University of Kerbala

College of Applied Medical Sciences

Department of Clinical Laboratories

The Relationship Between Type 2 Diabetes Disease and Aflatoxin B_1 and Their Effects on Some Human Biochemical Parameters in Baghdad Province

A thesis

Submitted to the Council of the

College of Applied Medical Science - University of Kerbala

In Partial Fulfillment of the Requirements for

the Degree of Master of Clinical Laboratories

Written by

Alaa Edan Sirhan

B.Sc. Medical Laboratory Science Technology
AL-Rasheed University College, 2018

Supervised by
Prof. Dr. Sami Abdul-Redha Al-Jumaily

(2023) September Safar (1445)

بسم الله الرحمن الرحيم

(قَالَ رَبِّ اشْرَحْ لِي صَدْرِي وَيَسِّرْ لِي أَمْرِي وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي يَفْقَهُوا قَوْلِي)

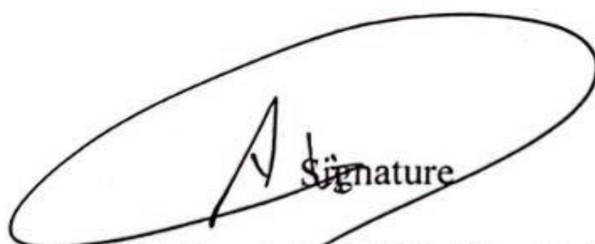
صدقَ اللهُ العلي العظيمُ

سورة طه الآية(٢٥-٢٨)

Supervisor's certification

Supervisor's Declaration

I certify the thesis entitled (**The Relationship Between Type 2 Diabetes Disease and Aflatoxin B_1 and Their Effects on Some Human Biochemical Parameters in Baghdad Province**) was prepared under my supervision by **Alaa Edan Sirhan** at the department of Clinical Laboratories\ College of Applied Medical Sciences\ University of Kerbala, in partial fulfillment of the requirements for the degree of Master in Clinical Laboratories.



Signature

Prof. Dr **Sami Abdul-Redha Al-Jumaily**

Supervisor

28 /٥/ 2023

Head of Department Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Assist. Prof. Dr. Linda Hammed Turki

Head of Clinical Laboratories Department

College of Applied Medical Sciences/ University of Kerbala

/ /2023

Linguistic Certification

We, the examining committee, certify that we have read the thesis entitled " **The Relationship Between Type 2 Diabetes Disease and Aflatoxin B_1 and Their Effects on Some Human Biochemical Parameters in Baghdad Province** " and have examined the student (**Alaa Edan Sirhan**) in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories.**

Signature

Prof. Dr. **Ibtihal Muiz Mahdi Al-hussaini**

(Chairman)

28 / 9/ 2023

Signature

Asst.Prof.Dr. **Mayada Sahib Hassan**

(Member)

28 / 9/ 2023

Signature

Asst.Prof. Dr. **Huda Abd-alredh Abdullah**

(Member)

28/ 9/ 2023

Signature

Prof. Dr. Sami Abdul-Redha Al-Jumaily

(Member & Supervisor)

28 /9/ 2023

I have certified upon the discussion of the examining committee .

Signature

Assist. Prof. Dr. Huda Abd-alredh Abdullah

Dean of the College of Applied Medical Sciences / University of Kerbala

1 / 10/ 2023

Approval Certification

We certify that the thesis entitled (**The Relationship Between Type 2 Diabetes Disease and Aflatoxin B_1 and Their Effects on Some Human Biochemical Parameters in Baghdad Province**) fulfills partial requirements of the degree of Master in Clinical Laboratories.

Signature

Head of Clinical Laboratories Department

Assist. Prof. Dr. Linda Hameed Turki

College of Applied Medical Sciences

University of Kerbala

\ \ 2023

Signature

Vice Dean Scientific Affairs

Assist. Prof. Dr. Huda Abd-alredh Abdullah

College of Applied Medical Sciences

University of Kerbala

\ \10\ 2023

Acknowledgements

*First of all, my thanks and praise to the cause of the reasons and the facilitator of matters, **lord of the Worlds**, who enabled us to do this Also, I also extend my sincere thanks and gratitude to the Dean of the College, the respected **Ass. Prof. Dr. Jawdat Nouri Ghaaib**, and the head of the Analytics Department, **Dr. Linda Hameed Turki,** and all the professors of the Department of Clinical Analysis.*

*I would like to express my gratitude, appreciation, and sincere thanks to my supervisor, **Prof.Dr. Sami Abdul-Redha Al-Jumaily,** for his generous efforts in planning and executing this piece of work. without their help, I can't a completed my work.*

My deepest thanks to all the staff of Al-Kindy Teaching Hospital and Endocrinology Center in Baghdad, Al-Rusafa. As well as Ministry of Science and Technology's Department of Environment and Water.

*Also, I would like to extend my thanks and appreciation to all my friends, especially **Mohammed Rabie and Zahraa Falah,** for their help to me and their sincere feelings. I offer them from the heart my heartfelt thanks, and I ask God to grant them success.*

Alaa 2023

Dedication

To.... The Al-Imam Al-Muntazar

To …the Martyrs of Iraq

To... the person we take as a role model, my dear father

To...the source of success in this world, my dear mother

To... my dear brothers

To every patient who is suffering from diabetes mellitus and needs our efforts

Special thanks to my close colleague: Dr. Ali Abd Hussein, Dr. Radhwan Mohammed, Dr.Abbas Aboud, Qais, Mohammed, Zainab Rahman, Sada Kareem, Zahraa,

Alaa 2023

Table of contents

No	Subject	page
	Acknowledgments	I
	Dedication	II
	List of Contents	III-V
	List of Table	VI
	List of Figures	VII
	List Abbreviations	VIII-X
	Summary	XI-XII
	Introduction	1-3
2.1	Diabetes Mellitus	4
2.1.1	History of Diabetes	5
2.1.2	Epidemiology	6
2.1.3	Types diabetes mellitus	6
2.1.3.1	Type 1 diabetes	6
2.1.3.2	Gestational diabetes	7
2.1.3.3	Other specific types of diabetes	7
2.1.3.3.1	Diabetes insipidus	7
2.1.3.3.2	Pre-diabetes	7
2.1.3.3.3	Maturity-Onset Diabetes of the Young (MODY3)	8
2.1.3.4	Type 2 Diabetes Mellitus (T2DM)	8
2.1.3.4.1	The Pathogenesis of T2DM	8
2.1.3.4.2	Other pathogenesis	9
2.1.4	diagnosis of T2DM	11
2.1.4.1	Fasting blood sugar	11
2.1.4.2	Glycated hemoglobin HBA1C	11
2.1.4.3	Random glucose test	12
2.1.4.4	Oral glucose tolerance test (OGTT)	12
2.1.5	Effect T2DM on thyroid hormone	11
2.1.6	The effect T2DM on liver	12
2.1.7	The effect T2DM on kidney	13
2.2	Mycotoxin	14

2.2.1	Aflatoxin (AF)	15
2.2.1.1	AflatoxinB_1 (AFB_1)	18
2.2.1.1.1	AFB1's Physiochemical Characteristics	20
2.2.1.1.2	Aflatoxin absorption, distribution, metabolism, excretion, and Mechanisms of action	24
2.2.2	Exposure of humans to Aflatoxin	29
2.2.3	Methods of detection of serum Aflatoxin B_1	31
2.2.3.1	Thin Layer Chromatography (TLC)	32
2.2.3.2	Enzyme-Linked Immunosorbent Assay	34
2.2.3.3	high-performance liquid chromatography (HPLC)	34
2.2.4	Effect of AflatoxinB1 on the Immune System	35
2.2.5	Effect of AflatoxinB1 on the Thyroid Glands	36
2.2.6	Aflatoxin B1 and renal impairment	37
2.2.6.1	Assessment of kidney function	38
2.2.7	Aflatoxin B_1 and liver impairment	39
2.2.7.1	Testing the liver's performance	40
3	Materials	42
3.1.1	Apparatus and instruments	42
3.1.2	Kits and chemicals	43
3.1.3	The Tools	44
3.2	Study design	45
3.2.1	Study groups	45
3.2.2	Exclusion criteria	46
3.2.3	Control Criteria	46
3.2.4	Patient Criteria	47
3.3	Methods	47
3.3.1	Blood collection and storage	47
3.3.2	Determination of HBA1C	48
3.3.3	Measurement of WBC, RBC, and HB by complete blood count (CBC test)	50

3.3.4	T3 enzyme linked immunosorbent assay (ELISA)	50
3.3.5	T4 enzyme linked immunosorbent assay (ELISA)	52
3.3.6	TSH enzyme linked immunosorbent assay (ELISA)	56
3.3.7	Routine Biochemical tests	59
3.4.1	Qualitative analysis of serum AFB_1 by TLC	59
3.4.2	Quantitative analysis of AFB1 by HPLC	62
3.4.2.1	preparation of standard AFB_1 for HPLC	62
3.4.2.2	Sample preparation	62
3.5	Statistical Analysis	63
3.6	Ethical Considerations	63
4.1	Measurement Qualitative and Quantitative of the AFB_1	64
4.1.1	Measurement Qualitative of AFB_1 by TLC	64
4.1.2	Measurement Quantitative of the AFB_1 by HPLC	66
4.2	Measurement of Biochemical Parameters of this Study	68
4.2.1	Measurement of AST and ALT enzymes in study groups	68
4.2.2	Effect of AFB1 and T2DM on Kidney Function Parameters	70
4.2.3	Effect of AFB1 and T2DM on thyroid hormone levels	74
4.2.4	Measurement of HBA1C and FBS in study groups	80
4.3	Assessment of the Physiological Parameters	83
4.4	Estimation of correlation coefficient	89
4.4.1	Estimation of correlation coefficient between the AFB1 and T2DM	89
4.4.2	Estimation of correlation coefficient between the AFB1 with parameters	90
4.4.3	Estimation of correlation coefficient between the T2DM with parameters	91
	Conclusions	93
	Recommendations	94
	References	95
	Appendix	122
	Summary in Arabic	131

List of Table

NO	Subject	Page
2.1	Physical and chemical properties of major aflatoxins	23
3.1	Apparatus and instruments	42
3.2	Kits and chemicals	43
3.3	the tools used in this study	44
3.4	Division of study groups	46
3.5	Reagents of T_3	52
3.6	T4 enzyme Kit composition	54
3.7	TSH enzyme Kit composition	59
4.1	Distribution of AFB_1 according to patient and control groups by using TLC	64
4.2	Distribution of AFB_1 according to Sex	65
4.3	Measurement concentration AFB1 by HPLC in patients and control groups	67
4.4	Distribution of AST levels in patient and control groups	68
4.5	Distribution of ALT levels in patient and control groups	69
4.6	Distribution of Blood Urea levels in patient and control groups	71
4.7	Distribution serum creatinine levels in patient and control groups	72
4.8	Distribution of HBA1C levels in patient and control groups	81
4.9	Distribution of FBS levels in patient and control groups	82
4.10	Distribution of HB levels in serum patient compare to the control groups	84
4.11	Distribution of RBC levels in patient and control groups	85
4.12	Distribution of WBCs levels in patient and control groups	88
4.13	Estimation of Correlation between T2DM and AFB1	90
4-14	Correlation between AFB1 and parameters levels in blood serum	91
4-15	Explained the correlation T2DM and parameters levels in blood serum	92

List of Figure

NO	**Subject**	Page
2.1	Mycotoxin classification and the major generating species	15
2.2	Chemical structure of aflatoxin B1 and aflatoxin M1	24
2.3	The adverse cellular effects of mycotoxins and their metabolites	25
2.4	Overview of AFB_1 toxicokinetics	28
2.5	Exposure source of humans to aflatoxins	30
4.1	Distribution of T3 according to patient and control groups	75
4.2	Distribution of T4 according to patient and control groups	76
4.3	Distribution of TSH according to patient and control groups	78

List of Abbreviations

Abbreviations	Full form
AD	Gregorian Year
ADA	American Diabetes Association
AFB1	Aflatoxin Blue $_1$
AFB2	Aflatoxin Blue 2
AFBO	AFB1-exo-8,9-epoxide
AFG1	Aflatoxin green1
AFG2	Aflatoxin green 2
AFL	Aflatoxicol
AFM_1	Aflatoxin milk 1
AFM_2	Aflatoxin milk 2
AFP_1	Aflatoxin binding protein
AFs	Aflatoxins
Alb	Albumin
ALT	Alanine transaminases
AOAC	the Association of Official Analytical Chemist
AST	Aspartate transaminases
ATP	Adenosine Triphosphate
AGE	Advanced glycation end-product
BMI	Body mass index
BUN	Blood urea nitrogen
CBG	Casual blood glucose
CNS	Central nervous system
CRP	C-Reactive Protein
CYP1A2	Cytochrome P450 1A2
CYP3A4	Cytochrome P450 1A4
D.W	Distal water
DCs	Dendritic cells
DM	Diabetes mellitus
ELISA	Enzyme linked immunosorbent assay
F, C, NTX	Female, control Non- AFB1 toxin
F, C, TX	Female, control with AFB1 toxin
F, D-2, NTX	Female, type 2 diabetes Non- AFB1 toxin
F, D-2, TX	Female, type 2 diabetes with AFB1 toxin
FBG	Fasting Blood Glucose
FDA	Food and Drug Administration
FFAs	Free fatty acids

GC	Guanine-cytosine
GHS	Glutathione-S-transaminases
HB	Hemoglobin
HBA1C	Hemoglobin A1c
HCC	Hepatocellular carcinoma
HPLC	High Performance Liquid Chromatography
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IR	Insulin resistance
LADA	Latent Autoimmune Diabetes
LDL	Low-density lipoprotein
M, C, NTX	Male, control Non- AFB1 toxin
M, C, TX	Male, control with AFB1 toxin
M, D-2, NTX	Male, type 2 diabetes Non- AFB1 toxin
M, D-2, TX	Male, type 2 diabetes with AFB1
mitDNA	Mitochondrial DNA
MRLs	Maximum residue limits
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate H
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NPN	Non-protein nitrogenous
OTA	Ochratoxin A
OGTT	Oral glucose tolerance test
OS	Oxidative stress
RBC	Red blood cells
RCTs	Randomized controlled trials
Rf	Relative flow
ROS	Reactive oxygen species
S.Cr	Serum creatinine
SD	Standard deviation
T2DM	Diabetes mellitus Type 2
T3	Triiodothyronine
T4	Tetraiodothyronine
TA	Thymine-adenine
Tg	Thyroglobulin

TGF-β	Transforming growth factor
TH	Thyroid hormone
TLC	Thin Layer Chromatography
TNF-α	Tumor necrosis factor-alpha
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
UV	Ultraviolet
WBC	White blood cells
WHO	World Health Organization

Summary

Type 2 diabetes mellitus is a type of metabolic disorder leading to hyperglycemia because of impaired insulin, insulin resistance, or both. insulin resistance condition, where people with T2DM produce insulin but are unable to adequately utilize it to move glucose to their cells, leading to elevated glucose levels. The estimated global prevalence of diabetes in individuals aged 20 to 79 years was 10.5% (536.6 million) in 2021 and was projected to increase to 12.2% (783.2 million) in 2045. Many of the risk factors leading to T2DM include oxidative stress, obesity, and contamination of food. aflatoxin B_1 secondary metabolites from the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, These toxins contaminate food, feed, and many foodstuffs such as rice, corn, canned food, vegetables, and peanuts. The aim of this study was the examination of aflatoxin B_1 levels in the blood serum of patients with type 2 diabetes and the correlation of those levels to several physiological and biochemical parameters.

Case-control research design Samples were donated by Diabetes Central/Baghdad, Endocrines, and Al-Kindy Hospital. A total of 177 participations were investigated; 93 (44 males and 49 females) had T2DM, and 84 (37 males and 47 females) were controls. Each subject had 10 ml of blood collected from a vein, and the serum was separated using centrifugation. These serum samples were subsequently used to perform enzyme-linked immunosorbent assay tests on triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH). Thin-layer chromatography and High Performance Liquid Chromatography methods for the qualitative and quantitative detection of aflatoxin B_1. Also, used complete blood count device measurements of white blood count, Hemoglobin, and red blood cells and fine-care measurements of HBA1C. Additionally, Fasting

Blood Sugar, aspartate aminotransferase, alanine transaminases,, urea, and creatinine can be measured in the hospital utilizing an automated device.

The study's data analysis was created using the IBM SPSS 26 statistical tool. The groups were compared on various levels using the ANOVA table and Duncan test. The link between the variables was determined by comparing the observed findings with the expected outcomes using the chi-square test. The association between the two was demonstrated and the relationship's enduring tendency was determined using the correlation persons coefficient (r).

The outcome demonstrated a relationship between AFB_1 both the patient and control groups. Male and female patients had the highest levels of toxin (3.98 ng/ml and 4.01 ng/ml, respectively). In addition, males and females in the control group had the highest level of toxin (0.14 ng/ml). AFB_1 also shown a positive correlation with T2DM (r= 0.528). High levels of aspartate aminotransferase, alanine transaminases, urea, and creatinine, as well as high levels of TSH, T4, and low levels of T3, were the effects of AFB_1 and T2DM on physiological and biochemical markers. Additionally, increased WBC_s counts and significantly decrease RBC_s and HB levels ($P< 0.05$).

The conclusion is that females more sensitive than male for AFB_1. The results of this study showed that the presence of toxins in the body at any level poses a risk to human health since they accumulate over time and interfere with a number of biological functions. T2DM and AFB_1 had negative effects on the biomarkers utilized in the experiment, though to varying degrees. The study groups that contained AFB_1 demonstrated how AFB_1 leads to the onset of diabetes by having higher FBS and HBA1C levels.

Chapter One

Introduction

1.Introduction

Diabetes mellitus type 2 (T2DM) is a common metabolic disorder characterized by chronic hyperglycemia. Development is generally brought about by a confluence of two main factors: impaired insulin secretion by pancreatic beta-cells and impaired insulin response in insulin-sensitive tissues (Roden and Shulman, 2019); (Hurtado and Vella, 2019). Insulin, a peptide hormone produced by the pancreatic beta-cells, plays a key role in the regulation of blood glucose levels and energy metabolism (Dirir *et al*., 2022).

When β-cells are unable to counteract peripheral insulin resistance, T2DM develops. T2DM is defined by the existence of peripheral insulin resistance in tissues such skeletal muscle, adipose tissue, and the liver. Insulin resistance causes an increase in insulin demand, β-cell compensation by increasing β-cell mass and insulin secretion, and hyperinsulinemia to develop. Hyperinsulinemia, in a vicious cycle, worsens the metabolic dysregulations that results in β-cell degeneration and the emergence of T2DM (Rachdaoui, 2020).

The global diabetes prevalence in 20–79-year-olds in 2021 was estimated to be 10.5% (536.6 million people), rising to 12.2% (783.2 million) in 2045. Diabetes prevalence was similar in men and women and was highest in those aged 75–79 years. Prevalence (in 2021) was estimated to be higher in urban (12.1%) than rural (8.3%) areas and in high-income countries (11.1%) compared to (5.5%) (Sun *et al*., 2022a).

Many factors lead to T2DM and insulin resistance; one of these factors is constant exposure to contaminated food, which contains a high level of toxins (Firmin *et al*., 2016).

Aflatoxins (AFs) are secondary metabolites produced mainly by the fungi, *Aspergillus flavus* and *Aspergillus parasiticus* , and widely contaminate various types of crops all over the world, such as maize, peanuts, wheat, barley, and rice. Approximately 20 AFs have been identified, and four of them occur naturally, including aflatoxin B_1 (AFB1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) (Rushing and Selim, 2019, Deng *et al*., 2018).

AFB_1 is the most toxic, having hepatotoxic, immunotoxic, mutagenic, carcinogenic, and teratogenic properties in humans and experimental and farm animals. has been classified as a Group I carcinogen by the International Agency for Research on Cancer. Consumption of food contaminated by AFB_1 can seriously affect the health of humans (Deng *et al*., 2018). AFB_1 is a potent hepatotoxic toxin, that can cause hepatitis, cirrhosis, and immunological damage to the liver. It has been involved in the etiology of human hepatocellular carcinoma (Fan *et al*., 2021).

Kareem *et al*., 2021 found AFB_1 contamination in the blood of 100% uncertain chronic kidney disease patients and healthy controls, respectively. Also, the concentration ranges in serum samples were 0.68–8.23 ng/ml for uncertain CKD patients.

AFB_1 is absorbed into the digestive tract, released into systemic circulation, and affects other organs. Eraslan *et al*., 2006 results showed a significant effect of AFB_1 on the thyroid gland. Also, another study showed the effect of AFB_1 on hematology parameters (Marijani *et al*., 2017).

AFB_1 was detected in human serum by several methods, including ELISA, TLC, HPLC, and others. All available analytical methods for the determination of

AFB1 principally included the same steps like sampling, sample preparation, detection, confirmation, and finally risk assessment. HPLC approaches are most commonly used today because of their high accuracy, precision, and sensitivity in detecting toxins (mushtaq *et al*., 2020).

Given the prevalence of T2DM in Iraq and its various causes, this study was conducted to identify the role of food contamination in the local markets in the exacerbation of T2DM and to investigate the possibility of a relationship between AFB_1 and this disease, T2DM.

Aim of the study

Investigation of aflatoxin B_1 in the blood patients with type 2 diabetes mellitus and correlation between them in some biochemical and physiological parameters.

Objectives of The Study

1- Investigating whether there is a relationship between type 2 diabetes and aflatoxin B_1.
2- Determining whether aflatoxin B_1 has an impact on the study's parameters.
3- Determine whether type 2 diabetes has an impact on study parameters.

Chapter Two
Literature Review

Literature Review

2.1. Diabetes Mellitus(DM)

Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is either disturbed insulin secretion or various grades of insulin resistance, or usually both (Schleicher *et al.,* 2022). Insulin, a hormone secreted by β-cells of the islets of Langerhans, controls the metabolism of carbohydrates, proteins, and fats by stimulating the absorption of molecules like glucose from the blood into fat, skeletal muscle cells, and the liver (Wondmkun, 2020a). The main feature of T2DM is the decrease in insulin sensitivity. The main causes of T2DM are obesity, oxidative stress, genes, and aging. Insulin resistance occurs first. The increase in visceral fat leads to an increase in fatty acids, which leads to an increase in gluconeogenesis and glucose levels. The increase in glucose level leads to decompensation of β cells, compensation and decompensation of β cells ultimately lead to impaired glucose tolerance, leading to the development of T2DM (Ma *et al.,* 2018). The symptoms of diabetes include thirst, polydipsia, polyuria, fatigue, constant hunger, weight loss, dry mouth, and blurred vision. These measures do not discriminate between T1DM and T2DM, and only one parameter is enough to define diabetes mellitus (DM). T2DM is mainly diagnosed with pancreatic β-cell dysfunction and peripheral insulin resistance (Kupai *et al.,* 2022).

Glucose is the primary source of energy for cells, which are the building blocks of life. It is given to the body by insulin, which carries out the metabolic tasks that keep people alive. A glucose level imbalance is a sign of DM, a common type of chronic disease. It leads to long-term complications, such as blindness,

kidney failure, and heart disease, which have a negative impact on one's quality of life (Gollapalli *et al.,* 2022).

2.1.1 History of Diabetes

The ancient Egyptians described clinical symptoms similar to diabetes 3000 years ago. The term "diabetes" was coined by Aretus of Cappadocia (81–133 AD). Later, the word mellitus (sweet honey) was added by Thomas Willis (Britain, 1675) after rediscovering the sweetness of the urine and blood of patients (first noticed by the ancient Indians). In 1776, Dobson (Britain) confirmed for the first time the presence of excess sugar in urine and blood as the cause of their sweetness. The history of diabetes in modern times coincides with the advent of experimental medicine. An important milestone in the history of diabetes is the identification of the liver's role in glycogen formation and the concept that diabetes is due to excess glucose production by Claude Bernard (France, 1857). Mering and Minkowski (Austria, 1889) discovered the role of the pancreas in the pathogenesis of diabetes. Later, this discovery formed the basis for the isolation and clinical use of insulin by Banting and Best (Canada, 1921). Trials of an oral anti-diabetic agent preparation ended successfully with the first marketing of tolbutamide and carbutamide in 1955 (Ahmed, 2019).

Ancient descriptions of diabetes emphasize the importance of monitoring and recording medical conditions as humans evolved. Early physicians used everything they could (smell or even taste) in pursuit of knowledge, skills, and diagnosis (Lakhtakia, 2013).

2.1.2. Epidemiology

Prevalence of DM recent findings suggest that the burden of DM has risen significantly over the past decade and may be considered a growing epidemic (Lovic *et al*., 2020). According to the 2021 International Diabetes Federation (IDF) report, the prevalence of T2DM in people aged 20–79 years is 537 million (10.5%) and is projected to reach 783 million (12%) by 2045. In Africa, approximately 24 million people will have T2DM in 2021. This number is predicted to increase to 55 million by 2045, an increase of 129% (Adamu *et al.,* 2023).

Around 1.4 million Iraqis have diabetes. Reported T2DM prevalence in Iraq ranges from 8.5% to 13.9%. A local study including more than 5400 people in the city of Basrah, southern Iraq, reported a 19.7% prevalence of diabetes in subjects aged 19 to 94 years.

In Iraq, there are insufficient epidemiological studies and randomized controlled trials (RCTs) related to diabetes; Therefore, it remains difficult to fully understand the prevalence of diabetes in Iraq and the most effective therapies for the Iraqi population (Abusaib *et al.,* 2020).

2.1.3 Types of Diabetes Mellitus

2.1.3.1 Type 1 Diabetes (T1DM)

It is a chronic autoimmune disorder. T1DM is the most common form of diabetes mellitus in children, and about 10% of all diabetes cases are type I. The body does not produce insulin. People of this type are called juvenile-onset diabetes mellitus or insulin-dependent diabetes mellitus, which results from a loss of the insulin-producing beta cells of the pancreas (Roep *et al.,* 2021).

2.1.3.2 Gestational Diabetes

Hyperglycemia that develops during pregnancy and resolves after birth has been described (McIntyre *et al.,* 2019). The prevalence of gestational diabetes has been increasing over the past decades. Several genetic, social, and psychological risk factors can contribute to the development of gestational diabetes, which leaves mothers and their children with many physical and mental complications (Mills *et al.,* 2020).

2.1.3.3 Other Specific Types of Diabetes

2.1.3.3.1 Diabetes Insipidus

It is, in fact, not diabetes but a case of high blood glucose levels because the kidneys excrete a lot of fluid (urine), (Christ-Crain *et al.,* 2019).

2.1.3.3.2 Pre-Diabetes

It is a medical condition when the level of glucose in the blood is above the normal level, but it is not diagnosed as T2DM. It was found that 5 to 10% of people develop T2DM. Prediabetes is also known as impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), (Mohammed *et al.,* 2022).

2.1.3.3.3 Maturity-Onset Diabetes of the Young (MODY)

Form of DM begin at a young age, while T2DM is more commonly diagnosed in people over 30 years of age. While MODY is not usually associated with being overweight or obese, an obese person with MODY may develop symptoms sooner than people who are not affected by it (Garcia-Gonzalez *et al.*, 2018). MODY disorders that affect 1–5% of all patients with diabetes mellitus (Nkonge *et al.*, 2020).

2.1.3.4 Type 2 Diabetes Mellitus (T2DM)

T2DM, one of the most prevalent metabolic illnesses, is brought on by a combination of two main factors: impaired insulin production by pancreatic cells and improper insulin response in insulin-sensitive tissues. Because the processes of insulin release and activity are crucial for maintaining glucose homeostasis (Galicia-Garcia *et al*., 2020).

2.1.3.4.1 The Pathogenesis of T2DM

Insulin resistance (IR) impairs the ability of muscle cells to take up and store glucose and triglycerides, which results in high levels of glucose and triglycerides circulating in the blood. IR is commonly present in older adults but has become increasingly prevalent at all ages, including middle-aged individuals who are overweight and sedentary. IR is typically defined as decreased sensitivity and responsiveness to insulin-mediated glucose disposal and inhibition of hepatic glucose production (Kumar *et al.,* 2019).

Impaired islet cell function is associated with islet α and β cells. The number of islet β cells is significantly reduced in T2DM patients, and the ratio of α/β cells is significantly increased. In addition, the sensitivity of α cells to glucose is

decreased, which makes the glucagon level and liver sugar output increase and eventually leads to the incidence of T2DM. This is the classic theory of double hormone abnormalities (Ma *et al.,* 2018).

Oxidative stress diabetes is one of the most common metabolic disorders in the world. A huge body of evidence indicates a role for oxidative stress in the development of many human diseases, including diabetes. Oxidative stress refers to an imbalance between potentially harmful free radicals and the body's mechanisms to efficiently detoxify them in favor of the free radicals. Consequently, excess free radicals can attack and damage a wide range of biomolecules, including proteins, lipids, and nucleic acids (Nikooyeh and Neyestani, 2016).

Gene influences additionally, there is a ton of proof that T2DM has a solid genetic foundation. Offspring of one parent with T2DM have a lifetime risk of the condition of 40%, which increases if the mother is also affected, and approaches 70% if both parents have the condition (Lyssenko and Laakso, 2013).

An initiating factor for diabetes linked to insulin resistance is obesity. Adipose tissue in obese people releases increased levels of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines that may contribute to the emergence of insulin resistance. In addition, genetic predisposition, adipose tissue hypoxia, oxidative stress, lipodystrophy, and endoplasmic reticulum stress all contribute to insulin resistance (Wondmkun, 2020b).

2.1.3.4.2 Other Pathogenesis

Inflammation

Halim and Halim, The outcomes of his study suggested that inflammation is likely to have a direct impact on insulin resistance or blood glucose levels by

increasing them significantly (Halim and Halim, 2019). Proinflammatory molecules such as interleukin 6 (IL-6), C-Reactive Protein (CRP), tumor necrosis factor-alpha (TNF-α), or IL-1 are released into the bloodstream and into particular organs, inducing a condition known as metabolic inflammation. Since IL-1 inhibits β-cell function and activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor, it is true that IL-1 plays a role in the autoimmune response against β-cells in the pancreas *(*Galicia-Garcia *et al.,* 2020).

Gender

Diabetes is affected by psychological and behavioral changes in the individual, and the chance of developing diabetes increases with an increase in body mass index *(*Zhang *et al.,* 2019*a*). Women are more likely than men to acquire T2DM because they have less muscle mass, which limits their ability to absorb more glucose (Kautzky-Willer *et al.,* 2016). Also, they have relatively high amounts of estrogen and progesterone, both of which contribute to a loss in whole-body insulin sensitivity as well as the difference in fat storage sites in the case of excess weight (Mauvais-Jarvis, 2017).

Smoke

Smoking leads to more than 8 million deaths per year. This is from both active and passive uses, i.e., non-smokers exposed to smokers. Smokers are 30–40% more likely to develop T2DM as compared to non-smokers. When an individual smokes, the level of nicotine increases in his or her body, leading to a reduction in muscle glucose intake, developing insulin resistance, and T2DM (Ismail *et al.,* 2021).

