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Impact of Chlorine and Cadmium on Genetic Instability and Histological Changes in Male Mice

A Thesis

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By

Khowla Sadiq Bahjat

B.Sc. Biology (2004)

Supervised by

Assist. Prof. Dr. Maytham Abdulkadhim Dragh

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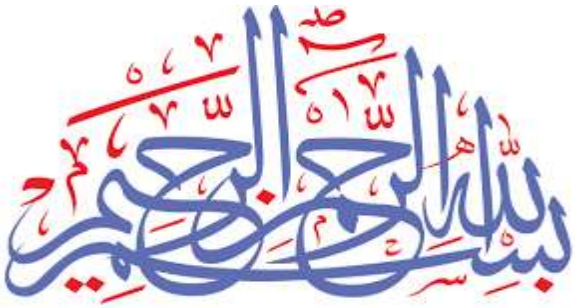

﴿ فَمَن يَعْمَلْ مِنَ الصَّالِحَاتِ
وَهُوَ مُؤْمِنٌ فَلَا كُفْرَانَ
لِسَعْيِهِ وَإِنَّا لَهُ كَاتِبُونَ ﴾

صَدَقَ اللهُ الْعَلِيُّ الْعَظِيمُ

سورة الانبياء الآية (٩٤)

Supervisor Certificate

I certify that this thesis entitled " Impact of Chlorine and Cadmium on Genetic Instability and Histological Changes in Male Mice) "

Submitted by (Khowla Sadie Bahjat) has been prepared under my supervision at the College of Science,
University of Misan; as partial fulfillment of the requirements for the Degree of Master in Biology.

Signature
Assist. Prof. Dr. Maytham Abdulkadhim Dragh

Department of Biology
College of Science/ University of Misan
Date: / /2023

Recommendation of Head of Biology Department
Because of the available recommendations; I forward this thesis to debate by the examining committee.

Signature
Assist. prof. Dr.Maytham Abdul Kadhim Dragh
Head of Department of Biology
College of Science/ University of Misan
Date: / /2023

Dedication

To my sunshine and moonlight, the man who taught me how to be, my beloved late father, may Allah have mercy on him.

To the one who has brought me more happiness and peace than her own, she is the epic of love, the joy of life, and the embodiment of devotion and selflessness... my tender mother.

To my support, my pride, my honor, and my inspiration... my dear brother Amir.

To my refuge, my dreams, and my companion on this journey... my beloved husband.

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Summary

Environmental pollutants significantly threaten living organisms, including humans and wildlife. Chlorine and cadmium are two such pollutants that have been extensively studied for their adverse effects on various biological systems. Chlorine is widely used in water treatment, industrial processes, and disinfection, while cadmium is a toxic heavy metal commonly found in industrial emissions, batteries, and contaminated soil. Exposure to these pollutants can lead to genetic instability and histological changes, which can have profound implications for overall health and well-being. In this study, we aimed to investigate the impact of chlorine and cadmium on genetic instability and histological changes in male mice, providing valuable insights into the potential risks associated with environmental exposure to these pollutants.

This study used seventy male mice obtained from the Alrazi Center in Baghdad as experimental animals. The mice were chosen as the animal model for their suitability for the research objectives. They had an average weight of 31 grams and were 10 to 12 weeks old. The mice were divided into seven groups, including those for chlorine and cadmium exposure and a control group. Under controlled environmental conditions, the animals were housed in cages and provided with standard rodent food and tap water ad libitum. Ethical considerations and proper

care were followed to ensure the welfare and ethical treatment of the animals. The mice were divided into seven groups: three subgroups for chlorine exposure (Cl_1, Cl_2, Cl_3), three subgroups for cadmium exposure (Cd_1, Cd_2, Cd_3), and one control group (Cg) not exposed to chlorine or cadmium. Chlorine exposure was administered at concentrations of 2.5 mg/l, 5 mg/l, and 10 mg/l, while cadmium exposure was performed at concentrations of 3 ppm, 6 ppm, and 12 ppm. The exposure duration was one month, during which the mice were given chlorine- or cadmium-contaminated drinking water.

Tissue samples from the testes and kidneys were collected for subsequent histopathological analysis and the evaluation of *TNF-α* gene expression. The exposure concentrations were adjusted based on the body weight of the mice to ensure accurate dosing and establish appropriate exposure levels. The expression levels of *TNF-α*, a pro-inflammatory cytokine, were analyzed to assess the impact of chlorine and cadmium exposure on genetic instability. The gene expression analysis revealed significant differences among the subgroups. In the chlorine exposure group, a dose-dependent increase in *TNF-α* expression was observed, with the highest expression at the chlorine concentration (Cl_3). An inverted U-shaped relationship was observed in the cadmium exposure group, with intermediate cadmium concentrations (Cd_1 and Cd_2) showing the highest *TNF-α* expression levels. These results indicate that chlorine and cadmium exposure can alter *TNF-α* gene

expression in male mice, suggesting the induction of inflammatory responses. Histopathological analysis of the testes and kidneys was performed to evaluate the histological changes induced by chlorine and cadmium exposure. The results showed significant alterations in tissue structure and morphology compared to the control group. In the chlorine exposure group, the higher chlorine concentrations (Cl_2 and Cl_3) caused severe damage, including degeneration, necrosis, and inflammatory cell infiltration. Similarly, in the cadmium exposure group, the higher cadmium concentrations (Cd_2 and Cd_3) resulted in extensive tissue damage, including tubular degeneration, interstitial inflammation, and fibrosis. These findings indicate that chlorine and cadmium exposure can lead to histological changes in male mice's reproductive and renal organs.

The present study provides important insights into the impact of chlorine and cadmium exposure on genetic instability and histological changes in male mice. The findings highlight the dose-dependent effects of chlorine exposure on *TNF-α* gene expression and the inverted U-shaped relationship observed with cadmium exposure. The histopathological analysis further confirms the detrimental effects of both pollutants on tissue structure and morphology. Understanding the adverse effects of chlorine and cadmium on genetic stability and tissue health is crucial for assessing the potential risks associated with environmental exposure.

Table of Contents

List of Tables

List of Figures

Table of Diagrams

List of Abbreviations

	Abbreviate	Definition
١	DNA	Deoxyribonucleic acid
٢	*TNF-∝*	Tumor necrosis factor alpha
٣	Gapdh	Glyceraldehyde3-phosphate dehydrogenase
٤	*IGF*	Insulin-like growth factor
٥	IGF-1-R	Insulin-like growth factor 1 receptor
٦	IGF-1-F	Insulin-like growth factor 1 factor
٧	PI3K	phosphoinositide 3-kinase (Li z.*et al.*,2022)
٨	AKT	is a serine/threonine kinase that play a critical role in the phosphoinositide3-kinase(PI3K)signaling pathway (Li z.*et al.*,2022)
٩	MAPK	Mitogen-activated Protein kinase
١٠	ERK	Extracellular signal-Regulated Kinase
١١	Cl	Chlorine
١٢	Cd	Cadmium
١٣	Cg	Control group
١٤	COPD	Chronic Obstructive Pulmonary disease
١٥	SSB_S	Single-Strand Breaks
١٦	DSB_S	Double-strand Breaks
١٧	BER	Base excision repair
١٨	NER	Nucleotide excision repair
١٩	HR	Homologous recombination repair
٢٠	NHEJ	Non-homologous end joining
٢١	HRR	Homologous recombination repair

