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Investigation of Aflatoxin B1 in the Blood of Diabetes types 2 patients and its effects on Kidney and liver functions

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بِسْمِ اللّهِ الرَّحْمنِ الرَّحِيمِ

﴿وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلاَّ قَلِيلاً﴾

صدق الله العلي العظيم

سورة الإسراء: الآية (٨٥)

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Dedications

To 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.

To My Mother, Brothers, and Sister

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After giving thanks and praise to the creator I extend my sincere thanks and gratitude to all those who helped me complete the research .

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Summary

This study was aimed to investigation of aflatoxin B1 in blood serum of patients with diabetes type_2 samples were collected from Al-Hussian hospital, Karbala province. From November 2021 till March 2022. The number was (86) samples; (42) samples were for patients with diabetes disease and (44) samples were for healthy people.

The results showed that 16 (38.1%) out 42 samples that collected from patients with diabetes type_2 contamination with aflatoxin B1, while 13 (29.5%) sample that collected from healthy persons were contamination with Aflatoxin B1 with no significant difference between them.

Also the amount of blood serum collected from females and males patients with diabetes type 2 and contamination with AFB1was 17 (19.7%) and 12 (13.9%) respectively.

The results illustrated the AFB1 concentration in blood serum of females and males patients with diabetes were 1.343 ng/ml and 0.684 ng/ml respectively with significant difference between them while the concentration of toxin in blood serum of females and males of healthy persons were 0.133 ng/ml and 0.135 ng/ml respectively with no significant difference between them.

Measurement coefficient (r) appearance there is correlation between gender and presence of AFB1 in blood serum (r=0.974 p value 0.324) it means the females more sensitive to AFB1 if compared with males groups. Males patients with diabetes and presence of AFB1 in blood serum of them lead to increased urea level to 42 mg/ml (abnormal level) compare 34 mg/dl in blood serum of males health. also creatinine

level increased in blood serum of males patients with diabetes and borne AFB1 to 1.88 mg/dl.

also uric acid level raised to 8.3 mg/dl in blood serum of males patients with diabetes and borne AFB1 compare with its level in males healthy without AFB1 was 5.87 mg/dl on the other hand alkaline phosphate (ALP) increased to abnormal level in (blood serum of patients male without Aflatoxin B1) group and (female patients without Aflatoxin B1) group reached to 145.8 U/L and 147.1 U/L respectively. Also alanine amino transferase (ALT) parameter raised to (18.15 and 15.08) U/L in blood serum of (males patients with diabetes and with Aflatoxin B1) group and (males patients with diabetes without Aflatoxin B1) group respectively.

The result of measurement of Aspartate aminotransferase (AST) in blood serum of males and females patients with diabetes type 2 without Aflatoxin B1 raised to 53.38 U/L and 49.82 U/L respectively.

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List of Abbreviations

No.	Abbreviation	Meaning
1.	AFB1	Aflatoxin B1
2.	AFG1	Aflatoxin G1
3.	AFM1	Aflatoxin m1
4.	ALP	Alkaline phosphate
5.	ALT	Alanine Amino transferase
6.	AST	Aspartate aminotransferase
7.	BUN	Blood urea Nitrogen
8.	CKD	chronic kidney disease
9.	DC	Dendrtic cells
10.	DM-2	Diabetes mellitus Type 2
11.	DNA	Deoxyribonucleic acid
12.	EIISA	Enzyme linked immuno sorbent assay

13.	**FBS**	**Fasting Blood sugar**
14.	**GSH**	**Gluta thione**
15.	**H UV**	**Ultraviolet**
16.	**HPLC**	**High Performance Liquid Chromatography**
17.	**OTA**	**Ochratoxin A**
18.	**PK**	**protinase k**
19.	**PPBS**	**post prandial Blood sugar**
20.	**RNA**	**Ribonucleic acid**
21.	**ROS**	**Reactive oxygen species**
22.	**SOD**	**superoxide dismutase**
23.	**TLC**	**Thin Layer Chromatography**
24.	**TLR**	**Toll- like receptors**
25.	**TLR-2**	**Toll_ like receptor 2**
26.	**TLR-4**	**Toll like receptory 4**
27.	**TYR**	**Tyrosinc**
28.	**WHO**	**World Health Organization.**

Chapter One

INTRODUCTION

1.1 Introduction

Mycotoxins is chemicals compounds produce by filamentous fungi that disrupting human and animals health by mycotoxicosis different from tradition mushroom poisoning, These fungi called" toxigenic fungi". All of those species are Deuteromycetes (asexuell) some of which have been known Ascomycetes (sexuall) stage. Mycotoxins are secondary metabolite of the fungi concerned, these are compounds that developed after one or more nutrient become limiting (Bu'look, 1975; and Miller, 1995).The production of mycotoxin are absolutely determined by the presence of situation that support the growth of the fungi concerned, under environmental conditions. Different fungi species are favored to cause disease on the crops in the farm, while saprophytes tend to grow on the stored crops or sometimes grow on other materials, (Wild and Turner, 2002)

The studies of toxigenic fungi reveal that they developing many compounds often form different biological functions the environmental conditions impact in the produceing of these compounds have been a fertile major of study in the recent because mycotoxin influence the human and animals (Prelusky *et al.,* 1994).

When Penicillin discovered and other fungal derived antibiotic, the scientists considered that these compounds active in nature. In the post war period, secondary metabolites were characterized as everything from waste product the consequence of "displacement activities" of the fungi. the compounds are significant as virulence factor and as mediators of interference competition with enzyme lead to inhibition activity of it's (Jolly *et al.,* 2007; Scott *et al.*,1997).

Aflatoxins are cancerous secondary metabolites produce primarily by *Aspergillus flavus* and *Aspergillus parasiticus* in agricultural food stuff such as peant, maize, cereals, and animal feed. Aflatoxins are produced at temperature of 12_40 C° and require 3_18 % moisture. Six out of 18 different types of aflatoxins that have been identified are considered important and are designated as B1, B2, G1, G2, M1 and M2. These aflatoxins groups exhibit molecular differences.

Aflatoxin B1 is the most common of the most widespread in the world and accounts for 75 % of all aflatoxins contaminated of food and feed. Aflatoxins are highly liposoluble compounds and readily absorbed from the site of exposure usually through the gastrointestinal tract and respiratory tract into blood stream. (AL. Jumiley, 2014)

Human and animals get exposed to aflatoxins by ingestion of aflatoxin _contaminated food or ingestion of aflatoxins carried over from feed into milk and milk products like cheese and powedered milk as well as other animal tissue. Also by inhalation of dust particles of aflatoxins especially AFB1. The aflatoxins are absorbed across the cell membranes where they reach the blood stream. They are distributed in blood to different tissue and to the liver, the main organ of metabolism. (Cast, 2003)

Taking a high amount of aflatoxin B1 in short time lead to acute toxicity. Most common signs and symptoms are vomiting, Abdominal pain, Edema, Nausea, Itching and death.

The contamination of foods with aflatoxin B1 can cause serious consequences in human health (Marchese *et al.*, 2018)

Type 2 diabetes mellitus (T2DM) is one of the major global diseases, however it's always linked with obesity. Patients with diabetes mellitus are exposed to serious complications such as (microvascular and nephropathy) and macrovascular complications such as (cardiovascular and comorbidities) owing to hyperglycemia and individuals condition of Insulin resistance metabolic syndrome occur. There is a lack of the study on this toxin and it’s related with one of the most common disease diabetes mellitus type _2. Because of the serious consequences result from AFB1 and it's effects on some chronic diseases, I decided to study this topic . (Libby *et al.,* 2021)

1.2 Aim of study

Investigation of Aflatoxin B1 in human blood serum of patients with diabetes mellitus type 2 and study of effects Aflatoxin B1 and diabetes on functional liver and kidney.

Chapter Two

LITERATURE REVIEW

2.1 Diabetes Mellitus type

Diabetes mellitus type 2 is similar to monogenic disease, which can be diagnosed by genetic test. Most cases of monogenic are wrongly diagnosed as diabetes mellitus type 1 or type 2, which are totally different from monogenic disease, monogenic disease are also called maturation onset diabetes of young(MODY), some type of monogenic disease can cause early renal failure,(Steck *et al.,* 2011), while most complication of diabetes mellitus type 2 are macrovascular and microvascular which are both result from atherosclerosis. If atherosclerosis result from big blood vessels cause macrovascular which ended up to stroke, myocardial infarction and muscle wasting. It also causes diabetic foot. Whereas microvascular cause retinopathy, nephropathy and encephalopathy. The most serious and common of diabetes mellitus type 1 is diabetic ketoacidosis (DKA).The precipitating factors are omission of insulin, infection, myocardial infarction, and trauma. All metabolic disturbance seen in DKA are the direct or indirect consequence of the lack of insulin. Early diagnosis and proper treatment should lead to control glucose level and improve health in a long term.(Vanden *et al.,* 1992)

Genetic testing is recommended if diabetes is diagnosed within first six months of age, children and young adult particularly those with strong history family, patients with diabetes who don't have typical features of diabetes type 1 or type 2 such as presence of autoantibodies for type 1 or obesity for type 2, a person has stable mild fasting hyperglycemia. Most form of diabetes mellitus are caused by autosomal dominant external link mutation. That means the condition can be passed on to the children when only one parents is carrier or has disease, a parent who carries the gene has a

50% percent chance of having an affected child with monogenic diabetes (Steck *et al*., 2011).

Diabetic is metabolic Syndrome, metabolic means affecting metabolism, syndrome means the symptoms that appear affecting more than one system, however the major character is hyperglycemia (increase in glucose blood level). The symptoms of diabetes are polyuria, polydipsia, polyphagia, and undesired weight loss. Type 2 diabetes mellitus also called NIDM (non-Insulin dependent diabetes mellitus) the main patient's problem with diabetes mellitus type 2 is Insulin resistance (Insulin receptors don't response to Insulin) in the onset of the disease there is hyperinsulinemia as attempt from the body to enforce the receptors to response. When glucose enter the cell (beta cell) of pancreas, by Glut_2 it undergoes glycolysis and produce ATP, this produced ATP in turn goes to ATP dependent potassium channel that present on Beta cell of Langerhans, next the channel closes, as it's well known potassium is mainly extracellular when potassium being inside the cell causes depolarization voltage gated calcium channel so calcium influx inside the cell catching the vesical which have Insulin and cause exocytosis of Insulin outside the cell. (Libby *et al*., 2011).

The effect of decrease the Insulin's action leads to hyperglycemia which means that glucose will get over renal threshold and begin to appear in urine it's estimated by (170_180 mg/dL). when glucose releasing from the kidney it take water, vitamin(water soluble vitamin) and minerals. Insulin hormone is anabolic hormone decrease of its action leads to increase lipolysis and decrease lipogenesis and that's why undesired weight loss happens, defect of this hormone increasing catabolism and decrease

anabolism so it's effects on the production of antibodies and decreases the immunity.(Berbudi *et al.*, 2020)

Insulin is protein hormone produced from beta cell of Islet of Langerhans in pancreas, it has no role in diagnosis of diabetes mellitus. The normal fasting Insulin should be blow(10µU /ml). (Libby *et al.*, 2011)

Table (2-1) Normal and Diabetic value of blood glucose.

	Normal value of blood glucose	**Diabetic value of blood glucose**
FBS	70 100mg/dl	Equal or less 126mg /dl
PPBS	5. 6 mmol/L	7mmol/L
PPBS	<140mg/dl	200 mg/dl

(Bozkaya *et al.*, 2010)

2.2 Homeostasis of glucose level in the blood

The regulation of glucose level in the blood is attributed to organic chemical substance which called Hormones. Hormones are secreted from endocrine system. These hormone are involved in many Functions such as regulation of body system , reproduction a metabolism and energy a growth and homeostasis.

The original word of hormone comes from Greek which means (to excite), our bodies entirely depend upon the endocrine system and nervous system to control upon the different systems in our bodies and these two system (endocrine and nervous system interferer with each others to regulation the functions of the body. (Chao *et al.*, 2019)

Some glands of endocrine systems are included in the brain such as Hypothalamus and pituitary gland which induced by nervous signal. Endocrine system has slow response but long term effect in compare with nervous system which have fast response and short term effects . Hormones need to specific receptor on the surface of the cells to influence on them.

Those receptor are different according to the characteristic of hormones . By signaling pathway We could be classified hormons into three groups paracrine, endocrine and autocrine.

Hormones are also classified according to their chemical nature to amino acid such as T3 and T4, peptide such as glucagon, protein like insulin. And the lipid derivatives like estrogens (Libby *et al.* , 2011)

Insulin is the most important hormone in regulation of blood glucose level, which is secreted from pancreas - pancreas is heterocrine which contain two types of tissue endocrine and the other one exocrine endocrine producing tissue made of two cells alpha cells and B cells The more significant cells a alpha cells responsible for secreasing hormone glucagon and β cells responsible for secreting hormone Insulin (Sharma ,2021) Insulin is responsible for bring glucose level down, it's stimulus is hyperglycemia glucagon hormone is release when glucose level is low (hypoglycemia).(Chao *et al.*, 2019)

The second type of Pancreas's tissue is named exocrine which secreted Acini that related to directional function. (Matschinsky and Wilson, 2019)

Insulin synthesis in the nucleus of β cell , Insulin consists of (51 Damino acid and two peptides chain A peptide and B peptide, Two peptides are binding to each other by disulphide bond (Menting *et al.*, 2013)

Inside the nucleus there is specific gene undergoing transcription into mRNA and then mRNA comes to the cytoplasm where there are ribosomes and translation to protein, after that the Protein goes to the Rough Endoplasmic reticulum to undergo specific modification and then go to the structure called Golgi apparatus in Golgi apparatus it gets pakaged-out of Golgi apparatus there is insulin in specific vesicles. (pedersen *et al.*, 1990).