2.1.4. Effect T2DM on Thyroid Hormone

The thyroid hormone is well known for controlling metabolism, growth, and many other bodily functions. The thyroid gland, anterior pituitary gland, and hypothalamus comprise a self-regulatory circuit called the hypothalamic-pituitary-thyroid axis. The main hormones produced by the thyroid gland are thyroxin, or tetraiodothyronine (T_4), and triiodothyronine (T_3). Thyrotropin-releasing hormone (TRH) from the hypothalamus, thyroid-stimulating hormone (TSH) from the anterior pituitary gland, and T4 work in synchronous harmony to maintain proper feedback mechanisms and homeostasis (Núñez *et al.,* 2017).

Thyroid hormone (TH) production is a tightly regulated process controlled by a classic negative feedback loop involving the hypothalamus, the pituitary, and the thyroid, which has led to the common name hypothalamus-pituitary-thyroid axis. Thyrotropin-releasing hormone (TRH) is produced in the hypothalamus. Once released, TRH reaches the pituitary gland, binds to the TRH receptor, and stimulates the production and secretion of thyroid-stimulating hormone (TSH), also known as thyrotropin (Gauthier *et al.,* 2020).

Diabetes impairs thyroid function by changing thyroid-stimulating hormone (TSH) levels and by disturbing the conversion of thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues. In euthyroid diabetic patients, the nocturnal TSH peak can be absent or diminished, and the TSH response to thyrotropin-releasing hormone (TRH) can be compromised. Ketoacidosis can results in a drop in T_3 and T_4 levels, while TSH levels can stay normal. Moreover, hyperinsulinemia and insulin resistance promote thyroid tissue proliferation, increase the prevalence of nodular thyroid disease, and results in a goiter (Mohammed Hussein and AbdElmageed, 2021).

Both hyperthyroidism and hypothyroidism can affect insulin resistance, although through different mechanisms. Hyperthyroidism precipitates impaired fasting glucose and/or diabetes and worsens glycemic control in pre-existing T2DM. Hypothyroidism results in impaired glucose absorption from the gastrointestinal tract, delayed peripheral glucose assimilation, and gluconeogenesis (Eom *et al.,* 2022).

2.1.5.The Effect of T2DM on the Liver

The liver is by far the most important metabolic organ, with essential roles in regulating homeostasis and mediating glucose and lipid metabolism. The metabolic activities of the tissue are precisely controlled by the actions of metabolic substrates, including free fatty acids (FFAs) and hormones (Daryabor *et al.,* 2020).

The metabolic disorder (diabetes) affects many organs, including the liver, which plays a key role in the regulation of carbohydrate, lipid, and protein metabolism. Elevated serum aminotransferase levels, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were commonly observed in diabetes. ALT and AST are the most specific markers of hepatic injury, which are located in the hepatocellular cytosol and mitochondria, respectively. A recent report shows a significant association between increased ALT and AST and insulin resistance in T2DM (Shibabaw *et al.,* 2019). As a results, individuals with T2DM have an increasing risk of moderate to severe liver damage and a higher chance of developing liver cancer (Tanase *et al*., 2020).

2.1.6 The Effect T2DM on Kidney

DM is the most common cause of chronic kidney disease in the world, leading to multiple complications including end-stage renal disease, cardiovascular disease, infection, and death. Chronic kidney disease in the setting of diabetes or diabetic kidney disease (DKD) manifests clinically as albuminuria, reduced glomerular filtration rate (GFR), or both (Afkarian *et al.,* 2016).

Hyperglycemia is the primary etiological factor responsible for the development of diabetic kidney disease. Once hyperglycemia becomes established, multiple pathophysiological disturbances, including hypertension, altered tubuloglomerular feedback, renal hypoxia, lipotoxicity, podocyte injury, inflammation, mitochondrial dysfunction, impaired autophagy, and increased activity of the sodium-hydrogen exchanger, contribute to progressive glomerular sclerosis and the decline in glomerular filtration rate (DeFronzo *et al.,* 2021).

The kidneys excrete metabolic waste products and regulate the serum concentration of a variety of substances. At the stage of renal disease, these substances often become abnormal, and the extent of the abnormality depends on the severity of the disease. Serum creatinine and urea concentrations change inversely with changes in GFR, So these are useful to detect the degree of renal dysfunction. Urea and creatinine are useful parameters for the functioning of the kidney. Changes in serum creatinine concentrations more reliably reflect changes in GFR than changes in serum urea concentrations. Several studies reported the relationship between blood glucose, serum creatinine, and serum urea levels in T2DM patients (Mishra *et al.,* 2015).

2.2. Mycotoxin

The presence of toxic compounds such as mycotoxins in human food and animal feedstuffs is a significant issue globally, and it is considered a risk for humans and animals. Mycotoxins are recognized as secondary metabolites, and their presence in food affects the quality and safety of food, results in substantial economic losses. Mycotoxin comes from the Greek terms ‘mykes’ and ‘toxicum’, ‘meaning’ fungus/mold and poison. The formation of these toxins occurs under certain conditions, such as moisture content, temperature, constituents of food material, and the presence of water vapor in the air (Iqbal, 2021). The continuous and anticipated proliferation of mycotoxigenic *Aspergillus species* has raised the prospect of mycotoxin contamination in the feed and food production chains as a results of Earth's changing climate. Since their discovery, these dangerous mycotoxins have led to significant health and economic issues. Aflatoxin, ochratoxin, gliotoxin, fumonisins, sterigmatocystin, and patuling are mycotoxins that are derived from *Aspergillus* (Figure 2.1). Some of them are present in dairy products, primarily milk and cheese, as well as fresh and particularly dried fruits and vegetables, nut products, usually groundnuts, oil seeds, coffee beans, and various grain products, such as rice, wheat, barley, rye, and frequently maize. They are also present in the livers of animals that have been fed mycotoxin-contaminated forage (Ráduly *et al*., 2020).

The ideal temperature for the growth of *Aspergillus* in maize is between 33 °C and 35 °C. The major types of aflatoxins present in food are AFB_1, AFB_2, AFG_1, and AFG2 (Pokharel *et al.,* 2021). AFs are the most toxic and have been extensively studied (Norlia *et al.,* 2019).

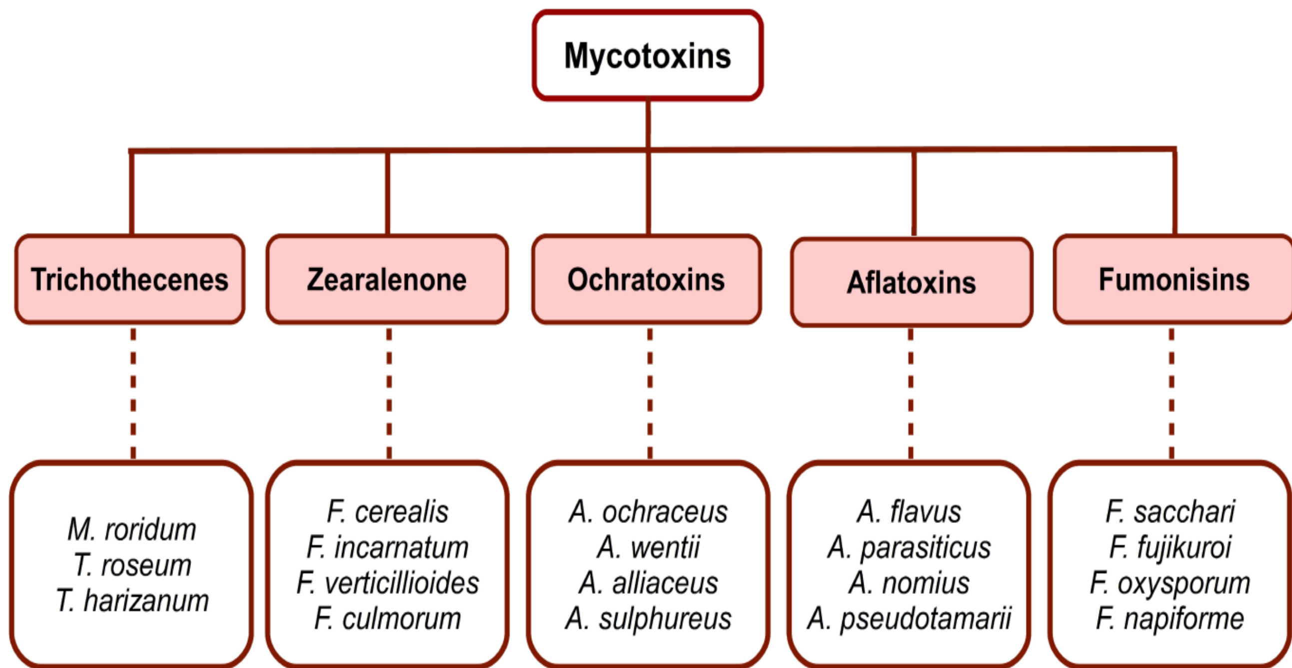


Figure.2.1 Mycotoxin classification and the major generating species (Popescu *et al*., 2022)

2.2.1. Aflatoxin (AF)

Aflatoxins (AFs) are secondary metabolites produced by toxigenic strains of molds, mainly *Aspergillus flavus* and *Aspergillus parasiticus*, which grow in soil, hay, decaying vegetation, and grains. AF toxicity occurs due to acute or chronic exposure to aflatoxin. The term "aflatoxin" is derived from the name *Aspergillus flavus*. It was named around 1960 after its discovery as the source of a disease in Turkey called Turkey X disease (Dhakal *et al.*, 2022). The genus *Aspergillus* contains four subgenera and 339 species. The mycotoxins produced by *Aspergillus* spp. are known as aflatoxins. AFs are commonly

produced by *Aspergillus flavus* and *A. parasiticus,* but some other species, such as *A. nomius*, A. pseudotamarii, *A. parvisclerotigenus*, and *A. bombycis* of section *Flavi*; *A. ochraceoroseus* and *A. rambellii* from section *Ochraceorosei; Emericella astellata* and *E.venezuelensis* from *Nidulatans*, have also been reported as aflatoxin producers (Shabeer *et al.,* 2022).

There are mainly six different types of aflatoxins: aflatoxins B1 (AFB1), aflatoxins B2 (AFB2), aflatoxins G1 (AFG1), aflatoxins G_2 (AFG_2), aflatoxins M_1 (AFM_1), and aflatoxins M_2 (AFM_2). Out of these, B1, B2, G1, and G2 are found in food crops or their products, while M_1 (a metabolite of B_1) and M_2 are found in the milk of some animals' by-products, such as dairy products. Aflatoxin B_1 and B_2 are produced by *A. flavus,* while Aflatoxin G_1 and G_2 are synthesized by *A. parasiticus* and largely contaminate a wide range of food commodities, including cereals (maize, sorghum, pearl millet, rice, and wheat), oilseeds (peanut, soybean, sunflower, and cotton), spices (chilies, black pepper, turmeric, coriander, and ginger), nuts (almond, Brazil nut, pistachio, walnut, and coconut), yam, and various milk products (Kumar *et al.,* 2021).

AFB_1, AFB_2, AFG_1, and AFG_2 are the four main naturally occurring aflatoxins produced by the *Aspergillus species* of mold. The letters "B" and "G" stand for the blue and green fluorescent colors produced under ultraviolet (UV) light on thin-layer chromatography plates. The subscript numbers 1 and 2 denote major and minor compounds, respectively. The B designation of aflatoxins B_1 and B_2 is due to their blue fluorescence when exposed to UV light, whereas the G designation refers to the related structures' yellow-green fluorescence when exposed to UV light (Kareem *et al.,* 2021).

These fungi usually infect cereal crops, including wheat, walnuts, corn, cotton, peanuts, and tree nuts, and can pose serious threats to human and animal

health by causing various complications such as hepatotoxicity, teratogenicity, and immunotoxicity (Kumar *et al*., 2017).

Among the mycotoxins affecting food and feed, AF is the major one in food that ultimately harms human and animal health. The level of toxicity associated with AF varies with the types present, with the order of toxicity being $AFB_1 > AFG_1 > AFB_2 > AFG_2$ (Kumar *et al*., 2017). The food and Drug Administration (FDA) set a tolerance limit of 20 ppb for aflatoxins in view of their toxic effects on foods (Sarma *et al.,* 2017).

Temperature and humidity are only two examples of the many variables that affect how much AF is produced in food. Customers experience health problems when these molds contaminate crops, especially in hot and humid weather. The toxins may cause immunosuppression in humans and may also be mutagenic, teratogenic, estrogenic, neurotoxic, hepatotoxic, nephrotoxic, and neurotoxic (Misihairabgwi *et al.,* 2019). AF is a potent carcinogen and mutagen with hepatotoxic and immunosuppressive properties, as well as the ability to disrupt various metabolic activities, results in liver and kidney damage (Mahjoory *et al.,* 2023).

There is a threat to human health represented by toxin-producing fungi, especially AFs. In Iraq, many studies have been conducted on the fungi associated with cereals, particularly wheat grains and their derivatives. Since the wheat crop has great importance, especially with regard to human and animal sustenance, it is necessary to periodically investigate the safety of this crop and its by-products, starting from the field, passing through the silos, until reaching the mills, and before reaching the consumer (FADHIL *et al.,* 2022).

Darwish *et al.*, AFs are the most common mycotoxins (43.75%) in Africa, followed by fumonisin (21.87%), ochratoxins (12.5%), zearalenone (9.38%), deoxynevalenol (6.25%), and beauvericin (6.25%). They reported high levels of aflatoxin in samples collected from several African countries, including South Africa, Lesotho, Egypt, Tunisia, Morocco, Sudan, Tanzania, Zambia, Uganda, Kenya, Ethiopia, Nigeria, Ghana, Benin, Mali, Togo, and Burkina Faso (Darwish *et al.,* 2014).

AFB_1, is the most common and abundant form of aflatoxin, has the greatest carcinogenic potential. Thus, AFB_1 has been the most extensively studied of these molecules in terms of metabolic pathways, biomarkers of exposure, and mutagenic and carcinogenic analyses (McCullough and Lloyd, 2019).

2.2.1.1. AflatoxinB$_1$ (AFB_1)

AFB_1 is one of the most deleterious types of mycotoxins (Madbouly *et al.,* 2023). is the most toxic and carcinogenic of the aflatoxins, being far more hazardous than cyanide, arsenic, and organic pesticides (Wang *et al.,* 2022a). AFB_1 has been classified as a group I human carcinogen by the International Agency for Research on Cancer (Guo *et al.,* 2020). AFB_1 is a kind of mycotoxin primarily produced by the secondary metabolism of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. which is the most commonly occurring and toxic one of all the aflatoxins. AFB_1 is confirmed to be widely distributed in nature, especially in a variety of food commodities including groundnuts, maize, rice, sorghum, milk, and oils (Dai *et al.,* 2017). AFB_1 is the most toxic mycotoxin, causing harmful effects on humans (Wang *et al.,* 2019). AF overexposure poses a threat to almost 4.5 billion people globally, and these toxins are responsible for 4.6% to 28.2% of all occurrences of hepatocellular carcinoma (Min *et al.,* 2021).

AFB_1 is recognized as being hepatotoxic, carcinogenic, and mutagenic. AFB_1 is the third-most important cause of liver cancer, especially in sub-Saharan Africa and developing countries in Asia (Fouad *et al.,* 2019). The target organs of AFB_1 are the liver, kidney, spleen, bone marrow, intestine, testis, and ovary (Park *et al.,* 2020).

The human body absorbs AFB_1 through the oral and respiratory systems, which affects the body's antioxidant defenses and immune system and harms internal organs, especially the liver, which can results in AFB1 hepatitis, cirrhosis, and immunological damage (Fan *et al.,* 2021).

Humans are frequently exposed to low concentrations of AFs due to their daily intake of rice, corn, peanuts, etc. AFB_1 in crops enters the human body through the food chain and seriously harms human health, causing chronic liver damage, further inducing liver cancer. The metabolism process of AFB_1 in the liver causes lipid peroxidation in hepatocytes. The activation of the CYP450 enzyme system would produce a large amount of reactive oxygen species (ROS), which can cause oxidative stress damage and even apoptosis (Zhang *et al*., 2020, Jiang *et al*., 2019).

Excessive apoptosis may produce the immunodeficiency syndrome, severe hepatitis, and degenerative neurological diseases. In vitro experiments show that AFB_1 would induce hepatocytes apoptosis by the mitochondrial pathway and the death receptor pathway (Wu *et al*., 2019).

AFB1 is categorized as class one in food toxicological importance by the World Health Organization (Keller, 2019). AFB_1 enters the blood stream from the gut and goes to the liver, where, upon metabolism, several end compounds are produced. AFB_1 is oxidized in the liver to the hydroxylated metabolite AFM_1,

which is found in urine, milk, and blood (Nduti and Njeru, 2017). AFM_1 is the hydroxylated metabolite of AFB_1 and is mainly found in individuals who are widely exposed to mycotoxins. The biotransformation of AFB_1 takes place within the liver (Akash *et al*., 2021a).

With the exception of the production of aflatoxicol, which is catalyzed by the coenzyme NADPH, the biotransformation is carried out by the liver's cytochrome P450 enzymatic system. A cytoplasmic enzyme found in the soluble portion of liver homogenates that is NADPH-dependent can also be used to reduce AFB_1 and produce aflatoxicol (AFL). After ingesting contaminated food, AFB_1 is partially metabolized and biotransformed into AFM1 in the liver, and AFM1 is subsequently eliminated in milk, which is then eliminated in milk, urine, tissues, and biological fluids (Zhang *et al*., 2021, Ramalho *et al*., 2018). The AFM_1 is non-degradable and resistant to various industrial treatments, such as heat treatments, sterilization, or pasteurization (Badr *et al.,* 2022).

All these biotransformation products of AFB_1 may remain in the form of residues in the liver, as observed in several species. These residues are considered markers of toxin exposure in the diet (Ramalho *et al.,* 2018).

2.2.1.1.1. AFB_1's Physiochemical Characteristics

A family of highly substituted coumarin derivatives with a fused dihydrofurofuran molecule, the AFs are commonly categorized as difuranocoumarins. Particularly, AFB_1 exhibits high fluorescence emission in the blue area (thus the designation B) when exposed to ultraviolet light and the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure (Figure 2.2). The primary hydroxylated metabolite of AFB_1, known as AFM_1, is generated by cytochrome P450 1A2 (CYP1A2), It produces intensely fluorescent blue-violet

light (Marchese *et al.,* 2018). which can be divided into B groups (that is, can deliver blue fluorescence, such as AFB_1, AFB_2) and G groups (that is, can deliver green fluorescence, such as AFG_1, AFG_2) according to the fluorescence (Li *et al.,* 2022b).

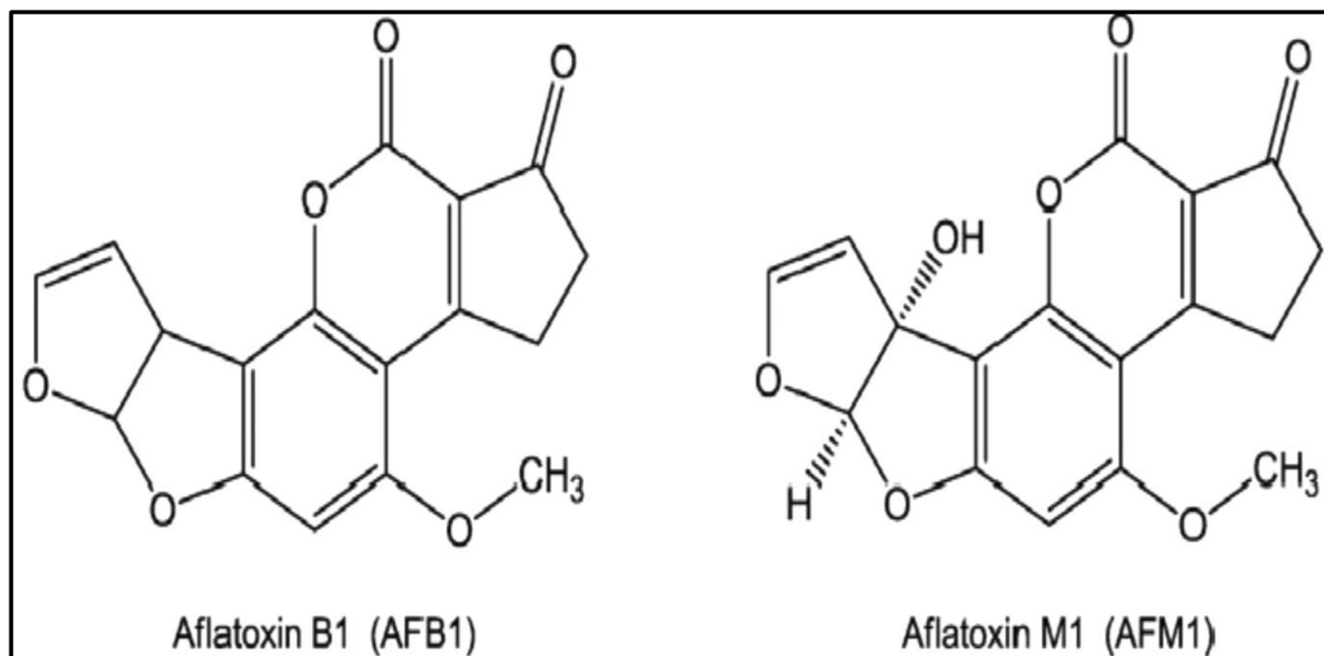


Figure.2.2. Chemical structure of aflatoxin B1 and aflatoxin M1 (Marchese *et al.*, 2018)

Both toxins share similar chemical characteristics in that they are easily soluble in polar organic solvents, only slightly soluble in water, and insoluble in nonpolar solvents (MdQuadri *et al.,* 2013). They resist thermal degradation during food production thanks to their remarkable thermal stability, even at high temperatures (>100 °C). Since pasteurization and other thermal treatments alone have shown to be ineffective at reducing aflatoxin food contamination, especially in milk and dairy products. This constitutes a significant barrier (Marchese *et al.,* 2018). Although most mycotoxins are easily degraded when heated, AFs show high thermal stability (Wang *et al.,* 2023). AFB_1 is given as $C_{17}H_{12}O_6$ based on the structure, with four protons in the difuran ring and a carbonyl group in the cyclopentenone cross-conjugated with the lactone ring (Li *et al.,* 2022b).

AFs are colorless to pale yellow crystals that exhibit fluorescence under UV light. They are slightly soluble in water (10–20 μg/ml) and freely soluble in moderately polar solvents such as chloroform, menthol, and dimethyl sulfoxide. They are unstable in UV light in the presence of oxygen, unstable in extreme pH (<3or>10). The lactone ring opens under alkaline conditions, and the aflatoxins are destroyed, but this reaction is reversible under acidification. Ammoniation results in the opening of the lactone ring at high temperatures, which causes the decarboxylation of aflatoxins, and this reaction is irreversible (physical and chemical properties of aflatoxins). Some important physical and chemical properties of major aflatoxins are given in table 2.1(Kumar, 2018).

Table.2.1: Physical and chemical properties of major aflatoxins (Kumar, 2018)

Aflatoxin Name	Molecular Formula	Moleculr Weight	Melting Point	Uv absorption (e)		Fluorescence Emission (nm)
				Λmax (nm)	ε (L. mol^{-1}. Cm^{-1}) × 10^{-3}	
B1	$C_{17}H_{12}O_6$	312	268-269	223	25.6	425
				265	13.4	
				362	21.8	
B2	$C_{17}H_{14}O_6$	314	286-289	265	11.7	425
				363	23.4	
G1	$C_{17}H_{12}O_7$	328	244-246	243	11.5	450
				257	9.9	
				264	10	
				262	16.1	
G2	$C_{17}H_{14}O_7$	330	237-240	265	9.7	450
				363	21	

AFM_1 is the 4-hydroxy derivative of AFB_1 and is secreted in the milk of mammals that consume AFB_1. AFM_1 has a relative molecular mass of 328 Da and has the molecular formula $C_{17}H_{12}O_7$ (Joint, 2001). AFM1, the principal hydroxylated metabolite of AFB1, is found in the milk (hence the designation M) of mammals fed with contaminated feedstuffs. The carry-over of AFB_1 as AFM_1 in the milk of dairy cows has been established to range from 0.3% to 6.2%. However,

AFM_1 was also found in lactating mothers' milk. Several studies reported carcinogenic and immunosuppressive effects similar to those of AFB_1 on both humans and other animals, even if with a less potent effect. However, AFM_1 is the only mycotoxin for which maximum residue limits (MRLs) in milk have been established (Marchese *et al.,* 2018).

2.2.1.1.2. Aflatoxin absorption, distribution, metabolism, excretion, and Mechanisms of action

AF enters the intestine after ingesting contaminated food. The liver is the first and most significant organ to be impacted after they are dispersed, processed, and expelled (They also gather in muscle), as shown in figure (2.3). In phase I of the biotransformation of xenobiotics, P450 Cytochromes are crucial (Antonissen *et al.,* 2017). CYP1A2 and CYP3A4 are the enzymes that convert AF and are the most highly expressed proteins in mammals. The metabolite produced by the oxidation reaction has the ability to bind to proteins and DNA, producing cytotoxicity and genotoxicity, respectively (Allocati *et al.,* 2018). For instance, AFB_1 generates AFB1 adducts when it binds to guanine residues in nucleic acids. that may cause DNA damage by converting guanine-cytosine (GC) to thymine-adenine (TA). The binding of AFB_1 to proteins is irreversible, with the most well-known adduct being AFB_1-lysine in albumin. An epoxy reactive intermediate, such as AFB_1-8,9-epoxide, is created, or this is hydrolyzed to a less hazardous form, AFM_1, during the first stage of metabolic oxidation in the liver (Allocati *et al.,* 2018).

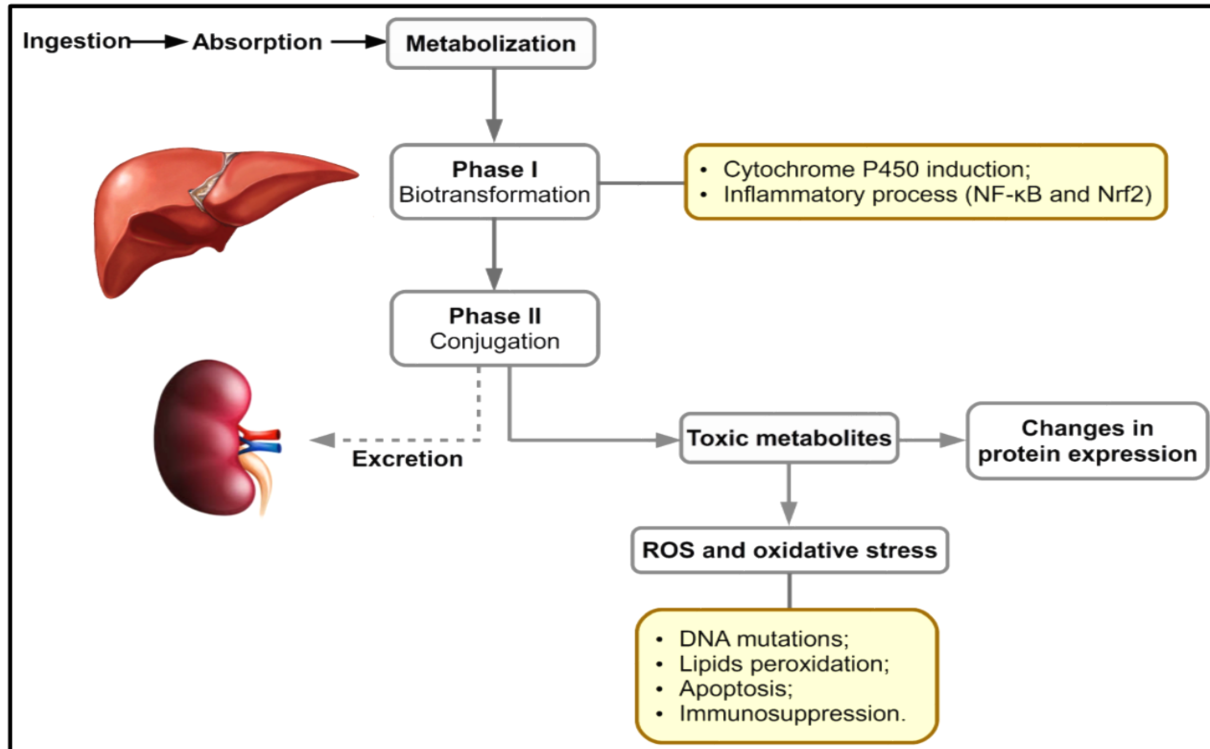


Figure. 2.3. The adverse cellular effects of mycotoxins and their metabolites.(Popescu *et al.,* 2022)

In order to add functional polar groups, the processes of hydroxylation, sulfoxidation, epoxidation, N, O, and S-dealkylation, oxidative aromatic hydroxylation, desulfuration, denitrosation, and dehalogenation are all catalyzed by phase I enzymes. The enzymes of the cytochrome P450 superfamily are responsible for these processes (Popescu *et al.,* 2022).

Phase II of metabolism is particularly active in the conjugation events of metabolites that were previously generated with glucuronic acid and sulfate. The phase I epoxide metabolite may subsequently be detoxified in phase II through reduction to a less harmful metabolite like AFM1 or AFQ1, glutathione

conjugation, hydrolysis by an epoxide hydrolase to AFB1-8,9-dihydrodiol, or any of these processes (Popescu *et al.,* 2022).

In addition to inhibiting protein synthesis, exposure to this mycotoxin also affects the levels of other metabolic pathway enzymes (Caloni and Cortinovis, 2011). Reactive oxygen species (ROS) are produced when cells are exposed to AFB1 in a way that impairs the respiratory chain. Oxidative stress results if these are not mitigated through enzyme and non-enzyme-based antioxidant processes (Ma *et al.,* 2021). DNA and proteins are also oxidized as a results of the overproduction of ROS, which also targets polyunsaturated fatty acids from glycerophospholipids. A crucial factor in the harmful effects of aflatoxins is lipid peroxidation and oxidative DNA damage (Popescu *et al.,* 2022).

AFB_1 is converted in vivo to an aflatoxin B_1-exo-8,9-epoxide by the liver enzyme cytochrome p450 oxidase. The epoxide that is formed is a highly reactive electrophile and has an extremely high regiospecificity for the N7 position of the guanine residue in DNA. It specifically binds to the p53 encoding region to form a DNA adduct. This results in nonfunctioning p53 proteins, which are important tumor suppressors in humans (Reid *et al.,* 2016). Another isoform of P450 (CYP3A4) metabolizes AFB_1 to AFB_1-endo-epoxide, which is less toxic considering that it cannot bind nucleic acids and can be excreted in different forms. Both the exo- and endo-epoxides can undergo rapid non-enzymatic hydrolysis to AFB_1-8,9 dihydrodiol, reacting with the α-amino group of lysine in serum albumin (Zhou *et al.,* 2019).

In addition, AFB_1 binds to DNA and induces structural DNA modifications that lead to gene mutations, as well as changes in telomere length and cell cycle control points. The binding of AFB_1 to DNA at the base of guanine in liver cells corrupts

the genetic code that controls the growth of cells, leading to tumor formation (Moore *et al*., 2018).