٢٢	MMR	Mismatch repair
٢٣	MGMT	06-Methyl-guanine-DNA-methltransferase
٢٤	8-Cl-Dg	8-Chloro-2-deoxyguanosinne
٢٥	ROS	Reactive oxygen species
٢٦	P53	Protein 53 Pathway
٢٧	G2	G for gap, is 4N (tetraploid), The G1, S, and G2 phases collectively make up the interphase in the Cell Cycle. (Vincent W. Yang,2012)
٢٨	M phase	mitosis, in which the cell's chromosomes are equally divided between the two daughter cells. (Vincent W. Yang,2012)
٢٩	NF-Kb pathway	Nuclear factor-κB (NF-κB) represents a family of inducible transcription factors, which regulates a large array of genes involved in different processes of the immune and inflammatory responses. (Liu. Et al.,2017)
٣٠	RT-PCR	Real time polymerase chain reaction technique
٣١	RNA-Seq	Ribonucleic acid sequencing technique
٣٢	IGF-1 Ea	Types of Insulin Growth factor hormone
	IGF-1 Eb	
	IGF-1 Ec	
٣٣	$XRCC_4$ $DNA\text{-}PK_{CS}$	DNA repair enes
٣٤	mg	Milligrams
٣٥	L,l	Liter
٣٦	B.W.	Body Weight
٣٧	kg	Kilo Gram
٣٨	Ppm	Part per million
٤٠	V.	Volume
٤١	Wt.	weight
٤٢	TBE	Tris/Borate/EDTA (Buffer solution)
٤٣	Ct Values	Threshold Cycle Values
٤٤	SOD	Superoxide Dismutase
45	nM/L	Nanomolar /Liter
٤٦	NADPH	The nicotine amide Adenine Dinucleotide Phosphate

		Oxidase, Considered a major source of ROS in eukaryotic cell. (Vermot et al.,2021)
٤٧	C.T.	Connective Tissue
٤٨	c-JNK	c-Jun N terminal Kinase
٤٩	ARSD	Adult Respiratory Distress Syndrom
٥٠	WHO	The World Health Organization
٥١	THM_S	Trihalomethanes
٥٢	SNP	single nucleotide polymorphism
53	C	Cytosine
54	T	Thymine
55	A	adenine
56	G	Guanine
57	I	Isoleucine
58	S	Serine
59	A	Alanine
60	T	Threonine
61	D	Aspartic acid
62	G	Glysine
63	L	leucine
64	In.Del.	insertion /deletion polymorphism
65	μL	Microliter
66	ng	Nano gram
67	dNTP	Deoxynucleotide
68	ddNTP	Di-Deoxynucleotide

1. Introduction

Environmental pollution is a growing concern worldwide, particularly with the increasing industrialization and urbanization in developing countries. Environmental pollutants such as heavy metals and chemicals pose a significant threat to human health, as well as the health of other organisms. Exposure to such pollutants has been linked to various health problems, including genetic instability and pathological changes (Gangwar *et al.*, 2020).

Genetic instability and pathological changes are two significant parameters that determine the health status of an organism (Oliveira *et al.*, 2012). The genetic material of an organism is constantly exposed to various environmental stressors, such as chemical toxins, radiation, and infectious agents (Andrawus *et al.*, 2020). These stressors can cause DNA, RNA, and protein synthesis alterations, leading to genetic instability and pathological changes (Mangerich *et al.*, 2012; Niedernhofer *et al.*, 2018). Among the chemical toxins, chlorine and cadmium are two widespread environmental pollutants that have been shown to exert harmful effects on living organisms (Abha and Singh, 2012). When concentrations of some heavy metals increase exceed the permissible limit in water, it may pose a risk to human health (Elhdad, 2019, Jazza *et al*., 2022, Prasad *et al*., 2014)

Chlorine is a highly reactive gas widely used for hygiene purposes in water treatment, swimming pools, and industrial processes. However, accidental releases and improper use of chlorine can lead to environmental contamination and exposure to humans and animals. Chlorine exposure has been associated with respiratory problems, skin irritation, and eye damage in mice (Martin *et al.*, 2003).

In other animals, chlorine exposure has been reported to cause lung inflammation, oxidative stress, and DNA damage (Zellner and Eyer, 2020). Furthermore , cadmium is a toxic metal that occurs naturally in the environment but is also released into the environment through industrial processes, mining, and fossil fuel combustion which that exposure can occur through inhalation, ingestion, or skin contact. As well as is known to accumulate in various body organs, particularly the liver , testis and kidney tissues, which can cause toxic effects on these organs (de Angelis *et al.*, 2017; Bhattacharya, 2020; Genchi *et al.*, 2020). Over more, the exposure has been associated with various health problems, such as lung cancer, prostate cancer, and kidney damage (Satarug *et al.*, 2023).

Tumor necrosis factor alpha (*TNF-α*), glyceraldehyde 3-phosphate dehydrogenase (Gapdh), insulin-like growth factor 1 receptor (*IGF-1-R*), and insulin-like growth factor 1 factor (*IGF-1-F*) are four primers that can be used to assess the impact of chlorine and cadmium on genetic instability and pathological changes in Male mice (Xiao, 2015).

The *TNF-α* is a cytokine that is crucial in regulating inflammation and immune response (Wang and He, 2020). Which is produced by various cell types, including immune cells, and can activate multiple signaling pathways involved in cell survival, proliferation, and apoptosis (Bashir *et al.*, 2009). Furthermore, the *TNF-α* has been shown to be involved in tumor development and progression, and its overexpression has been linked to various types of cancer (Cruceriu *et al.*, 2020).

The *IGF-1-R* and *IGF-1-F* are two insulin-like growth factor (IGF) family members, which play crucial roles in cell growth, differentiation, and survival (Cheng *et al.*, 2021). The *IGF-1-R* is a transmembrane receptor that binds to *IGF-*

1-F and activates multiple signaling pathways, including the PI3K/AKT and MAPK/ERK pathways, which are involved in cell proliferation and survival (Roy *et al.*, 2010; Xiao, 2015). Dysregulation of the *IGF* system has been associated with various types of cancer, including breast, prostate, and colorectal cancer (Vella *et al.*, 2022).

Additionally, *IGF-1-R* and *IGF-1-F* have also been shown to play a role in the development and progression of tumors in Male mice (Aljada *et al.*, 2014). The impact of chlorine and cadmium on genetic instability and pathological changes in male mice has been previously studied which the chlorine exposure has been shown to induce DNA damage, inflammation, and oxidative stress in the lungs of Male mice (Mahrous *et al.*, 2015). Cadmium exposure has been linked to an increased incidence of liver tumors and alterations in liver histology in male mice (Jabeen *et al.*, 2022).

However, the underlying molecular mechanisms by which chlorine and cadmium exert their effects on genetic instability and pathological changes in Male mice remain unclear. The use of *TNF-α*, *IGF-1-R*, and *IGF-1-F* can provide valuable insights into the molecular changes induced by chlorine and cadmium exposure in Male mice.

1.1. The aim:

This study investigates the impact of chlorine and cadmium exposure on genetic instability, pathological changes, the expression of *TNF-α*, and identify genotypes *IGF-1* gene in male mice. Specifically, pathological changes in exposed mice's kidney, and testes. Additionally, the study aims to investigate the expression of *TNF-α*, and use identification primers to identify genotypes of *IGF-1* gene, in these organs to determine their potential role in the genotoxic effects of chlorine and cadmium exposure. The data obtained from this study will be

analyzed statistically to determine the correlation between genetic instability, pathological changes, and gene expression. The ultimate goal is to contribute to developing new strategies for preventing and treating pollution-induced diseases.

2. Literature review

2.1. Background

Chlorine and Cadmium are two environmental pollutants that are of growing concern due to their widespread use and potential health impact (Genchi *et al.*, 2020; Zellner and Eyer, 2020). The Chlorine is a highly reactive gas used in various industries, including water treatment, paper and textile production, and plastics manufacturing (Jordan *et al.*, 2020). However, the Cadmium is a toxic heavy metal in the Earth's crust and is released into the environment by mining, smelting, and manufacturing activities (Mousawi *et al.*, 2020).

Exposure to chlorine and cadmium can lead to various health problems, including respiratory distress, liver and kidney damage, and cancer (Barsouk *et al.*, 2021; Genchi *et al.*, 2020; Hsu *et al.*, 2019). The male reproductive system is susceptible to these toxicants, as studies have shown that they can cause alterations in sperm morphology, motility, viability, and reductions in testosterone levels (Ali Hameed *et al.*, 2022).

Animal models are often used to study the effects of environmental toxins on health, as they can provide insights into mechanisms of toxicity and potential human health effects, which; previous studies have demonstrated that exposure to chlorine and cadmium can cause genetic instability and histological changes in various organs, including the testes and kidneys (El-Beltagy *et al.*, 2019; Ghadi *et al.*, 2019).