After release of insulin to the blood from the vesicles fused with cell membrane which contain in addition to insulin C-peptides and Amylin compound which is significant in Type 2 DM (Libby *et al.*, 2011)

Insulin bind to tyrosine kinase receptor . When it binds it stimulates specific phosphorylation of tyrosine residues and the overall result is to activate intracellular messenger called Pick and AKT (Protein kinase B). Glucose converts into a specific molecule, it's polymerize glucose into glycogen and to pyruvate by activating glycolysis a pyruvate turn into acetyl CoA and then acetyl COA enter Kerb's Cycle producing NADH and FADH2 which end in the electron transport chain producing ATP. (Stumvoll *et al.* , 2008 ; Leibowitz *et al.*, 1995)

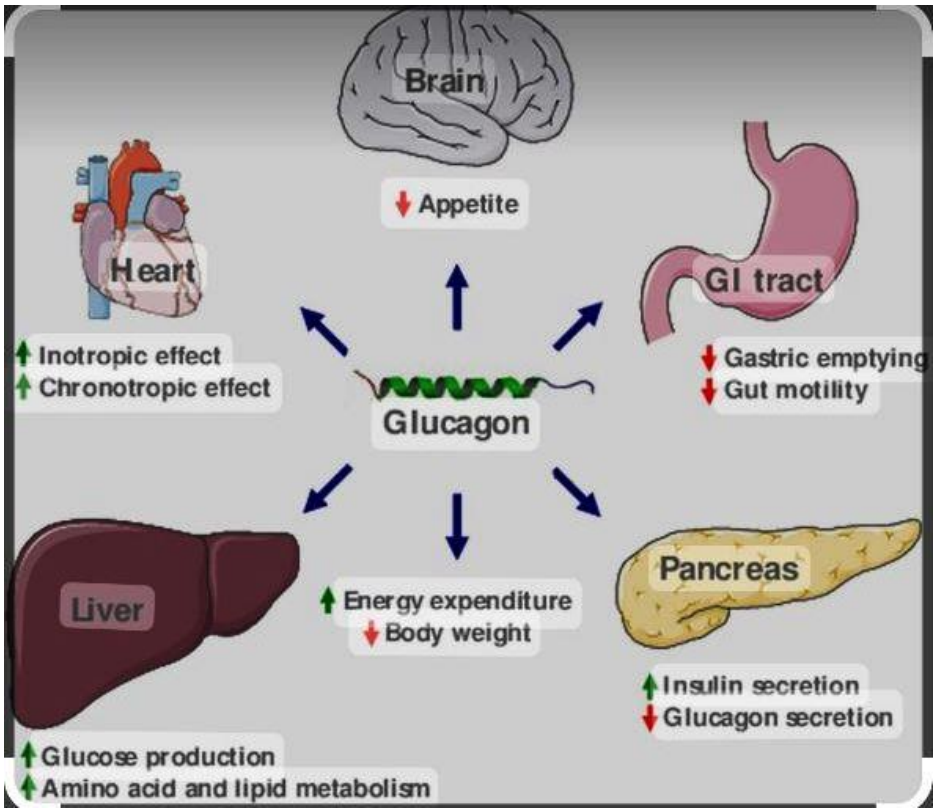

Figure: (2-1) Mechanisms of action Insulin Hormon (Burcelin *et al.*, 1996)

Glucagon is 29 - amino acid peptide it synthesis from gene present in the nucleus of alpha cells in a way that is similar to insulin (Stumvoll *et al.*, 2008).

Glucagon binds to the specific receptor. called G-protein receptor then it stimulates stimulatory protein which bind to the GpT protein which in turned activates an effector enzyme which called adenylate Cyclase that take ATP and convert it into cAMP that activates protein kinase A (PKA).

Glycogenolysis process converts glycogen to glucose so glucose level in the blood will (Emhoff *et al.*, 2013)

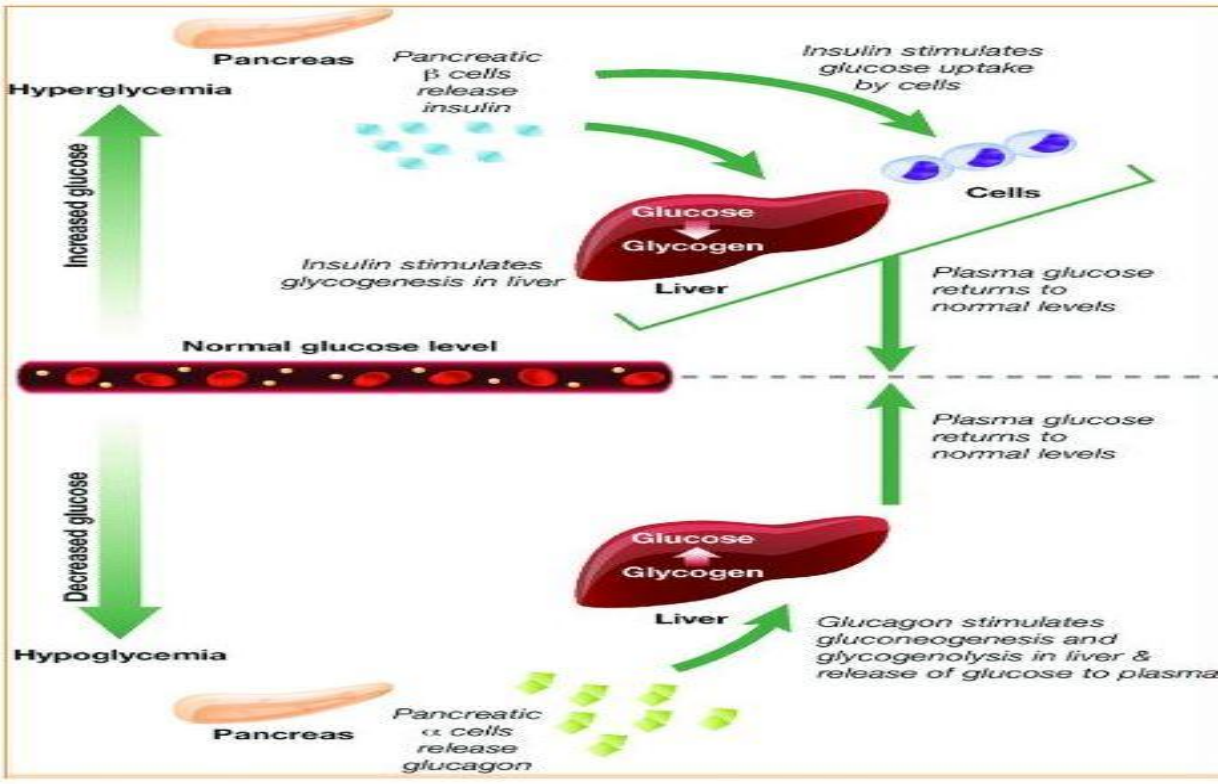

Figure (2-2): Mechanisms of action Glucagon Hormone(Christophe, 1995)

2_3Mycotoxin concept.

Mycotoxicosis is the name has called upon the group of illness and disturbances in human and animals caused by secondary metabolite produced by some fungi, mycotoxins are polyketones compounds yield under specific physical, chemicals and biological circumstance that occur by reduction of ketone group in the biosynthesis of fatty acid carried out by mould, mycotoxins are produced at the end of the exponential phase or at the beginning of stationary phase of the mould growth more than 200 mycotoxins have been identified up to now, the production of mycotoxins are subjected to three factors humidity or free water, water and temperature. Mycotoxins is regarded as the most dangerous, if we compare mycotoxins

with bacterial toxin, since bacterial toxin is protein in it's chemicals nature it will be recognized once it get entry into the body by the immune system, while mycotoxins are toxic chemicals compounds of low molecular weight the immune system can not recognize them and that lead no reaction occurs (Pitt, 1989). Mycotoxins are toxic structurally diverse, secondary metabolites produced by a wide range of moulds and they infect food and feed and agricultural commodities (scott *et al* ., 1997)

Characteristics of mycotoxin.

1. Mycotoxin are complex structure and most of them are phenolic and alkaloids compound
2. Low molecular weight (less than 10^4 Dalton)
3. Mycotoxin cannot induce of immune system, so that it's not able to cancel mycotoxin activity
4. Most of mycotoxin are heat stable

Classification of mycotoxin.

The mycotoxin are classified into many groups but classified bases are different between mycotoxin classification system and another

Classification depended on target organisms affect

1. The mycotoxin effected on liver called Hepatotoxins
2. The mycotoxin effected on neuro tract called neuro toxin.
3. The mycotoxin effected on kidney referred nephrotoxin

Classification depended on chemical nature of mycotoxin

1. The mycotoxins consist of coumarin lactones called polyketides.
2. Mycotoxin derived from amino acid referred amino acid derived

Classification depended on diseases caused by mycotoxin

1. Reyes syndrome mycotoxins.
2. Stachbotry toxicosis.

Classification dependent on fungal produce of mycotoxins.

1. mycotoxin produced by *Aspergillus* called *Aspergillus toxin.*
2. mycotoxin produced by *pencillium* referred *penicillium toxin.* (Garies and wernery, 1994)

Effect of mycotoxin.

1. Biochemical and physiological effects of mycotoxin. The biochemical of mycotoxin including;

A. They are inhibition of protein synthesis.
B. They are inhibition of RNA and DNA synthesis.
C. some of mycotoxins are able to increase of ALT and AST levels in blood addition of sugar, on the other hand some mycotoxin decrease the number of RBC and Hb level and also the hormones level decreased (Li_Huiying and Zhang, 2018)

2. Nephrotoxicity :_ the kidney is major of mycotoxin induced toxicity where it's principle on the middle and terminal segments of the proximal convoluted tubules studies have shown that Ochratoxin-A exposure can lead to increased urine volume, blood urea nitrogen, urine glucose and protein urea as well as to reduction in the activity

of enzymes in the kidney such as alkaline phosphate, leucine amino peptidase.

3. Carcinogenicity :_the carcinogenicity of mycotoxin with kidney and liver. Aflatoxin B1 and Ochratoxin have been implicated in the development of cancers of human liver and urinary tract respectively. (Meerding,2004)
4. Genotoxicity: A few mycotoxin induced DNA damage have been reported. DNA single strand breaks have been observed consistently in mammalian cell culture. DNA strand breaks have also been observed in vivo assay in spleen, liver, kidney cell of mice intraperitoneal Injection of mycotoxin such as OTA and AFB1.
5. Immunotoxicity mycotoxin have been shown to affect a number of parameters of immune function. The number of splenocytes are reduced . The animals lab treated with mycotoxins become susceptible to infection with *salmonella choleraesuis.* The mechanism of mycotoxin_ induced immunosuppression in animals are unclear although studies suggest that it may be related to the inhibitory effect of mycotoxin on DNA and protein synthesis in lymphocytes macrophages, and other immune system types. (Magan and Olesen, 2002)
6. Neurotoxicity :_

A. the potential neurotoxicity of mycotoxins has been investigated in a limited number of animal revealed lesions in the ventral mesencephalon, hippocampus, striatum and cerebellum of the brain were found after four rats were administered (29 mg OTA /kg b. w) oral gavages for eight days
B. Increase Gamma glutamyl transferase activity in the brain

C. OTA inhibit protein and DNA synthesis. (Zhang *et al.*, 2020)

Toxic effects of mycotoxin on tissue.

Mycotoxin caused pathogenic changes in many organs tissue as small intestine represented by shrinkage of intrastructure of it, and increases lymphocytes cells, while in liver severe vascular congestion and necrosis occur and in kidney highly proliferation of cell endothelial layer of Bowman's capsules in addition to hemorrhage and focal aggregate inflammatory cell. (Tai and pestka, 1999).

The major of mycotoxins.

2.4 Aflatoxin B1:

Aflatoxin B1 is mycotoxins produced by the fungi Asper

Aspergillus flavus and *Aspergillus parasiticus*, *Aspergillus* are opportunistic molds wide spread in the environmental and contaminate a wide range of agricultural products such as cereals, nuts, pean and egg.

Aflatoxin belong to a group of difuranocoumaries that are categorized into two groups in agreement with their chemicals structures and the difuranocoumaries cyclopentenone series (AFB1, AFB2, AFB2A, AFM1, AFM2 and aflatoxicol) and difurocoumarolactone series (AFG2, AFG2A, AFGM1, AFGM2, AFB3). The most important aflatoxins produced by Aspergillus species contain (AFBI, AFB2, AFG1, AFG2). (B) and (G) indicated to the blue and Green fluorescent under UV light on thin Layer chromatography plates respectively, while subscript number 1 and 2 referred to the major and minor compounds respectively, B1 and B2 confer to the

sight of blue fluorescence under the UV while the G assignation of yellow green fluorescent under UV light.

The metabolic product of Aflatoxin m1 and M2 were first separated from lacting animals feed on grains contaminated with Aflatoxin B1 and and G2 were founded as the dihydroxy, among four major Aflatoxin (B1, B2, Gland G2) G2 happens with high amount done with less toxic, while AFB 1 is referred to highest degree of toxicity (Meerdink, 2004).

Bbosa. Godfrey *et al*., (2013) studied the biological and health effects of of Aflatoxin B1 on the body organs and body system, the exposure of food to the fungal troubles occurs during preharvest, transport, in the depots, production of mycotoxin in cereal crops in Inadequate temperature and humidity both in field and storage.

To prevent fungal contamination some researchers applied method in which they use formic and ctric acid to forbid biosynthesis of Aflatoxin in poutry feed with variable moisture conditions. Pollution agricultural products with aflatoxin representing a grave proplem from the point of view food security and as well as economic losses, these economic losses are estimated with 52 million dollar per year only in corn crops (Singh and Mandel, 2014).

The degree of toxicity rely on the duration of exposure to the aflatoxins, the health problems of aflatoxins may include carcinoma, liver cirrhosis, nephropathy. (Darwish *et al*., 2014)

There is association between urinary AFB 1 and contaminated dietary with such toxin, that was indicated by prevalence of toxin among adult whose diet was contaminated, and that should be assured the extremely

important to prevent contamination of food with AFB1 . It's essential to determine the level of AFB1 and the frequency of it's exposure, around 4.5 billion people are estimated being stand for AFBI in the world Wide. AFB1 also have serious effect on pregnant women especially those who have a high viral load and abnormal liver function (lauer *et al*., 2020). The wallop of Aflatoxin B1 in health has been reinforcement by trials in China and Africa countries which have high proportion of hepatitis B infections where food exposed to Aflatoxin also common exposure to Aflatoxin is prevalent in west Africa and tests that carried out displayed high rate of people were positive to Aflatoxin. It has been linked to liver and kidney tumor (Yilmas *et al*., 2018), human exposed to AFBI may result from ingestion of animal product such as milk, egg and meat, it has been found that all AFB1 are speedily absorbed because of low molecular weight the AFB1 absorbed quickly by gastrointestinal tract through non_known passive mechanisms and then quickly demonstrate as metabolites in blood after only 15 minutes while in the milk it takes around 12 hours the main source of aflatoxins exposure is from food, maize is crops that play an essential role in the diet of many countries around the world, the contamination of diet considered a health and economic dilemma, not just food contamination intake is the source of AFB1.,but it can also get entry by inhalation and by dermal which both are considered important path ways (Malik *et al*., 2014)

2.5 Chemicals properties of AFB1.

Aflatoxin B1 (C17H1206, MW312) is crystalline compounds soluble in moderately polar solvent such as Methanol, chloroform, dimethylsulfoixide and dissolve in water in the range 10_20 mg/litter. AFB1 fluoresce under UV radiation. The crystalline structure is permanent

in lack of light especially UV radiation, up to temperature 100C°. Chemically AFBI is a really substituted coumarin structure with fused dihydrofurofuran moiety (Kensler *et al*., 2011). Aflatoxin M1(C17H1207, MW 328), one of the hydroxylated metabolite of AFB1, it occurs in milk of mammals that intake contaminated feed of concerned toxin, Aflatoxin also release in urine. In specific AFBI is characterized by the fusion of cyclopentenone ring to the lactone ring of the coumarin structure (figure 1)and by strong fluorescence emission in the blue region (Marchese *et al*., 2018).

Both toxin have similar structure properties they are lightly soluble in water, insoluble in nonpolar solvents and readily soluble in polar organic solvents (Danesh *et al*., 2018)

They have steadily structures in high temperature, even over 100C°, so the high temperature using through food preparing does not destroy the toxins, This represents a really problem in attempts to reduce food contamination with such toxin, especially in milk and dairy products, as pasteurization and other thermal process appeared to be poorly effective (Mahmood *et al*., 2018).