Damage to mitDNA is induced by mitochondrial membrane adduction and mutations, results in increased cell death (apoptosis) and energy output disruption (ATP production) (Thrasher and Crawley, 2012). Reactive aflatoxin-8, 9-epoxide can influence the mitotic (M) phase, the growth process (G_1 and G_2 phase) and the production of DNA (S phase) in the cell cycle by disrupting the different control points that regulate the growth and proliferation of the cell cycle, leading to cell deregulation and cancer production (Hu *et al*., 2018). AFB_1 causes the base G to turn into the base T in the third position of codon 249. Hepatocellular carcinoma (HCC) has also been linked to high levels of AFB_1 contamination in food in parts of East Asia and Africa (Bbosa *et al.,* 2013). AFB_1 is metabolized in humans and animals by cytochrome P450 enzymes, and its metabolites include AFM_1, AFP_1, AFQ_1,and AFBO. AFM_1 accumulates in milk, whereas other metabolites are excreted through urine, feces, and bile and can also enter the bloodstream as shown in figure (2.4) (Li *et al.,* 2022a).

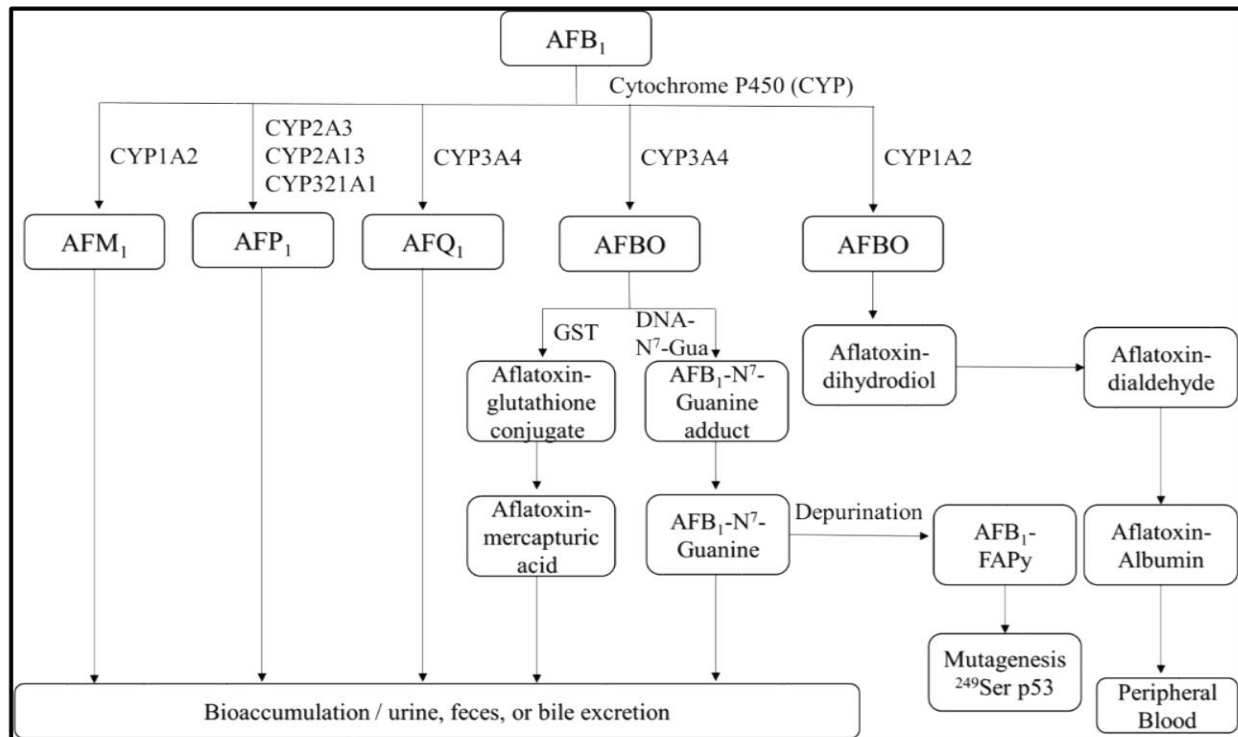


Figure.2.4. Overview of AFB_1 toxicokinetics.(Li *et al.,* 2022a)

Different CYP450 isozymes are responsible for the bioactivation of AFB1 depending on the host, the organ, and the sub-cellular component. The microsomal CYP1A2, 3A4, 3A5, 3A7, 2A3, and 2B7, the hepatocytic 3A3, and the lung CYP2A13 are the main isozymes responsible for AFB1 bioactivation in the aforementioned organs in humans out of the 57 CYP450 recognized isoenzymes (Benkerroum, 2020).

The major CYP enzymes involved in human AFB_1 metabolism in the liver are CYP3A4, 3A5, and 1A2 (Gilbert-Sandoval *et al.,* 2020). While the lipoxygenases and prostaglandin H syntheses appear to be important in its biotransformation in the lung (Dohnal *et al.,* 2014). AFB_1 metabolism involves several pathways that are depicted. Other reactions are odealkylation to AFP_1, hydroxylation to AFM_1 and AFQ_1, or ketoreduction to AFL. AFB_1 metabolites can serve as biomarkers of human exposure to this mycotoxin: AFM_1, AFQ_1, and AFP1

have been detected in human urine, as well as AFB1-N7-guanine, the depurination product of the DNA adduct. Moreover, the AFB_1-albumin adduct in blood or AFB1-lysin are useful biomarkers of exposure (Groopman *et al.*, 2014).

2.2.2. Exposure of Humans to Aflatoxin

Aflatoxisis is a type of poisoning that can be either chronic or acute and is brought on by ingesting tainted food or animal feed, according to Bankole and Adebanjo., Therefore, the term is not just restricted to human poisoning and may also refer to aflatoxin toxicity in other organisms like agricultural animals, birds, fish, and other critters. Chronic aflatoxicosis results from ingesting low to moderate quantities of aflatoxins. Aflatoxin intake from food is a major cause of hepatocellular cancer (Bankole and Adebanjo, 2003).

Human intoxication by aflatoxins may occur via contact, ingestion, or inhalation. They can affect the liver, kidneys, stomach, lungs, salivary glands, colon, and skin. As shown in figure (2.5), once ingestion of AFB_1 has taken place, the gastrointestinal tract rapidly absorbs it along with other aflatoxins, and the circulatory system transports them to the liver (Lalah *et al.,* 2019). Approximately 1–3% of the ingested aflatoxins irreversibly bind to proteins and DNA bases to form adducts such as aflatoxin B_1-lysine in albumin. Disruption of protein and DNA bases in hepatocytes disrupts their functions and causes liver toxicity. This results in chronic exposure, which is defined as the ingestion of very small doses of aflatoxins over a long period of time (Kowalska et al., 2017b). Exposure to AFB_1 is reported to be both acute and chronic; the main exposure pathway to AFB_1 is through the intake of contaminated food. In addition, inhalation and dermal pathways have also been reported in populations exposed to this compound. As mentioned above, the main target of AFB_1 is the liver, most likely because CYP enzymes are mainly produced by the liver (Diaz de Leon-Martinez *et al.,* 2020).

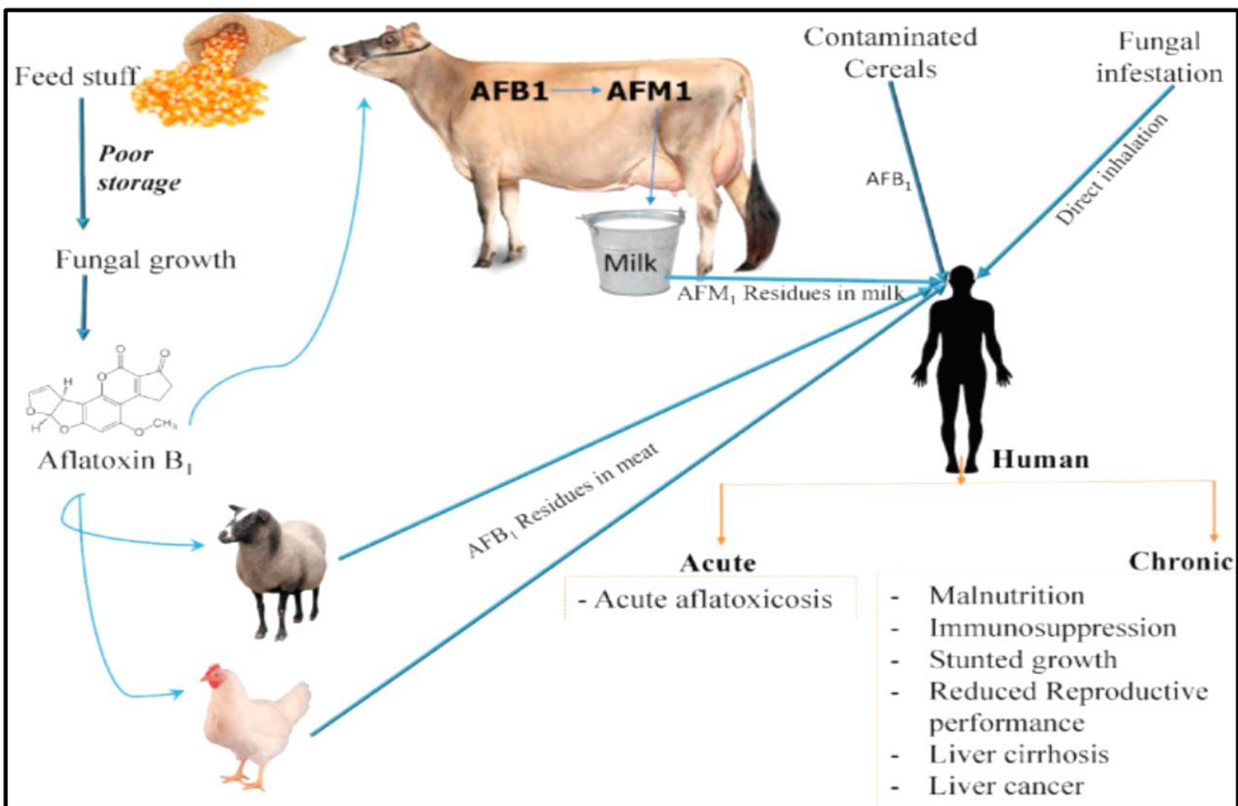


Figure.2.5. Exposure source of humans to aflatoxins (Adegbeye *et al.,* 2020)

AF exposure can results in acute symptoms including nausea, vomiting, cramping, and convulsions, as well as chronic problems like hepatotoxicity, immunotoxicity, and teratogenicity. One of the main causes of hepatocellular cancer in developing countries is aflatoxin (Kew, 2012).

AFB_1 could cause central and peripheral nervous system deterioration. AFB_1 exposure to the brain has been linked to acute central nervous system (CNS) symptoms, including coma, cerebral edema, and even death. Additionally, a shortage of these neurotransmitters after AFB1 exposure has been linked to symptoms of brain deterioration such as dullness, restlessness, muscular tremor, convulsions, loss of memory, epilepsy, stupidity, loss of motor coordination, and aberrant sensations (Richard *et al.*, 2020).

AFB1 was able to affect the proteins' final products as well as the metabolism of amino acids, which resulted in hyper-ammonemia that easily crossed the blood-brain barrier and triggered the production of glutamate neurotransmitters, which are cytotoxic to brain cells and cause encephalopathy. Critical cellular components like DNA, lipids, and proteins were destroyed as a results of glutathione (GHS) depletion via the 8,9 epoxides of AFB1 (Richard *et al*., 2020). Numerous other aflatoxin-related pathologies have also been linked to nutritional diseases, delayed physical and mental development, changes in reproduction, and diseases of the nervous system, among others, in both people and animals (Martínez *et al.,* 2023).

Children face chronic exposure to high aflatoxin levels in regions with endemic food contamination. Exposure starts in utero, continuing throughout the early stages of life, although breastfeeding offers certain respite from high intakes per day (Awuchi *et al.,* 2022). In Gambia, a correlation was reported between aflatoxin exposure in utero and growth impairment in children within the first 12 months of life. On the basis of these findings, the consumption of aflatoxin-contaminated foods by pregnant mothers may have significant effects on the growth and development of children after delivery (Awuchi *et al.,* 2022).

2.2.3. Methods of Detection of Serum Aflatoxin B_1

AFB_1 has received significant attention in the past few years. Because of its hepatocarcinogenic nature, a number of analytical, diagnostic, and immunological procedures are available for assessment and estimation of AFB1 in different feeds, such as enzyme-linked immunosorbent assay (ELISA), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LC-MS/MS), and electrochemical immunosensors (ECI) (mushtaq *et al*., 2020). All available analytical methods for the determination of AFB1 principally included the same steps like, sampling, sample preparation,

detection, confirmation, and finally risk assessment. HPLC approaches are most commonly used nowadays because of their high accuracy, precision, and sensitivity (MUSHTAQ *et al.,* 2020).

2.2.3.1.Thin Layer Chromatography (TLC)

TLC continues to be one of the most straightforward, quick, and reliable procedures for screening aflatoxins in foods and feeds. It is 100% as effective as the enzyme-linked immunosorbent assay approach for assessing aflatoxins in fungal cultures. It has been utilized in a number of recent investigations to detect and quantify aflatoxins in foods, feeds, and a variety of different matrices (Salisu *et al*., 2021). TLC was invented by De Iongh *et al*., and since 1990, the Association of Official Analytical Chemists (AOAC) has regarded it as the method of choice (Wacoo *et al*., 2014).

TLC, one of the first planar chromatographic techniques, is currently used to separate and differentiate organic analytic in a mixture (Jain *et al*., 2017). Due to its advantages, including (i) low solvent usage, (ii) simplicity of use, (iii) high sample throughput (e.g., analysis of 8–10 samples simultaneously using a single development solvent), and (iv) affordability, it is acknowledged as a sustainable chromatographic technique. (v) TLC relies on solvent capillary flow and does not require pressure controls, pumps, valves, etc., so there is no wear and tear and no requirement for spare parts; (vi) Additionally, no particularly trained personnel are needed. In this sense, sustainability refers to the possibility of system failure and the availability of resources to make the system functional again. When failure is less common and restoration supplies are more easily accessible, chromatographic procedures are more durable (Jain *et al*., 2021, Jain *et al*., 2020).

We looked at the solvent mixtures that are widely used in systematic toxicological study, including ethyl acetate-ethanol (8:2 v/v), chloroform-acetone (8:2 v/v), chloroform-methanol (8:2 v/v), and ethyl acetate-acetone (8:2 v/v). Additionally, saturation periods ranging from 10 to 30 min were also investigated because they had a substantial impact on the chromatographic separation. A 15-minute saturation time produced a promising performance. Thus, a chloroform-methanol (8:2 v/v) mixture with a 15-minute saturation duration was chosen as the developing system (Jain *et al.,* 2023).

TLC is a method that can be used to separate, evaluate the purity of, and identify organic molecules. One of the most popular separation methods used in aflatoxin analysis is TLC, which is also known as flat-bed chromatography or planar chromatography. A stationary phase like silica, alumina, or cellulose immobilized on a glass or plastic plate serves as the normal-phase TLC's mobile phase. The sample is deposited as a spot on the stationary phase, whether it is liquid or dissolved in a solvent. By running standards beside the unidentified spot, a sample's composition can be determined. The solvent then travels up the plate through capillary action after being positioned vertically in a tank with one edge of the plate in it. The plate is withdrawn from the tank once the solvent has reached the other edge, and the separated spots are seen using UV, fluorescence, or other methods because the components have various partitioning behaviors (Pilařová *et al.,* 2019).

TLC can identify and quantify aflatoxins at levels as low as 1 ng/g. The Rf value for each spot is calculated. It is the ratio of the distance (cm) from the start to the center of the sample spot and the distance (cm) from the start to the solvent front. Rf stands for "ratio of fronts" or retardation factor is characteristic for a given compound to be on the same stationary phase using the same mobile phase

under the same conditions of plate development. For identification purposes, the Rf values of standards are compared to those of unknown samples (Hussain, 2011).

2.2.3.2. Enzyme-Linked Immunosorbent Assay

ELISA is used for detecting and quantifying the presence of an antigen (aflatoxin) in a sample using an enzyme-labeled toxin and antibodies specific to the aflatoxin test kit (Jangampalli Adi and Matcha, 2018). ELISA has the advantage of being a cheap, rapid method with low sample volumes and relatively fewer preparation procedures than other methods, which at the same time have high specificity and sensitivity and fast responses with high repetition capabilities. However, the accuracy of ELISA can sometimes be influenced by the nature of the mycotoxin, the sample preparation process, and the nature of the material, although its accuracy and reproducibility can be improved by having a previous separation procedure (Maggira *et al.,* 2022).

2.2.3.3. High-Performance Liquid Chromatography (HPLC)

HPLC is commonly used for determining aflatoxins because of its high selectivity and sensitivity (Chen *et al.,* 2022). provides fast and accurate aflatoxins detection results within a short time. A sensitivity of detection as low as 0.1 ng/kg using FLD has been reported (Wacoo *et al*., 2014).

HPLC mainly utilizes a column that holds packing material (the stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used (Malviya *et al.,* 2010).

HPLC is considered the reference method for the qualitative and quantitative determination of mycotoxins (Maggira *et al.,* 2022).

2.2.4. Effect of AflatoxinB1 on the Immune System

Four major toxic forms of aflatoxin: B_1, B_2, G_1 and G_2 are known to be hepatotoxic, cytotoxic and immunosuppressive. Compared with the other AFs, AFB_1 is considered as the predominant and most toxic naturally occurring form. The immune system can be the target of many chemicals, inducing severe adverse effects on host health. Chronic aflatoxin exposure has been reported to play a direct immunosuppressive effect on immune system for both humans and animals (Mohsenzadeh *et al.,* 2016).

Due to the complexity of the immune system, there is no single parameter that can completely identify and assess the immunotoxicity of mycotoxins. The immunotoxicity of mycotoxins needs to be studied with respect to different aspects of the innate and adaptive immune responses, including the proliferation, differentiation, or maturation of immune cells such as lymphocytes, dendritic cells (DCs), and macrophages, cytokine production, antibody levels, and even susceptibility to pathogens including bacteria and viruses. Generally, the immunotoxicity of mycotoxins is defined as the adverse effects on the functioning of both local and systemic immune systems that results from exposure to mycotoxins. It can suppress the immune response, results in decreased host resistance to infections or tumors, or enhance the immune response, leading to the production of autoimmune effector cells and auto-antibodies that induce a pathological immune response against tissues, thereby causing damage to the structure of the tissue (Sun *et al*., 2022b).

AFB_1 is an external toxin and has documented pathologic effects on apoptosis in the liver, kidney, thymus, spleen, and gastrointestinal tract immune systems. Based on the dose and time of effect, one report indicates that AFB_1 may reduce the number of B and T lymphocytes and disturb cytolysis activity in neutrophils. A research has confirmed that AFB_1 can induce apoptosis and potential ATP depletion in various cells, which might occur through caspase-3/7 activation and ATP depletion (Mehrzad *et al*., 2020b).

2.2.5. Effect of Aflatoxin B_1 on the Thyroid Glands

Thyroid hormones affect development, growth, and metabolic control, therefore being indispensable to normal development and body energy expenditure (Louzada and Carvalho, 2018). Thyroid hormone synthesis is regulated by feedback mechanisms mediated by the hypothalamus-pituitary-thyroid (HPT) axis. Thyroid hormone synthesis requires active iodide uptake through sodium/iodide symporter, thyroglobulin (Tg) production, and Tg iodination by the thyroid peroxidase (TPO) enzyme. Thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), are released by Tg proteolysis. T_4 is released from the thyroid gland in a much larger amount (in a ratio of approximately 14:1) (Babić Leko *et al.,* 2021). In humans, approximately 80% of the amount of TH secreted by the thyroid is in the form of T_4, while 20% is the active form of T_3 (van der Spek *et al.,* 2017).

the major part of T_3 is generated locally from T_4 by deiodinase 2 (DIO2) in most tissues of the body and in the brain, especially at the hypothalamus-pituitary level (Dunn *et al.,* 2015). The liver and the kidney are the principal organs responsible for T_4 deiodination and the synthesis of T_3, which accounts for up to two-thirds of T_3 production and release into the blood. However, most of the organs and tissues are also efficient in producing T_3 (Sirakov and Plateroti, 2011).

The toxins can induce OS, which also impacts negatively on various hormonal influences, e.g., by causing antioxidant imbalance and impairing the functions of the deiodinase enzymes. For example, OS reduces the capacity of DIO2 to convert thyroxin (T_4) into its biologically active form, T_3. Different defense mechanisms that protect against free radical damage have been characterized in various cellular localizations, including the endoplasmic reticulum, mitochondria, plasma membrane, peroxisomal, and cytosol (Somppi, 2017).

Lower T_3 and T_4 concentrations stimulate thyroid gland T_3 and T_4 receptors, stimulating the synthesis and release of TSH. Increased TSH levels stimulate the utilization of iodine by the alimentary tract and its diffusion in the thyroid gland. Iodine is accumulated in the thyroid and forms a complex with thyroglobulin molecules. Afterwards, each thyroglobulin molecule binds to one or two molecules and forms T_3 and T_4 (Valchev *et al.,* 2014).

AFB_1 has been stated to inhibit the function of different endocrine glands by disturbing the enzymes and their substrates that are responsible for the synthesis of different hormones (Saeed, 2020).

2.2.6. Aflatoxin B_1 and Renal Impairment

The kidneys maintain homeostasis of body fluids, electrolytes, osmolality, and pH, excrete metabolic waste products, and secrete hormones and bioactive molecules. The kidneys are composed of nephrons, small independent functional units with a glomerular part filtering fluid and small molecules from the blood and a single tubule that reabsorbs most filtered molecules and secretes metabolic waste products, concentrating the urine to 1-2 liters per day (Kellum *et al.,* 2021).

It is noteworthy that, in addition to the liver, AFB1 is also beginning to target the kidney. High quantities of poisonous chemicals are also deposited in the renal

medulla because drug or toxic substance metabolites are selectively taken up and concentrated by renal tubular cells before being eliminated in the urine. This implies that toxin AFB1 buildup may potentially occur in the kidney. According to research, oxidative stress is a significant risk factor for AFB1 toxicity. Exposure to AFB1 increases reactive oxygen species (ROS) levels, which can disrupt cellular redox balance and results in kidney damage from oxidative stress. Therefore, it is stressed that reducing oxidative stress is a successful method of treating AFB1 nephrotoxicity (Wang *et al.,* 2022b).

Li *et al*., These findings demonstrated that the kidney was one of the primary organs targeted by AFs and suggested that a number of metabolites might be transported, generated, or broken down in the kidney, including l-proline (which is used to detoxify kidney damage). Since AFB_1 directly damages kidney cells, increasing cell apoptosis and death, mycotoxin residues in the kidney represent a concern to both animals and humans (Li *et al*., 2018b). The toxic effects of AFB_1 are mainly mediated via OS; AFB_1 induces the formation of free radicals and inhibits the production of antioxidant enzymes, which leads to an imbalance between oxidation and anti-oxidation and increases oxidative damage (Tao *et al.,* 2021).

2.2.6.1. Assessment of Kidney Function

Urea, commonly referred to as blood urea nitrogen (BUN) when measured in the blood, is a product of protein metabolism. BUN is considered a non-protein nitrogenous (NPN) waste product. Amino acids derived from the breakdown of protein are deaminated to produce ammonia. Ammonia is then converted to urea via liver enzymes. Therefore, the concentration of urea is dependent on proteins intake, the body’s capacity to catabolize protein, and adequate excretion of urea by the renal system (Burtis and Bruns, 2014).

Urea accounts for the majority (up to 80%–90%) of the NPNs excreted by the body. The body’s dependency on the renal system to excrete urea makes it a useful analyte to evaluate renal function. An increase in BUN can be the results of a diet that is high in protein content or decreased renal excretion (Burtis and Bruns, 2014).

Creatinine, also a NPN waste product, is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function. Creatine is synthesized in the liver, pancreas, and kidneys from the transamination of the amino acids’ arginine, glycine, and methionine. Creatine then circulates throughout the body and is converted to phosphocreatine by the process of phosphorylation in the skeletal muscle and brain. The majority of creatinine is produced in the muscle. As a results, the concentration of plasma creatinine is influenced by the patient’s muscle mass. Compared to BUN, creatinine is less affected by diet and more suitable as an indicator of renal function (Burtis and Bruns, 2014).

2.2.7. Aflatoxin B_1 and Liver Impairment

The liver is the main target organ for AF toxicity and carcinogenicity (Karamkhani *et al.,* 2020). AFB_1 is a potent hepatotoxic toxin that can cause hepatitis, cirrhosis, and immunological damage to the liver. It has been involved in the etiology of human hepatocellular carcinoma. AFB_1 can cause oxidative stress in the body's metabolism process and then cause cytotoxicity, such as apoptosis and DNA damage (Fan *et al.,* 2021). Although AFB_1 can be detoxified in the liver, high-level exposure induces acute hepatic necrosis, eventually leading to cirrhosis or carcinoma. The carcinogenic mechanism, AFB_1 metabolites may bind DNA and alkylate the bases via an epoxide metabolite, inducing cell cycle disorder and mutation of the DNA protective p53 gene (Zhou *et al.,* 2019). Also, AFB_1

increased lipid peroxidation, inflammation, and apoptosis in the liver (Karatekeli *et al.,* 2023).

AFB_1 increases the risk of cirrhosis in the absence of viral infection. Cirrhosis is a severe chronic liver disease featuring encapsulation or replacement of the damaged liver tissue by scar tissue with distortion of the hepatic vasculature and architecture. The disease is often asymptomatic until complications such as variceal bleeding, ascites and jaundice occur (Alvarez *et al.,* 2020). As well as the effect on the function of the liver and increased the serum activity of AST, ALT and ALP enzymes (Ismail *et al.,* 2020).

The AF albumin (Alb) adduct in the blood reflects exposure over the previous 2-3 months. This alb adduct is formed following the metabolism of aflatoxin in the liver, and its level correlates with both dietary aflatoxin exposure and other exposure sources (percutaneous and airborne). The AFB1-albumin adduct is the major protein adduct found in peripheral blood due to exposure to AFB_1 (Saad-Hussein *et al.,* 2016).

2.2.7.1. Testing the Liver's Performance

The liver is the body's largest single, discrete organ. It has four major functions: metabolism and synthesis; excretion; storage; and the detoxification of potential poisons(Blann, 2014). Clinicians are required to assess abnormal liver chemistry on a daily basis. The most common liver enzymes ordered are serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and bilirubin. These tests should be termed liver chemistry" or liver tests (Kwo *et al.,* 2017). There is a consensus that liver function enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are

biomarkers that reflect disease severity in a number of chronic liver diseases (Liu *et al.,* 2018).

Whether acute or ongoing, liver injury eventually results in an increase in serum aminotransferase levels. The enzymes AST and ALT play an important role in the citric acid cycle because they catalyze the transfer of -amino groups from aspartate and alanine to the-keto group of ketoglutaric acid to create oxalacetic and pyruvic acids, respectively. Both enzymes require the vitamin B6 compound pyridoxal-5'-phosphate to complete this action, but the effect of pyridoxal-5'-phosphate deficiency is greater on ALT than on AST activity. Due to the possibility that pyridoxal-5'-phosphate deficiency may lower ALT serum activity and contribute to patients' elevated AST/ALT ratio, this has therapeutic implications for those with alcoholic liver disease (Giannini *et al.,* 2005).

Both aminotransferase are highly concentrated in the liver. AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain, and red blood cells, and ALT has low concentrations in skeletal muscle and kidneys. An increase in ALT serum levels is, therefore, more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity)(Giannini *et al.,* 2005).

Chapter Three

Material and Methods

Materials and Methods

3.1. Materials

3.1.1. Apparatus and Instruments

The equipment's and instruments, used in this study are described in table (3.2)

Table:(3.2) Apparatus and instruments

NO	Apparatus	Company	Origin
1	Autoclave device	Lap Tech, Korea	Korea
2	Becman coulter	AU480	UK
3	Centrifuge	Human Humax4K	Germany
4	Complete blood picture (CBC)	Sysmex, Spin	Germany
5	Deep Freeze	Royal	England
6	Electric oven	Memmert	Germany
7	Electric shaker	Bioneer	,England
8	ELISA assay	Human Reader, HS	Germany
9	Fine care plus	Wondfo	China
10	High-Performance-Liquid Chromatography(HPLC)	Model Sykamn	Germany
11	Thin layer chromatography plate (TLC)	Chmlab	Spanish
12	Uv source	Cleaver	England

3.1.2. Kits and chemicals

The kits and chemicals, used in this study were presented in table (3.3)

Table (3.3) Kits and chemicals

NO	Kits and Chemical	Company	Origin
1	ALT	Randox	UK
2	AST	Randox	UK
3	Chloroform	Alpha chemik	India
4	Creatinine	Randox	UK
5	HBA1C	Wondfo	China
6	Methanol	Alpha chemik	India
7	Proteinase k kit	Intron Biotechnology	Korea
8	Standard Aflatoxin B1	Sigma	USA
9	Sugar	Randox	UK
10	Thyroid Stimulating Hormone (TSH)	Mybiosource	USA
11	Thyroxine (T4)	Mybiosource	USA
12	Triiodothyronine (T3)	Mybiosource	USA
13	Urea	Randox	UK

3.1.3. The Tools

The tools used in this study were present in table (3.4)

Table (3.4) the tools used in this study

NO	Tools	Company	Origin
1	Beaker	Volac,	England
2	Capillary tube	Vitrex	China
3	EDTA tube	Plastilab	China
4	Eppendrof tube 1.5 ml	China Mheco	China
5	Gell tube	Plastilab	China
6	Graduated cylinder	Volac	England
7	Jar for candle	Mheco	Chinese
8	Micro-Hematocrit	Vitrex	Chinese
9	Micropipette (10_100 µL)	Human Humapette	Germany
10	Micropipette (100_1000µL)	Human Humapette	Germany
11	Multi-channel-pipette	Capp	Denmark
12	Screwed glass test tube	Mheco	China
13	Stand	Mheco	China
14	Test tube	Mheco	China
15	Test tube rack	Mheco	China
16	Volumetric flask	Volac,	England

3.2. Study Design

A case-control study conducted from November 2022 to January 2023. Samples were selected from the patients attending Al-Kindy Hospital and Endocrines and Diabetes Central/Baghdad. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire), including age, gender, BMI, family history, blood pressure, liver and kidney disease, thyroid gland disease, dietary habits, and duration of diabetes. They were also exposed to a medical examination for signs and symptoms of diabetes type 2 by a doctor based on the World Health Organization (WHO) criteria.

For relationship purposes, patients with T2DM were divided into males and females with AFB_1 and males and females without AFB_1. Patient groups were compared with control males and females with AFB_1 and control males and females without AFB_1.

3.2.1. Study Groups

A total of 177 subjects were studied, 93 (44 males and 49 females) of whom were T2DM and 84 (37 males and 47 females) of whom were controls. These totals are divided according to table (3.1).

The ethical study protocol was approved by the Research Committee of the College of Applied Medical Sciences, Kerbala University, and the Rusafa Health Department in Baghdad.

Table (3.1): Division of study groups.