2.2. Chlorine and Cadmium

2.2.1. Chlorine

Chlorine is a chemical element with the symbol Cl and atomic number 17, it is a highly reactive, toxic gas that is widely used in industry, agriculture, and water treatment (Achanta and Jordt, 2021; van der Veen *et al.*, 2021). Chlorine is commonly used as a disinfectant in water treatment, as it is highly effective at killing bacteria, viruses, and other microorganisms that can cause disease (Jin *et al.*, 2020). Furthermore, Chlorine is a highly reactive gas widely used in industry and as a bleaching agent for paper and textiles, as well as in the production of solvents, pesticides, and plastics (Adane *et al.*, 2021). Chlorine is highly toxic and can cause respiratory distress, eye and skin irritation, and respiratory and central nervous system damage (Morim and Guldner, 2021).

Generally, the Chlorine gas is released into the environment during accidents or leaks in industrial facilities, as well as during the transportation of hazardous materials, and can also be released into the environment through the use of chlorine-based disinfectants in water treatment plants, as well as in swimming pools and other recreational water sources. Moreover, the exposure to gas can cause a range of health effects, including irritation of the eyes, nose, and throat, as well as coughing, wheezing, and shortness of breath. Interestingly, at high concentrations, chlorine gas can be lethal. “Exposure to levels of 800 ppm proves lethal to half of all exposed animals” (Morim and Guldner, 2021). In addition to acute toxicity, chronic exposure to chlorine gas has been associated

with an increased risk of respiratory diseases, such as asthma and chronic bronchitis (Carder *et al.*, 2019).

Chlorine can also enter the body through the ingestion of contaminated food or water, which the ingestion of high chlorine levels can cause gastrointestinal distress, including nausea, vomiting, and diarrhea (Mitchell, 2021). As well as, long-term exposure to high chlorine levels in drinking water has also been associated with an increased risk of bladder and colon cancer (Mohsen *et al.*, 2019).

2.2.2. Cadmium

Cadmium is a naturally occurring element with the symbol Cd and atomic number 48. It is a heavy metal that is widely used in industry, including in the production of batteries, pigments, and plastics (McLaughlin *et al.*, 2021). Furthermore, it is a toxic heavy metal in the Earth's crust and is released into the environment by mining, smelting, and manufacturing activities (Suhani *et al.*, 2021). On the other hand, cadmium exposure can lead to various health problems, including respiratory distress, kidney and liver damage, and cancer (Genchi *et al.*, 2020). Cadmium can enter the body by inhaling contaminated air or ingesting contaminated food and water, which can also be absorbed through the skin (Shanmugaraj *et al.*, 2019; Wang, Chen, *et al.*, 2021). Moreover, Exposure to cadmium can occur through inhalation of dust or fumes, ingesting contaminated food or water, or skin contact with contaminated soil or water. Studies provide the smokers are particularly at risk for cadmium exposure, as tobacco plants are known to absorb cadmium from the soil (Li, Wallin, *et al.*, 2020; Shanmugaraj *et al.*, 2019)

Cadmium is highly toxic, and exposure can cause various health effects, including damage to the kidneys, lungs, and reproductive system. As well as, Chronic exposure to cadmium has been associated with an increased risk of lung cancer and prostate cancer (Fatima *et al.*, 2019).

Cadmium is particularly toxic to the kidneys, and long-term exposure can lead to renal dysfunction and kidney disease; furthermore, it can also accumulate in the liver, causing damage to this vital organ (Sotomayor *et al.*, 2021). In addition, exposure to cadmium can cause various other health effects, including anemia, hypertension, and bone disease (Satarug and Phelps, 2020).

2.3. Impact of Chlorine and Cadmium Exposure on Human Health

Exposure to these toxins has been linked to a variety of adverse health effects in humans, including respiratory problems, renal damage, and cancer which, Chronic exposure to chlorine has been associated with an increased risk of respiratory disease, such as asthma and chronic obstructive pulmonary disease (COPD), as well as skin irritation and eye damage (Morim and Guldner, 2021). Similarly, chronic exposure to cadmium has been associated with an increased risk of kidney disease, lung cancer, and prostate cancer (Satarug and Phelps, 2020).

The impact of chlorine exposure on human health has been a subject of interest in various experimental studies. One cross-sectional study by Font (Font-Ribera *et al.*, 2010) examined the respiratory effects of chlorine exposure in swimmers, revealing a correlation between higher levels of chlorine in pool water and increased respiratory symptoms as well as decreased lung function. Another cohort study conducted by Brown (1997) investigated the long-term health effects of occupational chlorine exposure among wastewater treatment plant workers.

Their findings indicated a significant association between chronic exposure to chlorine and the development of respiratory symptoms, along with a decline in lung function. Additionally, Nozaic (2004) conducted a randomized controlled trial focusing on individuals with chlorine sensitivity, where chlorine removal systems demonstrated a reduction in symptoms and improved overall quality of life. These studies highlight the potential adverse health consequences of chlorine exposure, emphasizing the need for further research and interventions to mitigate its impact on human health. The health effects of chlorine and cadmium exposure can be particularly significant for vulnerable populations such as children, pregnant women, and individuals with pre-existing health conditions. In addition, certain occupational groups such as industrial workers and agricultural workers may be at increased risk of exposure to these toxins. Several previous experimental studies have investigated this relationship and provided valuable insights. For instance, Viaene *et al.* (2000) conducted a cross-sectional analysis, revealing a negative association between urinary cadmium levels and kidney function. Similarly, a longitudinal cohort study by Wang *et al.* (2021)found an inverse relationship between urinary cadmium levels and bone mineral density in postmenopausal women. Furthermore, Kim *et al.* (2021) conducted a case-control study, indicating a positive correlation between blood cadmium levels and the risk of developing lung cancer. These studies highlight the potential health implications of cadmium exposure and underscore the need for further research in this area. By examining these previous experimental designs and their conclusions, this literature review aims to provide a comprehensive understanding of the impact of cadmium exposure on human health.

2.4. Animal Models for Studying Chlorine and Cadmium Exposure

The human health impact of exposure to chlorine and cadmium can be severe, depending on the level and duration of exposure.

The studies on Chlorine used animal models to investigate the acute and chronic effects of chlorine exposure (Oliveira *et al.*, 2012); for example, studies in rodents have demonstrated that exposure to high levels of chlorine gas can cause lung inflammation and oxidative stress, as well as alterations in gene expression in the respiratory tract (Achanta and Jordt, 2021; Zellner and Eyer, 2020). Chronic exposure to lower chlorine levels has been shown to cause respiratory dysfunction, including airway hyperresponsiveness, increased mucus production, and remodeling of the airway epithelium. The animal studies have also demonstrated that chlorine exposure can lead to systemic effects, including cardiovascular and immune dysfunction (Honavar *et al.*, 2011; White and Martin, 2010). On other hands, Animal models have been used to investigate the toxic effects of cadmium exposure on a range of organs and tissues, including the kidney, liver, lung, and reproductive system (White and Martin, 2010). Furthermore, last studies in rodents have shown that cadmium exposure can cause oxidative stress, inflammation, and cell death in these organs, leading to functional impairment and tissue damage. Animal models have also been used to study the effects of cadmium on bone health, cardiovascular function, and cancer development (Yang *et al.*, 2021).

2.5. Genetic Instability

Genetic instability is a condition that refers to the inability of the genetic material to maintain its integrity, leading to changes in the DNA sequence, chromosome number, or structure (Bristow and Hill, 2008; Yatsenko and Rajkovic, 2019). This instability can occur at different levels, including the nucleotide level,

where changes in the DNA sequence can result in base pair substitutions, insertions, or deletions, or at the chromosomal level, where mutations or alterations in chromosome number or structure can occur (Janz *et al.*, 2019; Wandt *et al.*, 2021).