They are sensitive to UV light especially in the presence of oxygen, sensitive to acidity PH (<3 or 10).the lactone ring open under alkaline conditions and the aflatoxin are damaged but this reaction is reversible on acidification. Ammooniation leads to opening of lactone ring at high temperature, causes decarboxylation of aflatoxins and this reaction is irreversible (kumar , 2018).

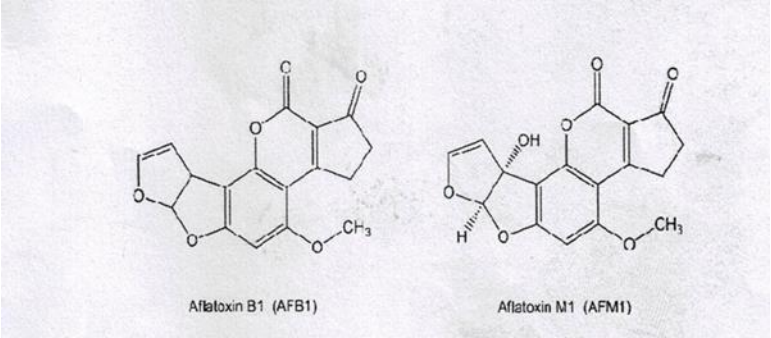

Figure 2-3 Chemical structures of Aflatoxin B1 and Aflatoxin M1 (Cast, 2003)

2.6 Aflatoxin absorption, distribution, metabolism, excretion and mechanisms of action.

Understanding the mechanism of aflatoxin requiring from us to be knowledgeable how these toxins are metabolized, the metabolite of these toxin have been reviewed in many studies (Eaton and Challenger , 1994)

function oxidase (MFO) enzymes belong to super family of cyp 450. By the action of these oxidase, AFB1 is transformed in the reactive 8,9.expoide occurring as two sterio, isomers exo and endo. The exo_8,9expoide has a high binding affinity to the DNA, so it forms the 8,9_dihydro_8_ (N7_guanyl)_9_hydroxy_AFBI (AFB1_N7_Gua) adduct hence causes DNA mutation (Verma, 2004). This expoideformis involved on other pathways conjugation with glutathione (GSH) catalyzed by Glutathione –s–Transferase (GST) with subsequence excretion as AFB_macrupturate, this pathway is vital for the detoxification of AFBI as a carcinogen, even if depletion of GHS could lead to high levels of reactive oxygen species (ROS) causing oxidative damage (Bbosa *et al.* , 2013) the others pathways are enzymatic and non_enzymatic transformation, AFB1

8,9 dihydroxydiol, that can transformed to dialdehyde form, aflatoxins dialdehyde can be released in urine as dialcohol by action of aflatoxin aldehyde reductase (AFAR) or can bind protein like albumin (Wild *et al*., 1992)

Or can binding to other macromolecules like protein or RNA leading disturbance of normal cellular functions and inhibition of protein, RNA, DNA synthesis (Verma, 2004) microsomal bioconversion of AFB1 include also hydroxylation of toxin causing to formation of more polar and less toxic metabolites, there are many studies that tried to know the role of CYP450 enzymes responsible for the formation of carcinogenic or detoxification metabolites CYPIA2 and CYP3A4 resulted to be more active isoenzyme of this family and have the ability to activate AFB1 (Gallagher *et al*., 1994) it's well known that AFB1 epoxide have the main role in the genotoxic process and thus carcinogenesis, the high affinity of epoxide intermediate for purine bases of DNA causes to the formation of AFB1_N7_Gua adduct, that promote adduct causes depuration and thus a purine site formation(wild and Turrner , 2002) on the other hand it's important addition that AFM1 is considered a detoxification Production of AFB1 metabolite appearing only 10% of mutagenicity comparing to it's precursors. The metabolic fate of AFM1 resulted to be similar to that of AFB1 with difference that AFM1 represents a less substance of expiration thus reflects the differences in genotoxic potencies, more ever some studies were indicated that CYP is not essential to AFMI to exert cytotoxic effect (Neal *et al*., 1998)

2.7 Aflatoxin B1 and liver Cancer in human

As indicated in many studies that AFBI induces the formation of DNA adducts which leads to liver cancer (Nugraha *et al*., 2018) AFBI considered one of the most important of hepatocellular carcinogensis, with 4.6 28.2% of HCC cases end up with liver cancer after exposure to AFB1 (kucukcakan and Hayruli, 2015) More ever hepatitis B virus (HBV) can increase the risk of HCC in AFB1_exposed people by 30_fold Lin and Wu, (2010). High exposure concentration causes acute hepatitis and, as a consequence the chronic exposure leads to liver cancer (Rapisarda *et al*., 2016) the recent studies reports that the correlation between AFB1 exposure can lead to expression change of autophagy related protein P62 and prognosis of patients with chronic HBV infection_ related HCC. However the genotoxic effects of AFBI were unknown and some authors treated human HL7702 hepatic cells with microcystin_LR (mc_LR) with AFB1 and demonstrated that me LR and AFB1 co_exposure induces DNA damage and increased the activating of superoxide dismutase and catalase, the level of glutathione and AFBI exposure showing that there is a clear correlation between AFB1 and liver cancer (Xiang *et al.*, 2017)

2.8 The effects of AFB1 on the immune system.

Pestkka and Bondy, (1994) showed that AFB suppressive immune system especially cell mediated immunity. That was clarified in different animal models. These research. have been demonstrated that AFB1 reduce T lymphocyte function and number, suppress lymphoblast genesis and impede delayed cutaneous hypersensitivity. AFB1 has also been proven to suppress phagocytosis activity (Pier, 1986). The remarkable decreasing in Splenic

CD4 (Th) cell number and IL2 production have been observed in mice treated with AFB1 (Hatori and Sharma, 1991 ;Moon *et al*., 1999) demonstrated that AFB1 impede the function of macrophage in different animal species. Also phagocytosis of alveolar macrophages and tumor necrosis factor alpha (TNF) released were suppressed in rats and mice exposed to AFB1 through the respiratory tract (Cusumbo *et al*., 1996 ;Jakob *et al*., 1994) pointed out to that AFB1 inhibit phagocytic cell in normal human peripheral blood monocyte .

The immunotoxic effects of AFB1 linked to frequency of exposure and to the doses, and this effects on immune surveillance molecule patterns recognition receptors (PRRS), still fuzziness. However the main role of PRR in inflammation to clear microbial infection is studied well (Biadja *et al*., 2010; Wang, 2011). Dendritic cells (DC) are antigen presenting cells (APC) and they linked innate and acquired Immunity (Joffre *et al*., 2009; Hangen *et al*., 2001). Expression of PRR allows sensing of P/DAMPS by TLR on DC trigger intracellular signals pathways. This induces production of proinflammatory cytokines, chemokines and interference and thus activates APC and other immune cells to link innate and adaptive part of the immune system for protection of human body. When exposure to AFB1 lead to the production of inflammatory molecules and free radicals and that causes damaging neighboring cells and tissue.

(Malvandi *et al*., 2013) . Although the contamination of AFB1 has decreased in developed countries it still heigh in developing countries, however it occurs in both developing and developed countries and since this toxin is hazard many researchers pointed out it will be considered as future's public health issue worldwide. Aflatoxins is hazard in animal feed and prose

a risk to human through unexpected consumption the scientists provide some information on how environmentally level of AFB1 modified TLR expression leading to functional impaired in DC. (Wild *et al.*, 2002)

AFB1 affect DNA in any cells in vivo and thus it can cause cancer AFB1 also impaired the redox status of immune cells and pathogen recognition receptors . (Mehrazed *et al*., 2011)

Cytochrome P450 with TLR4 activated in human lymphocytes and monocytes by AFB1 treatment, (Veigas *et al.,* 2013). deriving immune cells to pro-oxidant /inflammation status. (Garate *et al*., 2013) that thereby enhancing immune dysregulation and risk infection and cancer. Mehrazed *et al*., (2011) pointed to that researches have never been conducted on effect of AFB1 in human PRR.

2.9 Ochratoxin A

Ochratoxin-A (OTA) is a naturally occurring toxin produced by main types of fungi(molds), *Aspergillus ochraceus* and *Penicillium verrucosum.* Around the world OTA is found most frequently in stored grain. OTA makes its way into a variety of food and beverages, particularly cereal and grain products, but also dried fruit, wine coffee, beer, cocoa, juices, spices, pork, poultry and dairy products. There is some evidence that OTA can cause kidney tumors in both male and female rats as well as mammary gland (breast) tumors in female rats. OTA has been found in human blood, tissue and breast milk in several counteries. (Dusch *et al* ., 2003)

Types of Ochratoxin.

Several types of Ochratoxin occur naturally, namely, Ochratoxin A, Ochratoxin B and Ochratoxin C.

Ochratoxin A in human and animals.

Ochratoxin A(OTA) is an ubiquitous nephrotoxic and carcinogenic mycotoxin considered to be involved in the aetiology Balkan endemic nephropathy (BEN). The occurrence of this human fatal disease appears in regions of Bosnia and Herzegowina , Bulgaria, Croatia, Rumania, and Serbia. Several studies have shown that male mice develop kidney tumors after exposure to OTA in their diet. (Bashir *et al.*, 2020)

Carcinogenicity.

Ochratoxin A is potentially carcinogenic to human (Group 2B), and has been shown to be weakly mutagenic, possibly by induction of oxidative DNA damage. The evidence in experimental animals is sufficient to indicate carcinogenicity of Ochratoxin A. It was tested for carcinogenicity by oral administration in mice and rats. It slightly increased the incidence of hepatocellular carcinoma in male mice and in rats. In human very little histology data are available, so relationship between Ochratoxin A and renal cell carcinoma has not been found. However, the incidence of transitional cell (Urothrial) urinary cancers seems abnormally high in Balkan endemic nephropathy patients, especially for the upper urinary tract(Magan and Aldred, 2005)

Neurotoxicity

Ochratoxin A has strong affinity for the brain, especially the cerebellum ventral mesencephalon, and hippocampus structure. The affinity for the hippocampus could be relevant to the pathogenesis of Alzheimer's disease (Cast, 2003)

2.5.3. immunotoxicity.

Ochratoxin A can cause immunosuppression and immunotoxicity in animals. The toxin's immunosuppression activity in animals may include depressed antibodies responses, reduced size of immune organs (such as the thymus, spleen, and lymph nodes) changes in immune cells number and functions and altered cytokines production. (khatoon *et al* ., 2013)

Mechanisms of action of Ochratoxin A.

Ochratoxin A has a number of toxic effects in mammals, the most notable of which is nephrotoxicity . It is also immunosuppressive, teratogenic and carcinogenic. The biochemical and molecular aspects of it's action were first studies in bacteria. Ochratoxin A inhibits bacterial, yeast and liver phenylalanyl _tRNA synthetases. The inhibition is competitive to phenylalanyl alanine and is reversed by an access of this amino acid. As a consequence, protein synthesis is inhibited (Hassan, 2021)

2.10 Zearalenone (ZEA)

A macrocyclic beta_resorcyclic acid lactone, is a none _esteroidal estrogenic mycotoxin produced as a secondary metabolite by numerous specific of fusarium, including (*F. culmorum, F. equiseti, F. graminearum)* and others these fungi are present on almost all continents and they are

known to infect both pre_and post _harvested wheat, barley, rice, maize, and other crops, resulting in contamination of human food and animal feed worldwide.

There are many Zearalenone derivatives such as 7_dehydrozearalenone, zenralenic acid, and 8_hydroxyzearalenone. (Nash *et al* ., 1962)

Properties.

Zearalenone has fluorescent properties because it is a white crystalline solid that exhibits blue _green fluorescent when examined by UV light at a wavelength (360 nm)

The relative molecular mass of Zearalenone is 3l8.36 the melting point is 165 C°, and it has a good thermal stability.

1. Zearalenone produced by many certain strains of various species of the genus Fusarium (*F. Moniliforme*, *F. graminearum)*

it will not decompose when it is 120 C° for 4 hours.

ZEN will not dissolve in water, while easy to dissolve in alkaline solution such as sodium hydroxide and organic solvent such as methanol.

Estrogen receptors are the main target (Althali *et al.*, 2019)

The effects of toxicity.

This Zearalenone has the ability to bind estrogen receptors. This lead disturbances in the hormonal balance in the body, which may subsequently lead to many disease of the reproductive system such as

cancer to prostate, ovary, cervix or breast. Zearalenone can permeate through human skin however significant hormonal effect are not expected after skin contact in normal agricultural or residential environment. (Minervini *et al* ., 2005).

Detection of Zearalenone.

Detection of Zearalenone is achieved by High performance liquid chromatography (HPLC). Thin layer chromatography is also used. These methods are more commonly used (Majerus *et al* ., 2009)

2.11 Patulin toxin

4-hydroxy-4H-furo{2,3-C}pyran-2{6H}-1) (clavacin; PAT), (molar mass 154.12) (molecular formula C7H6O4) . is a mycotoxin produced by many different moulds such as Penicillium, Aspergillus, and Byssochlamys species(Byssochlamys nivea)and are mainly produced by *Penicillium patulum* (later called *Penicillium urticae*, now *Penicillium griseofulvum*). *and Penicillium expansum;* PAT is often associated with fruits, juices, and derived products (The high water and sugar content in fruits promotes the occurrence of PAT in fruits) , including foods intended for young children,these patulin-producing fungi attack susceptible products during growth, harvest, storage, or food processing . Among different fungi species, Penicillium expansum, which is commonly present in many varieties of fruits, is the major producer of PAT. Patulin has been primarily associated with apple and apple-based products. However, the toxin may also contaminate other fruits, moldy feed, rotten vegetables, and wheat straw residue (Brase *et al*., 2009).

Chemical and physical properties:

1- colorless
2- crystalline
3- water-soluble at 105–125 ◦C with melting point of 110 ◦C .
4- polyketide lactone.
5- PAT is thought to exert its toxicity by reacting with thiol groups in the cellular system , or covalent binding to the sulfhydryl group of various amino acids in proteins.
6- belongs to a class of toxic compound with low molecular weight (154.121 g/mol) (Marin *et al*., 2013)**.**

Optimal conditions for growth and toxin produce:

1. temperature : Optimal growth for *p.expansum* observed at 25 degrees C for every strain tested. Patulin production was stimulated when the temperature decreased (from 20 to 10 or 4 degrees C), while a further decrease of the temperature to 1 degrees C caused a reduction in patulin production.
2. oxygen level: A reduction of the O(2) level from 20 to 3% O(2) could stimulate or suppress patulin production depending on the strain, while a clear decrease of the patulin production was observed when the O(2) level was reduced from 3 to 1%.(Misdana *et al.,* 2016)

Acute toxicity : PAT incidence

PAT toxicity relates to deleterious formation of adducts with sulfhydryl groups, producing acute and chronic toxicity problems in animals and humans. The adverse health effects of PAT have led to the establishment

of safe levels of PAT in foodstuffs. The Codex Alimentarius established the maximum level of PAT in fruits and juices at 50 µg/kg. According to Commission Regulation (EC) No. 1881/2006, the European Union (EU) fixed maximum levels of PAT in fruit juices (50 µg/kg), solid apple products (25 µg/kg), and foods intended for infants and young children (10 µg/kg). Countries such as China, USA, and Canada have also established maximum levels for PAT in foods, primarily in apple-based products, in the range between 25 and 50 µg/kg. Furthermore, the Joint Expert Committee for Food Additives has established a provisional maximum tolerable daily intake of 0.4 µg/kg body weight. The LD50 (lethal dose, 50%) for PAT is between 29 and 170 mg/kg body weight (bw) for rodents and poultry, respectively (Brase *et al*., 2009).