N.O	Study Groups	Characteristics Groups
1	M, D-2, TX	Male, type 2 diabetes with AFB1
2	M, D-2, NTX	Male, type 2 diabetes Non- AFB1 toxin
3	M, C, TX	Male, control with AFB1 toxin
4	M, C, NTX	Male, control Non- AFB1 toxin
5	F, D-2, TX	Female, type 2 diabetes with AFB1 toxin
6	F, D-2, NTX	Female, type 2 diabetes Non- AFB1 toxin
7	F, C, TX	Female, control with AFB1 toxin
8	F, C, NTX	Female, control Non- AFB1 toxin

3.2.2. Exclusion Criteria

In general, patients who reported kidney disease, liver disease, thyroid disease, type 1 diabetes, heart disease, and high blood pressure. The study excluded participants who were using medications at the time.

3.2.3. Control Criteria

The control groups consisted of 84 healthy subjects (37 males and 47 females). They were selected from among known volunteer participants. Blood samples were taken from volunteers; participants do not have chronic diseases. The percentage of adult female and male subjects was approximately the same in patients' frames, and the ages of the participants were also convergent in the whole study group. Demographic information about the participants was also collected through the self-reported technique (student question).

3.2.4. Patient Criteria

Patients were selected from Al-Kindy Teaching Hospital and Endocrines and Diabetes Central in Baghdad, Iraq. This study consisted of 93 patients with newly diagnosed type 2 diabetes. It was weight and height that were registered. Important sociodemographic variables, general health status, family history of chronic diseases, dietary practices, and the duration of diabetes were all assessed using a questionnaire.

3.3. Method

3.3.1. Blood Collection and Storage

10 ml of blood were drawn from each participant's vein using a sterile syringe, and they were all brought to the main lab in gell tubes. Serum was separated from samples by centrifuging them for 15 minutes at 3000 rpm after they had been settled for 15 minutes. Before analysis, each serum sample was divided into two parts and placed in a refrigerator set to -20 C. After that, the serum was micropipette-transferred into an Eppendrof 1.5 ml for storage. The following parameters were then assessed using these serum samples:

1- Qualitative and quantitative detection of serum Aflatoxin B_1 by TLC and HPLC techniques

2- Measurement of fasting blood sugar (FBS); liver function test (AST, ALT);

Renal Function Test (Urea, Creatinine) by using an automation device in the hospital.

3- Triiodothyronine (T3), thyroxin (T4), and thyroid stimulating hormone (TSH) tests were performed using ELISA techniques.

4- The blood sample in the EDTA tube used for measurement

- Measurement of HBA1C by fine-care
- Measurement of WBCs, RBCs and HB by complete blood count (CBC test).

3.3.2. Determination of HBA1C

Principle:

Based on fluorescence immunoassay technology, the Fine care HBA1C test is performed. The kit employs a sandwich immune detection method; when sample is added into the sample well of the test cartridge, detector HBA1C antibodies and HB antibodies on the sample pad bind to HBA1C antigens and HB antigens in blood specimens, respectively, and form immune complexes. As the complexes migrate on the nitrocellulose matrix of the test strip by capillary action, the complexes of the detector antibodies and HBA1C are captured to HBA1C antibodies that have been immobilized on the test strip as well as the complexes of the detector antibodies and HB, which are captured to HB antibodies that have been immobilized on the test strip. As a results, the more HBA1C and HB antigens are present in the blood sample, the more complexes accumulate on the test strip. The detector antibody fluorescence signal strength reflects the amount of HBA1C and HB collected, and fine care FIA shows the HBA1C ratio in the blood sample.

Procedure:

Preparation

Before testing, activate "use" in the setting, then saved. The lot number of the test cartridge matches the ID chip as well as the detection buffer. The ID chip was inserted into the fine care ™ FIA System.

Mixing: The lid of Detection Buffer was closed tube and the sample mixture was mixed thoroughly by shaking it for 1 minute.

Loading: A75 μL of sample mixture was pipetted out and loaded into the sample well of the Test Cartridge.

Testing: There are two test modes for Fine care ™ FIA System

a- **Standard Test mode:** The Test Cartridge was inserted onto the Test Cartridge holder of Fine-care FIA System right after adding sample mixture to the sample well. "**Test**" was pressed to start testing

b- **Quick Test mode:** The timer was set and count down right after adding sample mixture into the sample well and leaved at room temperature for 5 minutes. Then the Test Cartridge was inserted onto the Test Cartridge holder of Fine-care ™ FIA System. "Test" was pressed to start testing. Fine-care ™ FIA system would start scanning the sample-load Test Cartridge immediately.

Results was displayed on main screen or be printed by press "**Print"**

Normal Values: in blood; (Kang *et al*., 2015):

- HBA1C $< 5.7\%$ Normal
- HBA1C 5.7 -6.4% Pre-diabetic
- HBA1C $\geq 6.5\ \%$ Diabetic

3.3.3. Measurement of WBCs, RBCs, and HB by Complete Blood Count (CBC test)

The number of cells (RBC, WBC, and HB) is calculated automatically after the blood sample is drawn from the EDTA tube. The results are shown with units of measurement one minute after the blood sample is drawn.

3.3.4.T3 Enzyme Linked Immunosorbent Assay (ELISA)

Principle of the Assay

T_3 ELISA kit uses a monoclonal anti-T_3 antibody and a T_3-HRP conjugate to use the competitive enzyme immunoassay technique. T_3-HRP conjugate is incubated with the assay sample and buffer in a pre-coated plate for one hour. The wells are decanted and washed five times when the incubation period is over. The HRP enzyme substrate is then allowed to sit in the wells for a while. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. Due to competition between T3 from samples and T3-HRP conjugate for the anti-T3 antibody binding site, the intensity of the color is inversely proportional to the concentration of T3. Since there are only so many sites available, as more sites in the sample are taken up by T3, fewer sites are left that can bind the T3-HRP conjugate. A standard curve is drawn connecting the color's intensity (OD) and standard concentration. This standard curve is interpolated to determine the T3 concentration in each sample.

Materials

All reagents provided are stored at 2-8 C. Refer to the expiration date on the table (3.5).

Table (3.5): Reagents of T3

NO	Materials	Specification	Quantity
1	Microtiter plate	96 wells	Strip well
2	Enzyme conjugate	6.0ml	1 vial
3	Standard a	0ng/ml	1 vial
4	Standard b	1.0ng/ml	1 vial
5	Standard c	2.5ng/ml	1 vial
6	Standard d	5.0ng/ml	1 vial
7	Standard e	10ng/ml	1 vial
8	Standard f	25ng/ml	1 vial
9	Substrate a	6ml	1 vial
10	Substrate b	6ml	1 vial
11	Stop solution	6ml	1 vial
12	Wash solution(100x)	10ml	1 vial
13	Balance solution	6ml	1 vial
14	Instruction	1	

Sample and Reagents preparation

1) Serum: A serum separator tube was used, and samples were allowed to clot for 2 hours at room temperature or overnight at 2–8 °C. Centrifuge at approximately 1,000 x g (or 3,000 rpm) for 15 minutes. Serum and assay were removed immediately orally, and samples were stored at -20 °C or -80 °C.

2) All kit components and samples were brought to room temperature (20–25 °C) prior to use.

3) Washing solution: 1000 ml of washing solution (1x) was created by diluting 10 ml of concentrated washing solution (100x) with 990 ml of deionized water or

distilled water. If the concentrate started to crystallize, it was gently blended while being heated to room temperature. The crystals have entirely disintegrated. At 2-8 °C, the 1x washing solution is stable for 2 weeks.

Assay procedure

1) Desired numbers of plated wells were secured in the holder, and then 100 µL of standards or samples were added to the appropriate well of a microtiter plate pre-coated with antibodies. 100 µl of pbs (PH 7.0–7.2) was added into an empty control well.

2) Each well (not the blank control well) contains 50 ul of conjugate. blended successfully. It's crucial to mix thoroughly in this phase cover the dish and leave it to sit at 37 °C for an hour.

3) The microtiter plate was washed using the specific method described below:

Manual Washing: By emptying the contents of the dish into a trough or other suitable garbage container, the incubation mixture was removed. After thoroughly filling each well with 1x washing solution, the plate's contents were sucked into a trough or other suitable waste container. This process is repeated five times, totaling five washes. After cleaning, the board was flipped over and dried completely using absorbent paper or paper towels.

4) 50 µL of substrate A and 50 µL of substrate B were added to each well, including the empty control well, subsequently covered and incubated for 10–15 minutes at 20–25 °C. (avoid sunlight).

5) Added 50 ul of stop solution to each well, including the blank control well mixed well.

6) The optical density (O.D) was determined at 450 nm using a microplate reader on the spot.

3.3.5.T_4 Enzyme Linked Immunosorbent Assay (ELISA)

Detection principle of Human Thyroxine (T4) ELISA kit

This experiment use double-sandwich ELISA technique and the ELISA Kit provided is typical. Human T4 monoclonal antibody serves as the pre-coated antibody, while polyclonal antibody with biotin label serves as the detecting antibody. ELISA plate wells are filled with samples and biotin-labeling antibodies before being cleaned with PBS or TBS. Following an orderly addition of avidin-peroxidase conjugates, the TMB substrate is used to color the ELISA wells after the reactant has been fully removed with TBS or PBS. TMB is catalyzed by peroxidase to turn blue, and then under the influence of acid, it turns yellow. Sample testing variables and color depth have a positive correlation.

Kit composition:

Table (3.6): T4 enzyme Kit composition

Name	96 Tests
antibody pre-coated plate	8x12
Human T4 Standards	2 vial
Biotinylated antibody (1:100)	1 vial
Enzyme conjugate (1:100)	1vial
Enzyme diluents	1vial
antibody diluents	1vial
Standard diluents	1vial
Sample diluents	1vial
Washing buffer (1:25)	1 vial
Color Reagent A	1 vial
Color Reagent B	1 vial
Color Reagent C	1 vial

Test preparation

1. The Elisa Kit is removed from the refrigerator 20 minutes prior to the test's start, and the test is conducted once it has reached room temperature.

2. Diluted the concentrated washing solution with double distilled water (1:25).

3. Human T4 Standard Specimen: A 1.0 mL standard diluent was added to a lyophilized human T4 standard specimen and kepted 30 min after the sample.

Completely dissolved, mixed gently, labeled on the tube, and diluted as needed (following concentration values recommended for the standard curve: 1000, 500, 250, 125, 62.5, 31.2, and 15.6 nmol/L). The freeze-dried standard is completely thawed and mixed well.

4. Legend of standard sample dilution method: Seven clean tubes were taken and labeled with ②③④⑤⑥⑦⑧respectively. Added 300ul standard sample diluent into each tube. Pipette out 300µl diluent from tube ①to tube ②and mix well. Further Pipette out 300ul diluent from tube ②to tube ③ and mix well. Repeat steps above up to tube ⑦ Standard sample dilution in tube ⑧is negative control.

5.Biotinylated human T4 antibody liquid: Referring to needed amount, employ antibody diluent to dilute the concentrated biotinylated antibody (1:100) to form biotinylated antibody liquid. The preparation should be done 30 min in advance. And it's only for use on that day

6. Enzyme Conjugate Liquid: With reference to the required amount, the concentrated enzyme conjugate was diluted with enzyme conjugate diluent (1:100) to form enzyme conjugate liquid. Preparated 30 minutes in advance. It is only for use on that day.

7. Reagent liquid: Reagent liquid was prepared 30 minutes in advance with Reagent A and Reagent B in a ratio of 9:1.

Manual Washing: By aspirating the dish's contents into a basin or other suitable waste container, the incubation mixture was taken out. After thoroughly filling each well with 1x washing solution, the plate's contents were sucked into a trough or other suitable waste container. This process is repeated five times, totaling five washes. After cleaning, the board was flipped over and dried completely using absorbent paper or paper towels.

Procedure

1. The required strips were removed from a zip-top bag and allowed to acclimate. Used dried strips and material were put back into an aluminum foil-sealed bag with a storage temperature range of 2 to 8°C.

2. Empty wells set aside (if a dual-wavelength readout plate is used, empty wells can be discarded).

3. The corresponding wells (100 µl per well) were filled with a standard diluent and various samples or concentrations of standard human T_4 samples. Using adhesive tape to seal them, reaction wells are placed in an incubator set at 37 °C for 90 minutes.

4. Biotinylated human T4 antibody fluid was prepared 30 min in advance.

5. Elisa's dish was washed three times.

6. A biotinylated human T4 antibody fluid was added to each well (100 µU each). Wells were sealed with adhesive tape and incubated in the incubator at 37°C for 60 minutes.

7. The enzyme conjugate liquid was prepared 30 min in advance.

8. Elisa's dish was washed three times.

9. Liquid enzyme conjugate was added to each well except for the empty wells (100 µL each). Reaction wells were sealed with adhesive tape and incubated in the incubator at 37 °C for 30 minutes.

10. Elisa's dish was washed three times.

11. 100 µl of liquid reagent was added to the individual well (also in an empty well) and incubated in a dark incubator at 37°C. When the color of the high concentration of

the standard curve becomes darker and the color gradient appears, the incubation can be stopped, and the color reaction has been controlled within 30 minutes.

12. 100 μL of reagent C was added to the single well (also in the empty well) and mix well. The OD (450 nm) was read within 10 minutes.

3.3.6.TSH Enzyme Linked Immunosorbent Assay (ELISA)

Principle of the Assay

The quantitative sandwich immunoassay method is used in this ultra-sensitive TSH enzyme linked immunosorbent test (ELISA). A monoclonal antibody specific for TSH has been pre-coated on the microtiter plate included in this kit. The microtiter plate wells are then filled with standards or samples, and the antibody that has previously been coated on the wells will bind to any TSH that is present. To "sandwich" the TSH immobilized on the plate and quantify the amount of TSH in the sample, horseradish peroxidase (HRP)-conjugated monoclonal antibodies specific for TSH are added to each well. The microtiter plate is incubated, and the wells are meticulously cleaned to remove any unattached components. Then, each well is filled with the TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution. Just enough time is allowed for the enzyme (HRP) and substrate to react before the process may continue. Only the TSH- and enzyme-conjugated antibody-containing wells will change color.

The enzyme-substrate reaction is stopped using a sulphuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm.

Six calibration standards are included in this ultra-sensitive human TSH ELISA kit so that the levels of TSH in the sample may be determined. By measuring the calibration standards simultaneously with the samples, the operator can produce a standard curve for the relationship between optical density (O.D.) and TSH

concentration (lU/mL). The O.D. of the samples is then compared to the standard curve to determine the concentration of TSH in the samples.

The Kit Components

Table (3.7): TSH enzyme Kit composition

Microtiter plate	Pre-coated with anti-human TSH monoclonal antibody.
Conjugate	Anti-human TSH monoclonal antibody in a ready-to-use 12 ml volume conjugated to HRP.
Standard(St)-12 µlu/ml	1 vial of buffered protein base with preservative and lyophilized human TSH, which will reconstitute to contain 12 ul/ml.
St-6µlu/ml	One vial of human TSH that has been lyophilized and is stored in a buffered protein base with a preservative.
St-2 µlu/ml	1 viallyophilized human TSH in a buffered protein base including a preservative that, following reconstitution, will contain 2 lu/ml.
St-0.8 µlu/ml	One vial of lyophilized human TSH in buffered protein basis with preservative, which will reconstitute to contain 0.8 ul/ml.
St-0.2 µlu/ml	One vial of lyophilized human TSH in buffered protein basis with preservative, which will reconstitute to contain 0.2 ul/ml.
St-0 µlu/ml	1 vial of buffered protein base with preservative that has been lyophilized; reconstitution will results in a 0 ul/ml concentration.
substrate A	Buffered solution with $H2O2$ in 10 ml.
substrate B	solution with TMB in 10 ml of buffer.
stop solution	Sulfuric acid ($H2SO4$), 14ml. warning: corrosive substance

Kit and Sample preparation

The entire set of reagents was removed from the freezer and warmed to room temperature (20–25 °C). According to the following instructions, the following reagents were generated: Swirled slightly to mix properly before pipetting. TSH Standards: 1.0 mL of deionized or distilled water was used to reconstitute each TSH standard vial. With occasional moderate stirring, each solution was allowed to settle for at least 15 minutes. For three months, standard TSH stock solutions remain stable at 4 °C. Cycles of freeze-thaw were avoided.

Sample Preparation

Serum: Blood was drawn using standard venipuncture techniques, and serum was separated from blood cells as quickly as possible. Samples were left to clot for 1 hour at room temperature, centrifuged for 10 minutes at 4 °C, and serum extracted. Assay procedure.

1. All TSH parameters were prepared before the start of the screening procedure.

2. 100 ul of standards or samples were added to the appropriate well on a microtiter plate that had already been coated with antibodies after the required number of plated wells had been secured in the holder.

3. To each well, 100 mL of conjugate were added. Complete mixing is crucial in this step. covered and heated to 37 °C for three hours.

4. The microtiter plate was washed using the specific method described below:

Manual Washing: The incubation mixture was obtained by aspirating the contents of the plate into a basin or other suitable waste container. Each well was filled with distilled or de-ionized water using a spray bottle and then the contents of the plate were sucked into a trough or other suitable garbage container. This was repeated four

times, for a total of five washes. After a final wash, the dish was turned over and dried completely with absorbent paper or paper towels.

5. To each well, 100 mL of the substrate solution were added. Cover and incubate for 15 minutes at 37 °C

6. To each well, 100 mL of Stop Solution were added.

7. Within 30 min, the optical density (OD) was determined at 450 nm with a microtiter plate reader.

3.3.7. Routine Biochemical tests

Chemical analyzes such as FBS, AST, ALT, B. urea, and S.Creatinine were measured by the Becman-Coulter device, a fully automated device where a tube containing serum was placed in the place designated for it and then pressed on the Start button. It took 10 minutes to obtain the results.

3.4.1. Qualitative analysis of serum AFB_1 by TLC:

According (Kareem *et al*., 2021) The steps taken to conduct this study are as follows. Prior to beginning work, all glasses were sterilized in an autoclave equipment for 15 minutes at 120°C every day. Isopropyl alcohol 70 percent was also used to disinfect all work surfaces.

3.4.1.1Standard AFB_1 preparation for TLC

By dissolving 1 mg of AFB_1 in 5 mL of chloroform a stock solution of the AFB_1 standard (200 µg/mL) was prepared.

3.4.1.2Proteinase k solution

Proteinase k solution was created by mixing 22 mg of proteinase K powder with 1.1 ml of pure, sterile, distilled water.

3.4.1.3 AFB_1 Extraction from Serum Samples:

Serum samples were placed 700 ul in sterile test tubes, and each one received 20 ul of proteinase K solution. The test tubes were then allowed to respond for 10 minutes.

The mixture was then centrifuged for 15 minutes at 3000 x g, after which the filtrate was removed from each sample and the precipitate was left behind.

Chloroform (1 ml) was added to each filtrate (twice its size) and shaken violently in the electric shaker device, where it separated into two layers (chloroform layer and serum layer). The chloroform layer was then separated by a separating funnel and placed in a sterile other glass tube and allowed to evaporate.

3.4.1.4 Detection of AFB_1 by Thin Layer Chromatography (TLC):

Silica gel-coated TLC plates with a dimension of 20 cm x 20 cm were utilized after being activated in an electric oven at 120 °C for an hour prior to use. At a distance of 1.5 cm from the bottom and top of the plate's base, a thin straight line was drawn. The top line was used for numbering and the bottom line for loading samples. Chloroform: Methanol (98:2) served as the mobile phase for the separation of AFB_1.

AFB_1 stock standard (15 ul) was put as a spot on the TLC plate using a capillary tube, followed by the addition of 15 ul from each extracted sample on the plate, spaced 2 cm apart. The spots were then allowed to dry at room temperature. The plate was then placed in the tank for separation, which contained a mobile phase. Till the mobile phase was 2 cm from the upper plate edge, the thin layer plate was left in the tank. TLC plate was taken out of the tank and allowed to dry in the ambient air. Then, the plate was inspected using UV light (360 nm) to compare the extracted samples' color and relative flow (RF) to a reference toxin.

3.4.2. Quantitative Analysis of AFB_1 by HPLC

According (Liu *et al*., 2012) HPLC model SYKAM (Germany), It was utilized for thiamethoxam analysis and detection.

3.4.2.1 preparation of standard AFB_1 for HPLC

AFB_1 standard was prepared by dissolving 0.025 mg of AFB_1 in 100 ml of acetonitrile solution leading to a final concentration of 0.25 ng/ml.

3.4.2.2 Sample Preparation:

After gently mixing 500 uL of serum sample with 1 ml of hexane, the mixture was centrifuged at 2000 g for five minutes. After centrifugation, the upper hexane layer containing the serum lipids was taken out, and 1 ml of hexane was then injected once again to the serum sample. These serum samples were mixed vigorously for 4 minutes with 1 ml of chloroform before being centrifuged at 2000 g for 10 minutes. A 50 ul solution of methanol, water, and acetonitrile (25:25:50) was used to dissolve the lower layer of chloroform after it had been scraped off and dried using nitrogen steam.

The mobile phase was an isocratic acetonitrile**, D.W** (30 : 70 v/v) flowing at a rate of 0.7 mL/min

The detector was Florescent (Ex = 365 nm, Em = 445 nm), **and the column** was C18-ODS (25 cm * 4.6 mm).

Injection volume :50ul

3.4.2.3 Calculation

$$\textbf{Con. Sample (ng/ml)} = \frac{\text{Con. Standard * absorption sample}}{\text{absorption. Standard}} * \frac{\text{Dilution factor}}{\text{Volume sample}}$$

3.5. Statistical Analysis

Information from the questionnaire and every patient and control test results. A data sheet was used to record the samples. The IBM SPSS 26 statistical program was used to create the data analysis for this study. On the participant data for each group, descriptive statistics were run.

The ANOVA table and Duncan test were used to make multiple comparisons between the groups. A P-value of < 0.05 indicates that there is a statistically significant difference between the groups.

The chi-square test was used to compare the observed results with the expected results to reveal the relationship between the variables.

The correlation coefficient (r) was used to show the correlation of the relationship and determine the continuing trend of this relationship. It is expressed as a positive or negative number between -1 and 1. The value of the number indicates the strength of the relationship, and $r = 0$ means no correlation.

3.6. Ethical Considerations

Both the Baghdad Rusafa Health Department and the Ethical Committee of the College of Applied Medical Sciences at the University of Karbala gave their approval to the study protocol. The patients' permission was required in order to collect samples.

Chapter Four

Results and Discussion

Results and Discussion

4.1 Measurement Qualitative and Quantitative of the AFB_1

Analysis of AFB_1 was performed qualitatively by TLC and quantitatively by HPLC (All results of the analysis by TLC and HPLC were documented in the appendix).

4.1.1. Measurement Qualitative of AFB_1 by TLC

The results showed that the number of sample serums collected from patients where contamination with AFB_1 occurred was 46 (49.5%), and the number of sample serums collected from controls where contamination with AFB_1 occurred was 42 (50.0%), while the number of sample serums collected from patients without AFB_1 was 47(50.5%), and the number of sample serums collected from controls without AFB_1 was 42(50.0%). The results illustrated in table (4-1).

Table 4-1: Distribution of AFB1 according to patient and control groups by using TLC

Case		Number of samples with AFB1	Number of samples without AFB1	Total
Patient	F	46	47	93
	%	49.5%	50.5%	100.0%
Control	F	42	42	84
	%	50.0%	50.0%	100.0%
Total	F	88	89	177
	%	49.7%	50.3%	100.0%
*Chi-Square Tests; F= Frequency; AFB1= AflatoxinB1 X^2 Calculate = 41.76, X^2 table (0.05) = .005				

These results were approached with Abd AL-Redha *et al*., 2017 who found a relationship between toxins and patients as well as controls. The reason is due to the effect of AFB_1 on patients, which causes damage or may exacerbate the disease. Kadhum *et al*., 2022 reported that T2DM increased with increasing AFB_1 concentrations. In the control groups, the results indicate the presence of AFB_1 in them, suggesting that the presence of the toxin in healthy people may lead to diabetes mellitus or the development of hepatitis and kidney disease.

The distribution of AFB_1 according to sex showed that the number of females whose serum blood was contaminated with AFB_1 was 51 (58%). while the number of males whose serum blood was contaminated with AFB_1 was 37 (42%), Also, the number of females whose serum blood was without AFB_1 was 45 (50.6%). while the number of males whose serum blood was without AFB_1 was 44 (49%). The results illustrated in table (4-2).

Table 4-2: Distribution of AFB_1 according to Sex.

Sex		Number of samples with AFB_1	Number of samples without AFB_1	Total
Femal	F	51	45	96
	%	58.0	50.6	54.2
Male	F	37	44	81
	%	42.0	49.4	45.8
Tot:	F	88	89	177
	%	100	100	100

*Chi-Square Tests; F= Frequency; X^2 Calculate = 40.27, X^2 table (0.05) = .974

The results indicate both males and females were affected by AFB_1, but the results showed that females are more exposed than males, and this is agreement with many of the study including (Abdullah and Aljumaili, 2018);(Kadhum *et al*., 2022). Perhaps the reason is due to the activity of the enzyme glutathione S-transferases, whose activity in males differs from that of females.

Cytochromes P-450 3A4 and 1A2 are major liver enzymes that are responsible for converting AFB_1 to AFBO. Glutathione S-transferase (GST), the detoxifying enzyme that catalyzes AFBO conjugation with glutathione, provides protection against the liver-damaging effects, A main AFBO detoxification pathway is via its enzymatic conjugation with glutathione S-transferase (Awuchi *et al*., 2021).

Singhal *et al*., 1992 reported that GST activity in males was higher than in females in a study that evaluated GST activity in the human colon.

4.1.2. Measurement Quantitative of the AFB_1 by HPLC

AFB_1 was quantitatively measured by using an HPLC device to measure toxin concentrations in the study groups. The results showed that there was a highly significant difference between the study groups, and the *P*-value was <0.001. The highest concentration of toxin was in male patients (3.985 ng/ml) and female patients (4.016 ng/ml). Also, the highest concentration of toxin in the control (0.14 ng/ml) for both males and females. These concentrations are considered high compared to healthy people. The AFB_1 concentration range in diabetic patients was (3.985- 4.016 ng/dl) while the AFB_1 concentration range in the control group was (0.13- 0.14 ng/dl), as shown table (4-3).

Table 4-3: Measurement concentration AFB1 by HPLC in patients and control groups

Groups	Mean ng\ml	SD	Duncan test	*P*-value
M,D-2,TX	3.985	0.445	a	0.001*
F,D-2,TX	4.016	0.401	a	
M,D-2,NTX	0.005	0.021	b	
F,D-2,NTX	0.004	0.015	b	
M,C,TX	0.140	0.006	b	
F,C,TX	0.140	0.007	b	
M,C,NTX	0.010	0.011	b	
F,C,NTX	0.002	0.001	b	
*= significant p< 0.001; The difference between the letters indicates that there is a significant difference between the study groups.				

The results of the study indicate an increase in the concentration of toxin in groups with T2DM in both females and males, compared to groups that carry toxin in the non-diabetic control, and this indicates that T2DM increases the concentration of AFB_1, which is agreed with Kadhum *et al.*, 2022 who found the concentration of AFB_1 in the blood serum of patients with T2DM was 1.34 ng/ml and the concentration of AFB_1 in healthy blood serum was 0.13 ng/ml. Also, another study, our findings were in line with those of Kareem *et al.*, 2021 who discovered that the concentration ranges of AFB_1 in blood samples were 0.68–8.33 ng/mL for unsure CKD patients, 1.21–5.6 ng/mL for certain CKD patients, and 0.11–1.30 ng/mL for healthy controls.

The reason for this is due to continuous exposure to contaminated foods, undercooked meat, vegetables and many contaminated foods. Hassan *et al.,* Record high contamination of rice in Lebanon with AFB_1. Exposure to AFB1 from rice consumption in Lebanon was calculated as 0.1 to 2 ng/kg of body weight per day (Hassan *et al*., 2022).

4.2. Measurement of Biochemical Parameters of this Study

4.2.1. Measurement of AST and ALT enzymes in study groups

AFB1 positivity was distributed in the AST enzyme measurement among four groups. It included (M, D-2, TX), (F, D-2, TX), and (M, C ,TX), (F, C, TX), whose means were (41.69, 41.76, and 36.12, 36.24 mg/dl), respectively, compared with other groups, which included (M, D-2, NTX), (M,C,NTX), and (F,C, NTX), (F, D-2, NTX) whose means were (23.52, 15.62, and 18.12, 22.34 mg/dl), respectively, with a significant different between them, as shown in table (4-4).

Table 4-4: Distribution of AST levels in patient and control groups.

Groups	Mean mg/dl	SD	Duncan test	*P*-value
M,D-2,TX	41.69	3.41	a	0.001*
F,D-2,TX	41.76	6.58	a	
M,D-2,NTX	23.52	5.27	c	
F,D-2,NTX	22.34	5.72	c	
M,C,TX	36.12	7.54	b	
F,C,TX	36.24	3.63	b	
M,C,NTX	15.62	5.10	d	
F,C,NTX	18.12	4.81	d	
The difference between the letters indicates that there is a significant difference between the study groups.				

AFB_1 positive in ALT was distributed into four groups (M, D-2,TX), (F, D-2, TX), (M, C, TX), and (F, C, TX), whose means were (41.92, 40.20, 39.39, and 35.90 mg/dl), respectively, compared with the other groups which included (M, D-2, NTX), (M,C,NTX), and (F,C, NTX), (F, D-2, NTX) whose means were (25.23, 20.02, 15.45, and 16.66 mg/dl), respectively. Also, there was a significant different between them, with $P < 0.001$, as shown in table (4-5).

Table 4-5: Distribution of ALT levels in patient and control groups

Groups	Mean mg/dl	SD	Duncan test	*P*-value
M,D-2,TX	41.92	6.54	a	.001*
F,D-2,TX	40.20	4.31	ab	
M,D-2,NTX	25.23	5.29	c	
F,D-2,NTX	20.02	3.79	d	
M,C,TX	39.39	16.18	ab	
F,C,TX	35.90	6.73	b	
M,C,NTX	15.45	5.23	e	
F,C,NTX	16.66	5.00	de	
The difference between the letters indicates that there is a significant difference between the study groups.				

The results was in agreement with the many studies included (Karamkhani *et al*., 2020), (Navale *et al*., 2021) (Li *et al*., 2022a), and, which showed during their studies an increase in the levels of ALT and AST in groups that contain AFB1 compared to the control groups.

AST and ALT rise after ingestion of AFB_1; The gastrointestinal tract rapidly absorbs it, and the circulatory system transports it to the liver (Lalah *et al*., 2019).

AFB_1-lysine in albumin is an example of an adduct formed when 1-3% of the ingested AFs attach to proteins and DNA bases in an irreversible manner. Hepatocytes' functions are disrupted and liver toxicity is caused when protein and DNA bases are damaged. Chronic exposure is the outcome of ingesting little amounts of AFs repeatedly over an extended period of time (Kowalska *et al*., 2017a).

As for the effect of T2DM on the liver enzymes AST and ALT, there are many studies that are in line with our study, as a recent study in Rafsanjan showed an elevation of liver enzymes in patients with T2DM (Noroozi Karimabad *et al*., 2022). Similarly, with the study Mandal *et al*., 2018, which indicated elevated liver enzymes in patients with type 2 diabetes.

