6.2	0.16	
3	5.748	3990.740	212.285	28.0	25.5	0.32	
	Total	14274.384	832.669	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 15	Amount	: 0
Sample	: 15	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\15 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	6132.651	488.436	69.1	70.9	0.23	
2	3.388	430.939	43.840	4.9	6.4	0.14	
3	5.748	2299.351	157.046	26.0	22.8	0.28	
	Total	8852.941	689.322	100.0	100.0		

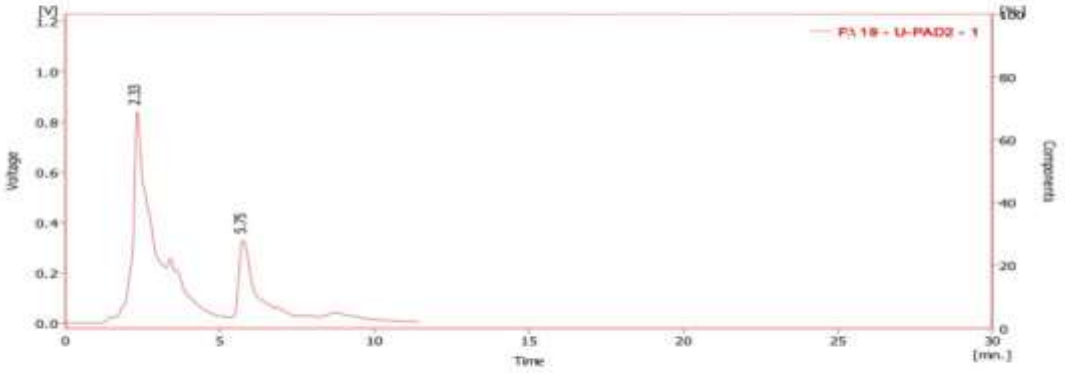
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 19
Sample : 19
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 nV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\19 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	5982.322	435.793	68.6	70.9	0.21	
2	5.748	2738.126	178.978	31.4	29.1	0.29	
	Total	8720.448	614.771	100.0	100.0		

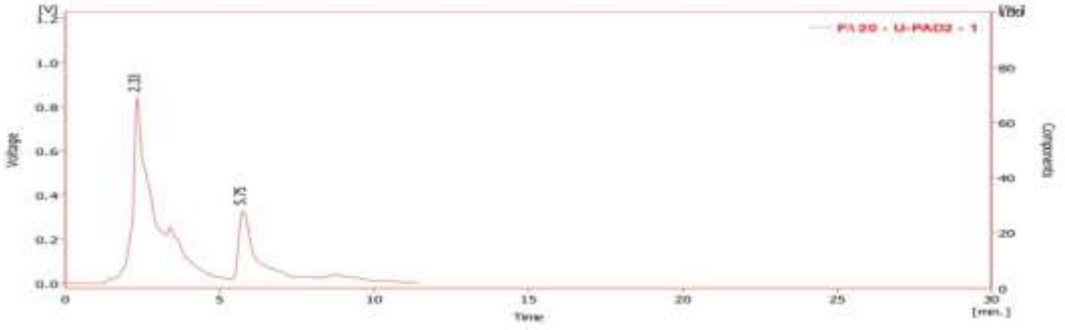
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 20
Sample : 20
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 nV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\20 - U-PAD2 - 1)

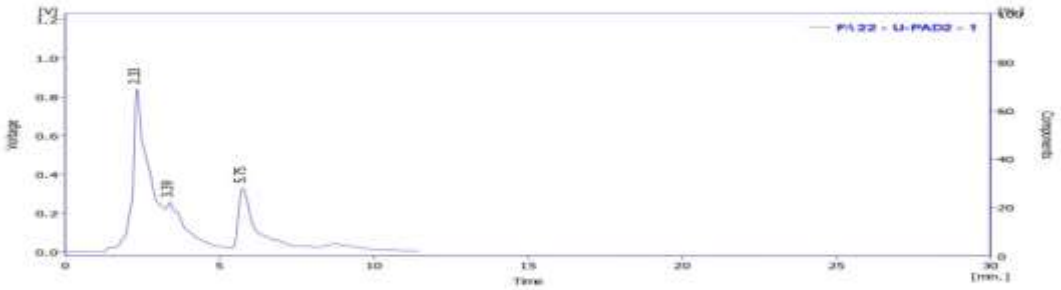
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	3532.501	327.695	70.1	75.3	0.17	
2	5.748	1510.152	107.614	29.9	24.7	0.22	
	Total	5042.653	435.309	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 22	Amount	: 0
Sample	: 22	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\22 - U-PAD2 - 1)

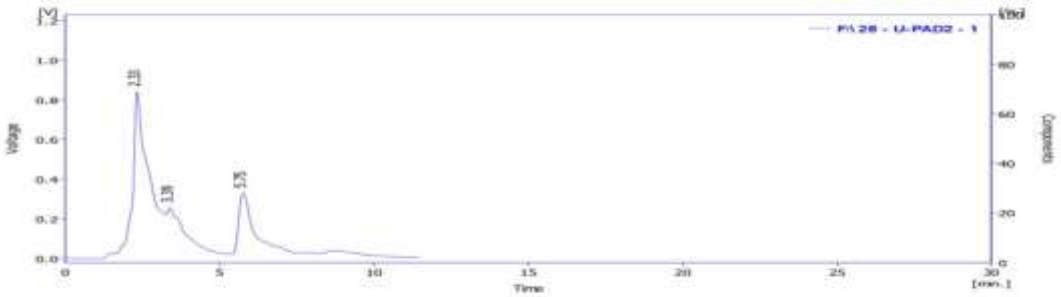
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.326	11858.751	598.039	74.6	70.4	0.30	
2	3.388	638.123	48.836	4.0	5.7	0.16	
3	5.748	3396.203	202.995	21.4	23.9	0.32	
	Total	15893.077	849.869	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 26	Amount	: 0
Sample	: 26	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