Toxic effects of patulin :

Body organs affected by PAT include kidney, liver, intestine, spleen, and stomach .PAT toxicity in mammalian cells and animals includes genotoxicity, teratogenicity, embryotoxicity, and immunotoxicity. According to the International Agency for Research on Cancer (IARC), PAT is classified in the group 3 as "not classifiable as to its carcinogenicity to humans".

1- patulin induced cell toxicity

A. Involvement of reactive oxygen species In several studies, it has been concluded that reactive oxygen species (ROS) is a key player in PAT mediated toxicity. PAT being electrophilic in nature forms covalent adducts with nucleophilic moieties particularly cellular thiols including glutathione(GSH) , which is important in neutralizing free radicals and

oxidants. PAT induced ROS reported to cause cell death through the activation of the endoplasmic reticulum stress pathway and disruption in mitochondrial function in HCT116 and HEK293.

B. PAT may cause damage to proteins through the reaction of electrophilic groups on PAT with sulfhydryl groups on proteins. Increased oxidative stress may also be a mechanism of cellular toxicity, with targets of PAT shown to include cell junction proteins, mitochondrial proteins, cytoplasmic proteins, and DNA fragmentation.

C. Inhibition of different enzymes: many enzymes with a sulfhydryl group in their active site are sensitive to PAT. Na+ -K+ dependent ATPase, RNA polymerase, aminoacyl-tRNA synthetase, and muscle aldolase have all been shown to be inhibited by PAT. However, enzymes that lack the sulfhydryl group are also sensitive to PAT, like urease (Saleh and Goktepe, 2019).

D. Immunotoxicity: it reduces the expression of IL-23, IL-10 and TGF-β in bovine macrophages. PAT, showed increased Th2 cytokine levels and decreased IFN-gamma production. PAT also causes airway hyperactivity and eosinophilic lung inflammation thereby increasing allergic immune response. apoptosis in dendritic cells. 7 PAT exposure leads to the reduced expression of IL-4, IL-13, IFN-gamma, IL-10 and intracellular GSH depletion in human peripheral blood mononuclear cells(Brase et al.,2009).

2- Patulin induced organ toxicity

1. Intestinal toxicity : PAT is reported to cause intestinal ulcers, inflammation and bleeding. Two human intestinal epithelial cell lines (HT29 and Caco-2), when exposed to a micromolar concentration of

PAT, showed the reduction in the TEER mediated by inactivation of protein tyrosine phosphatase. A study suggested that PAT may take part in initiating intestinal inflammation. PAT can enhance the passage of commensal bacteria and increased the effect of IL-1 beta on IL-8. PAT causes a decrease in goblet cells and an increase in apoptosis, which is extremely toxic to the intestine but on the other hand ascladiol, a metabolite of PAT, is relatively safe for the intestine (Cast, 2003) .

2. Hepatotoxicity :PAT causes increase in activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) and caused lipid peroxidation. furthermore causes increase Alkaline Phosphatase (AP), urea, creatinine and uric acid .
3. Neurotoxicity: PAT traces were also detected. Animal necropsies showed neuronal degeneration in the CNS, axonal degeneration in the PNS and nervous lesions. increased levels of GSSG, reactive oxygen species, thiobarbituric acid reactive substances and protein carbonyl levels and downregulated protein thiol and total thiol groups. Furthermore, PAT also reduces the activities of glutathione peroxidase and glutathione reductase. In neuro-2a cells, PAT causes ATP depletion and mitochondrial and lysosomal dysfunction.
4. renal disorder : PAT causes degeneration of glomeruli and hemorrhage in the tubules of the cortical region in kidney tissues. PAT disordered the arrangement of renal cells and reduced the dextran clearance abilities. PAT affects the growth of human embryonic kidney (HEK293) cells suggesting that it causes increased oxidative stress which may lead to apoptosis in HEK293 cells (Zhang *et al*., 2018).

2. 12 The effect of diabetes mellitus type 2 on kidney and liver functions

Diabetes mellitus type 2 is a common disease that result from increase of blood glucose level, it effects on many organs on the body and causes many complication. The effect of diabetes mellites type 2 on kidney and liver according to the studies a patients with diabetes type 2 are predisposed to develop complication such as end stage renal disease (ESRD) and Cardio vascular disease (CVD), both of which are serious complication and could cause death these disorder required early identification and diagnosis, ESRD and CVD have a number of clinical characteristic and risk features one of the most important risk factor is microalbuminuria of (ESRD) and (CVD), and reduction of urinary albumin excretion (UAE) (Schmieder *et al*., 2011). Dinnen *et al*, (1992) had shown that high release of glucose into the circulation is main reason which cause fasting hyperglycemia in 2 diabetes mellitus.

Since the liver is considered to be the exclusive source glucose in postabsorive human (Felig and Bergman, 1995) liver and kidney had been thought to be the only organ responsible for releasing glucose, because their ability for activation of glucose 6-phosphate (Weber and Canter, 1995). The body should defend it self from hyperglycemia. Nathan *et al*., (2005) pointed out to many chronic disease associated with hyperglycemia such as retinopathy, Neuropathy and Nephropathy , on other hand there are many complication associated with hypoglycemia such as neurological dysfunction, Coma, seizures and death . Patients with T2 DM have an increased of glucose into the circulation by liver in the first and kidney in the second (Meyer *et al*., 1998).

2.13 Measurement of liver function

The liver is the most important and largest organ in the body, it has many function such as production of protein and detoxification both of which are facilitated by enzyme. We can assess the liver functions by these enzymes and evaluate the liver health liver functions test (LFTs) are one of the most commonly screening blood test they use for investigation of liver disease or they also used for routine blood test (Dillon *et al*., 2019). The routine liver test that evaluate liver injury (ALT), asp (AST) and (ALP) the excretion functions of the liver can be assesses by bilirubin and prothrombin are useful in estimated of liver Functions whereas test that describe injury of liver such as (AST) and (ALP) have been mi stabled.

The other enzymes used to asses liver function are Gamma-glutamyl transpeptidase and Gamma-glutamyl transferase . these enzymes reveal obstructions in the biliary system either within the liver or in the large bile channels outside the liver. Presence of the Jaundice and neurological disorders due to the brain damage causing to hepatic encephalopathy and are accompanied with liver failure. While chronic liver failure ensues to accumulation of metabolites in circulation for example ammonia and fatty acid that may causes brain damage and hence hepatic encephalopathy (Kasaralar and Tillman, 2016).

2.14 The effect of aflatoxin B1 on the liver.

Gallanger *et al.*, (1994) demonstrated effects of AFB1 in the human body especially in the liver, which is the most organ responsible detoxifying chemical agent and poisons. In acute aflatoxicosis, binding of AFB1 exposed to various cellular macromolecules leads to hepatocellular injury

and death. In animal experiment and in human acutely exposed to aflatoxicosis, liver specimen showed significant necrosis of parenchymal cells and extensive proliferation of bile duct.

In past we observed abnormal liver function (low / high total protein, low albumin and high alanine aminotransferase (AST) level in approximately 30-40% of HIV negative. (Jolly *et al.,* 2007)

Ghanianiens exposed to aflatoxin B1 in food has been appear to interfere with metabolism protein (Reobuck and Maxuteuko, 1994). Also Zhou *et al*., (1997) showed that HBV and AFBI work synergistically to increase the risk of liver cancer.

On the other hand wild *et al*., (1992) observed that 31% of HIV negative Ghanianiens were positive for hepatitis infection (16% for HBV-14-13% for HCV) thus certain clinical factor may predict high aflatoxin level in human especially in HIV-positive individuals who already suffer the severe compromising health effect of HIV. positive people are shown to have high rate of liver injury (indicated by liver enzymes and /or bilirubin level) that may be due to medication HBV, HCV Co-infection (Bonacini, 2004)

2.15 Function parameters of kidney

Serum creatinine is parameters using to assess chronic kidney diseases (CKD) status. These parameters are also used to evaluate status in susceptible diabetic and hypertension patients. chronic kidney disease (CKD) causes severe reduction in renal function (Ventktapathy *et al*., 2014) It's the case when renal lose their normal function especially excretory and

regulatory, function due to infection, autoimmune diseases, diabetes, hypertension, cancer and toxic chemicals (Abdullah *et al*., 2012). Urea it's also referred to as blood urea nitrogen (BUN) it's non protein nitrogenous which is one of the important parameters to assess renal functions but it's concentration dependent on protein intake and it's also dependent upon the ability of the body to catabolize protein and ability of renal to excreation of urea by the renal system. Creatinine also non-protein waste product is formed from breakdown of creatinine and it's considered an important indicatior to assess renal funcions. creatinine is compound produce primarily in muscle and affected by the muscle mass of the patients (Chang and Holcomb, 2016)

2.16 Detection of blood serum AFB1.

The aflatoxins contamination of food and feed at very low concentration. So There is an urgent need to applied selective, sensitive, and high convenient methods for the detection of aflatoxins, The most recent and progress method is nano material because of it’s high performance and versatile properties they also offers great prospects of highly sensitive, selective and simple method to detection AFB1, its different from traditional methods in process - complicated-time consuming-labour intensive and instrument expensive (Xue *et al*., 2019). The traditional procedures used to detection of AFB1 involved the chemical analytical techniques such as TLC, HPLC and immunological assay for identification and quantification.

The complex procedures used to purify the aflatoxins before analysis have limited the application of these approaches, the monitoring techniques

which have been developed is immunoassays. by using monoclonal anti-bodies (Hangen *et al*., 1981)

These antibodies have been proven to be use ful for quantitalying the aflatoxins in biological fluid- TLC and HPLC are quantitative and qualitative methods used in routine analysis of aflatoxins detection.

These techniques required skilled operators and the sample must be intensive process before subjected. Also these techniques are highly expensive. Thin layer Chromatography is techniques applied to separate organic compounds normal phase TLC consists of stationary phase such as silica or settled or aluminum, and cellulose glasses or plastic plate and solvent mobile phase.

The sample is set as a spot on the stationary phase, the constitutent of sample can be identified by simutaneously running standard with unknown spot the plate is placed in a tank contain proper solvent, after one hour we removed the plate and exposure it to UV light to visualized the toxin (kareem *et al.,* 2021).

2.17 The effect of aflatoxin B1 in kidney functions.

AFB and AFMI effects in kidney functions the related studies about their mechanism are rare, studies have been indicated that excretion of AFB1 and AFM1 occurs first through biliary pathway and second by the urinary pathway, AFB1 could be detected in different level in kidney and urine of two claves with dosage of 0.8 ng/kg and 1-8 ng/kg although many studies are conducted on the mechanism of AFB 1 toxicity and metabolite but it's still unclear.

Direct evidence of exposure of human to AFB1 by intake food or by inhalation are observed in number of countries by detection AFB1 and their meta bolities in human biological samples. In animals model the exposure to AFB1 is accompanied with renal adverse effects such as high level in creatinine and urea. Total protein decrease in renal tubular reabsorption and increase in sodium and potassium excretion (wei *et al*., 2014) The kidney need high oxygen and nutrient because of their Workload and it's role in Filtering one- third of the blood reaching them and reabsorbed 98-99% of the salt and Wale AFB1 exposed part of nephron suffer from nephrotoxicity. The aflatoxins induces reduction in protein content and increase necrosis of kidney and many studies have revealed that AFB1 induces tumor in experimental animals model (Lakkawar *et al*., 2004) the other effect of AFB1 is renal lesion with characteristic of megalocytosis in the proximal renal tubule .(kareem *et al*., 2021)

Chapter Three

MATERIALS AND METHODS

3. Materials and Method.

3.1 Design Of The study.

The present work included a case_control study from November 2021 till March 2022, samples were collected from patients attending Al_Hussein City. The sociodemographic aspects of the patients were collected through the self reported techniques (study questionariae) which including gender and diabetes mellitus type 2.

To find the relationship between aflatoxin B1 and diabetes mellitus type 2 patients were divided certain etiology of diabetes mellitus type 2 group which compared to group who don't have disease (appearantlly healthy) as a control subject. The age of both patients and healthy groups ranged from 40_50 years old.

3. 1 Materials and Instruments used in study

The following materials, instruments and tools that used in conducted of this study

Table 3.1 Materials and Instruments used in study

No.	Apparatus	Manufacture
1.	Autoclave EPTA tube	Lab Tech, Korea Plastilab, China
2.	Beaker	Volac, England
3.	Candle jar	China mhco China
4.	Capillary tube	Vitrex made in China
5.	Centrifuge	Hittich /EBAZO England
6.	Distelled water for HPLC	Schorlau Spain
7.	Electeric shaker	Bioneer, England

8.	Electric oven	Memmert, Germany
9.	Eppendorf (1.5 ml)	Vitrex made in China
10.	Ethanol 70%	Schorlau Spain
11.	Filter0, 2TF	Whatman made in Germany
12.	Gel tube	Plastilab, China
13.	High performance liquid chromatography (HPLC)	SYKAM /Germany
14.	Latex Examination Gloves powder	Rz made in Malaysia
15.	Medical cotton	Medicare Hygiene limited lebanano
16.	Micropipette(100 -1000µl)	Human Humapipette Germany
17.	Refrigerator	Gallen kap/England
18.	Separating funnel	Simax, Czech Republic
19.	Sodium hydroxide	BDH, England
20.	Stand	China mhco, China
21.	Syringe (5 ml)	Provi, made PRC
22.	Test tube	China mhco, China
23.	Test tube rack	China mhco, China
24.	Thin Layer chromatography	China mhco,China
25.	Tip micropipette	AFCO DISPO made in Jordan
26.	UV source	Cleaver, England
27.	Volumetric flask	Volac, England

Table 3. 2 Chemicals and kits used in this study

No.	Chemicals and kits	Manufacture
1.	Acetonitrile	Supelco, USA
2.	AFB 1stander	Himedia/India
3.	Alanine Aminotransferase kit (ALT)	Giesse /Italy
4.	Alkaline phosphate kit (ALP)	Giesse /Italy
5.	Aspartate transaminase (AST) kit	Giesse /Italy
6.	Chloroform	Thomas, Baker/India
7.	Creatinine kit	Giesse/Italy
8.	Methanol	Thomas, Baker /India
9.	Phosphate buffer Sodium hydroxide	BDH, England
10.	Protinase kit	Korean junaid Company's
11.	Urea kit	Giesse/Italy

3.2 Methodology

3.2.1 Design of study

3.2.2 study groups

A total of 86 subjects were studied 42 (21 male and 21 female) of them patients with diabetes type 2 44 (22 male and 22 female) non diabetic (healthy individuals)

The study protocol was approved by the ethical research committee , college of applied medical science, university of Kerbala. Groups of this study includs eight groups as the following table .