The direct liver damage caused by fatty acids when they are generated in excess is thought to be the cause of the elevated levels of these enzymes in patients with T2DM. Mechanisms High-concentration cell membrane rupture, mitochondrial malfunction, the production of toxins, and the activation and inhibition of crucial metabolic control processes are just a few examples of what this might entail. Other factors that could account for higher levels in conditions of insulin resistance are transaminases, which are produced as a results of reactive lipid peroxidation, peroxisomal beta-oxidation, and the recruitment of inflammatory cells. Administering insulin Pro-inflammatory cytokines, such as tumor necrosis factor, are more common in the resistant state and may worsen hepatocellular damage (Mathur *et al*., 2016).

4.2.2. Effect of AFB1 and T2DM on Kidney Function Parameters

Kidney function, such as blood urea and serum creatinine (S.Cr), are biochemical markers that reflect disease severity in a number of kidney diseases.

AFB_1 positive in B.Urea was distributed into four groups (M,D-2,TX), (F,D-2,TX), (M,C,TX), and (F,C,TX), whose means (42.58, 41.32, 32.85, and 34.56 mg/dl), respectively, compared with other groups including (M,D-2, NTX), (F, D-2,NTX), (M,C,NTX), and (F, C,NTX), whose means (32.35, 30.64, 22.32, and 23.61 mg/dl), respectively, with significant different between them. As shown in table (4-6)

Table 4-6: Distribution of Blood Urea levels in patient and control groups

Groups	Mean mg/dl	SD	Duncan test	*P*-value
M,D-2,TX	42.58	4.64	a	.001*
F,D-2,TX	41.32	5.87	a	
M,D-2,NTX	32.35	7.83	b	
F,D-2,NTX	30.64	6.47	b	
M,C,TX	32.85	4.56	b	
F,C,TX	34.56	5.60	b	
M,C,NTX	22.32	6.29	c	
F,C,NTX	23.61	6.21	c	
The difference between the letters indicates that there is a significant difference between the study groups.				

While the distribution of AFB_1 in S.Cr levels was similar to that in B.Urea, as shown in table (4-7) when compared between study groups, there was a significantly increased between them.

Table 4-7: Distribution serum creatinine levels in patient and control groups

Groups	Mean mg/dl	SD	Duncan test	*P*-value
M,D-2,TX	1.03	0.17	a	.001*
F,D-2,TX	1.00	0.16	a	
M,D-2,NTX	0.81	0.13	b	
F,D-2,NTX	0.75	0.16	b	
M,C,TX	0.83	0.14	b	
F,C,TX	0.82	0.16	b	
M,C,NTX	0.60	0.19	c	
F,C,NTX	0.59	0.12	c	
The difference between the letters indicates that there is a significant difference between the study groups.				

The results are in agreement with the many studies (Karamkhani *et al*., 2020), (Kareem *et al*., 2021), (Navale *et al*., 2021), and (Li *et al*., 2022a) showed during their studies an increase in the levels of B.Urea and S.Cr compared to the control group.

Numerous investigations have found that AFB_1 harms renal tissue visibly and occasionally causes severe inflammatory cell infiltration and bleeding. The long-term administration of AFs has been proven to results in kidney damage and may involve toxicosis, cell necrosis, and inflammation, according to other investigations. These findings confirmed that the kidney was one of the primary target organs of AFs and showed that higher concentrations of creatinine and urea

were present. They also suggested that a number of metabolites might be transferred, produced, or degraded in the kidney, including proline, which was confirmed to be a unique metabolite in the kidney (Kareem *et al*., 2021), (Li *et al*., 2018a), (Matejova *et al*., 2017).

This study is agreement with Mathur *et al*., 2016 conducted in Iraq and showed an increase in urea and creatinine in the group with T2DM compared to the healthy group. The same results were reported in another study (Amartey *et al*., 2015).

Urea is the major nitrogenous end product of metabolic breakdown of protein in humans. It is dissolved in the blood and transported and excreted by the kidney as a component of urine. Creatinine is the breakdown product of creatine phosphate released from skeletal muscle at a steady state. It is filtered by the glomerular and a small amount is also secreted into the glomerular filtrate by the proximal tubules. Serum creatinine and blood urea nitrogen concentrations are the best guidelines for estimating progression, prognosis, instituting dietary restrictions in the renal disease in T2DM (Kene *et al*., 2021).

Hyperglycemia may directly cause mesangial expansion and injury by increasing the mesangial cell glucose concentration. Initially, glomerular mesangium expands by cell proliferation and later by cell hypertrophy. Transforming growth factor β (TGF-β) is important in the mediation of expansion and later fibrosis by the stimulation of collagen and fibronectin. Glucose can bind reversibly and finally irreversibly to proteins in the kidneys and circulation form advanced glycation end-products (AGEs). Due to long standing hyperglycemia, AGEs can form complex cross-links over years and contribute to renal damage. Furthermore, TGF-β, platelet-derived growth factor and vascular endothelial growth factor are elevated in diabetics nephropathy, thereby acting as mediators of proliferation and expansion, contributing to further renal and microvascular

complications (Thomas and Ford Versypt, 2022). Type 2 diabetic patients will accumulate waste products and thereby increase serum creatinine and urea levels. Impaired function of the nephron in diabetic Patients have a high serum creatinine level (Thomas and Ford Versypt, 2022).

4.2.3. Effect of AFB_1 and T2DM on thyroid hormone levels

The results showed significant a decrease in T3 levels serum groups, (M, D-2, TX) and (F,D-2,TX), whose means were (7.37±1.89 and 6.74± 1.67 ng/dl), respectively, compared with (M, D-2, NTX) and (F, D-2, NTX), whose means are (7.64± 2.1 and 7.63±2.3 ng/dl), respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), whose means are (12.7± 1.1 ng/dl) and (12.4± 1.2 ng/dl), compare with (M, C, NTX), and (F,C, NTX), whose means are (14.11 ± 0.42 and 13.82 ± 0.39 ng/dl), as shown in figure (4-1)

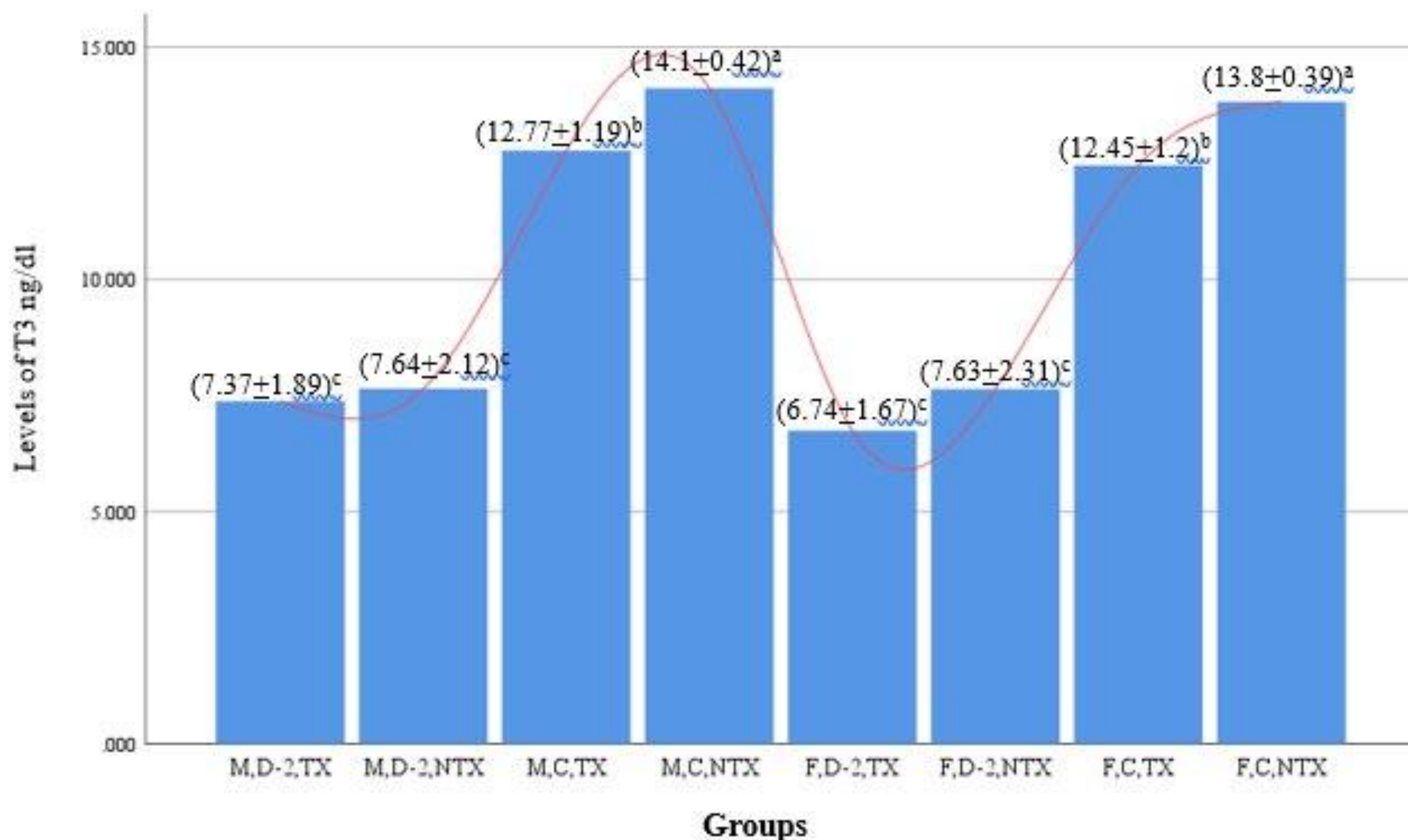


Figure 4-1: Distribution of T3 according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups

The results approach with Di Paola *et al*., 2022, who found a decrease in the level of T_3 hormone when fish were exposed to AFB_1. Another study conducted on broiler chicks after AFB_1 injection showed a decrease in the level of T_3 (Elwan *et al*., 2021). Also, another study was conducted on ducks after they were exposed to a diet containing AFB_1 results of the study showed a significant decrease in the level of the T_3 hormone (Valchev *et al*., 2014).

The results showed that T4 levels in the serum of the groups (M, D-2, TX) and (F, D-2, TX), whose means were (268.1 ± 40.6 ng/dl) and (277.9 ± 69.4 ng/dl), respectively, compare with the levels of T4 hormones in the serum of the groups (M, D-2, NTX) and (F, D-2, NTX), whose means ± SD were (241.1 ± 69.4 and 247.7 ± 83.3 ng/dl), respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), whose means ± SD were (180.7 ± 53.2 ng/dl) and (185.3 ± 52.5 ng/dl), respectively, with groups of (M, C, NTX), and (F, C, NTX), whose means are (173.2 ± 36.8 ng/dl) and (174.9 ± 35.9 ng/dl), respectively, with significantly increased between them, as shown in figure (4-2).

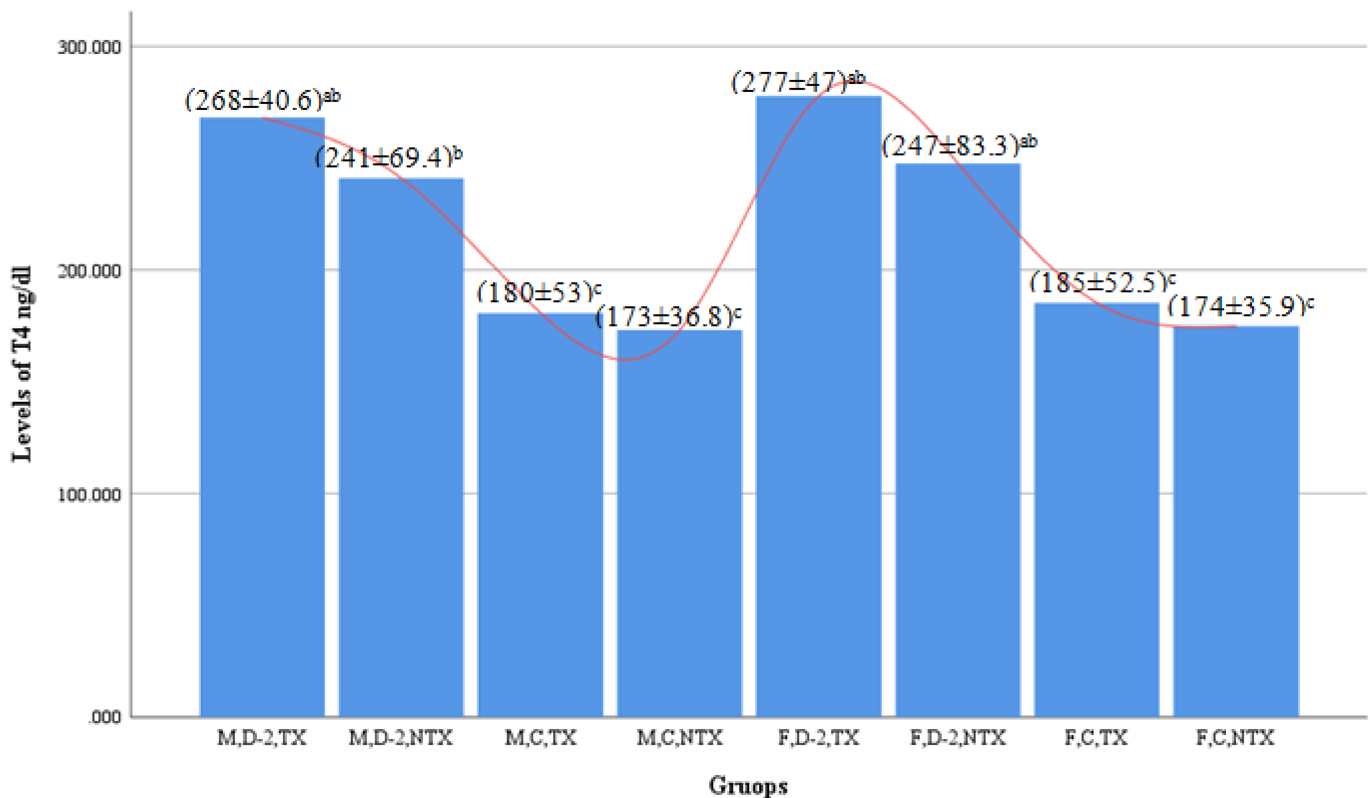


Figure 4-2: Distribution of T4 according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups

The results approach with study Eraslan *et al*., 2006 who found increase in T4 levels of quail when exposed to AFB1. Other studies showed a decrease in T4 levels ,which were (Valchev *et al*., 2014) (Elwan *et al*., 2021) (Di Paola *et al*., 2022), and. Also, the results showed an increase in levels of TSH in blood serum of (M, D-2, TX), and (F, D-2, TX) groups , whose means ± SD were (3.48 ± 0.37 and 4.15 ± 1.7), respectively, compare with the levels of TSH hormones in the serum of the groups (M, D-2, NTX) and (F, D-2, NTX) whose means ± SD were (3.23 ± 0.87 uIU/mL and 3.07 ± 0.59 uIU/mL), respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), compare with (M, C, NTX), and (F,C, NTX), with significantly increased between them, as shown in figure (4–3).

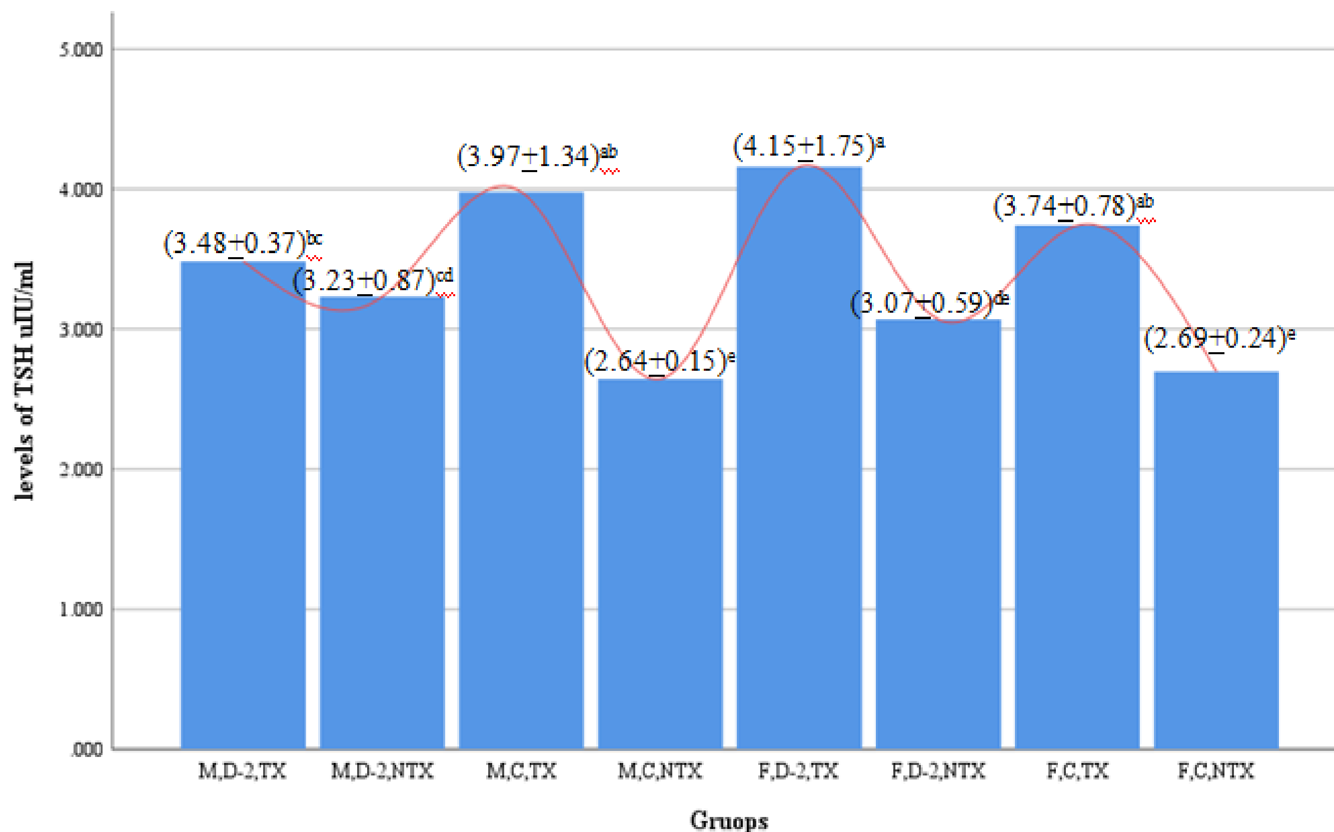


Figure 4-3: Distribution of TSH according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups

The results approached with study Wang *et al*., 2021a, who demonstrated an increase in TSH levels zebra fish upon exposure to AFB_1. To the best of our knowledge, this study is the first to show how AFB1 affects thyroid gland levels in humans.

Thyroid hormones regulate many of the body's functions, including metabolism, body temperature, and heart rate. Thyroid hormones include T_3, T_4, and TSH. Malfunctions in these hormones directly affect the general situation of the living being (Shahid *et al*., 2022). In this study, when the effect of AFB_1 toxin on thyroid hormones was examined, a decrease in the level of T_3 hormone and a

significant increase in T4 hormone were revealed compared to groups that did not contain AFB_1. However, changes in T_3 and T_4 should cause a change in TSH Even if indirectly (Eraslan *et al*., 2006). Our study showed that TSH was significantly affected by AFB1 compared with control groups. Lower T3 and T4 concentrations stimulate the thyroid gland T3 and T4 receptors, stimulating the synthesis and release of TSH (Valchev *et al*., 2014). In this study, there was an increase in levels of T4. There might have been a slowdown in the conversion of T_4 to T_3 in peripheral tissues. While some of the T3 diffused into the bloodstream is synthesized in the thyroid gland, a major proportion of it occurs as a results of the conversion of T4, synthesized in the thyroid gland, into T3 in peripheral tissues. 5'-deiodinase is the enzyme primarily responsible for this conversion. On the other hand, malic enzymes and 6-phosphogluconate dehydrogenase also take part in this process. These enzymes convert NAD into NADP, which is responsible for this conversion (Noyan, 1993). Type II and III deiodinases are selenoproteins, which indicates a role for selenium in the metabolism of thyroid hormones that are essential for growth and development. This enzyme catalyzes the conversion of T4 to its active metabolite T3 in the liver and kidney, and selenium deficiency results in an increase in levels of plasma T4 and a corresponding decrease in levels of more active T3. Also, thyroid peroxidase, also known as iodide peroxidase, may be affected by AFB_1. TPO is an enzyme that catalyzes the oxidation of iodide to produce iodine atoms, which are then attached to tyrosine residues on thyroglobulin to produce T4 or T3, or thyroid hormones (Habza-Kowalska *et al*., 2019).

Kalra *et al*., 2019 who found that T2DM impairs the conversion of thyroxine (T4) to triiodothyronine (T3) in the peripheral tissues. Both hypothyroidism and hyperthyroidism are more common in type 2 diabetes mellitus (T2DM) patients than in healthy humans.

Perros *et al*., 1995 found a decrease in TSH levels in T2DM, whereas our study reported an increase in TSH levels. Perhaps the reason for this is the low level of T3, which in turn stimulates TSH through the receptors. On the other hand, perhaps because the sample serum from patients collected in this study was newly diagnosed with type 2 diabetes and the proportions of toxins indicated by the HPLC (0.005, 0.004 ng/dl) were influencing the level of TSH in both diabetic males and females, Additionally, the BMI contributes to the elevated TSH levels.

4.2.4. Measurement of HBA1C and FBS in study groups

The results showed distribution of AFB_1 in the HBA1C levels of blood serum in groups (M, D-2, TX), (F, D-2, TX), (M, C, TX) and (F, C, TX), whose means were (7.64% , 7.76%,5.57% and 5.41%), respectively, compared with other groups. (M, D-2, NTX), (F, D-2, NTX), (M, C, NTX), and (F, C, NTX), whose means were (8.59%, 7.17%, 4.66%,and 4.66%) respectively, there was a significantly increased between them, as shown in table (4-8).

Table 4-8: Distribution of HBA1C levels in patient and control groups

Groups	Mean%	SD	Duncan test	*P*-value
M,D-2,TX	7.645	1.1138	bc	.001*
F,D-2,TX	7.762	1.0730	b	
M,D-2,NTX	8.596	1.7484	a	
F,D-2,NTX	7.178	.5697	c	
M,C,TX	5.576	.3308	d	
F,C,TX	5.416	.3804	d	
M,C,NTX	4.665	.4534	e	
F,C,NTX	4.668	.5046	e	
The difference between the letters indicates that there is a significant difference between the study groups.				

Also, the results showed an increase in levels of FBS in the blood serum of groups (M, D-2, TX) and (F, D-2, TX), whose means were (225 mg/dl and 210 mg/dl), respectively, compared with groups (M, D-2, NTX), and (F, D-2, NTX), whose means were (183 mg/dl and 176 mg/dl), respectively, with significant differences between them, while the levels of FBS were (116 mg/dl and 118 mg/dl), respectively, in groups (M, C, TX), and (F, C, TX), without significant differences between them, but significant differences with groups (M, C, NTX), and (F, C, NTX), as shown in table (4-9).

Table 4-9: Distribution of FBS levels in patient and control groups

Groups	Mean Mg/dl	SD	Duncan test	**P*-value
M,D-2,TX	225.98	50.177	a	0.001*
F,D-2,TX	210.53	103.093	ab	
M,D-2,NTX	183.88	39.639	bc	
F,D-2,NTX	176.20	76.743	c	
M,C,TX	116.91	9.160	d	
F,C,TX	118.44	7.317	d	
M,C,NTX	81.25	7.738	e	
F,C,NTX	86.89	13.791	de	
Groups with different letters indicate that there is a significant difference between them at $P<0.05$				

This study agreed with Alvarez *et al*., who found an increase in glucose level with continuous exposure to AFB_1. When assessing the association between AFB_1 and metabolic disorders (Alvarez *et al*., 2022). Also, another study showed a connection between AFB_1 and T2DM, identified an indication that linked AFB_1 to an increase in T2DM (Kadhum *et al*., 2022).

Many of risk factors, particularly inflammatory responses and OS, ultimately lead to the pathogenesis of T2DM and its associated Metabolic disorders. A study by Akash *et al*,. who showed that human exposure to AFM1 toxins (toxins metabolized from AFB1) leads to the development of T2DM by affecting the liver and kidneys and stimulating inflammatory responses and OS (Akash *et al*., 2021b).

The development of diabetes may, however, be influenced by mycotoxin exposure, as shown by a number of animal studies. As an illustration, a recent study in female rats found that chronic exposure to ochratoxin A (OTA), a mycotoxin related to AFB1, raises blood sugar levels while lowering insulin levels. Additionally, the pancreatic Langerhans islet cells may be harmed by OTA (Mor *et al*., 2017).

By altering the gut flora and results in dysregulation of intestinal function and weakened immune defenses, mycotoxins may also contribute to the onset of diabetes (Liew and Mohd-Redzwan, 2018). The alteration of intestinal barrier functioning and host metabolic and signaling pathways by gut dysbiosis may have a direct or indirect impact on the insulin resistance in diabetes (Sharma and Tripathi, 2019). According to numerous studies, the gut microbiome's dysbiosis contributes to the quick development of insulin resistance in people with diabetes (Sharma and Tripathi, 2019). After two weeks of oral AFB_1 exposure, a recent rodent study showed disturbance of the gut microbial metabolism (Wang *et al*., 2016). AFB_1 was found to be able to change the gut microbiota in rats in a dose-response way, according to a similar study (Wang *et al*., 2016). The scientists hypothesized that AFB_1 can cause harmful alterations in the community structure of the gut microbiota and serious disruption of numerous metabolic pathways involved in gluconeogenesis, the Krebs cycle, and the formation of lactic acid (Zhou *et al*., 2018).

4.3. Assessment of the Physiological Parameters

Assessment results of some physiological blood parameters showed effect of AFB_1 on some blood indicators. physiological parameters included types of WBC, RBC and HB levels.

Hemoglobin (HB)

Measurement levels of HB in study groups were compared between the groups (M, D-2, TX), (F, D-2, TX), (M, C, TX), and (F, C, TX), whose means were (13.0, 12.4, 13.2, and 13.0 g/dl), and with groups (M, D-2, NTX), (M, C,NTX), (F,C,NTX), and (F,D-2, NTX), whose means were (13.3, 13.5, 13.2, and 12.8 g/dl), respectively, with a significant difference between them. The results illustrated in table (4-10)

Table 4-10: Distribution of HB levels in serum patient compare to the control groups

Groups	Mean g/dl	SD	Duncan test	*P*-value
M,D-2,TX	13.075	1.2977	ab	.044*
F,D-2,TX	12.477	.6790	b	
M,D-2,NTX	13.396	1.0720	a	
F,D-2,NTX	12.883	1.1244	ab	
M,C,TX	13.229	1.6925	a	
F,C,TX	13.044	1.0054	ab	
M,C,NTX	13.565	1.2954	a	
F,C,NTX	13.273	.7642	a	
Groups with different letters indicate that there is a significant difference between them at $P<0.05$				

Red Blood Cells (RBCs)

RBC levels were measured in a similar manner when AFB_1 was supplied to study groups. When the groups (M, D-2, TX) and (F, D-2, TX) were compared to (M, D-2, NTX), the results showed a marginally significant difference, with a mean of (4.65 and $4.43*10^6$/ul) compared to (4.75 and 4.76 $*10^6$/ul). Furthermore,

when means (4.49 and 4.40 *10^6/ul), (4.70 and 4.92 *10^6/ul) were used to compare (M, C, TX), (F, C, TX), and (M, C, NTX), respectively. Consequently, the means of RBC in the groups that contained the toxin decreased relative to the groups that did not contain the toxin, as shown in table (4-11).

Table 4-11: Distribution of RBC levels in patient and control groups

Groups	Mean 10^6/ul	SD	Duncan test	*P*-value
M,D-2,TX	4.6525	0.64145	ab	0.045*
F,D-2,TX	4.4315	0.46754	b	
M,D-2,NTX	4.7550	0.53381	ab	
F,D-2,NTX	4.7691	0.37472	ab	
M,C,TX	4.4953	0.60004	b	
F,C,TX	4.4068	0.44768	b	
M,C,NTX	4.7065	0.71144	ab	
F,C,NTX	4.9250	0.53493	a	
Groups with different letters indicate that there is a significant difference between them at *P*<0.05				

This study approached with the study (Lei *et al*., 2021), which studied associations between serum AFB_1 and anemia in pregnant women in China. Another, study approach with this study conducted on pregnant women in Ghana which studied associations between serum AFB_1 and anemia (Shuaib *et al*., 2010).

Also, Hassan and Abdel-Reda., who found a decrease in RBC and HB levels in connection with nephropathy in patients and OTA toxins in human (Hassan and Abdel-Reda, 2022).

Animal studies have revealed that AFB_1 has impaired the morphology of erythrocytes by inducing oxidant stress and causing a decrease in hemoglobin concentration (Lei *et al*., 2021). In humans, it is not clear how aflatoxins may cause anemia; However, the fact that they act as toxins suggests that they may, like most other toxins, cause anemia through a hemolytic process. The accumulation of evidence suggests that AFs may cause DNA damage and mutations and suppress bone marrow functions (Verma, 2004).

Abdel-Wahhab *et al*., 2002 have reported a decrease in hemoglobin concentration and total RBC counts results in normocytic normochromic anemia in AF application alone. This decrease in the hematological parameters may be due to many factors, such as inhibition of Protein synthesis, as evidenced by lower serum albumin , decrease in the total iron binding capacity and the hemopoietic cellular defects of AF.

As for the effect of T2DM on HB and RBC, there are many studies that are consistent with our study, including (Ebrahim *et al*., 2022), (Dönmez *et al*., 2012). These studies showed that the levels of HB and RBC changed and led to anemia in patients with T2DM compared to healthy subjects.

In diabetic patients, multiple risk factors such as hyperglycemia, hyperosmolarity, OS, inflammation, and lipid metabolic disorders may affect RBC metabolism as they may increase aggregation, reduce cell deformability, and reduce membrane fluidity. Consequently, the overall alteration reduces the survival rate, morphology, size, and physiological functions of erythrocytes. Eventually, the

overall process affects the physiological functions of RBCs, which may in turn aggravate diabetic complications (Wang *et al*., 2021b).

White Blood Cells (WBCs)

The distribution of AFB_1 in the study groups is shown in table (4–12). The study compared groups (M, D-2, TX), (F, D-2, TX), and their respective means (8.7 and $8.6*10^3$/ul) with groups (M, D-2, NTX),(F, D-2, NTX) and their respective means (8.7 and $7.6*10^3$/ul). Additionally, when (M, C, TX), and (F, C, TX), whose means were (7.2 and $7.7*10^3$/ul) and (M, C, NTX),(F,C, NTX), whose means were (6.8 and $7*10^3$/ul), respectively, were compared, the results revealed a slight increase in the means of the groups that contain AFB_1, with a slight significantly decreased, $P< 0.05$.