2.5.1. Mechanisms of Genetic Instability

Genetic instability can result from various mechanisms, including DNA damage, DNA replication errors, and errors in DNA repair and recombination (Oliveira *et al.*, 2012). Furthermore, the DNA damage can occur due to exposure to mutagens such as chemicals, radiation, and viruses (Poetsch, 2020). These mutagens can cause DNA lesions, leading to changes in the DNA sequence (Nelson and Dizdaroglu, 2020). DNA replication errors can also occur due to mistakes in the DNA polymerase enzyme, which is responsible for copying the DNA during cell division (Vologodskii, 2022). These errors can result in the incorporation of incorrect nucleotides into the DNA sequence. DNA repair and recombination errors can occur due to defects in the enzymes responsible for repairing damaged DNA or maintaining genomic stability (Zhou *et al.*, 2021).

2.5.2. DNA Damage

The DNA damage can occur in different forms, including single-strand breaks (SSBs) (Mei *et al.*, 2020), double-strand breaks (DSBs) (Zhao *et al.*, 2020), base damage (Alhmoud *et al.*, 2020), and inter-strand crosslinks (Li *et al.*, 2022). Generally, the SSBs are the most common type of DNA damage, occurring up to 50,000 times per day in each human cell (Mei *et al.*, 2020; Wu *et al.*, 2021) . However, DSBs are less common but more severe, as they can lead to chromosome rearrangements and deletions (Schubert, 2021). Base damage can occur due to

chemical modification of DNA bases, which can lead to mispairing during replication (Lee and Kang, 2019). Inter-strand crosslinks occur when two strands of DNA are covalently linked together, which can prevent DNA replication and transcription (Renaudin and Rosselli, 2020). DNA damage can have various effects on cells, depending on the severity and type of damage and the efficiency of repair mechanisms. Mild DNA damage can be repaired efficiently, and the cell can continue with its normal functions (Lans *et al.*, 2019). However, if the damage is severe, it can lead to cell death or cancer. Furthermore, unrepaired DNA damage can also lead to mutations, accumulating over time and contributing to genetic instability (Kay *et al.*, 2019).

2.5.3. Repair Mechanisms for DNA Damage

To counteract DNA damage, cells have evolved various repair mechanisms that can recognize and fix different types of damage. These repair mechanisms include base excision repair (BER) (Caldecott, 2020), nucleotide excision repair (NER) (Duan *et al.*, 2020), homologous recombination (HR) (Sun *et al.*, 2020), non-homologous end joining (NHEJ) (Ensminger and Löbrich, 2020), and mismatch repair (MMR) (Gupta and Heinen, 2019). The BER and NER are involved in repairing base damage, while HR and NHEJ are involved in repairing DSBs (Tiwari and Wilson III, 2019). Furthermore, the MMR is engaged in correcting errors that occur during DNA replication (Bulock *et al.*, 2020),as shown in(figure-1) :

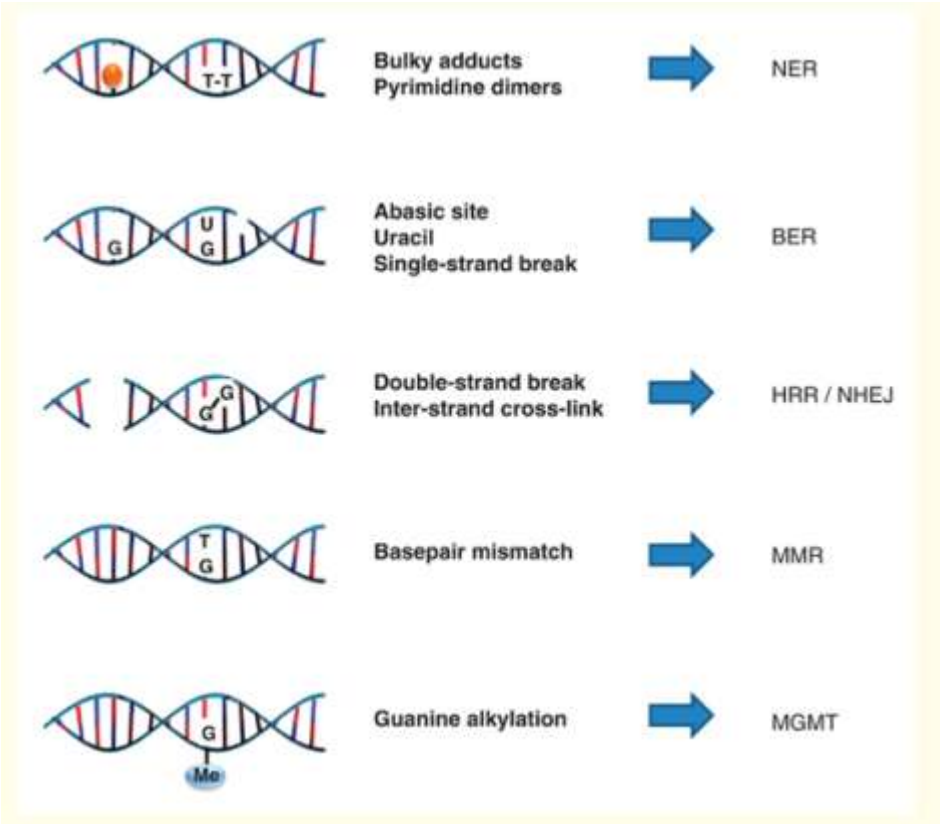


Figure 2-1: The DNA Repairing and its Mechanism in the Cell

Illustrates Mechanisms of DNA repair. Different classes of DNA damage and the cellular mechanisms involved in repairing these. NER, nucleotide excision repair; BER, base excision repair; HRR, homologous recombination repair; NHEJ, non-homologous end joining; MMR, mismatch repair; and MGMT, O6-methyl-guanine-DNA methyltransferase (Mitra, 2019)

2.5.4. Genotoxicity of Chlorine and Cadmium:

Genotoxicity is the ability of a substance to cause damage to genetic material, which can result in mutations, chromosomal aberrations, and ultimately, the development of cancer. Both chlorine and cadmium have been shown to exhibit genotoxic effects through various mechanisms.

Chlorine and cadmium can both induce genotoxicity through various mechanisms. As a powerful oxidizing agent, chlorine can cause oxidative damage to DNA, leading to the formation of DNA adducts and strand breaks (Caglayan *et al.*, 2019). Chlorine can also cause DNA damage through the formation of chlorinated DNA adducts, such as 8-chloro-2'-deoxyguanosine (8-Cl-dG) (Prasse *et al.*, 2020; Walker *et al.*, 2020). Additionally, chlorine can induce genotoxicity by promoting the formation of reactive oxygen species (ROS), which can also cause oxidative DNA damage (Feng *et al.*, 2023).

Similarly, cadmium can also cause genotoxicity through multiple mechanisms, including the production of ROS, inhibition of DNA repair mechanisms, and interference with DNA methylation (Waalkes, 2003). Forthermore, Cadmium exposure can lead to the formation of DNA adducts, DNA strand breaks, and oxidative DNA damage (Lee *et al.*, 1995). The Cadmium can also interfere with DNA repair mechanisms, accumulating unrepaired DNA damage, which can result in mutations and genomic instability (Manigandan *et al.*, 2015). Moreover, cadmium can induce epigenetic changes (Mahrous *et al.*, 2015). and (Figure 2-2) shows the effect of cadmium on mitochondria and the activity of enzyme:

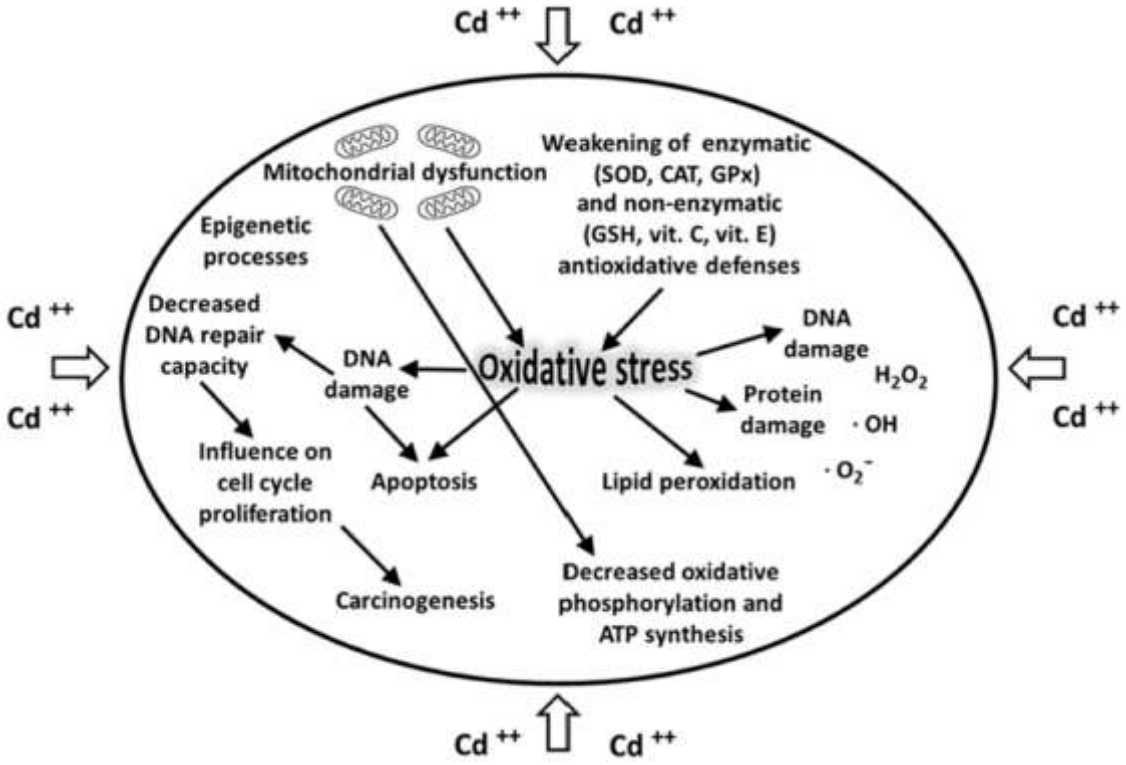


Figure 2-2: Cadmium acts on mitochondria, awakening the enzymatic and non-enzymatic antioxidative defenses . (Gench *et al.*,2020)

Mitochondria are the cell's energy-producing organelles and are particularly vulnerable to oxidative damage. Studies have shown that cadmium exposure can increase the production of reactive oxygen species (ROS) in the mitochondria. This increase in ROS production can cause damage to the mitochondrial DNA, lipids, and proteins to counteract the damage caused by ROS, cells have evolved antioxidative defense mechanisms (Gobe and Crane, 2010). These defense mechanisms include enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase and non-enzymatic antioxidants such as glutathione, vitamins C and E, and carotenoids (Shaito *et al.*, 2020). Cadmium

exposure has been shown to activate these antioxidative defense mechanisms in the mitochondria, both enzymatic and non-enzymatic, in an attempt to counteract the increased ROS production. However, if the level of ROS overwhelms the antioxidative defenses, it can lead to mitochondrial dysfunction and cell death. Therefore, while cadmium initially activates antioxidative defenses in the mitochondria, prolonged or excessive exposure can ultimately lead to mitochondrial damage and dysfunction (Luca *et al.*, 2015). Several studies have investigated the genotoxic effects of chlorine and cadmium exposure in various experimental models. For instance, a study conducted by Han et al. (2017) investigated the genotoxic effects of chlorine in vitro using human lung fibroblast cells. The study found that exposure to chlorine resulted in DNA damage, including DNA strand breaks and the formation of ROS. Additionally, a study by Manigandan *et al*.(2015) reported that chlorine exposure induced DNA damage and oxidative stress in human bronchial epithelial cells.

Previous studies have shown that exposure to heavy metals, such as cadmium, can activate protease caspases and induce apoptosis in hepatocytes, as illustrated in (Figure 2-3). Additionally, the shift in redox balance towards oxidative stress caused by Cd-toxicity can promote the transition process of mitochondrial permeability, which is also associated with apoptosis. A study on nickel toxicity observed that endoplasmic reticulum stress led to apoptosis via the protein 53 (p53) pathway, resulting in cell cycle arrest at the G2/M phase, and the activation of the NF-kB pathway induced an inflammatory response. This inflammatory response and cell cycle arrest in the G2/M phase may also contribute to apoptosis (Genchi *et al.*, 2020; Renu *et al.*, 2021).

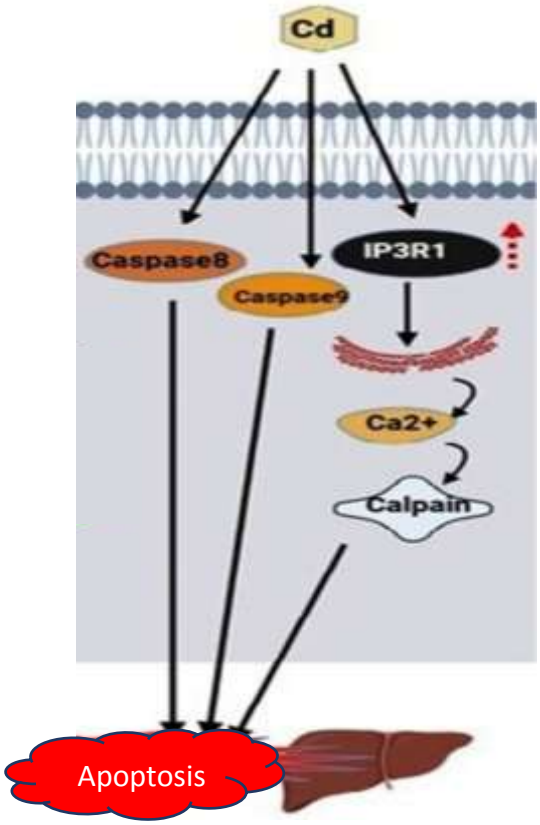


Figure 2-3 : shows As, Ni and Cd induced apoptosis in liver (Renu *et al.*, 2021)

2.6. Histopathological effect of cadmium and chloride

Environmental toxins like chlorine and cadmium exposure can lead to severe histological changes in the testes and kidneys (Damek-Poprawa and Sawicka-Kapusta, 2004; Nna *et al.*, 2017). These changes can negatively impact the functioning of these organs, leading to various health complications. In this section, we will discuss the effects of chlorine and cadmium on histology, specifically on the testicular and renal tissue.

2.6.1. Histopathological effect on Testicular Histology

The testes produce sperm and testosterone, and histological changes in this organ can lead to male infertility. Exposure to chlorine and cadmium has been

shown to cause histological changes in the testes, including reduced seminiferous tubule diameter, decreased numbers of germ cells, and impaired Leydig cell function (Chen *et al.*, 2023; Damek-Poprawa and Sawicka-Kapusta, 2004; Ghosh and Indra, 2018; Soni, 2017; de Souza Predes *et al.*, 2010).

2.6.1.1. Chlorine

Exposure to chlorine has been linked to significant testicular damage in both animals and humans, which, the animal studies, exposure to chlorine has been shown to cause a reduction in testicular weight and size, as well as a decrease in the number of spermatogonia, which are the cells responsible for sperm production (Franca *et al.*, 2006). This damage has been attributed to the ability of chlorine to induce oxidative stress and inflammation in testicular tissue, leading to DNA damage and cell death (Antar *et al.*, 2022). Furthermore, Chlorine exposure has also been shown to alter the expression of genes involved in testicular function, including those involved in spermatogenesis and steroidogenesis (Nasiri *et al.*, 2021; Zannino *et al.*, 2023).

In humans, exposure to chlorine has been associated with decreased sperm quality and motility, as well as a higher incidence of abnormal sperm morphology (Aghashahi *et al.*, 2020); furthermore, the study of male swimmers found that exposure to chlorinated swimming pools was associated with a decrease in sperm concentration and motility (Massányi *et al.*, 2020; Zeng *et al.*, 2014). Another study of male workers exposed to chlorine in a chemical plant found that they had a higher incidence of abnormal sperm morphology compared to unexposed workers (Jurewicz *et al.*, 2009).