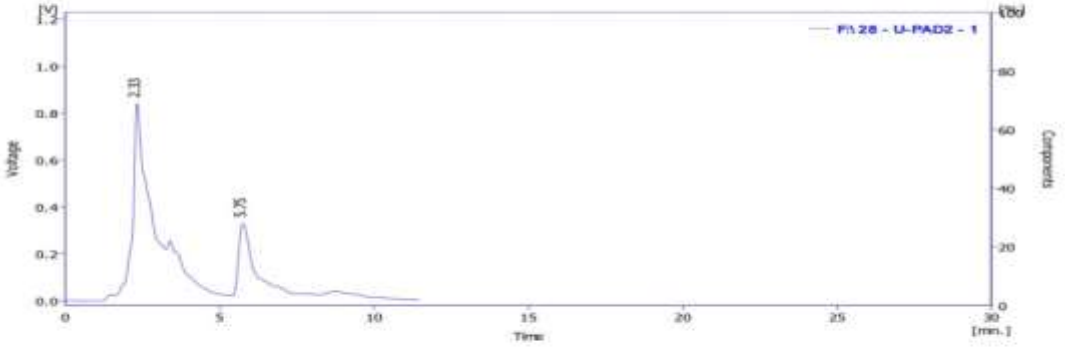
Result Table (Uncal - F:\26 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	7491.003	524.201	69.0	69.6	0.24	
2	3.388	359.044	41.961	3.3	5.6	0.14	
3	5.748	3003.347	186.623	27.7	24.8	0.30	
	Total	10853.393	752.785	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 28	Amount	: 0
Sample	: 28	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\28 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	14006.351	636.830	73.3	71.8	0.35	
2	5.748	5096.230	250.571	26.7	28.2	0.36	
	Total	19102.581	887.401	100.0	100.0		

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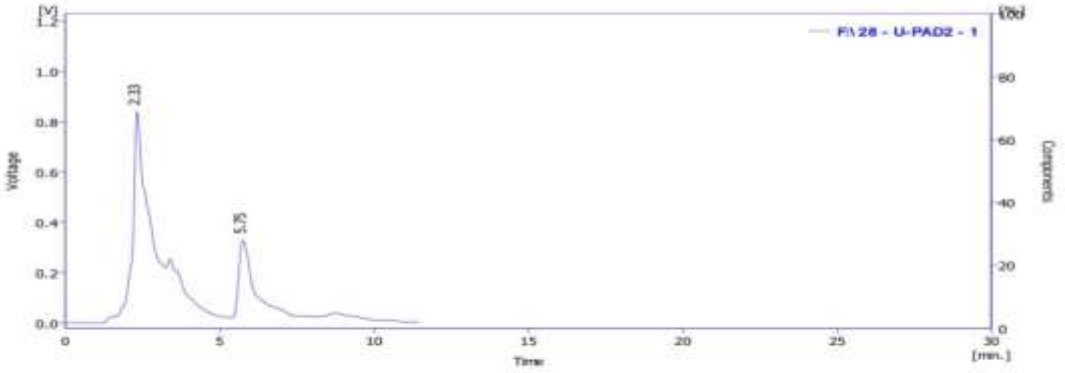
Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 28	Amount	: 0
Sample	: 28	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\28 - U-PAD2 - 1)

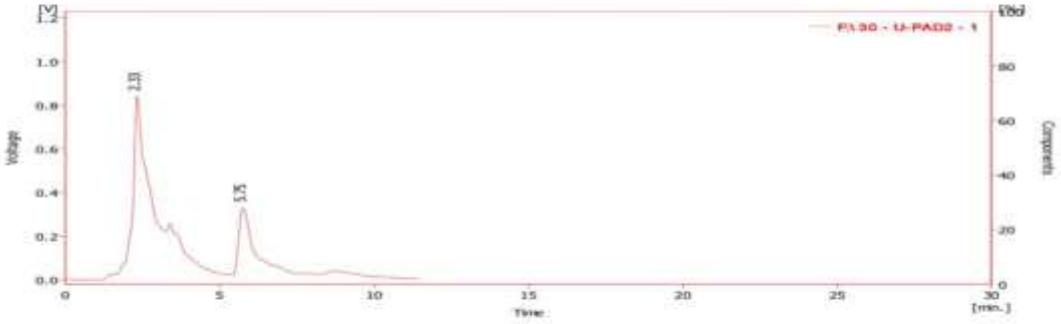
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	14006.351	636.830	73.3	71.8	0.35	
2	5.748	5096.230	250.571	26.7	28.2	0.36	
	Total	19102.581	887.401	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 30	Amount	: 0
Sample	: 30	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 nV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\30 - U-PAD2 - 1)