Table 4-12: Distribution of WBCs levels in patient and control groups

Groups	Mean 10^3/ul	SD	Duncan test	*P*-value
M,D-2,TX	8.74	2.21	a	0.042*
F,D-2,TX	8.60	2.82	a	
M,D-2,NTX	8.32	3.11	ab	
F,D-2,NTX	7.69	2.09	ab	
M,C,TX	7.22	2.02	ab	
F,C,TX	7.75	1.86	ab	
M,C,NTX	6.84	1.58	b	
F,C,NTX	7.03	1.88	b	
Groups with different letters indicate that there is a significant difference between them at $P<0.05$				

This results has agreement with Mehrzad *et al*., which found an increase in WBC levels in humans upon exposure to AFB_1(Mehrzad *et al*., 2020a). Additionally, there are researches that showed the effect of OTA toxins on WBC that are in line with our study, including Hassan and Abdel-Reda., who recorded an increase in the level of WBC when exposed to OTA, which is similar to AFB_1 (Hassan and Abdel-Reda, 2022).

There are many studies conducted on animals that were approached with our study, including Khaled and Thalij., that showed an increase in WBC in rats that were fed with AFB_1 (Khaled and Thalij, 2021). Likewise, a study showed that an increase in WBC when effects of AFB_1 combined with OTA and zearalenone on

metabolism, immune function, and antioxidant status in lactating dairy goats (Huang *et al.*, 2018). Additionally, Cao and Wang., suggested that AFB_1 exerted a direct toxic effect on the hemostasis blood system and hepatocytes (Cao and Wang, 2014).

AFB_1 is an external toxin and has documented pathologic effects on apoptosis in the liver, kidney, thymus, spleen, and gastrointestinal tract immune system. Based on the dose and time of effect, one report indicates AFB_1 may reduce the number of B and T lymphocytes and disturb cytolysis activity in neutrophils (Mehrzad *et al.*, 2020a).

As for the effect of T2DM on WBC, this study is consistent with Kheradmand *et al*., who reported a significant association between WBC count and diabetes (Kheradmand *et al*., 2021). Also, Zhang *et al*., his results showed that total WBC count, neutrophil count, and lymphocyte count were significantly increased in subjects newly diagnosed with diabetes compared to subjects without diabetes at baseline. The elevated WBC in T2DM patients is probably due to persistent inflammation results from diabetes. It is well accepted that type 2 diabetes is an inflammatory disease with chronic, low-grade activation of the immune system as a major component of the pathophysiology. A number of markers of inflammation, including white blood cells and cytokines, were found to be elevated, which activate the immune system (Zhang *et al*., 2017).

4.4. Estimation of correlation coefficient (r)

4.4.1. Estimation of Correlation Coefficient Between the AFB_1 and T2DM

Measurement of correlation coefficient between AFB_1 exposure and T2DM occur through the evaluation of coefficient r = 0.528, and P < 0.001. As shown in table (4-13)

Table (4-13) Estimation of Correlation between AFB_1 and T2DM

Correlation between AFB_1 and T2DM	Pearson Correlation	*P*-value
	r=0.528**	0.001

4.4.2.Estimation of correlation coefficient between the AFB_1 and parameters

The correlation between AFB_1 and physiological and biochemical parameters. The RBC results showed a strong negative correlation with p < 0.01 and r = -443. The biochemical markers of liver enzymes (AST and ALT) revealed a strong positive correlation between them and AFB_1, with p < 0.001 and r = 0.656 for AST and p < 0.001 and r = 0.682 for ALT. as well as the kidney function (B.Urea and S.Cr) revealed a strong positive correlation between them and AFB_1, with p < 0.001 and r = 0.644 for B.Urea and p < 0.001 and r = 0.634 for S.Cr.

The biochemical markers of thyroid hormone (T3 and T4) revealed a strong negative correlation between AFB_1 and T3 levels, with P < 0.001, r = -0.731, while T4 had a positive correlation with AFB1, P < 0.01, r = 0.460, as shown in table (4-14)

Table(4-14) : Correlation between AFB_1 and parameters levels in blood serum

Characteristics	Persons correlation coefficient	*P*-value
RBC	-0.443^{**}	0.006
AST	0.656^{**}	0.000
ALT	0.682^{**}	0.000
B.urea	0.644^{**}	0.000
S.Cr	0.634^{**}	0.000
T3	-0.731^{**}	0.000
T4	0.460^{**}	0.004

4.4.3. Estimation of correlation coefficient between the T2DM and parameters.

The correlation between T2DM and physiological and biochemical parameters. WBC results showed a strong correlation between them and T2DM, with $p < 0.001$ and $r = 0.409$.

The biochemical markers of liver enzymes (AST and ALT) revealed a strong positive correlation between them and T2DM, with $p < 0.001$ and $r = 0.368$ for AST and $p < 0.001$ and $r = 0.317$ for ALT. as well as the kidney function (B.Urea and S.Cr) revealed a strong positive correlation between them and AFB1, with $p < 0.001$ and $r = 0.485$ for B.Urea and $p < 0.001$ and $r = 0.477$ for S.Cr.

The correlation between T3 and T2DM revealed a strong negative correlation with $p < 0.001$ and $r = -0.615$, while the correlation between T4 and T2DM revealed a strong positive correlation with $p <0.001$ and $r = 0.346$. as well as the TSH results of correlation with FBS showed positive correlation with $p < 0.01$ and $r = 0.217$, as shown in table (4-15).

(4-15): Explained the correlation T2DM and parameters levels in blood serum.

Characteristics	Persons correlation coefficient	*p*-value
WBC	0.409^{**}	0.000
AST	0.368^{**}	0.000
ALT	0.317^{**}	0.000
B.urea	0.485^{**}	0.000
S.Cr	0.477^{**}	0.000
T3	-0.615^{**}	0.000
T4	0.346^{**}	0.000
TSH	0.217^{**}	0.004

Conclusion and Recommendation

Conclusion:

1- The present positive correlation between AFB_1 toxins and T2DM patient. The relationship between T2DM and $AFB_1$was synergism

2- According to the study's findings, females are more sensitive to AFB1 than males.

3- The study found that the AFB_1-borne groups had higher TSH and T4 levels and lower T3 levels.

4- The biomarkers used in the investigation were adversely impacted by T2DM and AFB_1, and their amounts varied.

5- Increased FBS and HBA1C levels in AFB_1-borne study groups to determine how AFB_1 affects contributes to the development of diabetes.

6- According to the findings of this study, the presence of toxins in the body at any level is a risk factor for human health since they build up over time and cause issues with a variety of bodily functions.

7- This study, to our knowledge, is the first to examine how AFB_1 affects the thyroid gland in humans.

Recommendation

1. The findings of this study suggest that more research into determining how AFB_1 affects enzymes such as thyroid peroxidase (TPO), deiodinase (selenium), and glutathione S-transferase (GST) enzymes is worthwhile.
2. According to this study, it would be beneficial to compare the effects of AFB1 on the hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH) in women who have delayed childbearing to those in newlyweds.
3. Work is being done to analyze and look into the relationship between AFB_1 and hepatitis patients.
4. According to the results of the study, it is useful to conduct research into the effect of AFB_1 on the testosterone hormone in patients with reproductive impairment and to compare it with healthy people who carry the toxin.
5. Investigation of the effect of AFM_1 on prolactin hormone in females.

The References

References

Abd Al-Redha, S., Falah, Z., Ahmed, F., Falah, G. & Hasson, A. 2017. Investigation Of Ochratoxin A In Blood And It's Relationship With Cancer Diseases.

Abdel-Wahhab, M., Nada, S. & Khalil, F. 2002. Physiological And Toxicological Responses In Rats Fed Aflatoxin-Contaminated Diet With Or Without Sorbent Materials. Animal Feed Science And Technology, 97, 209-219.

Abdullah, H. A. R. & Aljumaili, S. A. R. 2018. Investigation Of Patulin In Samples Blood Of Persons In Karbala Province.

Abusaib, M., Ahmed, M., Nwayyir, H. A., Alidrisi, H. A., Al-Abbood, M., Al-Bayati, A., Al-Ibrahimi, S., Al-Kharasani, A., Al-Rubaye, H. & Mahwi, T. 2020. Iraqi Experts Consensus On The Management Of Type 2 Diabetes/Prediabetes In Adults. Clinical Medicine Insights: Endocrinology And Diabetes, 13, 1179551420942232.

Adamu, U., Mpanya, D., Patel, A. & Tsabedze, N. 2023. Beyond HBA1C Cardiovascular Protection In Type 2 Diabetes Mellitus. Journal Of Endocrinology, Metabolism And Diabetes Of South Africa, 28, 7-13.

Adegbeye, M. J., Reddy, P. R. K., Chilaka, C. A., Balogun, O. B., Elghandour, M. M. M. Y., Rivas-Caceres, R. R. & Salem, A. Z. M. 2020. Mycotoxin Toxicity And Residue In Animal Products: Prevalence, Consumer Exposure And Reduction Strategies – A Review. Toxicon, 177, 96-108.

Afkarian, M., Zelnick, L. R., Hall, Y. N., Heagerty, P. J., Tuttle, K., Weiss, N. S. & De Boer, I. H. 2016. Clinical Manifestations Of Kidney Disease Among Us Adults With Diabetes, 1988-2014. Jama, 316, 602-610.

Ahmed, A. 2019. History Of Diabetes Mellitus. Saudi Medical Journal, 23, 375-378.

References

Akash, M. S. H., Haq, M. E. U., Qader, A. & Rehman, K. 2021a. Biochemical Investigation Of Human Exposure To Aflatoxin M1 And Its Association With Risk Factors Of Diabetes Mellitus. Environ Sci Pollut Res Int, 28**,** 62907-62918.

Akash, M. S. H., Haq, M. E. U., Qader, A. & Rehman, K. 2021b. Biochemical Investigation Of Human Exposure To Aflatoxin M1 And Its Association With Risk Factors Of Diabetes Mellitus. Environmental Science And Pollution Research, 28**,** 62907-62918.

Allocati, N., Masulli, M., Di Ilio, C. & Federici, L. 2018. Glutathione Transferases: Substrates, Inihibitors And Pro-Drugs In Cancer And Neurodegenerative Diseases. Oncogenesis, 7**,** 8.

Alvarez, C. S., Hernández, E., Escobar, K., Villagrán, C. I., Kroker-Lobos, M. F., Rivera-Andrade, A., Smith, J. W., Egner, P. A., Lazo, M., Freedman, N. D., Guallar, E., Dean, M., Graubard, B. I., Groopman, J. D., Ramírez-Zea, M. & Mcglynn, K. A. 2020. Aflatoxin B≪Sub≫1≪/Sub≫ Exposure And Liver Cirrhosis In Guatemala: A Case–Control Study. Bmj Open Gastroenterology, 7**,** E000380.

Alvarez, C. S., Rivera-Andrade, A., Kroker-Lobos, M. F., Florio, A. A., Smith, J. W., Egner, P. A., Freedman, N. D., Lazo, M., Guallar, E. & Dean, M. 2022. Associations Between Aflatoxin B1-Albumin Adduct Levels With Metabolic Conditions In Guatemala: A Cross-Sectional Study. Health Science Reports, 5**,** E495.

Amartey, N. A., Nsiah, K. & Mensah, F. O. 2015. Plasma Levels Of Uric Acid, Urea And Creatinine In Diabetics Who Visit The Clinical Analysis Laboratory (Can-Lab) At Kwame Nkrumah University Of Science And Technology, Kumasi, Ghana. J Clin Diagn Res, 9**,** Bc05-9.

Antonissen, G., Devreese, M., De Baere, S., Martel, A., Van Immerseel, F. & Croubels, S. 2017. Impact Of Fusarium Mycotoxins On Hepatic And Intestinal Mrna Expression Of Cytochrome P450 Enzymes And Drug Transporters, And On The Pharmacokinetics Of Oral Enrofloxacin In Broiler Chickens. Food And Chemical Toxicology, 101**,** 75-83.

Awuchi, C. G., Ondari, E. N., Nwozo, S., Odongo, G. A., Eseoghene, I. J., Twinomuhwezi, H., Ogbonna, C. U., Upadhyay, A. K., Adeleye, A. O. & Okpala, C. O. R. 2022. Mycotoxins&Rsquo; Toxicological Mechanisms Involving Humans, Livestock And Their Associated Health Concerns: A Review. Toxins, 14**,** 167.

Awuchi, C. G., Ondari, E. N., Ogbonna, C. U., Upadhyay, A. K., Baran, K., Okpala, C. O. R., Korzeniowska, M. & Guiné, R. P. 2021. Mycotoxins Affecting Animals, Foods, Humans, And Plants: Types, Occurrence, Toxicities, Action Mechanisms, Prevention, And Detoxification Strategies—A Revisit. Foods, 10**,** 1279.

Babić Leko, M., Gunjača, I., Pleić, N. & Zemunik, T. 2021. Environmental Factors Affecting Thyroid-Stimulating Hormone And Thyroid Hormone Levels. International Journal Of Molecular Sciences, 22**,** 6521.

Badr, A. N., Fouda, K., Mohamed, R. S., Farag, A.-R. H., Zaghloul, A. H. & Abdel-Salam, A. M. 2022. Protective Impact Of Encapsulated Probiotics And Ziziphus Spinachristi Against Liver Oxidative Stress And Dna Damage Induced By Aflatoxin M1. Journal Of Applied Pharmaceutical Science, 12**,** 082-094.

Bankole, S. & Adebanjo, A. 2003. Mycotoxins In Food In West Africa: Current Situation And Possibilities Of Controlling It. African Journal Of Biotechnology, 2**,** 254-263.

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W. & Kyegombe, D. B. 2013. Review Of The Biological And Health Effects Of Aflatoxins On Body Organs And Body Systems. Aflatoxins-Recent Advances And Future Prospects, 12, 239-265.

Benkerroum, N. 2020. Chronic And Acute Toxicities Of Aflatoxins: Mechanisms Of Action. Int J Environ Res Public Health, 17.

Blann, A. 2014. What Is The Purpose Of Liver Function Tests? Nurs Times, 110, 17-9.

Bogdanet, D., O'shea, P., Lyons, C., Shafat, A. & Dunne, F. 2020. The Oral Glucose Tolerance Test—Is It Time For A Change?—A Literature Review With An Emphasis On Pregnancy. Journal Of Clinical Medicine, 9, 3451.

Burtis, C. A. & Bruns, D. E. 2014. Tietz Fundamentals Of Clinical Chemistry And Molecular Diagnostics-E-Book, Elsevier Health Sciences.

Caloni, F. & Cortinovis, C. 2011. Toxicological Effects Of Aflatoxins In Horses. The Veterinary Journal, 188, 270-273.

Cao, J. & Wang, W. 2014. Effects Of Astaxanthin And Esterified Glucomannan On Hematological And Serum Parameters, And Liver Pathological Changes In Broilers Fed Aflatoxin-B 1-Contaminated Feed. Animal Science Journal, 85, 150-157.

Chen, M., Liu, X., Yang, S., Chen, Z., Di, B., Liu, W. & Yan, H. 2022. Hplc–Ms/Ms Method For The Simultaneous Determination Of Aflatoxins In Blood: Toxicokinetics Of Aflatoxin B1 And Aflatoxin M1 In Rats. Journal Of Analytical Science And Technology, 13, 27.

Christ-Crain, M., Bichet, D. G., Fenske, W. K., Goldman, M. B., Rittig, S., Verbalis, J. G. & Verkman, A. S. 2019. Diabetes Insipidus. Nature Reviews Disease Primers, 5, 1-20.

Dai, Y., Huang, K., Zhang, B., Zhu, L. & Xu, W. 2017. Aflatoxin B1-Induced Epigenetic Alterations: An Overview. Food And Chemical Toxicology, 109, 683-689.

Darwish, W. S., Ikenaka, Y., Nakayama, S. M. & Ishizuka, M. 2014. An Overview On Mycotoxin Contamination Of Foods In Africa. Journal Of Veterinary Medical Science, 76, 789-797.

Daryabor, G., Atashzar, M. R., Kabelitz, D., Meri, S. & Kalantar, K. 2020. The Effects Of Type 2 Diabetes Mellitus On Organ Metabolism And The Immune System. Frontiers In Immunology, 11.

Defronzo, R. A., Reeves, W. B. & Awad, A. S. 2021. Pathophysiology Of Diabetic Kidney Disease: Impact Of Sglt2 Inhibitors. Nature Reviews Nephrology, 17, 319-334.

Deng, J., Zhao, L., Zhang, N.-Y., Karrow, N. A., Krumm, C. S., Qi, D.-S. & Sun, L.-H. 2018. Aflatoxin B1 Metabolism: Regulation By Phase I And Ii Metabolizing Enzymes And Chemoprotective Agents. Mutation Research/Reviews In Mutation Research, 778, 79-89.

Dhakal, A., Hashmi, M. F. & Sbar, E. 2022. Aflatoxin Toxicity, Statpearls Publishing, Treasure Island (Fl).

Di Paola, D., Iaria, C., Capparucci, F., Arangia, A., Crupi, R., Cuzzocrea, S., Spanò, N., Gugliandolo, E. & Peritore, A. F. 2022. Impact Of Mycotoxin Contaminations On Aquatic Organisms: Toxic Effect Of Aflatoxin B1 And Fumonisin B1 Mixture. Toxins, 14, 518.

Diaz De Leon-Martinez, L., Rodríguez-Aguilar, M., Wong-Arce, A., Díaz-Barriga, F., Bañuelos-Hernández, B., Rosales-Mendoza, S. & Flores-Ramírez, R. 2020. Evaluation Of Acute And Chronic Exposure To Aflatoxin B 1 In Indigenous Women Of The Huasteca Potosina, Mexico. Environmental Science And Pollution Research, 27, 30583-30591.

Dirir, A. M., Daou, M., Yousef, A. F. & Yousef, L. F. 2022. A Review Of Alpha-Glucosidase Inhibitors From Plants As Potential Candidates For The Treatment Of Type-2 Diabetes. Phytochemistry Reviews, 21**,** 1049-1079.

Dohnal, V., Wu, Q. & Kuča, K. 2014. Metabolism Of Aflatoxins: Key Enzymes And Interindividual As Well As Interspecies Differences. Arch Toxicol, 88**,** 1635-44.

Dönmez, N., Dönmez, H., Keskin, E. & Kısadere, I. 2012. Effects Of Aflatoxin On Some Haematological Parameters And Protective Effectiveness Of Esterified Glucomannan In Merino Rams. The Scientific World Journal, 2012.

Dunn, J. D., Alvarez, L. A., Zhang, X. & Soldati, T. 2015. Reactive Oxygen Species And Mitochondria: A Nexus Of Cellular Homeostasis. Redox Biology, 6**,** 472-485.

Ebrahim, H., Fiseha, T., Ebrahim, Y. & Bisetegn, H. 2022. Comparison Of Hematological Parameters Between Type 2 Diabetes Mellitus Patients And Healthy Controls At Dessie Comprehensive Specialized Hospital, Northeast Ethiopia: Comparative Cross-Sectional Study. Plos One, 17**,** E0272145.

Elwan, H., Xie, C., Miao, L. P., Dong, X., Zou, X. T., Mohany, M., Ahmed, M. M., Al-Rejaie, S. S. & Elnesr, S. S. 2021. Methionine Alleviates Aflatoxinb1-Induced Broiler Chicks Embryotoxicity Through Inhibition Of Caspase-Dependent Apoptosis And Enhancement Of Cellular Antioxidant Status. Poultry Science, 100**,** 101103.

Eom, Y. S., Wilson, J. R. & Bernet, V. J. 2022. Links Between Thyroid Disorders And Glucose Homeostasis. Diabetes & Metabolism Journal, 46**,** 239-256.

Eraslan, G., Akdoğan, M., Liman, B. C., Kanbur, M. & Delibaş, N. 2006. Effects Of Dietary Aflatoxin And Hydrate Sodium Calcium Aluminosilicate On

Triiodothyronine, Thyroxine, Thyrotrophin And Testosterone Levels In Quails. Turkish Journal Of Veterinary & Animal Sciences, 30, 41-45.

Fadhil, W. F., Al-Saadoon, A. H. & Al-Moussawi, F. M. 2022. New Records Of Mycobiota Associated With Stored Wheat And Its By-Products In Iraq. Biodiversitas Journal Of Biological Diversity, 23.

Fan, T., Xie, Y. & Ma, W. 2021. Research Progress On The Protection And Detoxification Of Phytochemicals Against Aflatoxin B1-Induced Liver Toxicity. Toxicon, 195, 58-68.

Fayyaz, B., Rehman, H. J. & Minn, H. 2019. Interpretation Of Hemoglobin A1c In Primary Care Setting. Journal Of Community Hospital Internal Medicine Perspectives, 9, 18-21.

Firmin, S., Bahi-Jaber, N. & Abdennebi-Najar, L. 2016. Food Contaminants And Programming Of Type 2 Diabetes: Recent Findings From Animal Studies. J Dev Orig Health Dis, 7, 505-512.

Fouad, A. M., Ruan, D., El-Senousey, H. K., Chen, W., Jiang, S. & Zheng, C. 2019. Harmful Effects And Control Strategies Of Aflatoxin B_1 Produced By Aspergillus Flavus And Aspergillus Parasiticus Strains On Poultry: Review. Toxins (Basel), 11.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H. & Martín, C. 2020. Pathophysiology Of Type 2 Diabetes Mellitus. International Journal Of Molecular Sciences, 21, 6275.

Gauthier, B. R., Sola-García, A., Cáliz-Molina, M. Á., Lorenzo, P. I., Cobo-Vuilleumier, N., Capilla-González, V. & Martin-Montalvo, A. 2020. Thyroid Hormones In Diabetes, Cancer, And Aging. Aging Cell, 19, E13260.

Giannini, E. G., Testa, R. & Savarino, V. 2005. Liver Enzyme Alteration: A Guide For Clinicians. Cmaj, 172, 367-79.

Gilbert-Sandoval, I., Wesseling, S. & Rietjens, I. 2020. Predicting The Acute Liver Toxicity Of Aflatoxin B1 In Rats And Humans By An In Vitro-In Silico Testing Strategy. Mol Nutr Food Res, 64, E2000063.

Gollapalli, M., Alansari, A., Alkhorasani, H., Alsubaii, M., Sakloua, R., Alzahrani, R., Al-Hariri, M., Alfares, M., Alkhafaji, D. & Al Argan, R. 2022. A Novel Stacking Ensemble For Detecting Three Types Of Diabetes Mellitus Using A Saudi Arabian Dataset: Pre-Diabetes, T1dm, And T2dm. Computers In Biology And Medicine, 147, 105757.

Groopman, J. D., Egner, P. A., Schulze, K. J., Wu, L. S., Merrill, R., Mehra, S., Shamim, A. A., Ali, H., Shaikh, S., Gernand, A., Khatry, S. K., Leclerq, S. C., West, K. P., Jr. & Christian, P. 2014. Aflatoxin Exposure During The First 1000 Days Of Life In Rural South Asia Assessed By Aflatoxin B_1-Lysine Albumin Biomarkers. Food Chem Toxicol, 74, 184-9.

Guo, Y., Qin, X., Tang, Y., Ma, Q., Zhang, J. & Zhao, L. 2020. Cota Laccase, A Novel Aflatoxin Oxidase From Bacillus Licheniformis, Transforms Aflatoxin B1 To Aflatoxin Q1 And Epi-Aflatoxin Q1. Food Chemistry, 325, 126877.

Halim, M. & Halim, A. 2019. The Effects Of Inflammation, Aging And Oxidative Stress On The Pathogenesis Of Diabetes Mellitus (Type 2 Diabetes). Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 13, 1165-1172.

Hassan, H. F., Kordahi, R., Dimassi, H., El Khoury, A., Daou, R., Alwan, N., Merhi, S., Haddad, J. & Karam, L. 2022. Aflatoxin B1 In Rice: Effects Of Storage Duration, Grain Type And Size, Production Site, And Season. J Food Prot, 85, 938-944.

Hassan, W. S. & Abdel-Reda, S. 2022. Study Possibility Of Some Biomarkers Determind On Poisoning Of Human By Ochratoxin A.

Hu, P., Zuo, Z., Li, H., Wang, F., Peng, X., Fang, J., Cui, H., Gao, C., Song, H., Zhou, Y. & Chen, Z. 2018. The Molecular Mechanism Of Cell Cycle Arrest In The Bursa Of Fabricius In Chick Exposed To Aflatoxin B (1). Sci Rep, 8**,** 1770.

Huang, S., Zheng, N., Fan, C., Cheng, M., Wang, S., Jabar, A., Wang, J. & Cheng, J. 2018. Effects Of Aflatoxin B1 Combined With Ochratoxin A And/Or Zearalenone On Metabolism, Immune Function, And Antioxidant Status In Lactating Dairy Goats. Asian-Australas J Anim Sci, 31**,** 505-513.

Hurtado, M. D. & Vella, A. 2019. What Is Type 2 Diabetes? Medicine, 47**,** 10-15.

Hussain, I. 2011. Aflatoxin Measurement And Analysis. Aflatoxins–Detection, Measurement And Control. 1st Ed. Rijeka, Croatia: Intech**,** 129-146.

Iarc. 2012. Aflatoxins. Monographs On The Evaluation Of Carcinogenic Risks To Humans. Chemical Agents And Related Occupations: A Review Of Human Carcinogens. Proceedings Of The International Agency For The Research On Cancer, 100**,** 225-244.

Iqbal, S. Z. 2021. Mycotoxins In Food, Recent Development In Food Analysis And Future Challenges; A Review. Current Opinion In Food Science, 42**,** 237-247.

Ismail, I. E., Farag, M. R., Alagawany, M., Mahmoud, H. K. & Reda, F. M. 2020. Efficacy Of Some Feed Additives To Attenuate The Hepato-Renal Damage Induced By Aflatoxin B1 In Rabbits. Journal Of Animal Physiology And Animal Nutrition, 104**,** 1343-1350.

Ismail, L., Materwala, H. & Al Kaabi, J. 2021. Association Of Risk Factors With Type 2 Diabetes: A Systematic Review. Computational And Structural Biotechnology Journal, 19**,** 1759-1785.

Jain, B., Jain, R., Jaiswal, P. K., Zughaibi, T., Sharma, T., Kabir, A., Singh, R. & Sharma, S. 2023. A Non-Instrumental Green Analytical Method Based On

Surfactant-Assisted Dispersive Liquid&Ndash;Liquid Microextraction&Ndash;Thin-Layer Chromatography&Ndash;Smartphone-Based Digital Image Colorimetry(Sa-Dllme-Tlc-Sdic) For Determining Favipiravir In Biological Samples. Molecules, 28**,** 529.

Jain, R., Kumari, A. & Khatri, I. 2021. Simple And Rapid Analysis Of Acetaminophen In Human Autopsy Samples By Vortex-Assisted Dispersive Liquid–Liquid Microextraction-Thin Layer Chromatography-Image Analysis. Separation Science Plus, 4**,** 92-100.

Jain, R., Singh, R., Sudhaker, S., Barik, A. & Kumar, S. 2017. Coupling Microextraction With Thin Layer Chromatography–Image Processing Analysis: A New Analytical Platform For Drug Analysis. Toxicology And Forensic Medicine Open Journal, 2**,** 17-25.

Jain, R., Tripathi, R. M., Negi, A. & Singh, S. P. 2020. A Simple, Cost-Effective And Rapid Method For Simultaneous Determination Of Strychnos Nux-Vomica Alkaloids In Blood And Ayurvedic Medicines Based On Ultrasound-Assisted Dispersive Liquid–Liquid Microextraction–Thin-Layer Chromatography-Image Analysis. Journal Of Chromatographic Science, 58**,** 477-484.

Jangampalli Adi, P. & Matcha, B. 2018. Analysis Of Aflatoxin B1 In Contaminated Feed, Media, And Serum Samples Of Cyprinus Carpio L. By High-Performance Liquid Chromatography. Food Quality And Safety, 2**,** 199-204.

Jiang, Y., Hansen, P. J., Xiao, Y., Amaral, T. F., Vyas, D. & Adesogan, A. T. 2019. Aflatoxin Compromises Development Of The Preimplantation Bovine Embryo Through Mechanisms Independent Of Reactive Oxygen Production. Journal Of Dairy Science, 102**,** 10506-10513.

Joint, F. A. O. W. H. O. E. C. O. F. A. 2001. Safety Evaluation Of Certain Mycotoxins In Food / Prepared By The Fifty-Sixth Meeting Of The Joint Fao/Who Expert Committee On Food Additives (Jecfa). Rome: Fao.

Kadhum, G. M., Al_Jumaili, S. A. & Al_Hashemi, H. A. 2022. Investigation Of Aflatoxin B1 In Blood Of Diabetes Mellitus Type 2 Patients. Hiv Nursing, 22**,** 3632–3634-3632–3634.

Kalra, S., Aggarwal, S. & Khandelwal, D. 2019. Thyroid Dysfunction And Type 2 Diabetes Mellitus: Screening Strategies And Implications For Management. Diabetes Ther, 10**,** 2035-2044.

Kang, S. H., Jung, D. J., Choi, E. W., Cho, K. H., Park, J. W. & Do, J. Y. 2015. HBA1C Levels Are Associated With Chronic Kidney Disease In A Non-Diabetic Adult Population: A Nationwide Survey (Knhanes 2011–2013). Plos One, 10**,** E0145827.

Karamkhani, M., Asilian-Mahabadi, H., Daraei, B., Seidkhani-Nahal, A. & Noori-Zadeh, A. 2020. Liver And Kidney Serum Profile Abnormalities In Workers Exposed To Aflatoxin B1 In Urban Solid Waste Management Centers. Environmental Monitoring And Assessment, 192**,** 1-12.

Karatekeli, S., Demirel, H. H., Zemheri-Navruz, F. & Ince, S. 2023. Boron Exhibits Hepatoprotective Effect Together With Antioxidant, Anti-Inflammatory, And Anti-Apoptotic Pathways In Rats Exposed To Aflatoxin B1. Journal Of Trace Elements In Medicine And Biology, 77**,** 127127.

Kareem, S. A., Hameed, R. M., Abd Al-Redha, S. & Al-Khatt, H. 2021. Aflatoxin B1 As A Threshold Concept Of Uncertain Etiology Of Chronic Kidney Diseases. Indian Journal Of Forensic Medicine & Toxicology, 15**,** 737-744.

Kautzky-Willer, A., Harreiter, J. & Pacini, G. 2016. Sex And Gender Differences In Risk, Pathophysiology And Complications Of Type 2 Diabetes Mellitus. Endocrine Reviews, 37**,** 278-316.

Keller, N. P. 2019. Fungal Secondary Metabolism: Regulation, Function And Drug Discovery. Nature Reviews Microbiology, 17, 167-180.

Kellum, J. A., Romagnani, P., Ashuntantang, G., Ronco, C., Zarbock, A. & Anders, H.-J. 2021. Acute Kidney Injury. Nature Reviews Disease Primers, 7, 52.

Kene, K., Wondimnew, T., Welde, M., Mateos, T., Adugna, T., Gerema, U., Abdisa, D. & Abera, D. 2021. Prevalence And Determinants Of Impaired Serum Creatinine And Urea Among Type 2 Diabetic Patients Of Jimma Medical Center, Jimma, Southwestern Ethiopia. Endocrine And Metabolic Science, 3, 100096.

Kew, M. C. 2012. Hepatocellular Carcinoma In Developing Countries: Prevention, Diagnosis And Treatment. World J Hepatol, 4, 99-104.