2.6.1.2. Cadmium

Exposure to cadmium has also been shown to cause significant damage to testicular tissue (Bhardwaj *et al.*, 2021). In animal studies, exposure to cadmium has been linked to reduced testicular weight and size and decreased spermatogonia and Leydig cells responsible for testosterone production (de Souza Predes *et al.*, 2010). This damage has been attributed to the ability of cadmium to induce oxidative stress, inflammation, and apoptosis in testicular tissue (Bhardwaj *et al.*, 2021; Momeni and Eskandari, 2020). In humans, exposure to cadmium has been associated with decreased sperm quality, including reduced sperm concentration, motility, and viability. Cadmium exposure has also increased the incidence of abnormal sperm morphology and DNA damage. One study of infertile men found that they had higher levels of cadmium in their seminal plasma compared to fertile men, suggesting a possible link between cadmium exposure and male infertility (Wang *et al.*, 2020).

2.6.2. Histological Changes in Kidney

Both chlorine and cadmium exposure have been shown to cause histological changes in the kidney; since, the Chlorine exposure has been reported to cause tubular injury and necrosis, as well as interstitial fibrosis and inflammation, on the other hands, Cadmium exposure has been shown to cause proximal tubular damage, glomerular damage, and interstitial fibrosis (Kandemir *et al.*, 2020; Wang *et al.*, 2020). The severity of these changes depends on the dose, duration, and route of exposure.

2.6.3.1 Chlorine

Several studies have investigated the effects of chlorine exposure on renal histology. A study by Kuzuwa (Kuzuwa *et al.*, 2012)found that exposure to chlorine gas caused renal tubular injury and necrosis in rats. Another study Gritzka and Trump (Gritzka and Trump, 1968) reported that exposure to chlorine caused tubular damage and inflammation in mice. In addition, Sram et al. (2014) found that individuals exposed to chlorine at a water treatment plant had increased urinary biomarkers of kidney injury (Siew *et al.*, 2011).

2.6.3.2 Cadmium

Cadmium exposure has also been shown to cause histological changes in the kidney. A study by Embaby (Embaby *et al.*, 2020) found that cadmium exposure caused glomerular damage and proteinuria in rats. Another study by Alshammari (Alshammari *et al.*, 2021) reported that exposure to cadmium caused renal tubular damage and interstitial fibrosis in rats. Furthermore, studies reported that cadmium exposure caused proximal tubular damage and interstitial fibrosis in humans (Dudley *et al.*, 1985).

2.7. Gene Expression Analysis

2.7.1. Role of Gapdh in Gene Expression Analysis

Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) is an important enzyme involved in the glycolytic pathway, which catalyzes the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (Li, Tan, *et al.*, 2020). Apart from its metabolic functions, Gapdh has been widely used as a reference gene in gene expression analysis studies due to its stable expression across different tissues and cell types (Jeon *et al.*, 2020).

Gapdh is involved in several other cellular functions, including DNA repair, apoptosis, and transcriptional regulation. Recent studies have suggested that Gapdh can also act as a signaling molecule and modulate cellular responses to various external stimuli, including environmental toxins (Chaudhary *et al.*, 2021; Jedrzejczak-Silicka *et al.*, 2021).

2.7.2. Techniques Used for Gene Expression Analysis

Several techniques are used for gene expression analysis, including real-time PCR and RNA sequencing.

2.7.2.1 Real-Time PCR

Real-time PCR is a sensitive and specific method for quantifying gene expression levels, which, in this technique, a fluorescent signal is generated during the amplification of the target gene, which is measured in real-time using specialized instrumentation. The fluorescence intensity is directly proportional to the amount of product formed during each PCR cycle, and the target gene's relative expression levels are calculated using reference genes, such as Gapdh (Fraga *et al.*, 2008).

2.7.2.2. RNA Sequencing

RNA sequencing (RNA-seq) is a high-throughput sequencing technique that identifies and quantifies all RNA molecules in a given sample. RNA-seq can provide a comprehensive view of the transcriptome and help identify differentially expressed genes in response to various environmental stimuli (Kukurba and Montgomery, 2015).

2.8. *TNF-α*

Tumor Necrosis Factor alpha (*TNF-α*) is a cytokine that is crucial in regulating the immune response to infections and inflammation (Laha *et al.*, 2021). Additionally, produced by various immune cells, including macrophages, monocytes, and T-cells, and acts as a pro-inflammatory mediator; furthermore, *TNF-α* is involved in multiple physiological and pathological processes, such as the development of cancer, autoimmune diseases, and infectious diseases (Shapouri&Moghaddam *et al.*, 2018).

2.8.1. *TNF-α* and Genotoxicity

Exposure to chlorine and cadmium has been shown to induce the production of *TNF-α* in various tissues. In particular, exposure to cadmium has been shown to increase *TNF-α* expression in the kidney and liver of experimental animals(de Genaro *et al.*, 2021). Chlorine exposure has also been shown to induce the production of *TNF-α* in the lung tissue of mice (Lai *et al.*, 2019). Exposure to chlorine and cadmium has been shown to induce the production of *TNF-α* in various tissues. In particular, exposure to cadmium has been shown to increase *TNF-α* expression in the kidney and liver of experimental animals. Chlorine exposure has also been shown to induce the production of *TNF-α* in the lung tissue of mice (Bhardwaj *et al.*, 2021; Mawas *et al.*, 2022).

2.9. *IGF-1-F* and *IGF-1-R*

Insulin-like growth factor-1 (*IGF-1*) is a polypeptide hormone that regulates cell growth, differentiation, and apoptosis in various tissues. *IGF-1* exerts its effects by

binding to its receptor (*IGF-1R*), which is a transmembrane tyrosine kinase receptor that activates several downstream signalling pathways. In addition to *IGF-1R*, *IGF-1* can bind to a family of six insulin-like growth factor-binding proteins (IGFBPs), modulating *IGF-1* bioavailability and activity (Imrie *et al.*, 2012; Rigiracciolo *et al.*, 2020).

IGF-1R is expressed in various tissues, including liver, muscle, adipose tissue, and bone. *IGF-1R* activation has been shown to promote cell proliferation, survival, and migration. In addition, *IGF-1R* signaling is involved in regulating glucose and lipid metabolism, and it has been implicated in the pathogenesis of various diseases, including cancer and diabetes (Boucher *et al.*, 2016; Coleman *et al.*, 1994).

IGF-1-F (also known as *IGF-1Ea*) is one of the two major isoforms of *IGF-1*, the other being *IGF-1-EC* (also known as *IGF-1Eb*). *IGF-1-F* is expressed predominantly in the liver, and its expression is regulated by growth hormone. *IGF-1-F* has been shown to play a role in liver regeneration, muscle hypertrophy, and bone growth (Bailey-Downs *et al.*, 2012).

2.9.1. *IGF-1-F* and *IGF-1-R* and Chlorine/Cadmium Exposure

IGF-1 and *IGF-1R* signaling have been shown to play a role in the DNA damage response and repair processes. In particular, *IGF-1R* signaling has been shown to activate the DNA damage response kinases ATM and ATR, leading to the phosphorylation of several downstream targets involved in DNA repair, such as p53 and BRCA1. *IGF-1* has also been shown to regulate the expression of several DNA repair genes, including XRCC4 and DNA-PKcs (Hinkal and Donehower, 2008; Poreba and Durzynska, 2020).

Some evidence suggests that exposure to environmental toxins, such as chlorine and cadmium, may affect *IGF-1* and *IGF-1R* signaling and disrupt DNA repair processes. For example, one study showed that exposure to cadmium decreased *IGF-1R* expression in the liver, which was associated with impaired DNA repair capacity. Another study showed that exposure to chlorine led to a decrease in *IGF-1* expression in the liver, which was associated with oxidative stress and DNA damage (Malin Igra *et al.*, 2019; Mazhitova *et al.*, 1998).

Further research is needed to fully understand the mechanisms by which chlorine and cadmium affect *IGF-1* and *IGF-1R* signaling and DNA repair processes and their potential implications for health and disease.