Table 3.3 group of this study

No group	Groups	Characteristics Groups
1.	M,D-2,TX	male, diabetes type 2 with AFB1
2.	M, D-2, NT	male, diabetes type 2 without AFB1 toxin
3.	M, H, TX	male, healthy with AFB1 toxin
4.	M, H, NT	male, healthy without AFB1 toxin
5.	F, D-2, TX	female, diabetes type 2 with AFB1 toxin
6.	F, D-2, NT	female, diabetes type 2 without AFB1 toxin
7.	F, H, TX	female, healthy with AFB1 toxin
8.	F, H, NT	female, healthy without AFB1 toxin

3.2.3 Patients criteria

The study consists of 42 patients with diabetes mellitus type 2 who were selected from Al Hussein medical hospital in the Karabla governorate. A questionariae was applied in order to identify important sociodemographic characteristics. Gender health status family history of diabetes mellitus and diatery habit.

3.2.4 Exclusion criteria

Generly patients who reported congential disease in kidney and liver,, Diabetes mellitus type 1, pregnant women, children.

3.2.5 Control Criteria

Control group of an appearantlly healthy 44 subject (22 male and 22 female) were chosen from well know volunteers that had no history of diabetes mellitus type 2 the percentage of female and male adult individuals were equal to patients. The age of both patients and contol (healthy persons) ranged from 40_50 years old. Demographic information of the volunteers was also collected through the self reported techniques [student questionariae]

3.2.6 Approval of ethical

The protocol of the study was approved by ethical committee of the collge of Applied Medical Sciences in karbala and karabla health Department's tune in the medical city of Karbala. Samples were obtained after the consent from patients or the patient's relatives

3.2.7 Blood collection and storage.

Each individual was given 5 ml from the vein by the syringe which was sterile and the blood samples were put in a gel tube. Samples were left for 15 minutes and then centerifuged for 15 minutes at 3000 rpm to get serum. The serum was transported by micropipette to the sterile tube (Eppendorf 1.5 ml) each serum sample was divided into two parts and then stored at - 20 C°. The stored samples used to assess the following :

1. Liver functions test (ALT), (AST), (ALP) activity.
2. Renal functions test (S. creatinine, S. Uric acid, S. urea)
3. Qualitative and quantitative detection of AFB1.

3.2.8 Measure of qualitative of serum AFB 1

Measurment of qualitative of serum AFB1 was carried out by thin layer chromatography. (Kareem, 2021)

3.2.8.1 Steps Preparation of Protinase K solution.

Protinase k solution was prepared according to instruction of the Korean junaid Company. It was prepared by adding 1.1ml streile distelled water to 22 mg of protinase powder

3.2.8.2 Standard AFB1 Preparation for TLC

Preparation of AFB1 stander by disolved 500 microgram from AFB1 in two ml of chloroform become the AFB1 concentration is 250 mg /ml.

3.2.8.3 Extraction of AFB1 from blood serum samples.

1.5 ml were taken from each individuals by streile micropipette and transported to streile test tube and added to each one sample 50µl from Protinase k solution and left react for 10 minutes. After that the mixture was exposed to centerifugation for 15 minutes at 2500 rpm. Then from each sample the filtrate was taken and the preciptate was neglacted. Then 1mL chloroform was added to each filtrate and shake vigorously in the electeric shaker device, where it formed tow (blood serum layer and chloroform layer). Chloroform layer was separated by sparating funuel and put in sterile other glasses tube and let to evaporate.

3. 2.8.4. Detection of AFB1 in human blood serum

A thin layer chromatography plate was coated with silica gel. Dimension of (20×20) cm was used after activated it in electeric oven at 120C° before using a light straight line was made at a distance of 1.5 cm from the bottom and top of the plates the bottom line was used for loading samples and the top line was used for numbering. The mobile phase used to separate AFB1 was chloroform 95: methanol 5. Stander of AFB1 (10µl) was added as a spot on TLC plate by capillary tube then 20µl from each extracted sample were added on the plate with a distance of 2cm between samples after that these spots were left to dry in the room temperature. The plate finally were put in the separation tank which containing mobile phase.(Kareem, 2021)

Then thin layer plate was left in the tank until the mobile phase reached 2cm from upper edge of the plate . After that TLC plate was removed from the tank and left dry in the room temperature.

Then plate was examined under UV light (365nm) and compare the color and relative flow (RF) of extracted sample with stander toxin.

$$Rf = \frac{distance\ of\ solute}{distance\ of\ solvent}$$

1ml chloroform was added to each filtrate and shake vigorously in the electeric shaker device, where it formed two (blood serum layer and Chloroform layer) Chloroform layer was separated by sparating funuel and put in sterile other glasses tube and let to evaporate.

3.2.9 Measure of quantitative AFB1 in blood serum

Analysis of AFB1 by high performance liquid chromatography The separation carried out by liquid chromatography model SYKAM (Germany) by Fx 10 A_flouerscent detector (Liu *et al.*, 2012).

3. 2.9.1 Preparation of AFB1 standard

Taken 0.5 mg sample powder was dissolved in 30mL 0.1%. Trichloro acetic acid then agitated in ultrasonic bath for 5 minutes. The standard aflatoxin was separated on fast liquid chromatographic. Next, the extract were filtered on dispoable sepak filtred paper 0.5 milpore to remove the fibers.

3.2.9.2 calculation of concentration of AFB1

Average of duplicate reading of samples were performed measurement were prepared using the average blank _corrected for each sample standard AFB1 concentration in ng/mL to detemine the concentration of each unknown sample.

$$\text{Co. of sample (ng/m)} = \frac{\text{Area of sample}}{\text{Area of Standard} \times \text{conc. of standard}}$$

3.2.10.Measurment of Biochemical blood serum Parameters levels

The biochemical parameters were conducted in labs of Al_Hussein Medical City .

3.2.10.1 Method for quantitative blood serum Urea

The analysis were carried out according to the instructions of the Italien company GIESSE, correlation studies was preformed using PRECISENROM (REF. 6000) protocols and PRESICPATH (REF. 6001) Principle of procedure.

The urea assay is a modification of torly enzyme produce. The test is a kinetic assay in which the intial role of of the reaction is linear for limited period of time. Urea in the presence of glutamate dehydrogenase (GLDH) the formed ammonium ion react with alpha ketogluterate and NADH to form glutamate and NAD measured at 340 nm, NADH oxidation in unit time is proportion to the urea concentration in the sample.

3. 2.10.2 Method for quantitative of blood serum Creatinine.

The analysis was carried out according to the instructions of the Italien company GIESSE, Correlation studies were preformed using PRECISENROM (REF. 6000) protocols and PRESICPATH (REF. 6001) Principle of procedure.

Creatinine react in Alkaline environment with picric acid forming salt of yellow orange color. The intensity of of the color that develop in the specific time interval is proportion to the amount of Creatinine in the sample

3.2.10.3 Method for quantitative of blood serum Uric acid.

The analysis was carried out according to the instructions of the Italien company GIESSE. Correlation studies were preformed using PRECISENROM (REF. 6000) protocols and PRESICPATH (REF. 6001).

Principle of procedure.

Unicase transforms uric acid into allantion with formation of hydrogen peroxide (POD) reaction with 4 aminoantipyrine and 3 hydro 2.AStriiodobenzoic acid to produce color complex which it's intensity is directly proportion to the uric acid con entration in the sample.

3.2.10.4 Method for quantitative of blood serum ALT.

The analysis was carried out according to the instructions of the Italien company GIESSE. Correlation st dies were preformed using PRECISENROM (REF. 6000) protocols and PRESICPATH (REF. 6001).

Principle of procedure.

The present of alpha ketogluterate alanine is converted into pyruvate and glutamate by ALT/GPT in the sample in the presence of NADH and lactate dehydrogenase pyruvate is trancformed into lactate and NAD.

NADH Oxidation in time unit was measured at 340 nm is proportional to the concentration in the sample.

3.2.10.5 Method for quantitative of blood serum AST.

The analysis was carried out accordin, to the instructions of the Italien company GIESSE. Correlation studies were preformed using PRECISENROM (REF. 6000) protocols and PRESICPATH (REF. 6001).

Principle of procedure.

In the presence of alpha ketoglutamate AST/GOT in sample transforms Aspartate into oxaloacetate and glutamate in the presence of

NADH and malate dehydrogenase. Oxaloacetate is converted into malate and NAD.

Consuming of NADH per unit of time measured at 340 nm is proportional o the concentration of AST /GOT in the sample.

3.2.10.6 Method for quantitative of blood serum Alkaline phosphatase

The analysis was carried out according to the instructions of the Spanien company Spinreact. Correlation studies were preformed using protocols spintrol H calibrator spintrol normal and pathologic (REF. 1002011,100 2120) and (1002220).

Principle of procedure.

Alkaline phosphate ALP catalyses the hydrolysis of p_natrophenyl phosphate at PH 10.N liberating p_natrophenyl and phosphate. The rate of p_natrophenyl formation measure photoelectrically is proportion to the catlytic concentration of Alkaline phosphatase percent in the sample.

3.2.8.7. Statistical Of Analysis

Anova table and Duncan test were applied using for determine the statical significance of data p-values of under 0.05 was considered statically significant.(Paulson, 2008)

The correlation was done between:

Gender X blood toxin (AFB1) .

Health status X blood toxin (AFB1).

using chi _square (x^2)determination of statistically significant for the experiment of measurement qualitative of serum AFB 1 in blood serum (John and Sons., 2003).

Chapter Four

RESULT & DISCUSSION

4-1 Measurement of qualitative of serum AFB1

The results showed 17 (38.1%) out 42 samples of serum collected from patients with type -2 diabetes contaminated with AFB1 . While 13 (29.5%) samples collected from healthy persons (Control) were contaminated with Aflatoxin . Also the results appearance that 26(61.9%) collected from patients with diabetes not contaminated with aflatoxin B1. Also 31 samples collected from healthy person group were not contaminated with toxin (Table4_1) statistical analysis didn't showed significant difference between the number sample that contamination with AFB1 and the number samples that contamination with AFB1 which it's collection from healthy person.

Table(4-1) The number and percentage of Samples for patients and healthy that contaminated of AFB1.

Case	Without AFB1	With AFB1	Total
Healthy	31 (70. 5%)	13 (29. (5%	44
Patients	26 (61. 9%)	16 (38. 1%)	42
Total	57(60. 30%)	24 (33.7%)	86 (100%)

X^2 Calculate = 2.48

X^2 table = 3.84

This study showed serum male and female patients contaminated with AFB 1 were 17(19.7%) and 12(13.9%) without significant difference between them. While the number of the blood serum that collected from

females and males and without AFB1 was 27 (31. 39%) and 30 (34.88%) respectively (Table2_4).

Table (4-2) The number and percentage of Serum Samples. that collected from males and female contaminated AFB1

Gender	Without AFB1	With AFB1	Total
Males	30 (34. 88%)	12(13. 9%)	42
Females	27(31. 39%)	17(19. 7%)	44
Total	57(66. 3%)	29 (33. 37%)	86(100%)

X^2 Caculate = 0.87

X^2 table (0.05) = 3.84

This results agreement with (Kareem, 2021) showed that the investigated population were exposed to AFB1 was detected in 100% of uncertain chronic kidney disease (CKD) while in certain CKD patients and healthy was 24% and 20% respectively.

Abdullah and ALJumaili, (2018) found 22 out 36 Sample (61. 1%) of blood collected from persons were contain patulin by using Thin layer chromatography. The percentage of blood samples that collections from females and males was 54. 5% and 95. 5% respectively.

Also others study illustrated the percentage of blood serum sample that collected from Patients (with nephropathy) and contamination with Ochratoxin A was 90% while the percentage of the blood serum that collected from healthy group and contamination with Ochratoxin A was 3% With significant difference between them.

As well as the percentage of males and females blood serum contamination With mycotoxin (OTA) was 48.8% and (51.1%) respectively with no significant difference between them (Hassan, 2021)

4-2 Measurement of quantitative AFBI in blood serum.

The results of this study showed that blood samples collected from males and females patients with diabetes type_2 the AFB1 concentration reached to 1.343 ng/mL with significant difference from other group. Also the samples that collected from males patients with diabetes AFB1 was 0. 684 ng/mL with significant difference from AFB1 concentration in blood serum of Healthy group (Table-3_4)

This results approched with (kareem,2021) result who found the concentration ranges of AFB1 in serum samples were (0.68-8.33) ng/mL for uncertain CKD patients (1.21-5.6) ng/mL for certain CKD patients and (0.11-1.30) ng/mL for healthy control.

Table (4 _ 3) AFB1 Concentration (ng/ml) in blood Serum of groups study.

group	Statistical Notation	mean	SD±
Healthy, female	a	0.133	0.0035
Healthy, male	a	0.135	0.0033
Patients, males	b	0.684	0.7458
patients, females	c	1.343	0.299

Number with difference letters that means there are significant difference between them at p< 0.05 mean.

Bashir *et al.,* (2020) who found that mycotoxin (OTA) in blood serum of 82 person with 3.5_6.8 ng/mL

The incidence of positive values for AFB1 in blood was over 50% win respect to the levels found . The average where similar to those reported in the countries except in San Vicente de Tagua - Tagua where the woman's group presented values. higher than other reports(Cast, 2003)

Hassan, (2021) Showed that concentration OTA in blood serum of female and male patients, with nephropathy was (7.015 and 7.071) ng/ mL respectively While it was in both healthy females and males was (0-1, 0.009) ng/mL respectively

The increase levels of AFB1 in blood serum of females and males patients with diabetic type_2 belong to the diabetic disease which it's cause asignificant indication of kidney impairment and toxicity. Renal function decline to about 25-50% lead to less the AFB1 excretion (Sharma *et al*., 2011)

Increased levels of AFB1 in blood serum effected in functional of liver and kidney (Cast, 2003)

4-3 Estimation of correlason coefficient (r)

The result showed that there is correlation between gender and presence of AFB1 in blood serum (r = 0.974 P= 0.324) It means that The females more sensitive to AFB 1 compare with males group

Also there is correlation between healthy status and presence AFB1 toxin in blood the value of coefficient (r) was 0.703 and p value equal 0.402 (Table4_ 4) .It means that AFB1 concentration in blood serum of patients

with diabetes group highest compare of it's concentration in blood serum of healthy group.

The results approched with kukakcan *et al.,* (2015) who found correlation between presence of AFBI and liver disease

Kareem, (2021) showed that statically significant correlation observed between AFB1 level in blood serum and infected with chronic kidney disease (CKD).

Turner *et al*, (2013) found relationship between dose of AFB1 and grain. of infants in Gambia, Benin and Togo.

Table (4-4) Estimation of correlation coefficient (r)

Type of Correlation	Coefficient (r)	P. Value
Gender ×blood toxin (AFB 1)	0.974	0.324
Health Status X blood toxin (AFB 1)	0.703	0.402

4-4. Measurement of some blood biochemical levels of patients with diabetes type 2 groups and healthy groups.