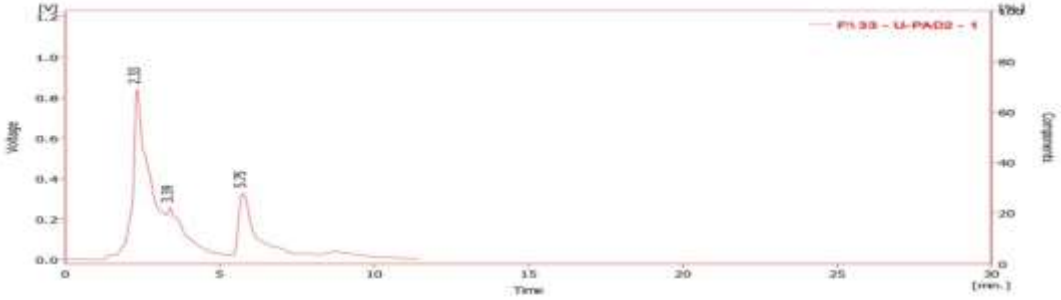
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	5311.227	460.612	69.8	74.6	0.22	
2	5.748	2299.351	157.046	30.2	25.4	0.28	
	Total	7610.578	617.658	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 33	Amount	: 0
Sample	: 33	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 nV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\33 - U-PAD2 - 1)

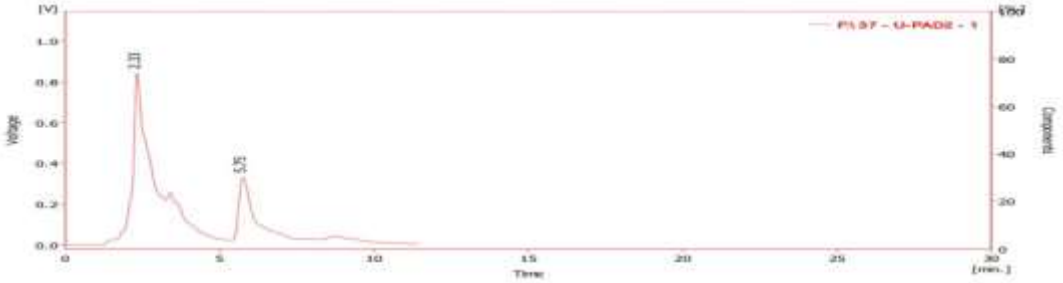
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.338	5341.342	451.612	80.1	67.3	0.21	
2	3.388	1154.978	58.206	13.3	8.7	0.18	
3	5.748	2318.768	160.754	26.6	24.0	0.28	
	Total	8715.087	670.572	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 37	Amount	: 0
Sample	: 37	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\37 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	7535.673	583.217	74.3	77.0	0.23	
2	5.748	2610.552	148.977	25.7	23.0	0.27	
	Total	10146.225	653.194	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 41	Amount	: 0
Sample	: 41	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change

Result Table (Uncal - F:\41 - U-PAD2 - 1)

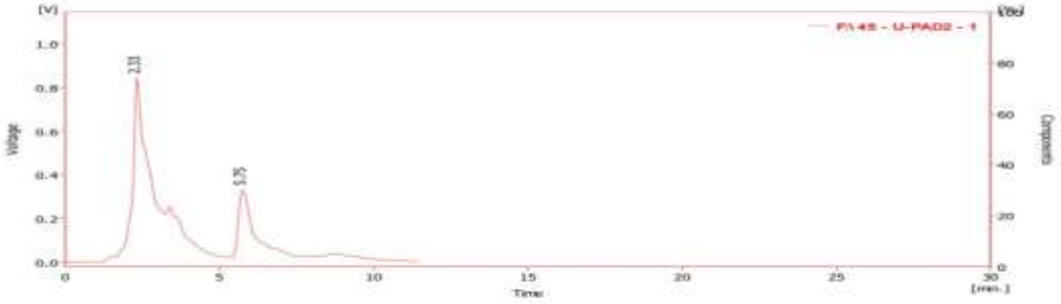
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	5241.342	451.512	79.3	84.0	0.21	
2	5.748	1366.804	85.824	20.7	16.0	0.18	
	Total	6607.146	537.434	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 45	Amount	: 0
Sample	: 45	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\45 - U-PAD2 - 1)

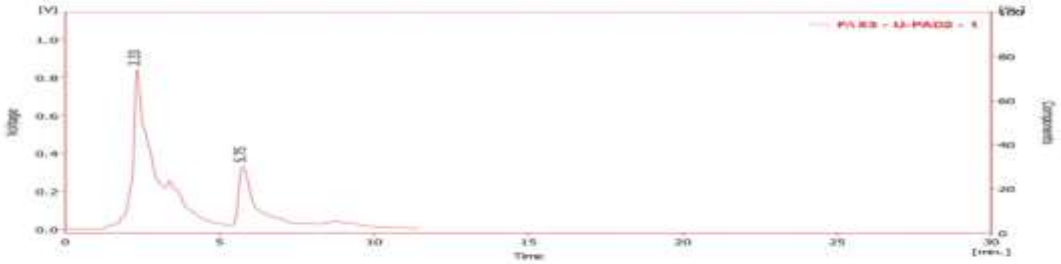
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	4681.257	433.924	46.6	63.0	0.21	
2	5.748	5356.850	255.342	53.4	37.0	0.36	
	Total	10038.106	689.266	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 53	Amount	: 0
Sample	: 53	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\53 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	7247.045	451.742	66.9	70.5	0.21	
2	5.748	3583.611	189.171	33.1	29.5	0.30	
	Total	10830.657	640.913	100.0	100.0		