3. Materials and Methods

3. Materials and Methods

3-1- Chemicals, Apparatus and Instruments used

3-1-1-Chemicals

Table (3-1): Shows the origin and names of the chemicals used in this study

No.	Name chemicals	Country	Company
1	Agarose LE	Korea	Intron
2	Absolute ethanol	Germany	
3	Canada Balsam	Germany	Roth
4	Chloroform	Switzerland	Sigma
5	DNA extraction kit (blood)	Iran	SIM Bio Lab,
6	Eosin	England	BDH
7	Formalin	England	BDH
8	G2 Master Mix		Denmark
9	Glucose kit	England	
10	HbA1c	England	
11	Human protein kinase A ELISA kit	China	
12	insulin ELISA kit	China	
13	Nuclease free water	Korea	Bioneer
14	Primers		Macrogen
15	Proteinase K		Promega
16	Red safe	Korea	Intron
17	Safe-Green 100bp Opti-DNA Marker	Canada	ABM
18	TBE buffer	China	Sun long
19	Xylene	England	BD

3-1-2- Instruments

No	Name instruments	Country	Manufacture Company
1	Afias		England
2	Agarose	England	Cleaver
3	Balance		(Germany)
4	Cotton	China	Citioglas
6	Cover slip	USA	Klempa
7	Disposable syringe (5 ml)		China
8	Distiller	Germany	GFL
9	EDTA tube	China	Vacuette
10	ELISA reader and washer	USA	Biotek
11	Eppendorf tube (1.5 µl)		China
12	Ethanol		Chia
13	Ethidium bromide		Korea
14	Filter paper	China	Whatman
15	Glove	China	Broche
16	Incubator	German	Fisher cient
17	Knife of Microtome	Korea	LG
18	master mix kit		Russia
19	Micro pipetes	Germany	DRAGON
20	Micropipettes (5-50 µl), (2-20 µl), (20-200 µl), (100-1000 µl) Slamed / Germany	Germany	Slamed

21	Multichannel micropipette (0-250 µl)	Germany	Slamed
22	PCR	Germany	Biometra
23	pipette tips 0.2 ml		China
24	pipette tips 1 ml		China
25	Plastic cage	Iran	Kajeen
26	Plastic cups	China	Shanghai Blopak
27	Round flask	Germany	ISOLAB
28	Shaker-centrifuge		Koria
29	Slide	China	Citioglas
30	Spectrophotometer		UK
31	Surgical kite	India	Hebson
32	Syringe	China	Citioglas
33	Test tube with separation gel	Jordan	AFCO
34	Vortex (Electronic)	Korea	Bionex
35	Water bath	Germany	Memmert

3.2. Experimental Animals

Seventy male mice were used as experimental animals in this study. The mice were obtained from the Alrazi center in Baghdad and housed in the College of Science, Misan University animal facility. The mice had an average weight of 31 grams and were 10 to 12 weeks old. They were chosen as the animal model due to their suitability for the research objectives. The mice were divided into seven groups, including those for chlorine and cadmium exposure and a control group. Proper care and ethical considerations were followed throughout the study to ensure the welfare and ethical treatment of the animals. The mice were housed in cages with a maximum of five mice per cage. The cages were maintained under

controlled environmental conditions, including a 12-hour light/12-hour dark cycle. The mice were provided with standard rodent food, which consisted of a mixture of 50% wheat, 30% fish, 13% greens, 2% sodium chloride (NaCl), and 5% raw fat. The food was available ad libitum throughout the study period. Additionally, tap water was provided to the mice for drinking purposes. The animals were handled in accordance with the institutional guidelines and the local animal ethics committee approved all experimental procedures to ensure compliance with ethical standards and animal welfare (Couto and Cates, 2019).

3.3. Design of the Study:

A total of 70 male mice, with an average weight of 31 grams and an age range of 10-12 weeks, were used in the study. The mice were divided into seven groups: three subgroups for chlorine exposure (Cl_1, Cl_2, Cl_3), three subgroups for cadmium exposure (Cd_1, Cd_2, Cd_3), and one control group (Cg) not exposed to chlorine or cadmium.

Chlorine Group:

Subgroup 1 (Cl_1): Mice exposed to chlorine at a concentration of 2.5 mg/l.

Subgroup 2 (Cl_2): Mice exposed to chlorine at a concentration of 5 mg/l.

Subgroup 3 (Cl_3): Mice exposed to chlorine at a concentration of 10 mg/l.

Cadmium Group:

Subgroup 1 (Cd_1): Mice exposed to cadmium at a concentration of 3 ppm.

Subgroup 2 (Cd_2): Mice exposed to cadmium at a concentration of 6 ppm.

Subgroup 3 (Cd_3): Mice exposed to cadmium at a concentration of 12 ppm.

Control Group:

Subgroup 1 (Cg): Mice not exposed to chlorine or cadmium.

Figure (3-1) mice groups divided

The chlorine exposure concentrations of 2.5 mg/l were converted according to body weight to 0.0016 mg/kg B.W., 5 mg/l to 0.0033 mg/kg B.W., and 10 mg/l to 0.0062 mg/kg B.W. . Similarly, cadmium exposure was performed at concentrations of 3 ppm, which corresponded to 1.56 mg/kg B.W., 6 ppm to 2.52 mg/kg B.W., and 13 ppm to 8.645 mg/kg B.W.. Concentrations were determined based on the body weight of the male mice to ensure accurate dosing and to establish appropriate exposure levels. Conversion to mg/kg B.W. considers each mouse's body weight, providing a more precise measure of the dosage administered. This approach enables the comparison of exposure levels between different mice and helps to establish a relationship between the administered dose and the physiological response. By adjusting the chlorine and cadmium concentrations to mg/kg B.W., the study aimed to standardize the exposure levels across the experimental groups. This approach ensures that the dosage is relative to the body weight of the mice, allowing for a more accurate assessment of the effects of chlorine and cadmium exposure on genetic instability and histological changes. These adjusted concentrations provide a basis for evaluating the impact of chlorine and cadmium exposure on the male mice and facilitate the interpretation of the study results in terms of their relevance to real-world scenarios and potential human health implications. The exposure to chlorine and cadmium was administered through the drinking water for a duration of one month. The concentrations of chlorine and cadmium were determined based on previous studies and their potential impact on genetic instability and histological changes (Flick *et al.*, 1971; Genchi *et al.*, 2020; Marettová *et al.*, 2015; Spencer *et al.*, 1998). After the one-month exposure period, the mice were euthanized following the approved institutional guidelines for animal experimentation. Tissue samples were collected from the testes and kidneys for subsequent histo-pathological analysis. Furthermore, the sample tissues to be collected and analyzed are the testes and kidneys. The expression of specific genes, namely *TNF-α* will be investigated in these tissues. The data collected from the histopathological analysis will be analyzed statistically to evaluate the effects of chlorine and cadmium exposure on genetic instability and histological changes in the male mice. The study design allows for the assessment of the impact of different concentrations of chlorine and cadmium on genetic instability and histological changes in male mice. By comparing the experimental groups with the control group, it will be possible to determine the effects of chlorine and cadmium exposure on the expression of *TNF-*

α genes in the testes and kidneys of the mice. In additionally identify the genotypes by *IGF-1* gene.

3.3.1. Dosage calculator

3.3.1.1. Cadmium

The doses were converted to units that are proportional to the body weight of the mice in each group using an equation $PPm = \frac{wt.(mg)}{V\ (L.)}$then dose calculation for each kg of body using an equation$se = \frac{wt.(g)}{mice\ \ (avarge\ \ wt.(g))}$.

Total average of mice group

1- Group Cd_1

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{232\ g}{9} = 25.77 \cong\ 26g$$

2- Group Cd_2

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{175\ g}{7} = 20.71 \cong\ 21g$$

3- Group Cd_3

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{199\ g}{6} = 33.1\ \cong 33g$$

Converted to units $PPm\ to\ wt/L$ (volume is 20)

1- Group Cd_1 (3 ppm)

$$PPm = \frac{wt.(mg)}{V\ (L.)} \rightarrow 3\ ppm = \frac{wt.(mg)}{20} = 60\ mg/L$$

2- Group Cd_2 (6 ppm)

$$PPm = \frac{wt.(mg)}{V\ (L.)} \rightarrow 6\ ppm = \frac{wt.(mg)}{20} = 120\ mg/L$$

3- Group Cd_3 (13 ppm)

$$PPm = \frac{wt.(mg)}{V\ (L.)} \rightarrow 13\ ppm = \frac{wt.(mg)}{20} = 261.98\ mg/L$$

Dose calculation $dose = \frac{wt.(g)}{mice\ \ (avarge\ \ wt.(g))}$

1- Group Cd_1 (3 ppm)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 1.56 \text{ mg/kg Bw}$$

2- Group Cd_2 (6 ppm)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 2.52 \text{ mg/kg Bw}$$

3- Group Cd_3 (13 ppm)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 8.645 \text{ mg/kgBw}$$

3.3.1.2. Chlorine

The doses were converted to units that are proportional to the body weight of the mice in each group using an equation $mg/l = \frac{wt.(mg)}{V\ (L.)}$ then dose calculation for each kg of body using an equation $se = \frac{wt.(g)}{mice\ \ (avarge\ \ wt.(g))}$.