Khaled, M. Q. & Thalij, K. M. 2021, Effect Of Aflatoxin B1 Contaminated Corn And Their Products On Some Physiology Parameters In Laboratory Rats. Iop Conference Series: Earth And Environmental Science. Iop Publishing, 012103.

Kheradmand, M., Ranjbaran, H., Alizadeh-Navaei, R., Yakhkeshi, R. & Moosazadeh, M. 2021. Association Between White Blood Cells Count And Diabetes Mellitus In Tabari Cohort Study: A Case-Control Study. International Journal Of Preventive Medicine, 12.

Klinken, S. P. 2002. Red Blood Cells. The International Journal Of Biochemistry & Cell Biology, 34, 1513-1518.

Kowalska, A., Hajok, I. & Piekut, A. 2017a. Assessment Of The Contamination Level By Fumonisins B 1 And B 2 Of The Corn Food Products Available On Polish Consumer Market. Polish Journal Of Environmental Studies, 26.

Kowalska, A., Walkiewicz, K., Kozieł, P. & Muc-Wierzgoń, M. 2017b. Aflatoxins: Characteristics And Impact On Human Health. Advances In Hygiene And Experimental Medicine, 71, 315-327.

Kumar, A., Pathak, H., Bhadauria, S. & Sudan, J. 2021. Aflatoxin Contamination In Food Crops: Causes, Detection, And Management: A Review. Food Production, Processing And Nutrition, 3, 1-9.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K. & Kang, S. G. 2017. Aflatoxins: A Global Concern For Food Safety, Human Health And Their Management. Frontiers In Microbiology, 7.

Kumar, V. V. 2018. Aflatoxins: Properties, Toxicity And Detoxification. Nutr. Food Sci. Int. J, 6, 555696.

Kumar, A. S., Maiya, A. G., Shastry, B., Vaishali, K., Ravishankar, N., Hazari, A., Gundmi, S. & Jadhav, R. 2019. Exercise And Insulin Resistance In Type 2 Diabetes Mellitus: A Systematic Review And Meta-Analysis. Annals Of Physical And Rehabilitation Medicine, 62, 98-103.

Kupai, K., Várkonyi, T., Török, S., Gáti, V., Czimmerer, Z., Puskás, L. G. & Szebeni, G. J. 2022. Recent Progress In The Diagnosis And Management Of Type 2 Diabetes Mellitus In The Era Of Covid-19 And Single Cell Multi-Omics Technologies. Life, 12, 1205.

Kwo, P. Y., Cohen, S. M. & Lim, J. K. 2017. Acg Clinical Guideline: Evaluation Of Abnormal Liver Chemistries. Am J Gastroenterol, 112, 18-35.

Lakhtakia, R. 2013. The History Of Diabetes Mellitus. Sultan Qaboos Univ Med J, 13, 368-70.

Lalah, J. O., Omwoma, S. & Orony, D. 2019. Aflatoxin B1: Chemistry, Environmental And Diet Sources And Potential Exposure In Human In Kenya. Aflatoxin B1 Occurrence, Detection And Toxicological Effects.

References

Lei, L., Liu, S., Ye, Y., Qiu, X., Huang, D., Pan, D., Chen, J., Qian, Z., Mcmillin, S. E., Vaughn, M. G., Luo, X., Wu, K., Xiao, S., Li, J., Liu, M., Yang, Y., Lai, M., Dong, G. & Zeng, X. 2021. Associations Between Serum Aflatoxin-B1 And Anemia In Pregnant Women: Evidence From Guangxi Zhuang Birth Cohort In China. Toxins (Basel), 13.

Li, C., Liu, X., Wu, J., Ji, X. & Xu, Q. 2022a. Research Progress In Toxicological Effects And Mechanism Of Aflatoxin B1 Toxin. Peerj, 10**,** E13850.

Li, H., Xing, L., Zhang, M., Wang, J. & Zheng, N. 2018a. The Toxic Effects Of Aflatoxin B1 And Aflatoxin M1 On Kidney Through Regulating L-Proline And Downstream Apoptosis. Biomed Research International, 2018.

Li, H., Xing, L., Zhang, M., Wang, J. & Zheng, N. 2018b. The Toxic Effects Of Aflatoxin B1 And Aflatoxin M1 On Kidney Through Regulating L-Proline And Downstream Apoptosis. Biomed Research International, 2018**,** 9074861.

Li, S., Yao, X., Wang, X., Tian, S. & Zhang, Y. 2022b. Reactive Molecular Dynamics Simulation On Degradation Of Aflatoxin B1 By Cold Atmospheric Plasmas. Innovative Food Science & Emerging Technologies, 80**,** 103101.

Liew, W.-P.-P. & Mohd-Redzwan, S. 2018. Mycotoxin: Its Impact On Gut Health And Microbiota. Frontiers In Cellular And Infection Microbiology**,** 60.

Liu, L., Jin, H., Sun, L., Ma, S. & Lin, R. 2012. Determination Of Aflatoxins In Medicinal Herbs By High-Performance Liquid Chromatography–Tandem Mass Spectrometry. Phytochemical Analysis, 23**,** 469-476.

Liu, Y., Zhao, P., Cheng, M., Yu, L., Cheng, Z., Fan, L. & Chen, C. 2018. Ast To Alt Ratio And Arterial Stiffness In Non-Fatty Liver Japanese Population:A Secondary Analysis Based On A Cross-Sectional Study. Lipids Health Dis, 17**,** 275.

Louzada, R. A. & Carvalho, D. P. 2018. Similarities And Differences In The Peripheral Actions Of Thyroid Hormones And Their Metabolites. Frontiers In Endocrinology, 9**,** 394.

Lovic, D., Piperidou, A., Zografou, I., Grassos, H., Pittaras, A. & Manolis, A. 2020. The Growing Epidemic Of Diabetes Mellitus. Curr Vasc Pharmacol, 18**,** 104-109.

Lyssenko, V. & Laakso, M. 2013. Genetic Screening For The Risk Of Type 2 Diabetes: Worthless Or Valuable? Diabetes Care, 36**,** S120.

Ma, J., Liu, Y., Guo, Y., Ma, Q., Ji, C. & Zhao, L. 2021. Transcriptional Profiling Of Aflatoxin B1-Induced Oxidative Stress And Inflammatory Response In Macrophages. Toxins, 13**,** 401.

Ma, Q., Li, Y., Wang, M., Tang, Z., Wang, T., Liu, C., Wang, C. & Zhao, B. 2018. Progress In Metabonomics Of Type 2 Diabetes Mellitus. Molecules, 23**,** 1834.

Madbouly, A. K., Rashad, Y. M., Ibrahim, M. I. & Elazab, N. T. 2023. Biodegradation Of Aflatoxin B1 In Maize Grains And Suppression Of Its Biosynthesis-Related Genes Using Endophytic Trichoderma Harzianum Aym3. Journal Of Fungi, 9**,** 209.

Maggira, M., Sakaridis, I., Ioannidou, M. & Samouris, G. 2022. Comparative Evaluation Of Three Commercial Elisa Kits Used For The Detection Of Aflatoxins B1, B2, G1, And G2 In Feedstuffs And Comparison With An Hplc Method. Veterinary Sciences, 9**,** 104.

Mahjoory, Y., Mohammadi, R., Hejazi, M. A. & Nami, Y. 2023. Antifungal Activity Of Potential Probiotic Limosilactobacillus Fermentum Strains And Their Role Against Toxigenic Aflatoxin-Producing Aspergilli. Scientific Reports, 13**,** 388.

Malviya, R., Bansal, V., Pal, O. & Sharma, P. 2010. High Performance Liquid Chromatography: A Short Review. Journal Of Global Pharma Technology, 2, 22-26.

Mandal, A., Bhattarai, B., Kafle, P., Khalid, M., Jonnadula, S. K., Lamicchane, J., Kanth, R. & Gayam, V. 2018. Elevated Liver Enzymes In Patients With Type 2 Diabetes Mellitus And Non-Alcoholic Fatty Liver Disease. Cureus, 10, E3626.

Marchese, S., Polo, A., Ariano, A., Velotto, S., Costantini, S. & Severino, L. 2018. Aflatoxin B1 And M1: Biological Properties And Their Involvement In Cancer Development. Toxins, 10, 214.

Marijani, E., Nasimolo, J., Kigadye, E., Gnonlonfin, G. J. B. & Okoth, S. 2017. Sex-Related Differences In Hematological Parameters And Organosomatic Indices Of Oreochromis Niloticus Exposed To Aflatoxin B1 Diet. Scientifica, 2017.

Martínez, J., Hernández-Rodríguez, M., Méndez-Albores, A., Téllez-Isaías, G., Mera Jiménez, E., Nicolás-Vázquez, M. I. & Miranda Ruvalcaba, R. 2023. Computational Studies Of Aflatoxin B1 (Afb1): A Review. Toxins, 15, 135.

Matejova, I., Svobodova, Z., Vakula, J., Mares, J. & Modra, H. 2017. Impact Of Mycotoxins On Aquaculture Fish Species: A Review. Journal Of The World Aquaculture Society, 48, 186-200.

Mathur, S., Mehta, D. K., Kapoor, S. & Yadav, S. 2016. Liver Function In Type-2 Diabetes Mellitus Patients. International Journal Of Scientific Study, 3, 43-47.

Mauvais-Jarvis, F. 2017. Sex And Gender Factors Affecting Metabolic Homeostasis, Diabetes And Obesity, Springer.

Mccullough, A. K. & Lloyd, R. S. 2019. Mechanisms Underlying Aflatoxin-Associated Mutagenesis–Implications In Carcinogenesis. Dna Repair, 77, 76-86.

Mcintyre, H. D., Catalano, P., Zhang, C., Desoye, G., Mathiesen, E. R. & Damm, P. 2019. Gestational Diabetes Mellitus. Nature Reviews Disease Primers, 5, 1-19.

Mdquadri, S., Niranjan, M., Chaluvaraju, K., Shantaram, U. & Enamul, H. 2013. An Overview On Chemistry, Toxicity, Analysis And Control Of Aflatoxins. Int. J. Chem. Life Sci, 2, 1071-1078.

Mehrzad, J., Fazel, F., Pouyamehr, N., Hosseinkhani, S. & Dehghani, H. 2020b. Naturally Occurring Level Of Aflatoxin B(1) Injures Human, Canine And Bovine Leukocytes Through Atp Depletion And Caspase Activation. Int J Toxicol, 39, 30-38.

Mills, G., Badeghiesh, A., Suarthana, E., Baghlaf, H. & Dahan, M. H. 2020. Polycystic Ovary Syndrome As An Independent Risk Factor For Gestational Diabetes And Hypertensive Disorders Of Pregnancy: A Population-Based Study On 9.1 Million Pregnancies. Human Reproduction, 35, 1666-1674.

Min, L., Fink-Gremmels, J., Li, D., Tong, X., Tang, J., Nan, X., Yu, Z., Chen, W. & Wang, G. 2021. An Overview Of Aflatoxin B1 Biotransformation And Aflatoxin M1 Secretion In Lactating Dairy Cows. Animal Nutrition, 7, 42-48.

Mishra, K., Mawar, A., Kare, P. K. & Verma, N. 2015. Relationship Between Fasting Blood Glucose, Serum Urea, Serum Creatinine And Duration Of Diabetes In Type-2 Diabetic Patients. Flora Fauna, 21, 127-32.

Misihairabgwi, J., Ezekiel, C., Sulyok, M., Shephard, G. & Krska, R. 2019. Mycotoxin Contamination Of Foods In Southern Africa: A 10-Year Review (2007–2016). Critical Reviews In Food Science And Nutrition, 59, 43-58.

Mohammed Hussein, S. M. & Abdelmageed, R. M. 2021. The Relationship Between Type 2 Diabetes Mellitus And Related Thyroid Diseases. Cureus, 13, E20697.

Mohammed, S. N., Mohammed, D. N. A., Jassim, D. W. E. & 2022 Evaluate The Interconnection Between Some Gut Hormones And Insulin Resistance In Iraqi Patients With Type 2 Diabetic Mellitus.

Mohsenzadeh, M. S., Hedayati, N., Riahi-Zanjani, B. & Karimi, G. 2016. Immunosuppression Following Dietary Aflatoxin B1 Exposure: A Review Of The Existing Evidence. Toxin Reviews, 35, 121-127.

Moore, M. M., Schoeny, R. S., Becker, R. A., White, K. & Pottenger, L. H. 2018. Development Of An Adverse Outcome Pathway For Chemically Induced Hepatocellular Carcinoma: Case Study Of Afb1, A Human Carcinogen With A Mutagenic Mode Of Action. Critical Reviews In Toxicology, 48, 312-337.

Mor, F., Sengul, O., Topsakal, S., Kilic, M. A. & Ozmen, O. 2017. Diabetogenic Effects Of Ochratoxin A In Female Rats. Toxins, 9, 144.

Mushtaq, S., Daş, Y. K. & Aksoy, A. 2020. Analytical Methods For Determination Of Aflatoxin B1 In Animal Feeds And Feedstuffs. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 17, 173-179.

Navale, V., Vamkudoth, K. R., Ajmera, S. & Dhuri, V. 2021. Aspergillus Derived Mycotoxins In Food And The Environment: Prevalence, Detection, And Toxicity. Toxicology Reports, 8, 1008-1030.

Nduti, N. & Njeru, P. 2017. Aflatoxin Variations In Maize Flour And Grains Collected From Various Regions Of Kenya. African Journal Of Food, Agriculture, Nutrition And Development, 17, 11743-11756.

Nikooyeh, B. & Neyestani, T. R. 2016. Oxidative Stress, Type 2 Diabetes And Vitamin D: Past, Present And Future. Diabetes/Metabolism Research And Reviews, 32, 260-267.

Norlia, M., Jinap, S., Nor-Khaizura, M. A. R., Radu, S., Samsudin, N. I. P. & Azri, F. A. 2019. Aspergillus Section Flavi And Aflatoxins: Occurrence, Detection, And Identification In Raw Peanuts And Peanut-Based Products Along The Supply Chain. Frontiers In Microbiology, 10**,** 2602.

Noroozi Karimabad, M., Khalili, P., Ayoobi, F., Esmaeili-Nadimi, A., La Vecchia, C. & Jamali, Z. 2022. Serum Liver Enzymes And Diabetes From The Rafsanjan Cohort Study. Bmc Endocrine Disorders, 22**,** 127.

Noyan, A. 1993. Yaflamda Ve Hekimlikte Fizyoloji. Hormonlar, 977**,** 1033.

Núñez, A., Bedregal, P., Becerra, C. & Grob, L. F. 2017. [Neurodevelopmental Assessment Of Patients With Congenital Hypothyroidism]. Rev Med Chil, 145**,** 1579-1587.

Park, S., Lee, J.-Y., You, S., Song, G. & Lim, W. 2020. Neurotoxic Effects Of Aflatoxin B1 On Human Astrocytes In Vitro And On Glial Cell Development In Zebrafish In Vivo. Journal Of Hazardous Materials, 386**,** 121639.

Perros, P., Mccrimmon, R., Shaw, G. & Frier, B. 1995. Frequency Of Thyroid Dysfunction In Diabetic Patients: Value Of Annual Screening. Diabetic Medicine, 12**,** 622-627.

Pilařová, V., Plachká, K., Khalikova, M. A., Svec, F. & Nováková, L. 2019. Recent Developments In Supercritical Fluid Chromatography–Mass Spectrometry: Is It A Viable Option For Analysis Of Complex Samples? Trac Trends In Analytical Chemistry, 112**,** 212-225.

Pokharel, A., Webb, P., Andrews-Trevino, J., Lamichhane, A., Shrestha, R., Acharya, S., Davis, D., Baral, K., Wang, J.-S., Xue, K., Paudel, K. & Ghosh, S. 2021. Prevalence And Associated Factors Of Breastmilk Aflatoxin M1 Levels In Mothers From Banke, Nepal. Food Control, 126**,** 108069.

Popescu, R. G., Rădulescu, A. L., Georgescu, S. E. & Dinischiotu, A. 2022. Aflatoxins In Feed: Types, Metabolism, Health Consequences In Swine And Mitigation Strategies. Toxins, 14, 853.

Rachdaoui, N. 2020. Insulin: The Friend And The Foe In The Development Of Type 2 Diabetes Mellitus. Int J Mol Sci, 21.

Ráduly, Z., Szabó, L., Madar, A., Pócsi, I. & Csernoch, L. 2020. Toxicological And Medical Aspects Of Aspergillus-Derived Mycotoxins Entering The Feed And Food Chain. Frontiers In Microbiology, 10, 2908.

Ramalho, L. N. Z., Porta, L. D., Rosim, R. E., Petta, T., Augusto, M. J., Silva, D. M., Ramalho, F. S. & Oliveira, C. A. F. 2018. Aflatoxin B1 Residues In Human Livers And Their Relationship With Markers Of Hepatic Carcinogenesis In São Paulo, Brazil. Toxicology Reports, 5, 777-784.

Reid, C. X., Sparks, D. L., Williams, W. P. & Brown, A. E. 2016. Single Corn Kernel Aflatoxin B 1 Extraction And Analysis Method. Natural Resources, 7, 405.

Richard, S. A., Manaphraim, N. Y. & Kortei, N. K. 2020. The Novel Neurotoxic And Neuroimmunotoxic Capabilities Of Aflatoxin B1 On The Nervous System: A Review. Advances In Bioscience And Clinical Medicine, 8, 1-8.

Roden, M. & Shulman, G. I. 2019. The Integrative Biology Of Type 2 Diabetes. Nature, 576, 51-60.

Roep, B. O., Thomaidou, S., Van Tienhoven, R. & Zaldumbide, A. 2021. Type 1 Diabetes Mellitus As A Disease Of The Β-Cell (Do Not Blame The Immune System?). Nature Reviews Endocrinology, 17, 150-161.

Rufo, D. D., Debelee, T. G., Ibenthal, A. & Negera, W. G. 2021. Diagnosis Of Diabetes Mellitus Using Gradient Boosting Machine (Lightgbm). Diagnostics, 11, 1714.

Rushing, B. R. & Selim, M. I. 2019. Aflatoxin B1: A Review On Metabolism, Toxicity, Occurrence In Food, Occupational Exposure, And Detoxification Methods. Food And Chemical Toxicology, 124, 81-100.

Saad-Hussein, A., Shahy, E. M., Shaheen, W., Taha, M. M., Mahdy-Abdallah, H., Ibrahim, K. S., Hafez, S. F., Fadl, N. N. & El-Shamy, K. A. 2016. Comparative Hepatotoxicity Of Aflatoxin B1 Among Workers Exposed To Different Organic Dust With Emphasis On Polymorphism Role Of Glutathione S-Transferase Gene. Open Access Maced J Med Sci, 4, 312-8.

Saeed, Y. 2020. Aflatoxin B1 As An Endocrine Disruptor Among Miller Flour Workers. Xiv, 2020.

Salisu, B., Anua, S. M., Ishak, W. R. W. & Mazlan, N. 2021. Development And Validation Of Quantitative Thin Layer Chromatographic Technique For Determination Of Total Aflatoxins In Poultry Feed And Food Grains Without Sample Clean-Up. Journal Of Advanced Veterinary And Animal Research, 8, 656.

Sarma, U. P., Bhetaria, P. J., Devi, P. & Varma, A. 2017. Aflatoxins: Implications On Health. Indian J Clin Biochem, 32, 124-133.

Sartore, G., Ragazzi, E., Caprino, R. & Lapolla, A. 2023. Long-Term HBA1C Variability And Macro-/Micro-Vascular Complications In Type 2 Diabetes Mellitus: A Meta-Analysis Update. Acta Diabetologica, 1-18.

Schleicher, E., Gerdes, C., Petersmann, A., Müller-Wieland, D., Müller, U. A., Freckmann, G., Heinemann, L., Nauck, M. & Landgraf, R. 2022. Definition, Classification And Diagnosis Of Diabetes Mellitus. Experimental And Clinical Endocrinology & Diabetes.

Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., Del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J. C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx,

C., Marko, D., Oswald, I. P., Piersma, A., Routledge, M., Schlatter, J., Baert, K., Gergelova, P. & Wallace, H. 2020. Risk Assessment Of Aflatoxins In Food. Efsa J, 18, E06040.

Shabeer, S., Asad, S., Jamal, A. & Ali, A. 2022. Aflatoxin Contamination, Its Impact And Management Strategies: An Updated Review. Toxins, 14, 307.

Shahid, M. A., Ashraf, M. A. & Sharma, S. 2022. Physiology, Thyroid Hormone. Statpearls [Internet]. Treasure Island (Fl): Statpearls Publishing; 2023 Jan-. Available From.

Sharma, A. J., Addo, O. Y., Mei, Z. & Suchdev, P. S. 2019. Reexamination Of Hemoglobin Adjustments To Define Anemia: Altitude And Smoking. Annals Of The New York Academy Of Sciences, 1450, 190-203.

Sharma, S. & Tripathi, P. 2019. Gut Microbiome And Type 2 Diabetes: Where We Are And Where To Go? The Journal Of Nutritional Biochemistry, 63, 101-108.

Shibabaw, T., Dessie, G., Molla, M. D., Zerihun, M. F. & Ayelign, B. 2019. Assessment Of Liver Marker Enzymes And Its Association With Type 2 Diabetes Mellitus In Northwest Ethiopia. Bmc Research Notes, 12, 1-5.

Shuaib, F. M., Jolly, P. E., Ehiri, J. E., Jiang, Y., Ellis, W. O., Stiles, J. K., Yatich, N. J., Funkhouser, E., Person, S. D., Wilson, C. & Williams, J. H. 2010. Association Between Anemia And Aflatoxin B1 Biomarker Levels Among Pregnant Women In Kumasi, Ghana. Am J Trop Med Hyg, 83, 1077-83.

Singhal, S. S., Saxena, M., Awasthi, S., Ahmad, H., Sharma, R. & Awasthi, Y. C. 1992. Gender Related Differences In The Expression And Characteristics Of Glutathione S-Transferases Of Human Colon. Biochimica Et Biophysica Acta (Bba)-Gene Structure And Expression, 1171, 19-26.

Sirakov, M. & Plateroti, M. 2011. The Thyroid Hormones And Their Nuclear Receptors In The Gut: From Developmental Biology To Cancer. Biochimica Et Biophysica Acta (Bba) - Molecular Basis Of Disease, 1812, 938-946.

Somppi, T. L. 2017. Non-Thyroidal Illness Syndrome In Patients Exposed To Indoor Air Dampness Microbiota Treated Successfully With Triiodothyronine. Front Immunol, 8, 919.

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J. & Magliano, D. J. 2022a. Idf Diabetes Atlas: Global, Regional And Country-Level Diabetes Prevalence Estimates For 2021 And Projections For 2045. Diabetes Research And Clinical Practice, 183, 109119.

Sun, Y., Huang, K., Long, M., Yang, S. & Zhang, Y. 2022b. An Update On Immunotoxicity And Mechanisms Of Action Of Six Environmental Mycotoxins. Food And Chemical Toxicology, 163, 112895.

Tanase, D. M., Gosav, E. M., Costea, C. F., Ciocoiu, M., Lacatusu, C. M., Maranduca, M. A., Ouatu, A. & Floria, M. 2020. The Intricate Relationship Between Type 2 Diabetes Mellitus (T2dm), Insulin Resistance (Ir), And Nonalcoholic Fatty Liver Disease (Nafld). Journal Of Diabetes Research, 2020.

Tao, W., Li, Z., Nabi, F., Hu, Y., Hu, Z. & Liu, J. 2021. Penthorum Chinense Pursh Compound Ameliorates Afb1-Induced Oxidative Stress And Apoptosis Via Modulation Of Mitochondrial Pathways In Broiler Chicken Kidneys. Frontiers In Veterinary Science, 8, 750937.

Thomas, H. Y. & Ford Versypt, A. N. 2022. Pathophysiology Of Mesangial Expansion In Diabetic Nephropathy: Mesangial Structure, Glomerular

Biomechanics, And Biochemical Signaling And Regulation. J Biol Eng, 16, 19.

Thomson, C. D. 2003. Selenium | Physiology. In: Caballero, B. (Ed.) Encyclopedia Of Food Sciences And Nutrition (Second Edition). Oxford: Academic Press.

Thrasher, J. & Crawley, S. 2012. Neurotoxicity Of Mycotoxins. R Etrieved From. Drthrasher. Org/Page189. Html.

Valchev, I., Lazarov, L., Hristov, T., Kanakov, D., Binev, R. & Nikolov, Y. 2014. Blood Triiodothyronine, Thyroxine And Thyroid-Stimulating Hormone Concentrations In Mulard Ducks With Experimental Aflatoxicosis. Bulgarian Journal Of Veterinary Medicine, 17.

Van Der Spek, A. H., Fliers, E. & Boelen, A. 2017. The Classic Pathways Of Thyroid Hormone Metabolism. Molecular And Cellular Endocrinology, 458, 29-38.

Verma, R. 2004. Aflatoxin Cause Dna Damage. International Journal Of Human Genetics, 4, 231-236.

Wacoo, A. P., Wendiro, D., Vuzi, P. C. & Hawumba, J. F. 2014. Methods For Detection Of Aflatoxins In Agricultural Food Crops.

Wang, C., Li, Y. & Zhao, Q. 2019. A Signal-On Electrochemical Aptasensor For Rapid Detection Of Aflatoxin B1 Based On Competition With Complementary Dna. Biosensors And Bioelectronics, 144, 111641.

Wang, J., Tang, L., Glenn, T. C. & Wang, J.-S. 2016. Aflatoxin B1 Induced Compositional Changes In Gut Microbial Communities Of Male F344 Rats. Toxicological Sciences, 150, 54-63.

Wang, S.-Y., Herrera-Balandrano, D. D., Shi, X.-C., Chen, X., Liu, F.-Q. & Laborda, P. 2023. Occurrence Of Aflatoxins In Water And Decontamination Strategies: A Review. Water Research, 232, 119703.

Wang, Y., Liu, F., Liu, M., Zhou, X., Wang, M., Cao, K., Jin, S., Shan, A. & Feng, X. 2022a. Curcumin Mitigates Aflatoxin B1-Induced Liver Injury Via Regulating The Nlrp3 Inflammasome And Nrf2 Signaling Pathway. Food And Chemical Toxicology, 161, 112823.

Wang, Y., Liu, F., Zhou, X., Liu, M., Zang, H., Liu, X., Shan, A. & Feng, X. 2022b. Alleviation Of Oral Exposure To Aflatoxin B1-Induced Renal Dysfunction, Oxidative Stress, And Cell Apoptosis In Mice Kidney By Curcumin. Antioxidants, 11, 1082.

Wang, Y., Wang, Q., Ji, C., Guo, X., Yang, G., Wang, D., Weng, H., Qian, Y. & Chen, C. 2021a. Mixture Toxic Impacts And The Related Mechanism Of Aflatoxin B1 And Deoxynivalenol On Embryonic Zebrafish (Danio Rerio). Environmental Sciences Europe, 33, 1-15.

Wang, Y., Yang, P., Yan, Z., Liu, Z., Ma, Q., Zhang, Z., Wang, Y. & Su, Y. 2021b. The Relationship Between Erythrocytes And Diabetes Mellitus. Journal Of Diabetes Research, 2021.

Wondmkun, Y. T. 2020a. Obesity, Insulin Resistance, And Type 2 Diabetes: Associations And Therapeutic Implications. Diabetes Metab Syndr Obes, 13, 3611-3616.

Wu, B., Mughal, M. J., Fang, J. & Peng, X. 2019. The Protective Role Of Selenium Against Afb 1-Induced Liver Apoptosis By Death Receptor Pathway In Broilers. Biological Trace Element Research, 191, 453-463.

Yadav, S. K., Pathak, R., Singh, R. K. & Mahato, R. V. 2018. Correlation Of Body Mass Index With Waist Circumference, Random Blood Sugar And Dietary Pattern As Predictors Of Diabetes Mellitus. International Journal Of Applied Sciences And Biotechnology, 6, 274-278.

Zhang, H., Ni, J., Yu, C., Wu, Y., Li, J., Liu, J., Tu, J., Ning, X., He, Q. & Wang, J. 2019a. Sex-Based Differences In Diabetes Prevalence And Risk Factors: A Population-Based Cross-Sectional Study Among Low-Income Adults In China. Frontiers In Endocrinology, 10, 658.

Zhang, H., Yang, Z., Zhang, W., Niu, Y., Li, X., Qin, L. & Su, Q. 2017. White Blood Cell Subtypes And Risk Of Type 2 Diabetes. Journal Of Diabetes And Its Complications, 31, 31-37.

Zhang, J., Wang, P., Xu, F., Huang, W., Ji, Q., Han, Y., Shao, B. & Li, Y. 2020. Protective Effects Of Lycopene Against Afb1-Induced Erythrocyte Dysfunction And Oxidative Stress In Mice. Research In Veterinary Science, 129, 103-108.

Zhang, Q., Zhao, G., Yang, N. & Zhang, L. 2019b. Fasting Blood Glucose Levels In Patients With Different Types Of Diseases. Progress In Molecular Biology And Translational Science, 162, 277-292.

Zhang, Y., Wang, P., Kong, Q. & Cotty, P. J. 2021. Biotransformation Of Aflatoxin B1 By Lactobacillus Helviticus Fam22155 In Wheat Bran By Solid-State Fermentation. Food Chemistry, 341, 128180.

Zhou, J., Tang, L., Wang, J. & Wang, J.-S. 2018. Aflatoxin B1 Disrupts Gut-Microbial Metabolisms Of Short-Chain Fatty Acids, Long-Chain Fatty Acids, And Bile Acids In Male F344 Rats. Toxicological Sciences, 164, 453-464.

Zhou, R., Liu, M., Liang, X., Su, M. & Li, R. 2019. Clinical Features Of Aflatoxin B1-Exposed Patients With Liver Cancer And The Molecular Mechanism Of Aflatoxin B1 On Liver Cancer Cells. Environmental Toxicology And Pharmacology, 71, 103225.