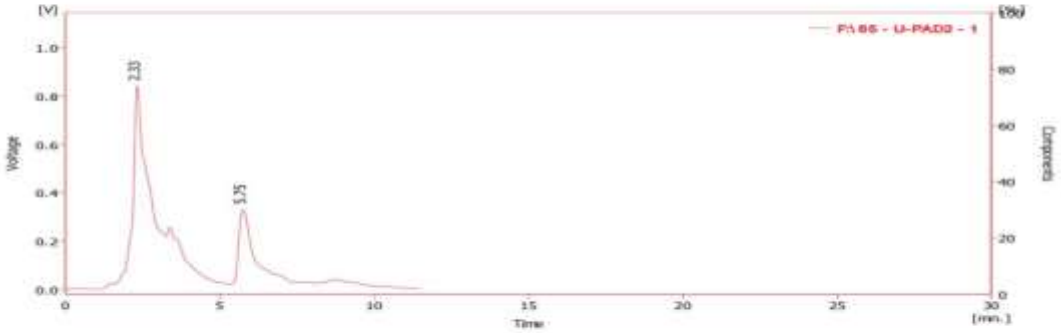
Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 65	Amount	: 0
Sample	: 65	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - P:\65 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	10058.450	577.832	72.4	72.5	0.27	
2	5.748	3841.293	219.050	27.6	27.5	0.33	
	Total	13899.743	796.883	100.0	100.0		

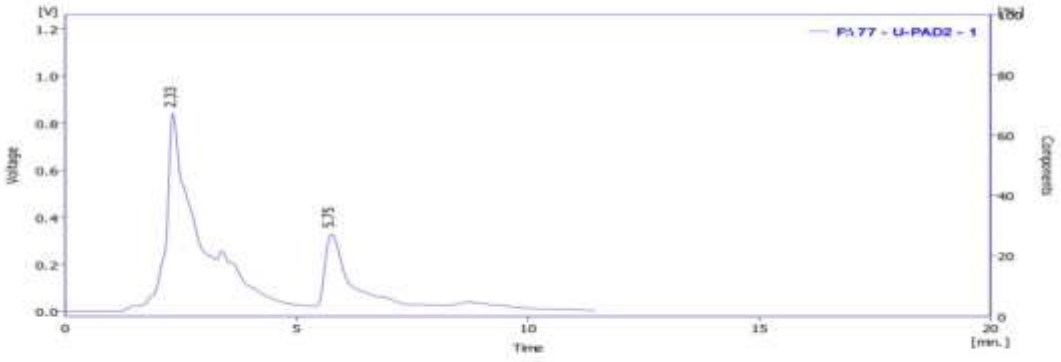
Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 77	Amount	: 0
Sample	: 77	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - P:\77 - U-PAD2 - 1)

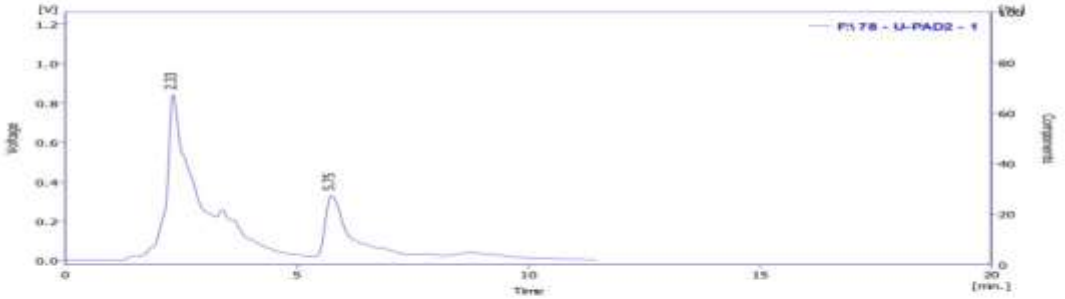
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	5809.433	448.923	71.4	76.5	0.21	
2	5.748	2329.728	138.123	28.6	23.5	0.25	
	Total	8139.161	587.046	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 78	Amount	: 0
Sample	: 78	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\78 - U-PAD2 - 1)

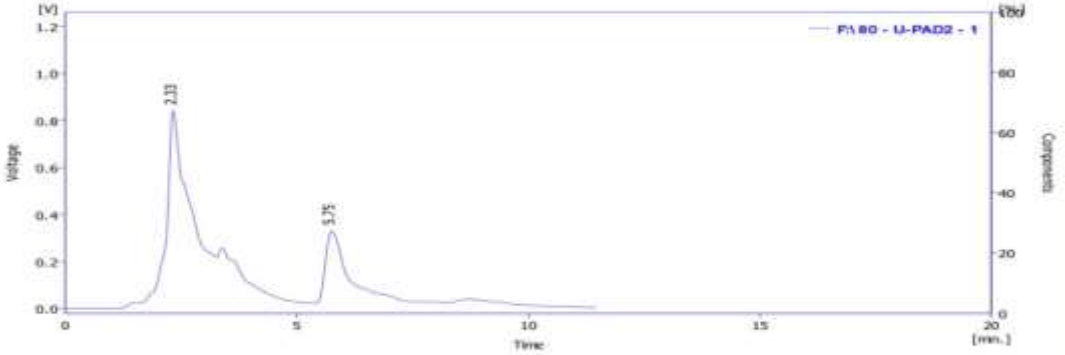
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	4523.989	426.157	63.1	70.9	0.21	
2	5.748	2646.554	174.994	36.9	29.1	0.29	
	Total	7170.543	601.151	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 80	Amount	: 0
Sample	: 80	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\80 - U-PAD2 - 1)