4.4.1. Creatinine.

The results illustrated that creatinine levels in blood (M, D-2, Tx) groups were 1.88 mg/dL while the levels of creatinine in blood of (F,D-2,Tx), (F,D-2,NT) and (F,H, NT) groups were (0.59,0.57, 60) mg/dL respectively with significant difference between them. on the other hand the creatinine levels in blood of (M , H , Tx), (M, H, NT) and (F, H, Tx) group were

(0.73,0.71,0.71) mg/dL respective with out significant difference (Table 5_4). several studies agreement with result of this study . Hassan, (2021) found that creatinine level in blood females patients with nephropathy and contaminated with Ochratoxin A increases to 3.73 mg/dL while the level of creatinine in blood healthy females was 0.57 mg/dL on the other hand the levels of creatinine in blood serum of males patients reached 4.64 mg/dL. Also other study illustrated that creatinine levels in blood serum of patients with chronic kidney disease and contaminated with AFB1 get 2.29 mg/dL (Kareem, 2021).

MateJova *et al*., (2017) demonstrated that AFB1 Caused injury in kidney tissue and inflammatory cell infiltration , hemorrhage, damage and necrosis.

It's also induced oxidative stress by peroxidation of lipid in the serum. Also Aflatoxin releasing free radicals especially superoxide anions in kidney tissue then activate oxidative reactions kidney which have a toxic effect on mouse kidney .

Mir, M.S and Dwived (2011) Found that OTA increased creatinine level in the blood is a significant indication of kidney impairment and toxicity.

Table (4-5) Creatinine levels in blood serum of studied groups.

No. of groups	groups	Statistical Notation	Mean (mg/dL)	SD±
1	M,D-2,Tx	b	1.88	0.13
2	M,D-2,NT	a	0.66	0.06
3	M,H,Tx	ab	0.73	0.28
4	M,H,NT	a	0.71	0.17
5	F,D-2,Tx	a	0.59	0.18
6	FD-2,NT	a	0.57	0.04
7	F,H,Tx	a	0.71	0.15
8	F,H,NT	a	0.60	0.14

Number with difference letters mean that there are significant difference between them at P < 0.05 mean.

4.4.2 uric acid

According statistical analysis of uric acid levels shown there is significant difference among the means of group study. Females patients with diabetic disease and it's blood contaminated with AFB1 the level uric acid was 8.3 mg/dl with significant difference of same group as 4,7 and 8 groups. Levels of uric acid in blood were (5.78 , 5.82 , 5.76) mg/dl respectively . while there is not significant difference in others groups (table 6_4). Uric acid is by product of purine metabolism, it's one of the non_nitrogenous protein compounds. Kidneys serve to rid the body of most undesirable waste products of metabolism as well as any excess of in organic substances. Uric acid test is used in specific to assess glomerular function. However it's affected by diet. Uric acid test is done to diagnosis

gout disease, monitor the efficacy of treatment given to lower uric acid level and to reveal the risk and cause recurrent kidney stones. Uric acid levels can vary based on sex. Normal uric acid are 1.5 to 6.0 mg/dl for women and 2.5 to 7.0 mg/dl for men. (Becker, 1993).

Table (4-6) uric acid levels in blood serum of groups study

No. of groups	groups	Statistical notation	Mean mg/dl	SD ±
1.	M,D-2,TX	b	8.3	3.44
2.	M,D-2,NT	ab	7.73	0.66
3.	M,H,TX	ab	7.76	2.62
4.	M,H,NT	a	5.87	1.37
5.	F,D-2,TX	ab	7.77	0.85
6.	F,D-2,NT	ab	6.68	0.54
7.	F,H,TX	a	5.82	0.33
8.	F,H,NT	a	5.76	1.39

Numbers with different letters means that there are significant differences between them at $p<0.05$ mean.

4-4-3 urea

The results that shown in table (6_4) illustrated the levels of urea in blood (M, D-2, TX) group and (M, D-2, NT) was 42 mg/dL and 40 mg/dL respectively. The levels of urea in blood of first group was significant difference from levels of other groups. Also levels of urea in blood of group one and two was abnormal. On other hand the levels of urea in blood of groups (2,3,4,5,6) were not significant difference between them. The levels

in blood groups 4, 7, 8 were (34, 32.62, 30.25) mg/dL respectively without significant difference between them.

The result of this study agreement with some studies that found urea and creatinine levels increased because the correlate with variations in glomerular rate. Blood urea levels become high levels when renal function decline to about 25 – 50 %. Sharma *et al.*, (2011).found that increase level of urea indicating serious kidney damage. Also blood levels of urea considerably higher, whereas protein level are lower than the control group indicating that kidney funcion is impaired.

In local study showed that level of urea increased in blood patients with chronic kidney disease contamination with AFB1 to 89. 59mg/dL compare with 27. 89mg/dL in blood of healthy group (kareem, 2021).

On other hand urea level in blood male patients with nephropathy disease and presence of OTA reached to 115 mg/dl while the level in blood females was 99.1 ml/dL (Hassan, 2021).

Table (4-7) urea levels in blood serum of studied groups.

No. of group	groups	Statistical Notation	mean mg/dL	SD ±
1	M,D -2,TX	a	42	17.82
2	M, D -2, NT	a	40	10.41
3	M,H ,T x	b	38	11.31
4	M, H, NT	c	34	9.06
5	F ,D -2, Tx	b	35	13.97
6	F,D -2, NT	b	36	10.41
7	F ,H ,Tx	c	32.62	5.62
8	F , H , NT	c	30.25	3.62

Numbers with different letters mean that there are significant difference between them at P < 0.05 mean.

4-4-4 Alkaline phosphatase (ALP)

The results of this test showed that the levels of ALP in blood of [M,H, Tx), [(M, D_2, NT), (F,D-2, NT), (F,D-2 Tx)] groups were (147.1,145. 28,145.8, 138.3) U/L respectively with significant difference of other groups. While the levels of ALP in blood serum of (M, H, NT) group and (F, H, NT) groups were (67.9 u/L) and (57.4 u/L) respectively

As well as the groups of number (1) and (7) was normal (Table 8_4).

These results agree with Zhang *et al*., (2016) who found that mycotoxin increased (ALP) levels in blood serum of patients with nephropathy and carrier toxin with significant difference of ALP level in blood of healthy groups.

also Torres,(2002) illustrated that ALP level in blood serum of patients with chronic kidney disease increase comparison of level in blood of healthy groups.

Kareem, (2021)/ found that mean ALP level in blood serum of patient with CKD and contamination with AFB1 was 110.18 U/L While the level in blood healthy group 78 U/L.

Rej *et al*., (1973) clarified that, ALP is derived from more than one tissue including bone, where one of the bone metabolites abnormalities such as chronic kidney and diabetic disease causes increased oestrogenic differentiation which lead to increased transit of many bound Protein compounds such as alkaline phosphatase which this leads to asignificant increase ALP in blood.

Table (4-8) ALP levels in blood serum of studied groups .

No. of groups	groups	Statistical Notation	Mean U/L	SD±
1	M,D-2,Tx	b	115.4	31. 45
2	M,D-2,NT	c	145.8	29. 05
3	M,H,Tx	c	147.1	33. 27
4	M,H,NT	a	67.9	27.7
5	F,D-2,Tx	c	138.3	31. 24
6	F, D-2 ,NT	c	145.28	36. 48
7	F, H , Tx	b	114.8	38. 11
8	F ,H, NT	a	57.4	27.8

Numbers with different letters mean that there are significant difference between them at P < 0.05 mean.

4-4-5. Aspartate aminotransferase (AST)

The results showed that AST level in male patients with diabetic type-2 and the blood serum not contamination with AFB1 (group/2) and females patients with diabetic type-2 and there is no toxin (AFB1) in blood (group/6) was 53.38 U/L and 49. 82U/L respectively. while the levels of AST in blood Serum of(males healthy without AFB1) group and the levels in blood serum of (females, healthy, without AFB1 toxin) decreased to (19.40) U/L and (21.50) U/L receptively with significant difference between them. Also the levels in blood serum of other groups were between (37. 20to 45. 44) U/L (Table 9_4)

This results agree with Zhang *et.al*., (2016) who found that level of AST increased in blood of patients with chronic kidney disease and those contamination of blood with OTA.

Also wells *et al*., (1986) showed that inhibiting AST activity in the liver resulted in mycotoxins poisoning.

In other study illustrated that AFB1 caused increased AST level in blood of patients with CKD to 22.45U/L Compare to 19.135U/L in blood healthy group.(Kareem, 2021)

Table (4-9). AST levels in blood serum of studied groups.

No. of groups	group	Statistical Notation	Mean (U/L)	SD±
1	M,D-2,Tx	ab	42.14	30. 48
2	M,D-2,NT	b	53. 38	29. 86
3	M,H,Tx	ab	37. 20	31. 85
4	M,H,NT	a	19. 40	5. 38
5	F,D-2,Tx	ab	45. 44	26. 94
6	F, D-2 ,NT	b	49. 82	34. 22
7	F, H , Tx	ab	41. 63	31.34
8	F ,H, NT	a	21. 50	2. 34

Numbers with different letters mean that there are significant difference between them at P < 0.05 mean.

4-4-6 Alanine Aminotransferase (ALT)

Regarding to statistical analysis of ALT it shows that there is significant differences among of study groups.

Alanine aminotransferase levels in the blood serum of (M. D_2,TX) group reached to (18.195) U/L while the levels of ALT in blood for (M, H, TX), (M, H, NT), (F, D_2,TX) and (F, H, TX) groups were (12, 10.38, 13.63, 11. 88) U/L respectively. without significant differences between them at P(0.023).(Table 10_4)

There are many studies results approached of this study. kareem, (2021) found that the range of ALT level in blood serum patients with CKD diseases and exposure to AFB1 was (5.51-23.84) U/L compare with level in blood serum of healthy persons (12.07-21.84)U/L. The results of local study demonstrated the ALT level in blood serum of Patients with CKD and exposure to OTA toxin was 9.98 U/L it compares with level of ALT in blood serum of healthy (7.86 U/L) with no significant difference between them.(Hassan, 2021)

Stove *et al*., (2012) proved that level of ALT become higher level , whereas protein levels are significant lower than in control group.

The ALT enzyme is typically found in liver cells/ it's presence in high level in Plasma implies tissue damaged or organ failure (Wells *et al*., 1986)

Table(4-10) ALT levels in blood serum of studied groups .

No-of groups	group	Statistical Notation	Mean (U/L)	SD±
1	M,D-2,Tx	a	18. 159	3. 21
2	M,D-2,NT	b	15. 08	9. 19
3	M,H,Tx	ab	12	7. 18
4	M,H,NT	ab	10.38	4. 48
5	F,D-2,Tx	ab	13. 63	5. 45
6	F, D-2 ,NT	b	14. 83	6. 73
7	F, H , Tx	ab	11.88	5. 28
8	F ,H, NT	ab	9. 5	2. 22

Numbers with different letters mean that there are significant difference between them at P < 0.05 mean.

CONCLUSION & RECOMMENDATION

Conclusions

1. Aflatoxin B1 contaminated blood serum patients with diabetes type 2 also AFB1 contaminated blood serum of healthy persons but with low concentration compare the diabetic patients.
2. Females are more sensitive to Aflatoxin B1 if compared with males.
3. Diabetic disease leads to increase the Aflatoxin B1 level in blood serum and this may be due to the ability of the AFB1 to cause tissue damage in the kidney tubules which leads to poor efficiency of the kidney function in excreting the AFB1.
4. The presence of aflatoxin B1 in blood serum of human at any concentration is a dangerous indicator of human health, because this toxin is accumulative has many vital goals in the human body.
5. The relationship between aflatoxin B1 and diabetes disease was synergism to increase levels of AST and ALT. Also AFB1 causes increasing levels of creatinine and uric acid, which means decrease renal functions.
6. According to available studies , this study is the first of it's kind locally and globally.

Recommendations

1. Checks on the food that is imported from, outside the country, and preventing the entry of any foods commodity contaminated with aflatoxin B1.
2. Taking care of stores for foodstuffs by building stores with technical speafications that prevent the occurrence of fungal infection and occurrence of contamination with its toxins.
3. Work to determine the permissible levels of aflatoxins in human food and animal feed.

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Appendix

Table (1) detection of AFB1 in human blood serum with diabetes mellitus type 2.

NO.	sample	result	NO.	sample	result
1	P	-	22	P	-
2	P	+	23	P	-
3	P	-	24	P	+
4	P	+	25	P	-
5	P	-	26	P	+
6	P	+	27	P	-
7	P	-	28	P	-
8	P	-	29	P	+
9	P	+	39	P	-
10	P	-	31	P	-
11	P	-	32	P	+
12	P	+	33	P	-
13	P	+	34	P	-
14	P	-	35	P	+
15	P	+	36	P	-
16	P	-	37	P	-
17	P	-	38	P	-
18	P	+	39	P	-
19	P	-	40	P	
20	P	+	41	P	+
21	P	+	42	P	+

P : Patient

+ : detection of AFB 1 in blood serum

- : no detection of AFB 1 in blood serum

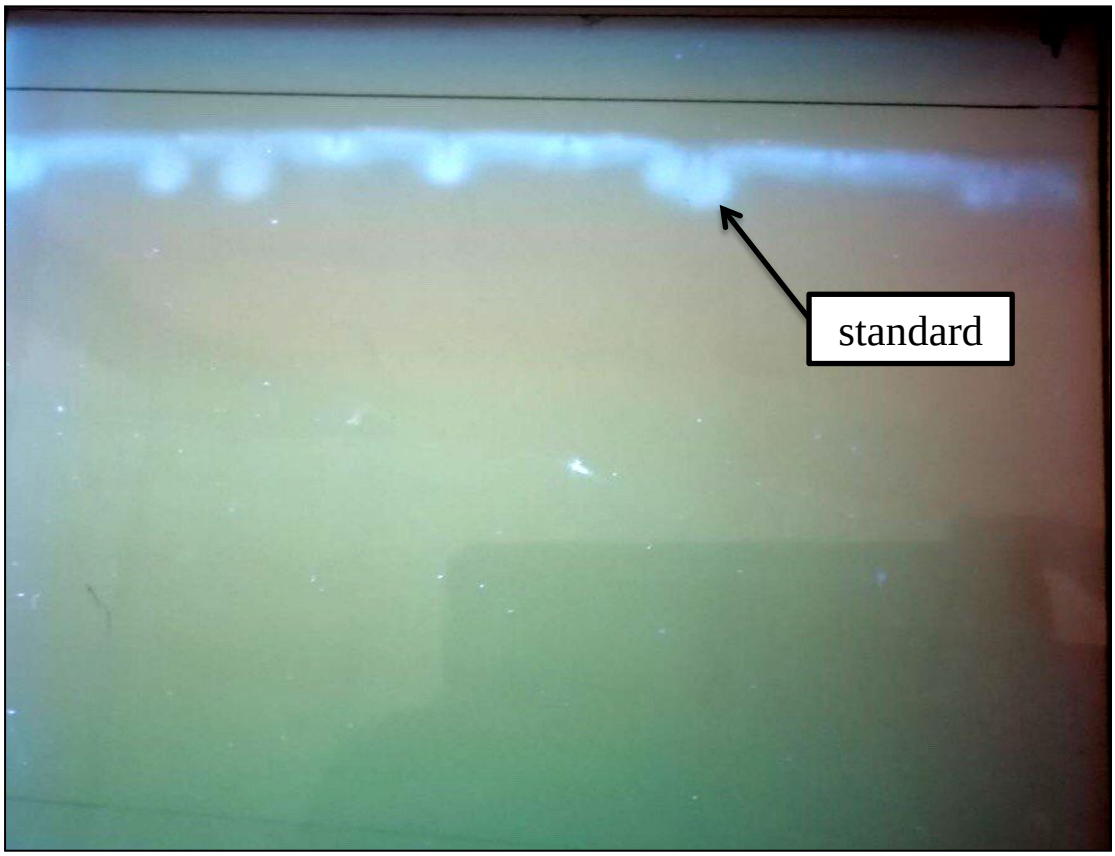
Table(2) detection of AFB 1 in human blood serum .