Appendix

Appendix 1- Questionnaire

Questionnaire

Name								
Phone								
Address								
code								
Age								
Sex	**Male**		**Female**					
BMI		**Weight**		**Height**				
Smoking	**yes**		**No**					
Genetics of this disease								
Blood pressure	**Yes**		**No**					
Liver disease	**Yes**		**No**		**Type**			
Thyroid gland disease	**Yes**		**No**					
Dietary habits								
Diabetes mellitus	**Yes**		**No**		**Type**		**Duration**	
Treatment	**Yes**		**No**		**Type**			
FBS								
HBA1C								
AST								
ALT								
CBC								

Appendix 2:TLC Method

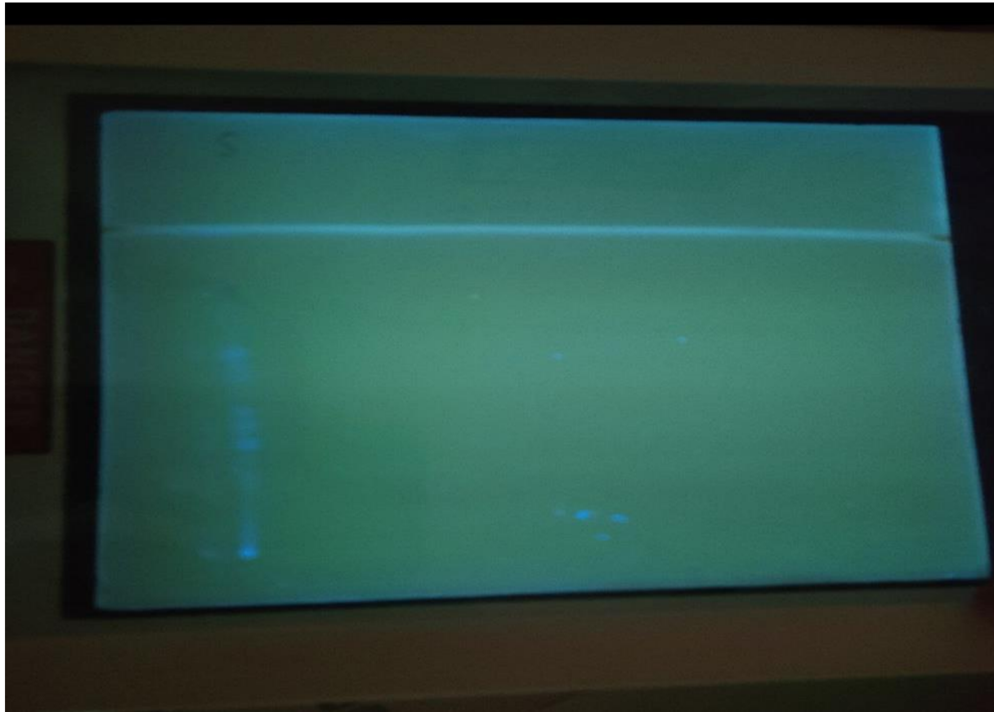


Figure.7.1 standard AFB1 by TLC

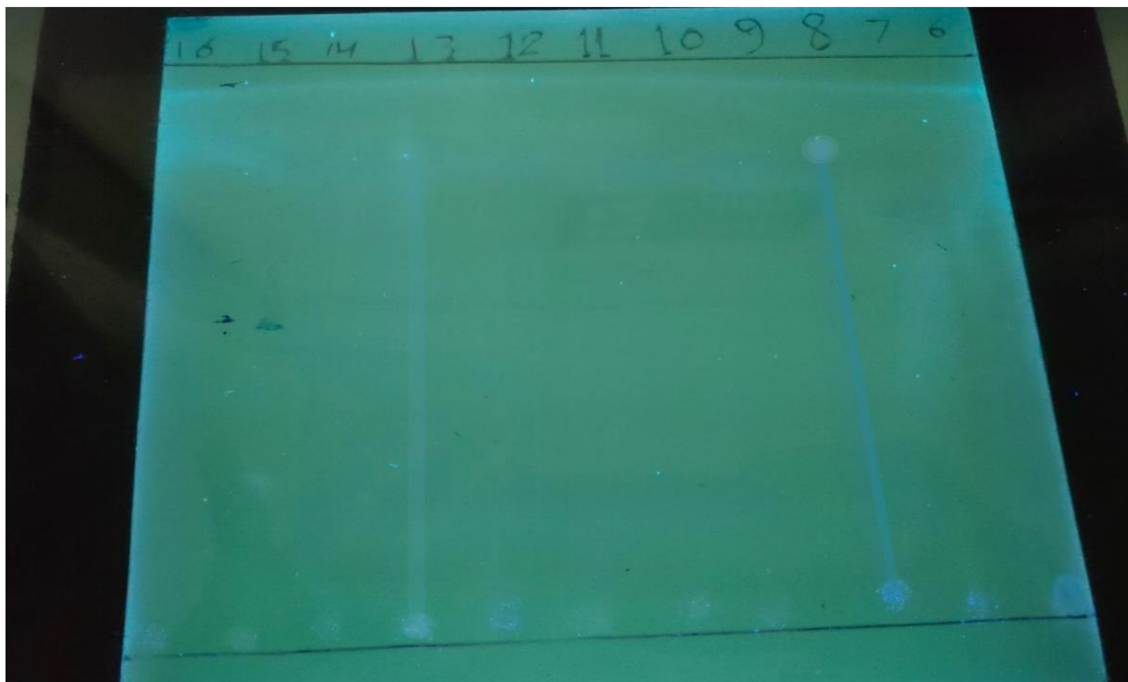


Figure 7.2 AFB1 positive sample by TLC in patient groups

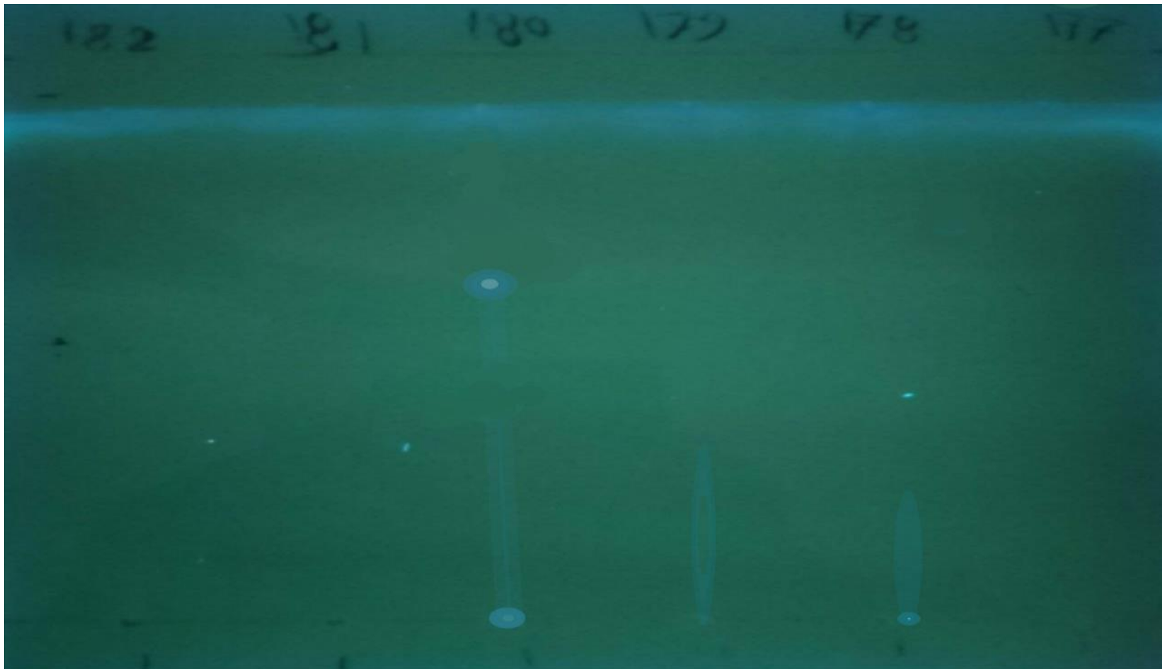


Figure 7.3 AFB1 positive by TLC in control groups

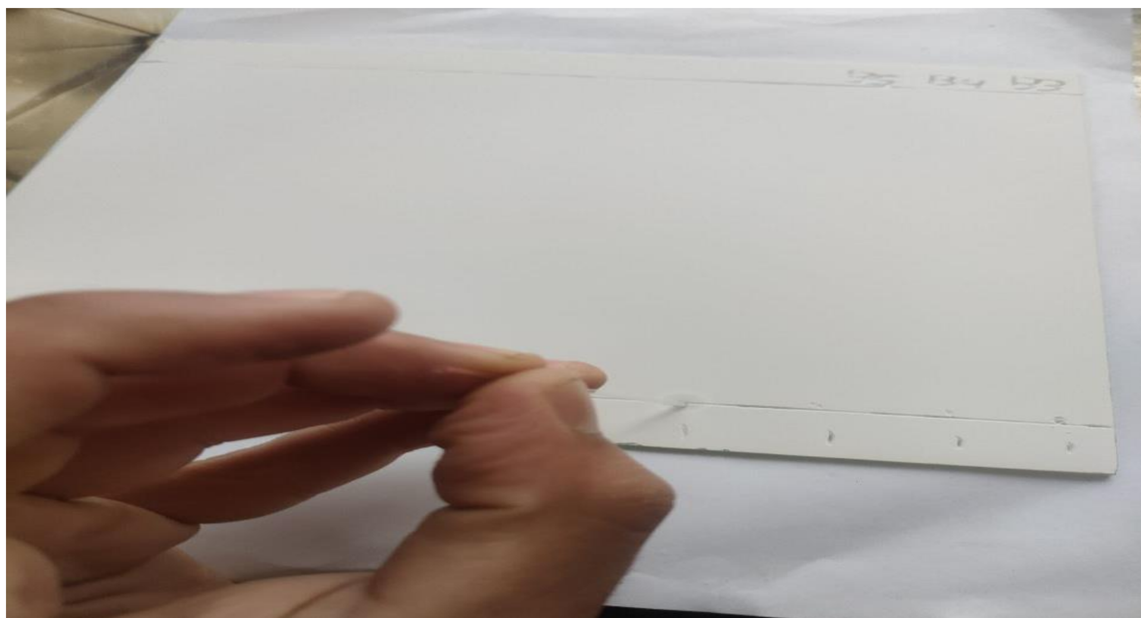


Figure 7.4 preparation of sample in TLC plate

Appendix 3 HPLC method

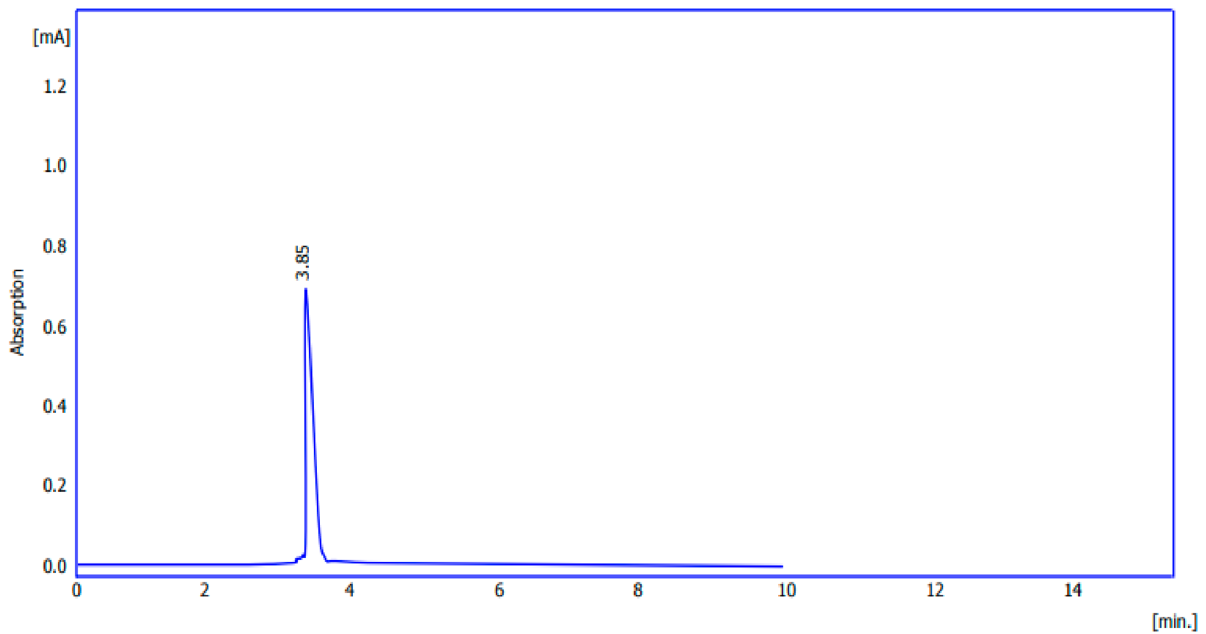
20/02/2023 01:40 م | Chromatogram F:\ aflatoxin B1 (0.25 ppb) .PRM | Page 1 of 1

Chromatography Laboratory

HPLC

Sample Info:

Sample ID	: aflatoxin B1 (0.25 ppb)	Amount	0
Sample	: aflatoxin B1 (0.25 ppb)	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ aflatoxin B1 (0.25 ppb)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.85	2598.08	788.08	100.00	100.00	0.25	
	Total	2598.08	788.08	100.00	100.00		

Figure 7.5 Standard AFB1 by HPLC method

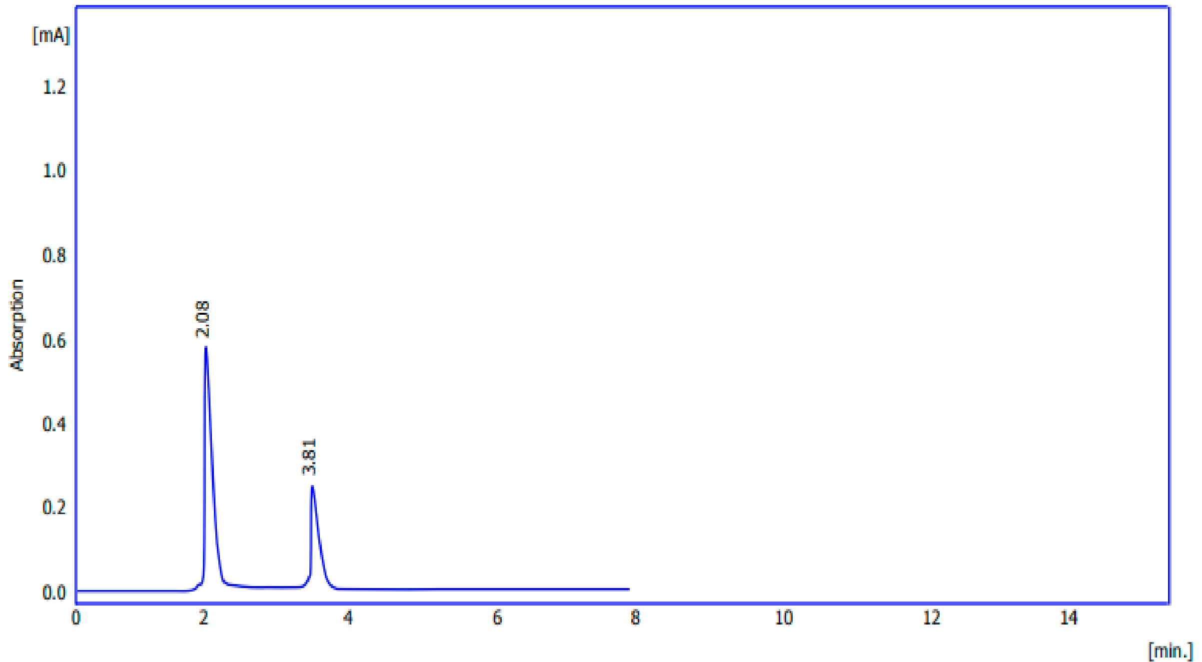
20/02/2023 01:40 م   Chromatogram F:\ control 1 .PRM   Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

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

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 1)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.08	8564.08	600.15	70.00	70.00	0.25	
2	3.81	1540.36	310.25	30.00	30.00	0.15	
	Total	10104.44	910.40	100.00	100.00		

Figure 7.5 concentration AFB1 positive in control groups

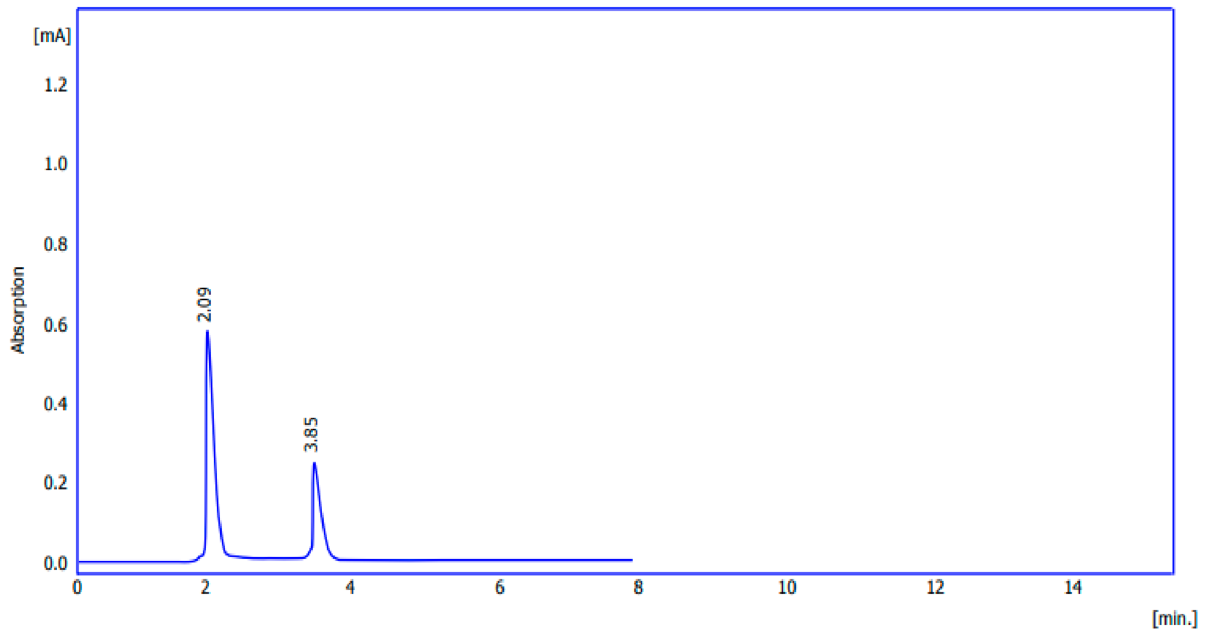
20/02/2023 01:40 م   Chromatogram F:\ control 2 .PRM   Page 1 of 1

Chromatography Laboratory

HPLC

Sample Info:

Sample ID	: control 2	Amount	0
Sample	: control 2	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 2)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.09	8620.14	600.01	70.00	70.00	0.25	
2	3.85	1398.08	310.33	30.00	30.00	0.15	
	Total	9951.06	910.34	100.00	100.00		

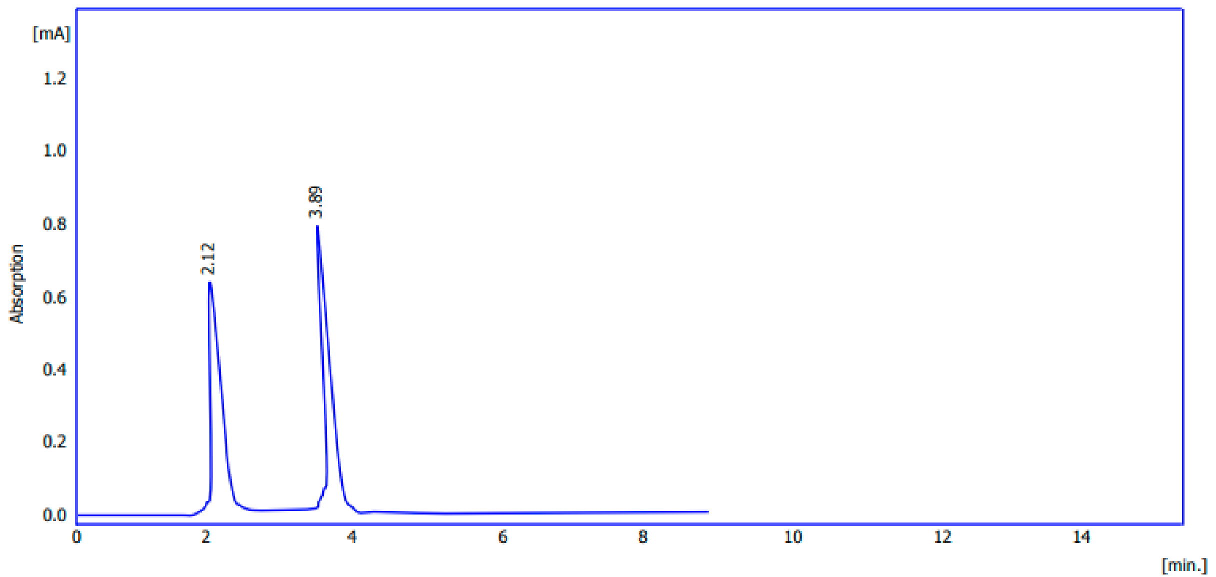
Figure 7.6 concentration AFB1 positive in control groups

21/02/2023 01:40 م   Chromatogram F:\ patient 1 .PRM   Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: patient 1	Amount	0
Sample	: patient 1	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 1)

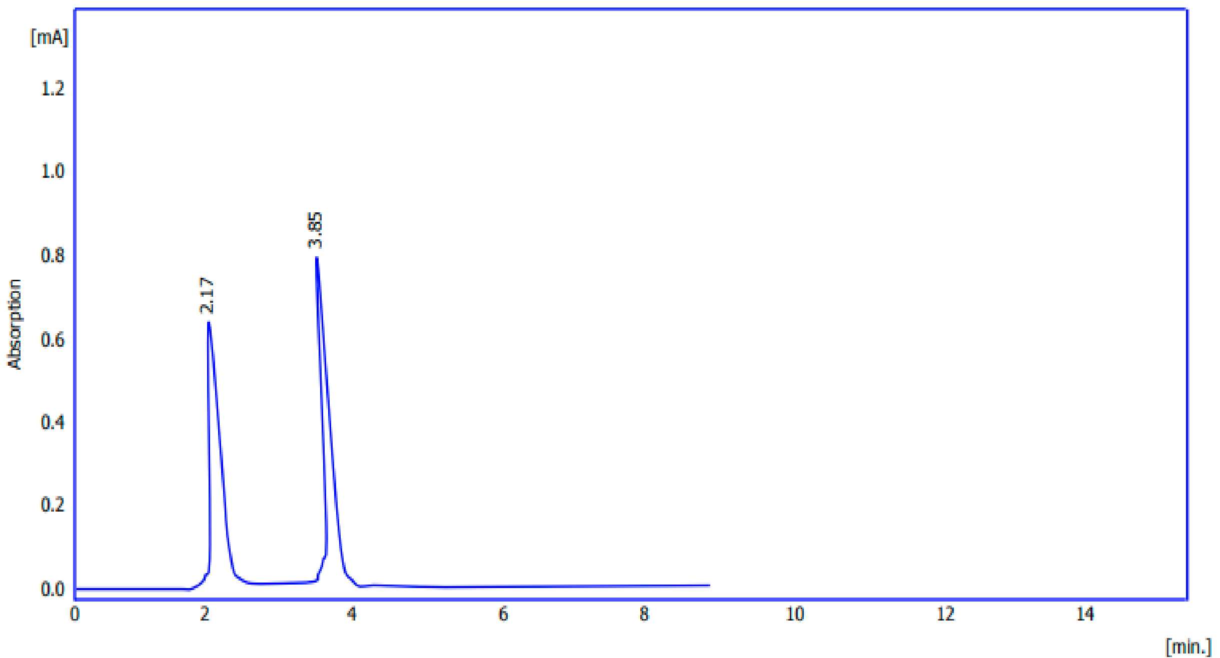
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.12	8230.12	611.49	45.00	45.00	0.25	
2	3.89	12652.08	812.65	55.00	55.00	0.30	
	Total	20882.20	1424.24	100.00	100.00		

Figure 7.7 concentration AFB1 positive in patient groups

21/02/2023 01:40 م Chromatogram F:\ patient 2 .PRM Page 1 of 1

Sample Info:

Sample ID	: patient 2	Amount	0
Sample	: patient 2	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ patient 2)

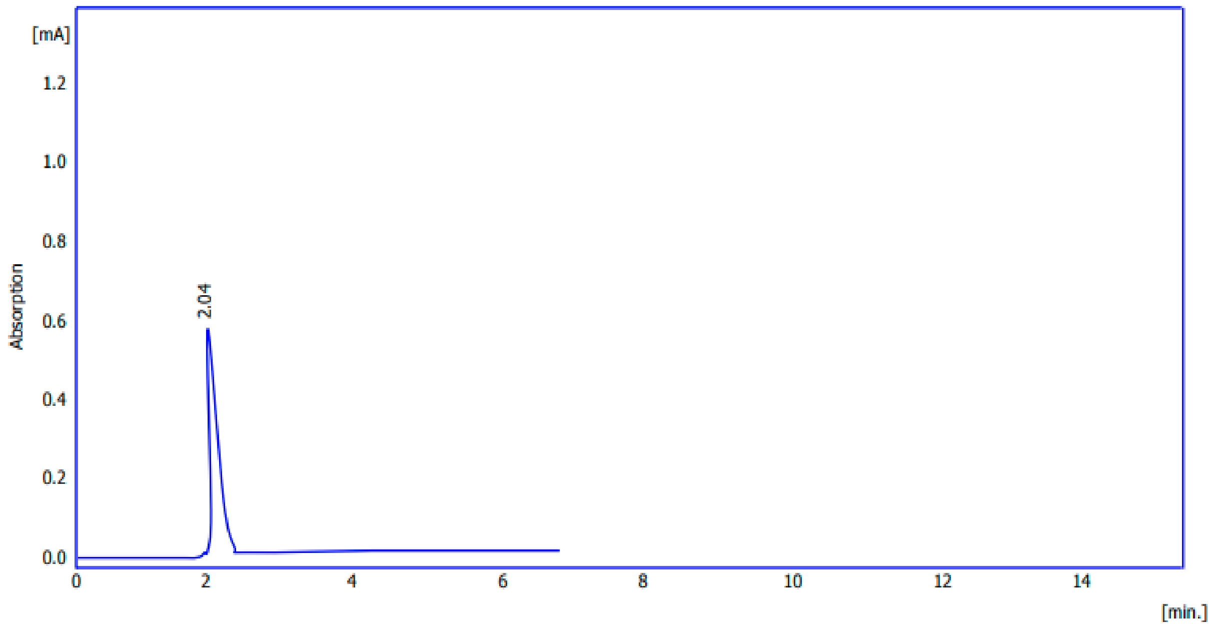
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.17	8451.08	611.04	45.00	45.00	0.25	
2	3.85	13652.00	812.11	55.00	55.00	0.30	
	Total	22103.08	1423.15	100.00	100.00		

Figure 7.8 concentration AFB1 positive in-patient groups

21/02/2023 01:40 م   Chromatogram F:\ control 11 .PRM   Page 1 of 1

Sample Info:

Sample ID	: control 11	Amount	0
Sample	: control 11	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ control 11)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.04	8611.08	591.25	100.00	100.00	0.25	
	Total	8611.08	591.25	100.00	100.00		

Figure 7.9 concentration AFB1 negative (zero) by HPLC

الخلاصة

مرض السكري من النوع الثاني هو نوع من الاضطرابات الايضية التي تؤدي الى ارتفاع السكر بسبب ضعف اما في انتاج الانسولين او مقاومة الانسولين او كلاهما. مقاومة الانسولين حالة تعني ان الاشخاص الذين لديهم السكري النوع الثاني ينتجون الانسولين لكن غير قادر على نقل الكلوكوز وإدخاله في خلاياهم. الانتشار العالمي لسكري يقدر في الاعمار من 20-79 سنة كان 10.5% (536.6 مليون) في سنة 2021 وربما يزداد الى 12.2% (782.2 مليون) في السنة 2045. العديد من العوامل تؤدي الى السكري النوع الثاني ومنها الاجهاد التاكسدي ، السمنة والطعام الملوث. سموم الافلاتوكسين1 B هي نواتج ثانوية ايضية من فطريات الرشاشيات بنوعيها فلافس والبراكتس هذه السموم تلوث الاغذية والأعلاف والعديد من المواد الغذائية مثل الارز والمعلبات و الفول السوداني وبعض الخضروات. الهدف من هذه الدراسة هو فحص مستويات الافلاتوكسين1 B في مصل دم مرضى السكري النوع الثاني والعلاقة بينهم في بعض المعلمات الفسلجية والكيميائية.

تصميم الدراسة كان الحالة-الضابطة وكان التبرع بالعينات من مستشفى الكندي التعليمي ومركز الغدد الصم. العدد كان 177 مشارك خضع للفحص 93 منهم مرضى السكري (44 ذكور و 49 اناث) و 84 (37 ذكور و 47 اناث) مجموعة ضابطة.

كل مشارك اخذ من وريده 10 مل من الدم وتم فصله في جهاز الطرد المركزي الى مصل. هذا المصل استخدم في تقنية الاليزا لفحص هرمونات الغدة الدرقية (T3,T4 and TSH) و تقنية (TLC,HPLC) استخدمت لقياس الكمي والنوعي لسموم الافلاتوكسين1 B بالإضافة الى استخدام جهاز صورة الدم الكاملة لقياس عدد الخلايا البيض والحمراء وأيضاً مستوى الهيموغلوبين و جهاز الفاين كير لقياس السكر التراكمي وكذالك تم فحص سكر الصائم و انزيمات الكبد (AST,ALT) و وظائف الكلى (S.Cr , B.Urea) بواسطة جهاز التحليل الذاتي الموجود في المستشفى. تم ادخال بيانات الدراسة في البرنامج الاحصائي (SPSS) اصدار 26 وتم مقارنة العديد من المجموعات باستخدام جدول انوفا (ANOVA) واختبار (Duncan) وكذالك تم المقارنة بين القيم المشاهدة والمتوقعة باختبار مربع سكوير (Chi-square) بالإضافة الى تحديد علاقة بين المجاميع باستخدام معامل الارتباط (r-Correlation).

اظهرت النتائج علاقة بين السموم الافلاتوكسين1 B وكل من مجموعات المرضى والضابطة. الذكور والإناث المرضى كان اعلى تركيز لديهم من سم الافلاتوكسين1 B (3.89 نانوغرام و 4.01 نانوغرام) على التوالي بينما الذكور والإناث في مجموعة الضابطة كان اعلى تركيز للسم (0.14 نانوغرام). الافلاتوكسين1 B اظهر علاقة ايجابية مع السكري النوع الثاني حيث سجلت الدراسة ارتفاع (AST, ALT ,B.Urea ,S.Cr , FBS and HBA1C) وكذالك ارتفاع (TSH, T4) وانخفاض (T3) بالإضافة الى زيادة في عدد الخلايا البيض ونقصان في الحمراء ومستوى الهيموغلوبين. كان هذا ناتج عن تأثير السكري النوع الثاني و الافلاتوكسين1 B على المعلمات الفسلجية والكيموحيوية.

المحصلة ان الاناث اكثر تحسس للسم من الذكور و اشارة نتائج الدراسة الى ان وجود اي نسبة من السم في الجسم ممكن ان تتراكم وتؤثر على الوظائف الحيوية للإنسان كذالك وجدت الدراسة التأثير السلبي في جميع المعلمات في التجربة وأظهرت مجموعات الدراسة التي احتوت على AFB_1 كيف يؤدي AFB_1 إلى ظهور مرض السكري من خلال ارتفاع مستويات FBS و HBA1C.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

العلاقة بين مرض السكري من النوع الثاني والأفلاتوكسين B1 وتأثيرهما على بعض المعايير الكيموحيوية للإنسان في محافظة بغداد

رسالة مقدمة إلى مجلس

كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل

شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

علاء عيدان سرحان

بكالوريوس تقنيات التحليلات المرضية / كلية الرشيد الجامعة 2018

بأشراف

أ.د سامي عبد الرضا الجميلي

صفر(1445)　　　　ايلول (2023)