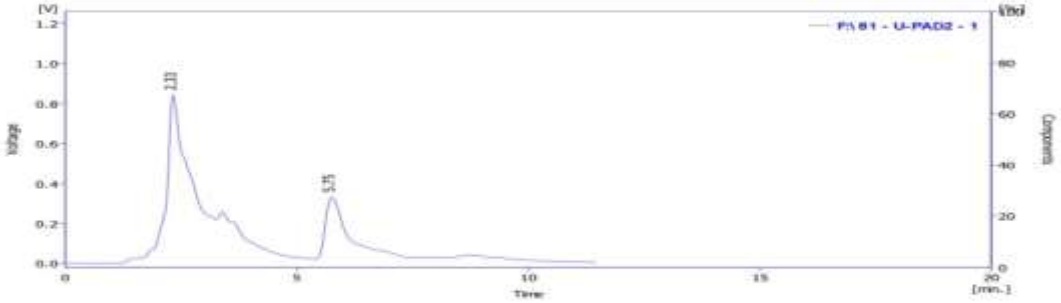
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	6916.196	513.160	81.7	80.4	0.24	
2	5.748	1544.951	124.919	18.3	19.6	0.24	
	Total	8461.146	638.079	100.0	100.0		

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 81	Amount	: 0
Sample	: 81	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - P:\81 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	6658.440	498.350	78.3	78.6	0.23	
2	5.748	1849.392	136.070	21.7	21.4	0.25	
	Total	8507.832	634.420	100.0	100.0		

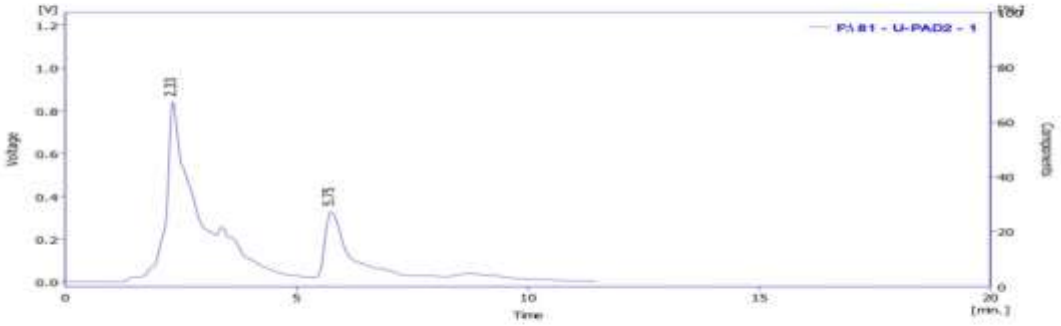
16/03/2021 01:30 م Chromatogram F:\81.PRM Page 1 of 1

Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 81	Amount	: 0
Sample	: 81	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - P:\81 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	6658.440	498.350	78.3	78.6	0.23	
2	5.748	1849.392	136.070	21.7	21.4	0.25	
	Total	8507.832	634.420	100.0	100.0		

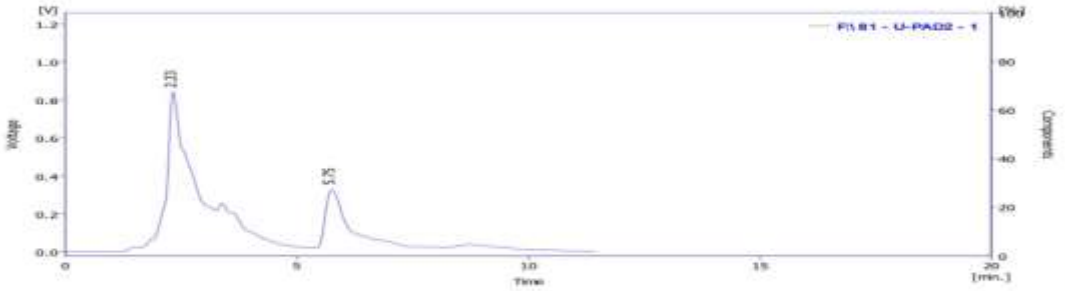
Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 81	Amount	: 0
Sample	: 81	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\81 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	6858.940	486.350	78.3	76.6	0.23	
2	5.748	1849.392	136.070	21.7	21.4	0.25	
	Total	8507.832	634.420	100.0	100.0		

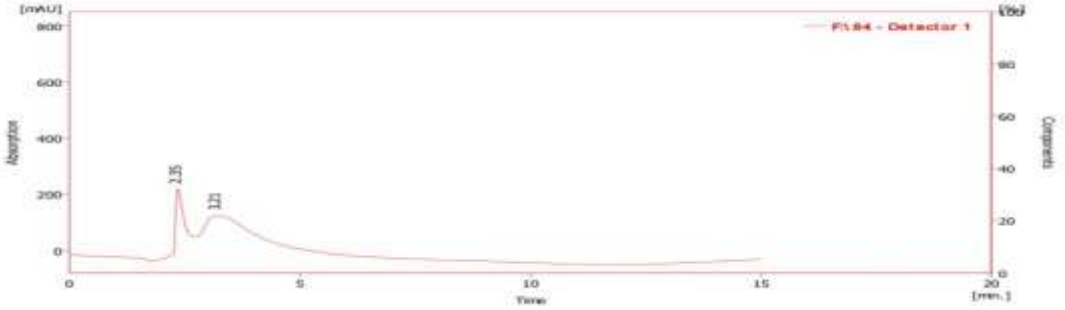
Chromatography Laboratory

HPLC , GC

Sample Info:

Sample ID	: 84	Amount	: 0
Sample	: 84	ISTD Amount	: 0
Inj. Volume [mL]	: 0.1	Dilution	: 1

Autostop	: 15.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 1	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\84 - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.353	1225.568	170.540	30.8	71.8	0.15	
2	3.207	2754.162	66.892	69.2	28.2	0.76	
	Total	3979.730	237.432	100.0	100.0		

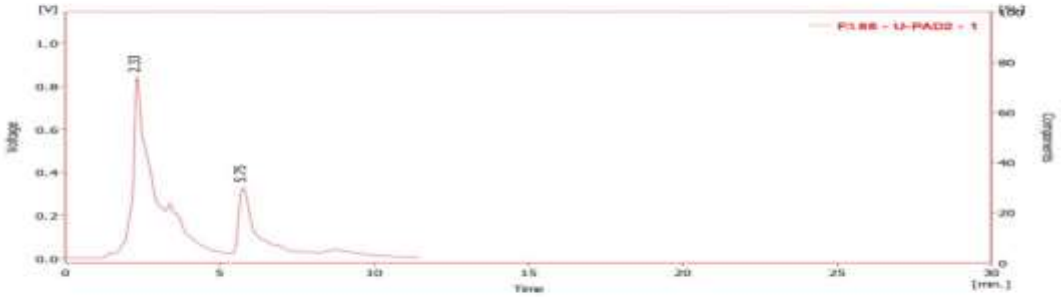
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 86
Sample : 86
Inj. Volume [mL] : 0.03

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

Amount : 0
ISTD Amount : 0
Dilution : 1

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\86 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	9574.543	561.466	77.7	76.6	0.26	
2	5.748	2744.149	171.086	22.3	23.4	0.29	
	Total	12318.692	732.552	100.0	100.0		

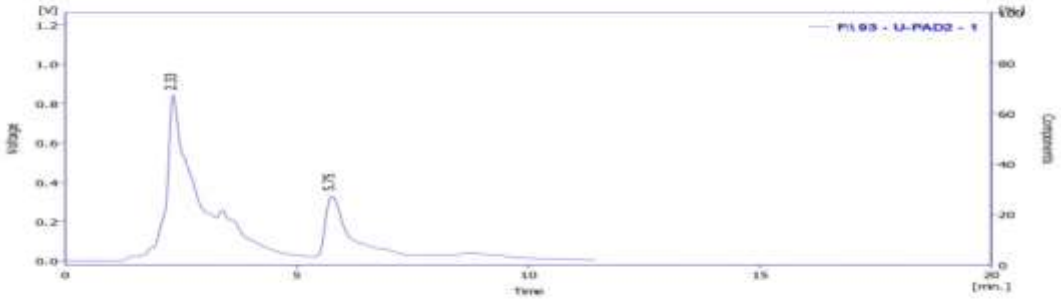
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 93
Sample : 93
Inj. Volume [mL] : 0.03

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

Amount : 0
ISTD Amount : 0
Dilution : 1

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\93 - U-PAD2 - 1)

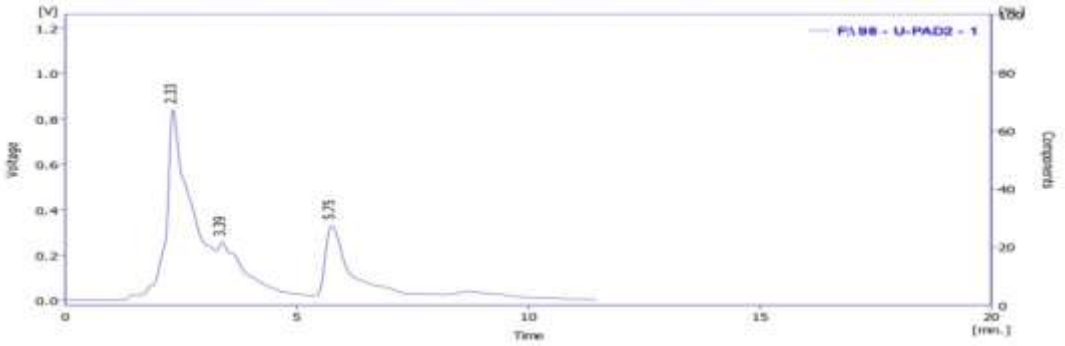
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	7505.673	503.217	80.3	78.7	0.23	
2	5.748	1840.302	136.070	19.7	21.3	0.25	
	Total	9385.066	639.287	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 98	Amount	: 0
Sample	: 98	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\98 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	8501.798	544.156	71.6	72.3	0.25	
2	3.388	344.016	40.965	2.9	5.4	0.14	
3	5.748	3035.864	167.249	25.6	22.2	0.28	
	Total	11881.678	752.370	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:

Sample ID	: 2	Amount	: 0
Sample	: 2	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1

Autostop	: 30.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change

Result Table (Uncal - F:\2 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	11616.417	626.808	73.2	72.4	0.31	
2	5.748	4260.983	239.969	26.8	27.6	0.34	
	Total	15877.400	866.776	100.0	100.0		