NO.	sample	result	NO.	sample	result
1	H	+	26	H	-
2	H	+	27	H	+
3	H	-	28	H	-
4	H	-	29	H	-
5	H	+	30	H	-
6	H	-	31	H	+
7	H	-	32	H	-
8	H	+	33	H	-
9	H	+	34	H	-
10	H	+	35	H	-
11	H	+	36	H	-
12	H	+	37	H	-
13	H	-	38	H	-
14	H	-	39	H	-
15	H	+	40	H	-
16	H	-	41	H	-
17	H	+	42	H	+
18	H	-	43	H	-
19	H	-	44	H	-
20	H	-			
21	H	-			
22	H	-			
23	H	-			
24	H	-			
25	H	-			

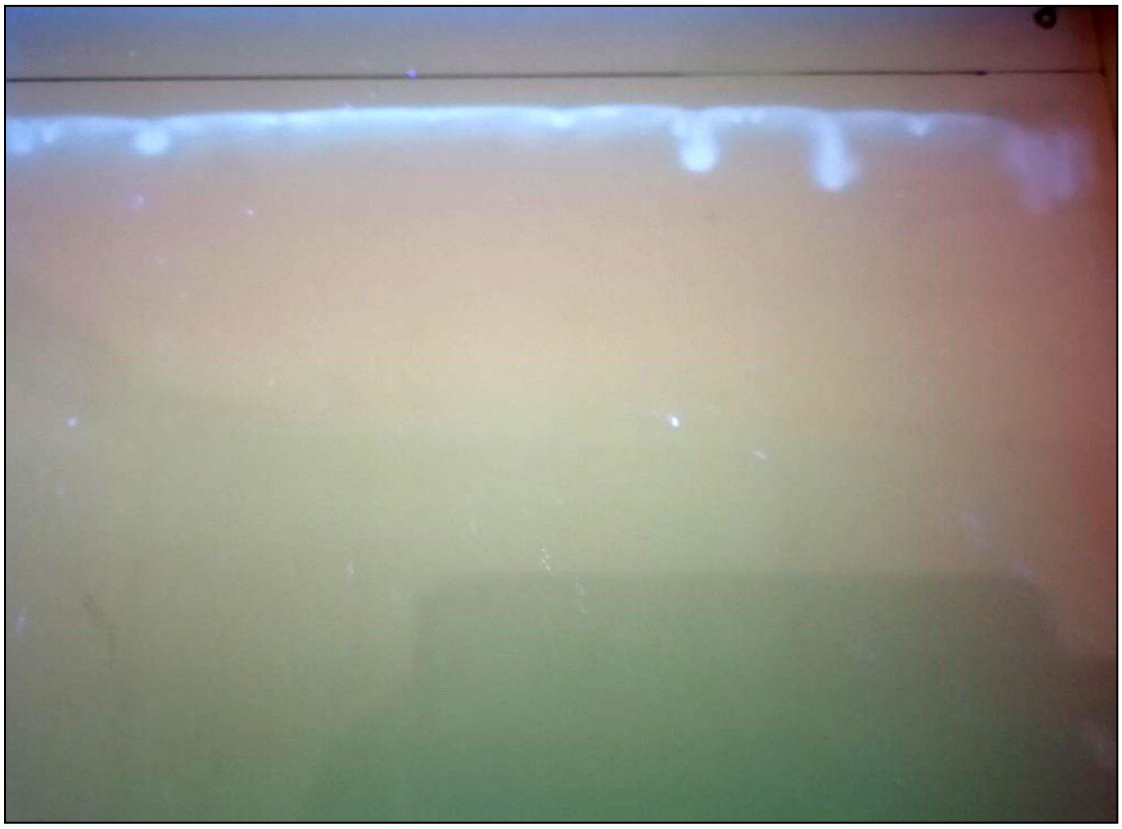
H : Healthy person

+ : detection of AFB 1

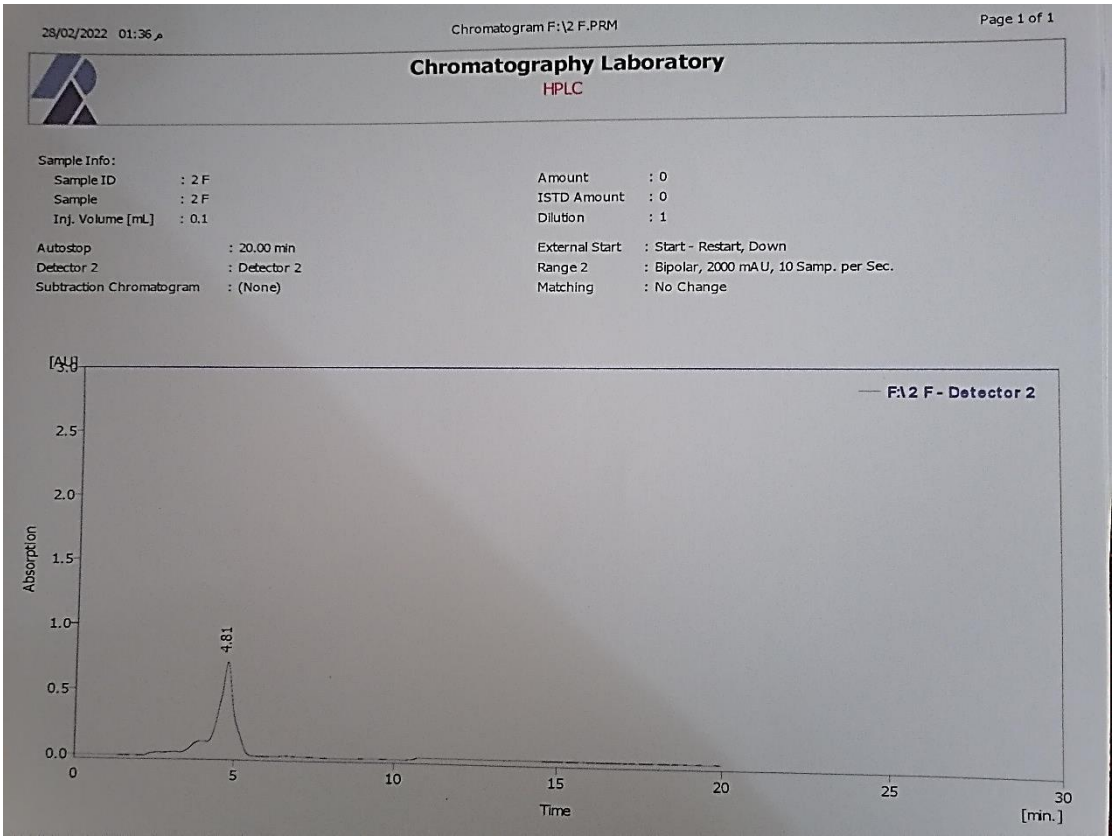
- : no detection of AFB 1



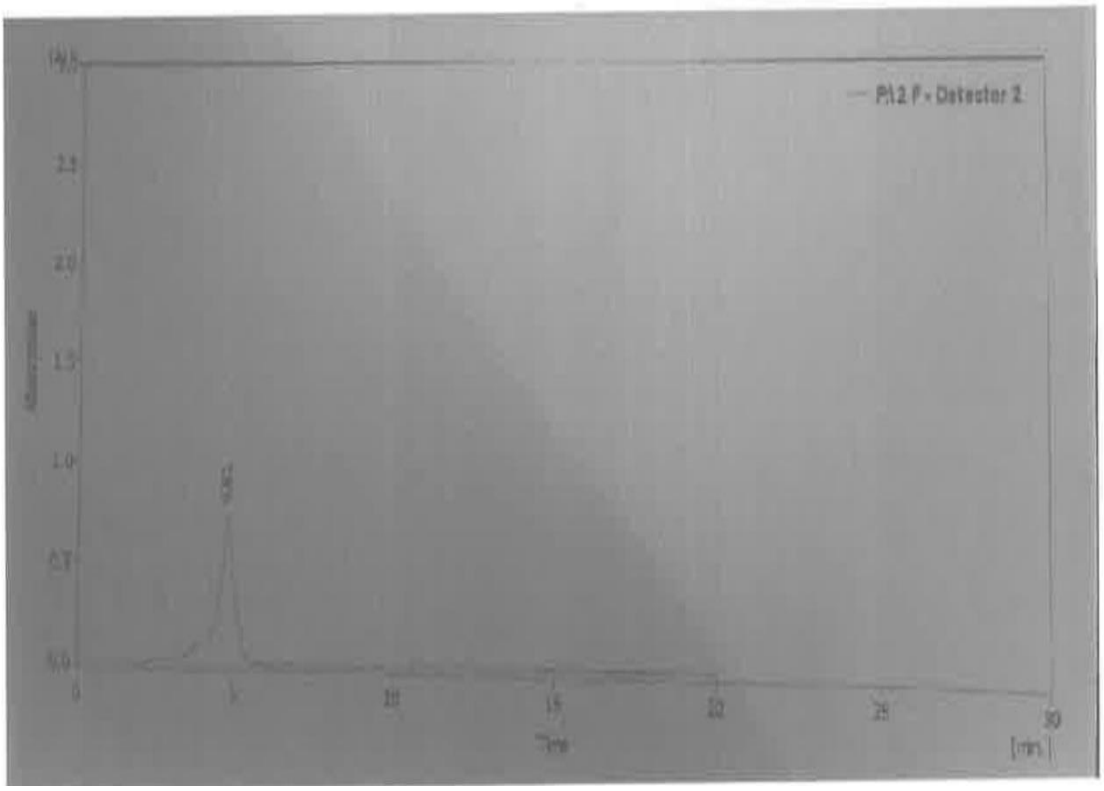
aflatoxins Loaded samples on the TLC plate under UV light



aflatoxins Loaded samples on the TLC plate under UV light



HPLC chromatography of positive AFB1 detection by TLC (No. 2fD).