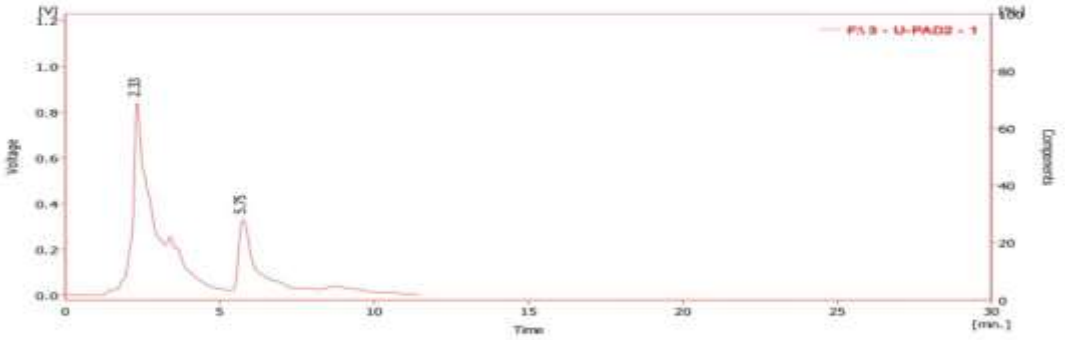
Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 3
Sample : 3
Inj. Volume [mL] : 0.03

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

Amount : 0
ISTD Amount : 0
Dilution : 1

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\3 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	5241.342	451.612	69.3	73.7	0.21	
2	5.748	2318.768	160.754	30.7	26.3	0.28	
	Total	7560.110	612.366	100.0	100.0		

Chromatography Laboratory
HPLC , GC

Sample Info:
Sample ID : 8
Sample : 8
Inj. Volume [mL] : 0.03

Autostop : 20.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

Amount : 0
ISTD Amount : 0
Dilution : 1

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change

Result Table (Uncal - F:\8 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.328	11438.091	615.955	75.6	74.4	0.32	
2	3.388	1074.616	57.035	7.1	6.9	0.18	
3	5.748	2621.551	156.106	17.3	18.9	0.27	
	Total	15134.257	823.114	100.0	100.0		

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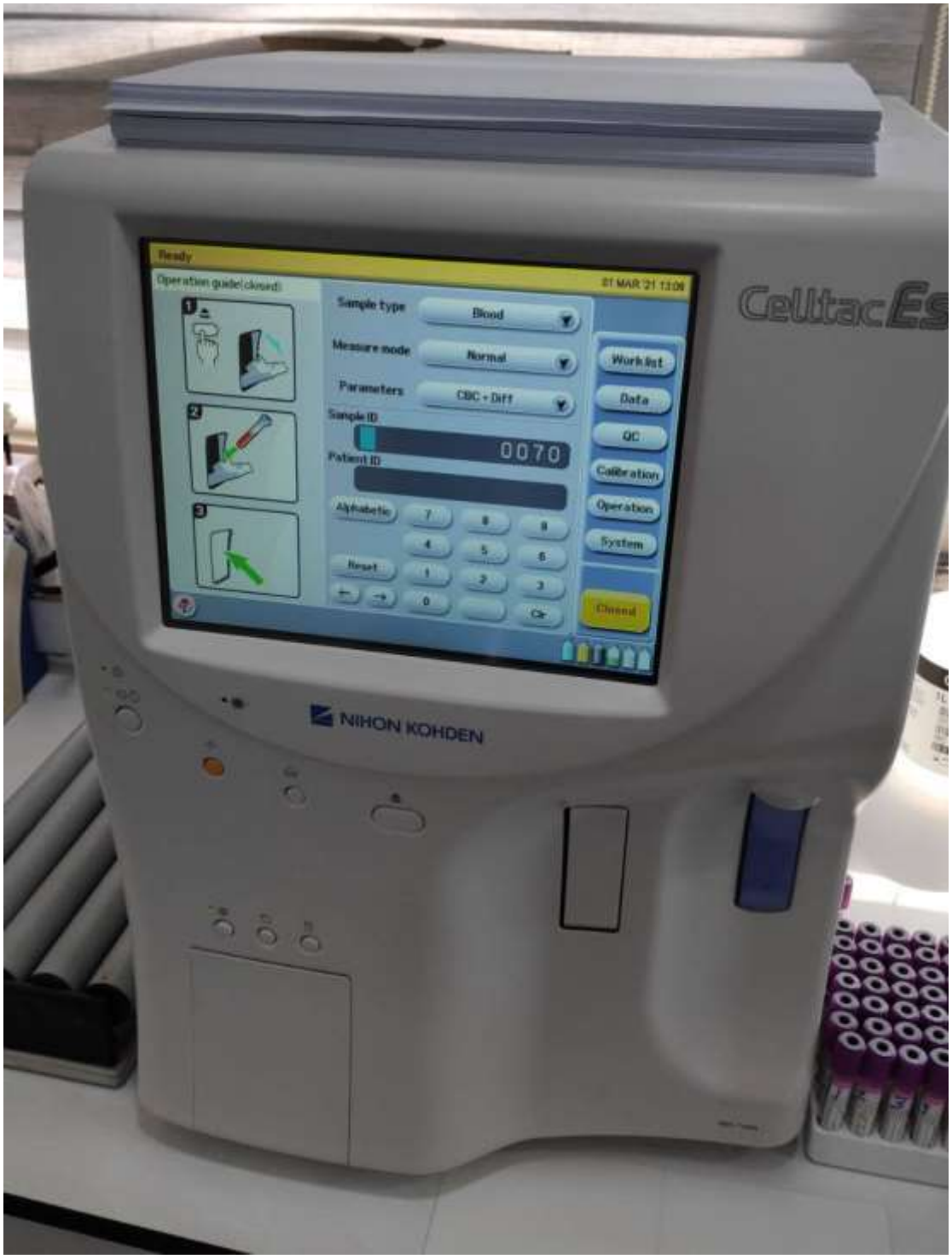
Made in Japan



Device\ Hematology Analyzer, NIHON KOHDEN, 00048

Device\ Hplc

الخلاصة

كان الهدف من الدراسة هو الكشف عن سم الـ Ochratoxin A في عينات دم الأشخاص الّذين يعانون من مرض الاعتلال الكلوي، لقد تم جمع العينات من مستشفى غازي الحريري/ مدينة الطب/ دائرة صحة بغداد/ الرصافة. وقد تضمن عدد تلك العينات (200) عينة، (100) عينة منها هي للمرضى الذين يعانون من مرض الاعتلال الكلوي، اما الـ (100) الأخرى فهي للأشخاص الاصحاء.

وقد بينت نتائج الكشف عن وجود سم الـ Ochratoxin A بنسبة 90% في بلازما دم مرضى الاعتلال الكلوي، بينما كانت نسبة وجوده في بلازما دم الاصحاء 3% بفارق معنوي كبير، كما أوضحت النتائج ان نسبة وجود سم الـ Ochratoxin A في بلازما دم المرضى الاناث يفوق نسبتهِ لدى المرضى الذكور، اذ كانت النسبة لدى الاناث هي 44%، في حين بلغت نسبته لدى الذكور 4%.

اظهرت النتائج ايضاً تفوق واضح في معدلات تركيز سم الـ Ochratoxin A في بلازما دم المرضى نسبة الى الاصحاء، اذ يبلغ معدل تركيزه لدى الاناث والذكور من المرضى **(7.015، 7.071)** نانوغرام/مل على التوالي، بينما بلغت في في كل من الاناث والذكور الاصحاء (0.1، 0.09) نانوغرام/مل على التوالي.

من جانب آخر أوضحت النتائج مصاحبة وجود سم الـ Ochratoxin A ارتفاع مستوى اليوريا لدى المرضى الذكور الى 115 مل/ديسيلتر، في حين بلغ المستوى لدى الاناث (99.1) مل/ديسيلتر، بينما كانت المستويات لدى كل من الاناث والذكور الاصحاء ضمن المستوى الطبيعي.

كما ارتفع معدل مستوى الكرياتين لدى مرضى الاعتلال الكلوي لدى الاناث او الذكور على حد سواء، الى (4.645، 3.73) مل/ديسيلتر على التوالي وبفارق معنوي كبير عن مستوياتهِ لدى الافراد الاصحاء.

اما مستويات الالبومين فقد ارتفعت قليلاً لدى مرضى الاعتلال الكلوي عن مستوياتها لدى الاصحاء وبفارق غير معنوي.

كذلك فقد تأثر مستوى انزيم AST لدى المرضى الاناث اذ بلغ نسبة (19.26) وحدة دولية/ لتر، في حين بلغ مستواه (15.4) وحدة دولية/ لتر لدى الاصحاء، وبفارق معنوي عند مستوى $P<0.05$.

وقد أوضحت النتائج ان مستويات انزيم ALT كانت ضمن المستوى الطبيعي لدى مرضى الاعتلال الكلوي والاصحاء.

من ناحية أخرى فقد تأثر انزيم ALP بالنسبة للإناث المرضى اذ بلغ نسبة (125.54) وحدة دولية/ لتر، في حين بلغ لدى الاناث الاصحاء نسبة (44.86)

وحدة دولية/ لتر، اما بالنسبة للمرضى الذكور فكانت النسبة مقاربة لما هو عليهِ لدى المرضى الاناث.

وقد أثبتت الفحوصات الخاصة بمعايير الدم الفسلجية وجود انخفاض بنسبة HCT لدى المرضى الذكور اذ بلغت (32.68)% وبفارق معنوي عالي عن نسبتهِ لدى الاصحاء.

وظهر انخفاض في مستوى الهيموغلوبين لدى المرضى الذكور مقارنة مع مستواه لدى الاصحاء، وبفارق معنوي بسيط $P<0.05$

وأشارت النتائج ايضاً الى انخفاض في عدد كريات الدم الحمر لدى المرضى مقارنةً بالاصحاء وبفارق معنوي عالي $P<0.001$

ولم تُظهر النتائج لمعايير الدم الفسلجية والمتمثلة بـ MCHC و MCH و WBC و PLT وجود اية فروقات معنوية على حد سواء لدى الاناث والذكور عن مستوياتها لدى الاصحاء.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء/ كلية العلوم الطبية
قسم التحليلات المرضية

دراسة إمكانية تحديد بعض الدلائل الحيوية على تسمم الإنسان بسم الاوكراتوكسين A

رسالة مقدمة

الى مجلس كلية العلوم الطبية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في الأدلة الجنائية

مقدمة من قبل

وائل سعد حسن

بكالوريوس علوم الحياة/ جامعة بغداد (٢٠٠٦)

بأشراف

ا.د. سامي عبد الرضا

١٤٤٣ هـ ٢٠٢٢ م