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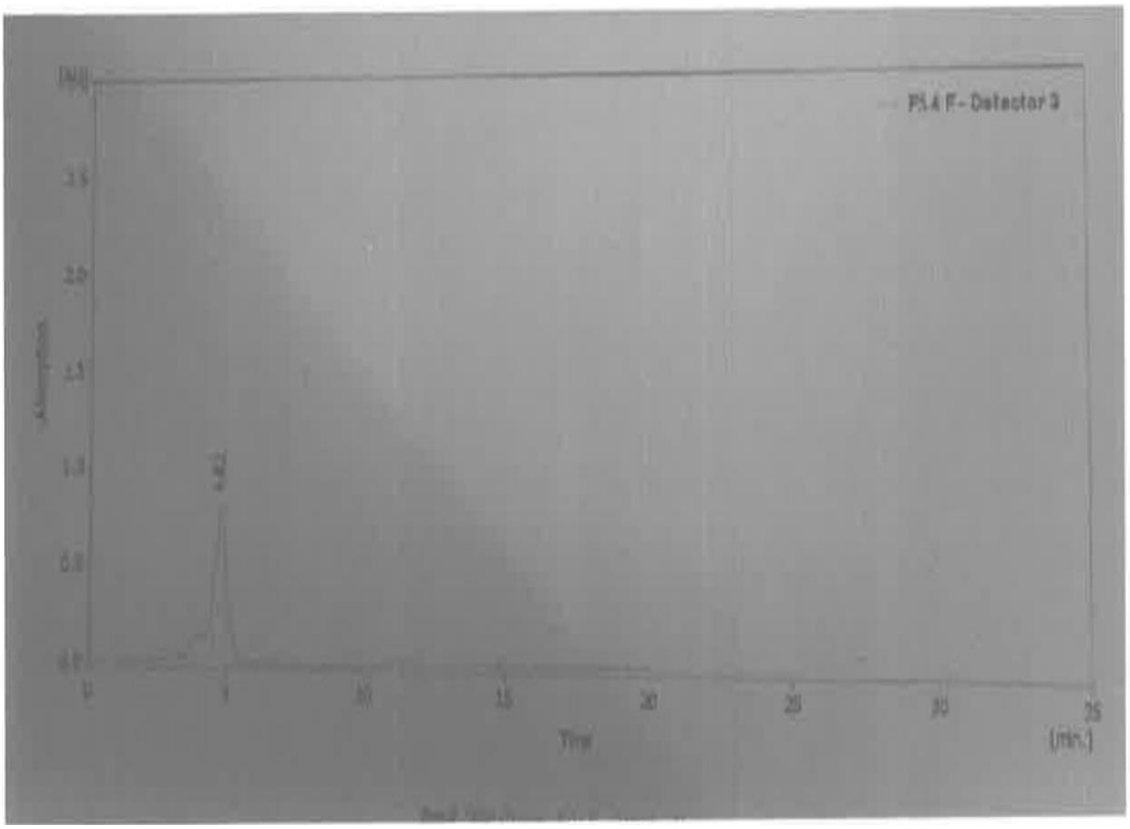
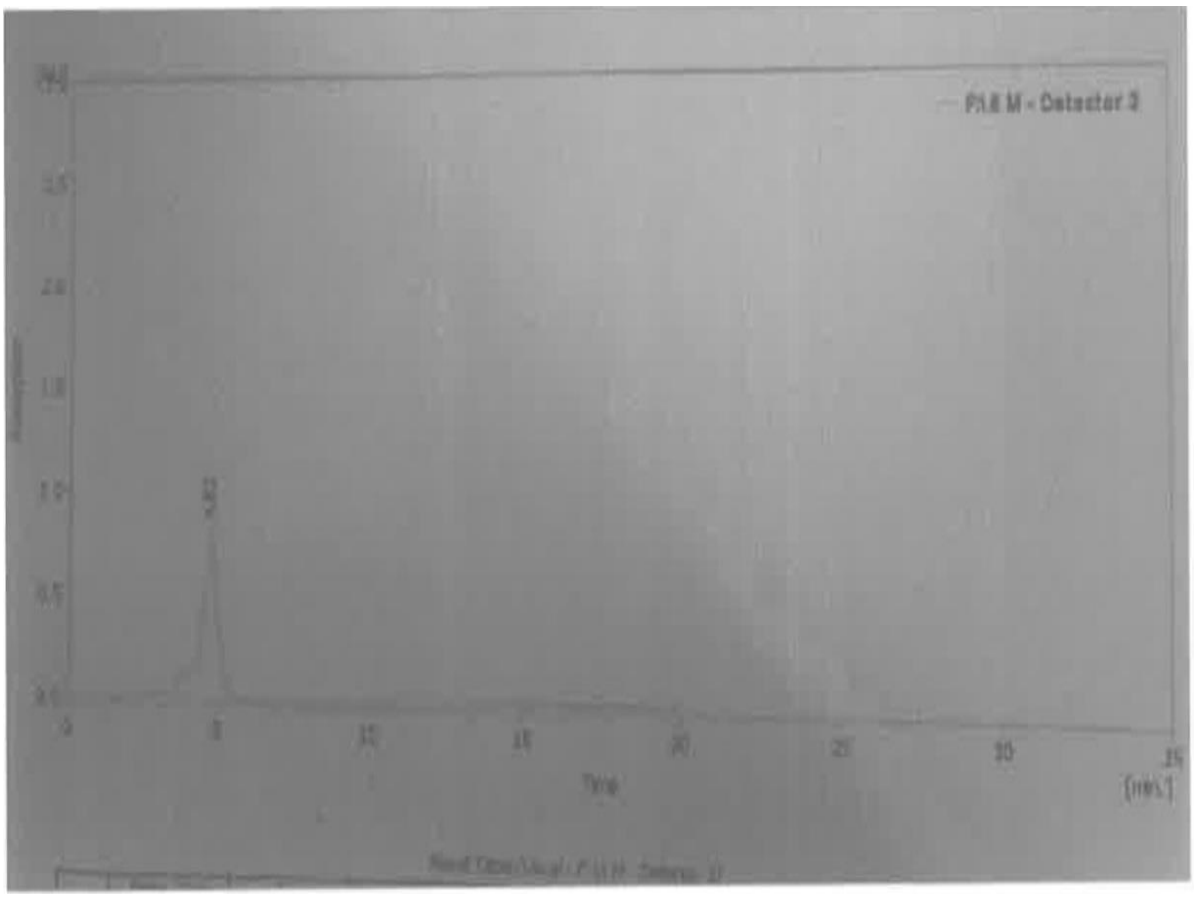
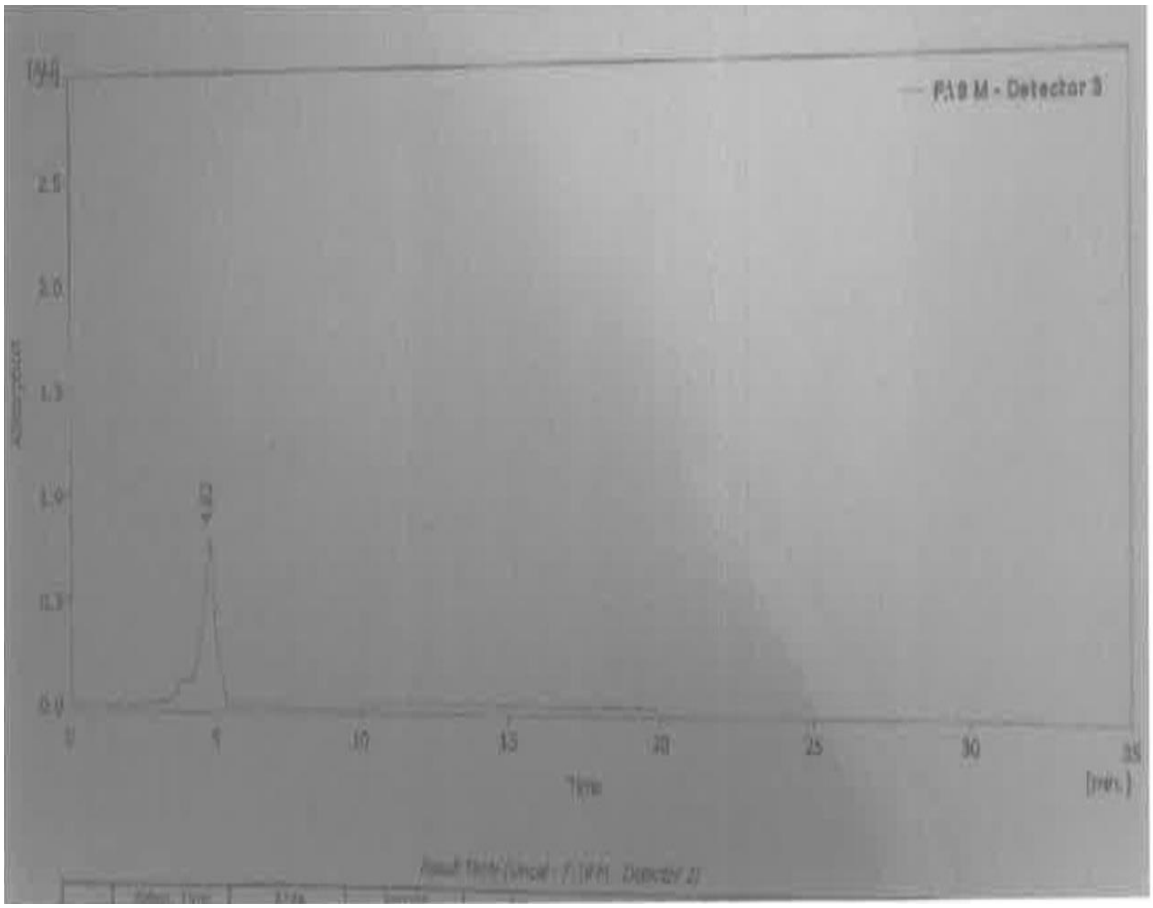


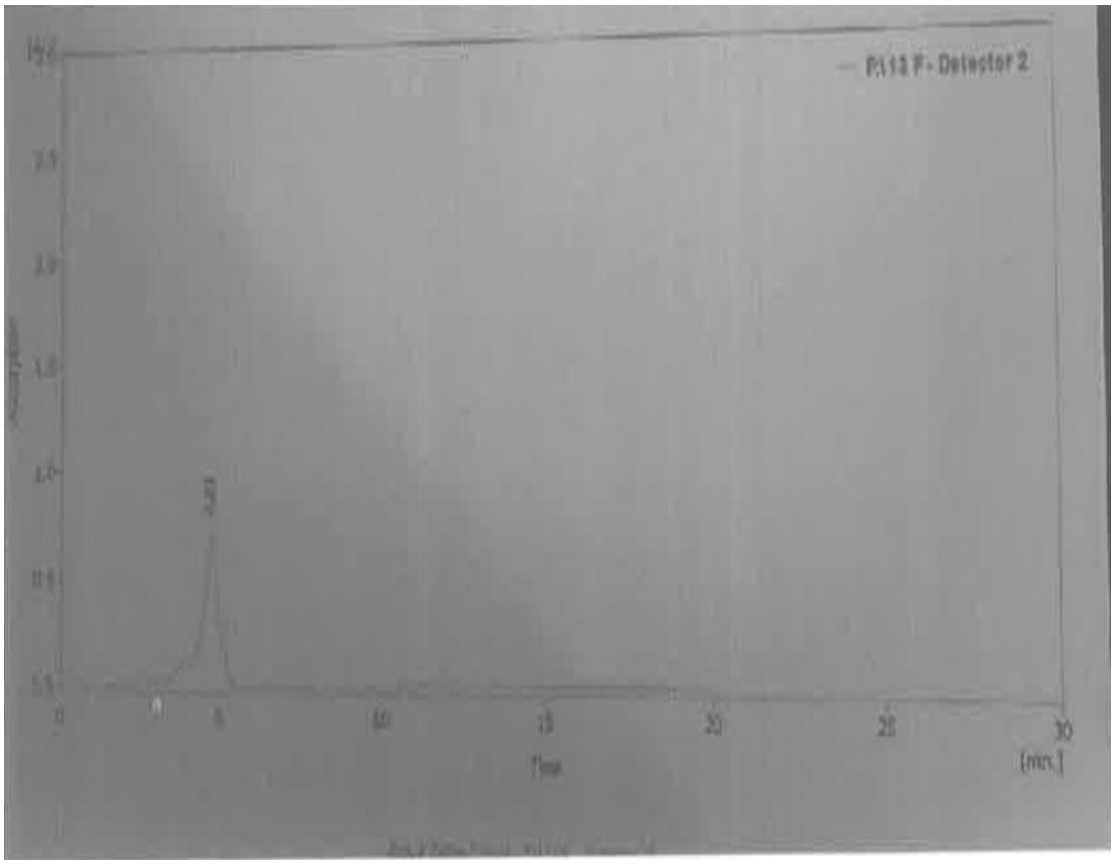
HPLC chromatography of positive AFB1 detection by TLC (No. 4fD).



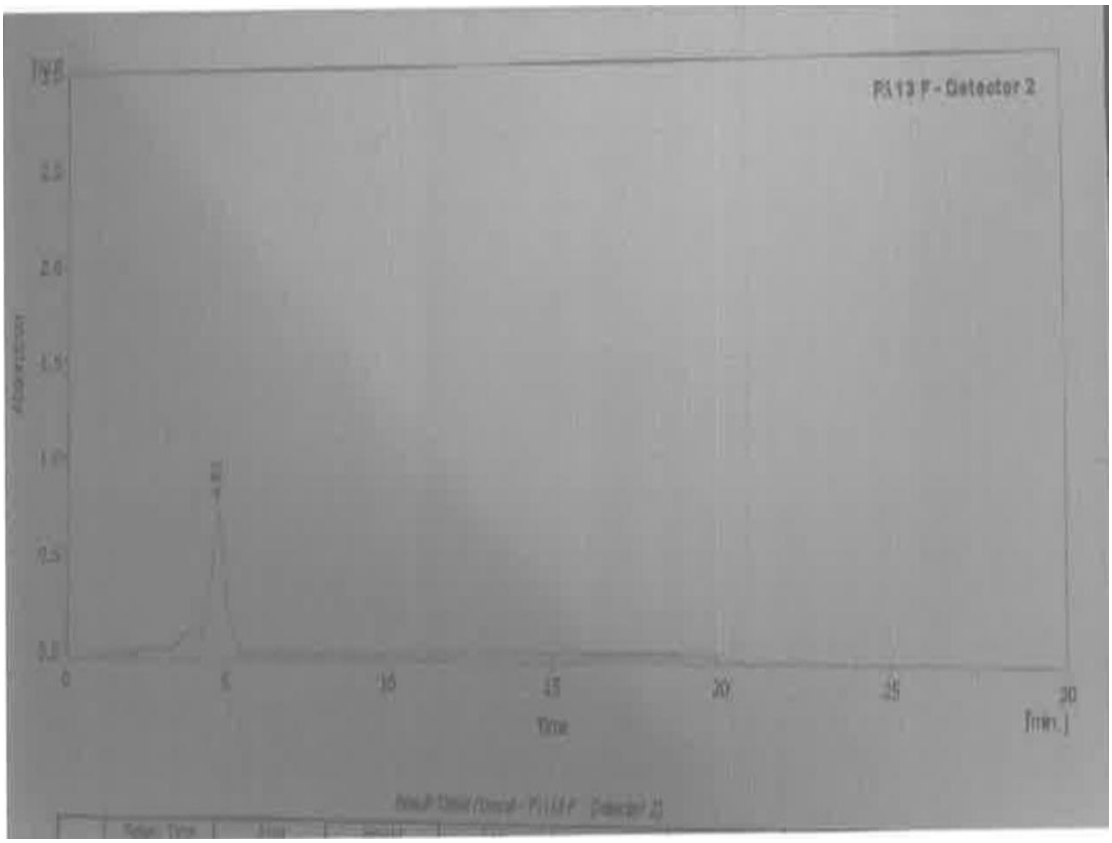
HPLC chromatography of positive AFB1 detection by TLC (No.8MD).



HPLC chromatography of positive AFB1 detection by TLC (No 9MD).



HPLC chromatography of positive AFB1 detection by TLC (No. 13fD).



HPLC chromatography of positive AFB1 detection by TLC (No. 13fD).

الخلاصة

الهدف من هذه الدراسة التحري عن وجود سم الافلاتوكسين B1 في عينات سيرم دم مرضى السكري من النوع الثاني ودراسة تأثيرهما على وظائف الكبد والكلى اذ تم جمع عينات الدم من المرضى الذين يراجعون مستشفى الحسيني في محافظة كربلاء وبواقع 42 عينة كذلك جمعت 44 عينة دم من افراد أصحاء.

أظهرت النتائج أن 16(38.1٪) من مرضى السكري كانت موجبة للسم Aflatoxin B1 (أي وجود السم في الدم) بينما كانت أعداد ونسب الاصحاء و الملوثة دمائهم بالسم ذاته 13 (29.5٪) وكانت الاناث اكثر حساسية من الذكور اذ بلغ عددهن 17 (19.7٪) في حين كان عدد العينات الملوثة بالسم من الذكور 12(13.9٪).

كذلك أظهرت النتائج ان تركيز السم الافلاتوكسين B1 في دم الاناث والذكور والمصابين بمرض السكري النوع الثاني (1.343) نانو غرام /مل و (0.684)نانو غرام /مل على التوالي وبفارق معنوي بينهما. من جانب آخر بينت نتائج حساب تركيز السم في دم الاناث والذكور الاصحاء بلغت (0.133) نانوغرام /مل (0.135) نانوغرام /مل على التوالي واوضحت حساب معامل الارتباط r وجود علاقة بين جنس الانسان وتركيز السم الافلاتوكسين B1 اذ كانت النساء اكثر حساسية للسم مقارنة بالذكور.

واشارت نتائج الفحوصات الكيموحيوية ان مستوى اليوريا لدى الأشخاص المصابين بالسكري والحاملين لسم الافلاتوكسينB1 بلغت 42 ملغم/ديسلتر مقارنة بمستواها لدى الذكور الاصحاء والبالغة 34 ملغم/ديسلتر من ناحية اخرى بلغ مستوى الكرياتينين في دم الذكور المصابين بالسكري 1.88 ملغرام/ديسلتر مقارنة بمستواه لدى الذكور الاصحاء والبالغ 0.71 ملغرام /ديسلتر.

اما مستوى حامض اليوريك uric acid في دم الذكور المصابين فبلغ 8.3 ملغرام /ديسلتر في حين بلغ 3. 87 ملغرام /ديسلتر في الذكور الاصحاء.

و أثبتت هذه الدراسة زيادة في مستوى Alanine Aminotransferase ALT في دم مجموعة الذكور المصابين بمرض السكري والملوثة دمائهم بسم الافلاتوكسين B1 اذ بلغت 18.15 وحدة دولية /لتر و15.08 وحدة دولية /لتر على التوالي.

وسجل اعلى مستوى لأنزيم Aspartate aminotransferase AST في دم الذكور المصابين بالسكري النوع الثاني وغير الحاملين لسم Aflatoxin B1 اذ بلغ 53.38 وحدة دولية /لتر وبفارق معنوي عن مستواه في دم الأشخاص غير المصابين بالسكري النوع الثاني وغير الحاملين لسم الافلاتوكسين B1

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كلية العلوم الطبية التطبيقية
قسم التحليلات المرضية

التحري عن سم الافلاتوكسين B1 لدى مرضى السكري النوع الثاني وتأثيره على وظائف الكلى والكبد

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية – جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في التحليلات المرضية

من قبل

غادة محمد كاظم

بكالوريوس علوم حياة / جامعة بابل (٢٠١٠)

بإشراف

أ.د. سامي عبد الرضا علي

أ.م.د. هدى عبد الرضا عبدالله

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