Total average of mice group

1- Group Cl_1

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{159\ g}{5} = 32g$$

2- Group Cl_2

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{228\ g}{7} = 32.57 \cong 33g$$

3- Group Cl_3

$$avarag = \frac{total\ \ wt.}{number} \rightarrow \frac{186\ g}{6} = 31g$$

Converted to units(volume is 20)

1- Group Cl_1 (2.5 mg/L)

$$\text{mg/L} = \frac{wt.(mg)}{V\ (L.)} \rightarrow 2.5\ \text{mg/L} = \frac{wt.(mg)}{20} = 0.05\ mg$$

2- Group Cl_2 (5mg/L)

$$\text{mg/L} = \frac{wt.(mg)}{V\ (L.)} \rightarrow 5\ \text{mg/L} = \frac{wt.(mg)}{20} = 0.1\ mg$$

3- Group Cl_3 (10 mg/L)

$$\text{mg/L} = \frac{wt.(mg)}{V\ (L.)} \rightarrow 10\ \text{mg/L} = \frac{wt.(mg)}{20} = 0.2\ mg$$

Dose calculation$dose = \frac{wt.(g)}{mice\ \ (avarge\ \ wt.(g))}$

1- Group Cl_1 (2.5 mg/L)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 0.0016\ \text{mg/kg Bw}$$

2- Group Cl_2 (5 mg/L)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 0.0033\ \text{mg/kg Bw}$$

3- Group Cl_3 (10mg/L)

$$dose = \frac{wt.(g/l)}{mice\ (avarge\ wt.(g))} = 0.0062\ \text{mg/kg Bw}$$

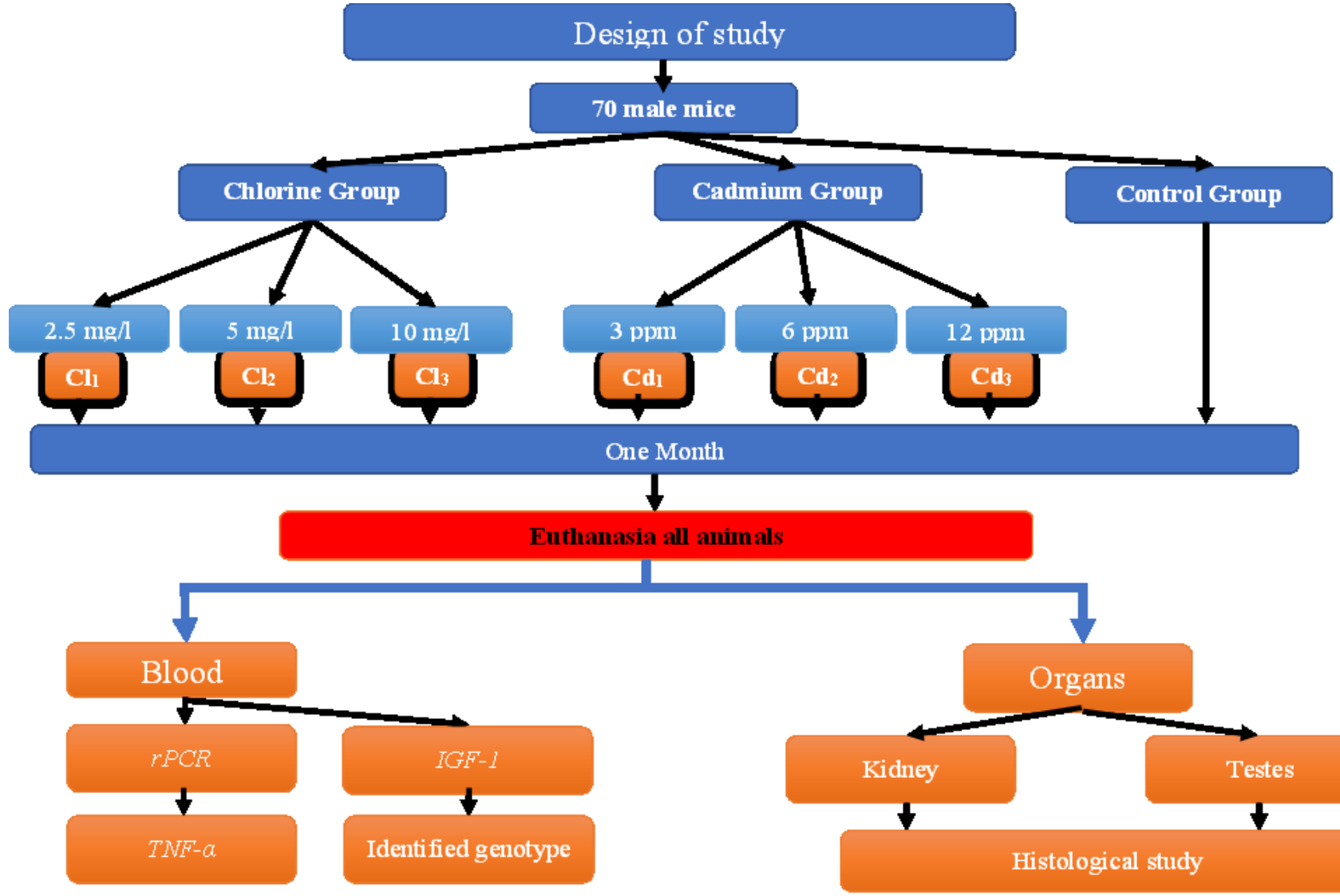


Diagram (3-1): Design of study

3.4. Collection of Samples and organ specimens

At the end of study, blood samples and organ specimens were collected from the male mice to assess various parameters and investigate the impact of chlorine and cadmium exposure on genetic instability and histological changes.

An appropriate anesthesia protocol was followed for tissue collection to ensure the humane handling of the mice. Ketamine and xylazine were used as anesthetic agents for the mice. The mice were carefully placed under anesthesia by administering a combination of ketamine and xylazine via intraperitoneal injection. The specific dosages and concentrations of ketamine and xylazine were determined based on the weight of the mice, following standard veterinary guidelines to achieve effective anesthesia while minimizing discomfort or distress.

Once the mice were under anesthesia, they were carefully positioned, and aseptic techniques were employed to maintain sterile conditions. The blood was collected from the heart, and the testes and kidneys were then surgically removed from each mouse using sterile instruments and handled with care to minimize tissue damage. The collected tissues were promptly transferred into containers containing 10% neutral buffered formalin to ensure proper fixation and preservation of the tissue morphology. The containers were securely sealed to prevent leakage or contamination during transportation to the laboratory.

Ethical considerations and guidelines for animal experimentation were strictly adhered to throughout the tissue collection process to ensure the welfare and minimize any potential distress or pain experienced by the mice. The procedures were conducted in accordance with the approved protocols by the institutional animal ethics committee to ensure compliance with ethical standards and regulatory requirements.

After the testes and kidneys were collected from each mouse, they were immediately transferred and carefully placed into separate containers filled with 10% neutral buffered formalin. The formalin solution helps to fix the tissues and preserve their cellular structures for subsequent Histopathological analysis. The containers were securely sealed to prevent any leakage or contamination during transportation and storage. Proper labeling was done on each container to ensure accurate identification of the samples. The containers were then stored in a suitable location with appropriate temperature and ventilation conditions to maintain the

integrity of the samples until they were processed for histopathological examination.

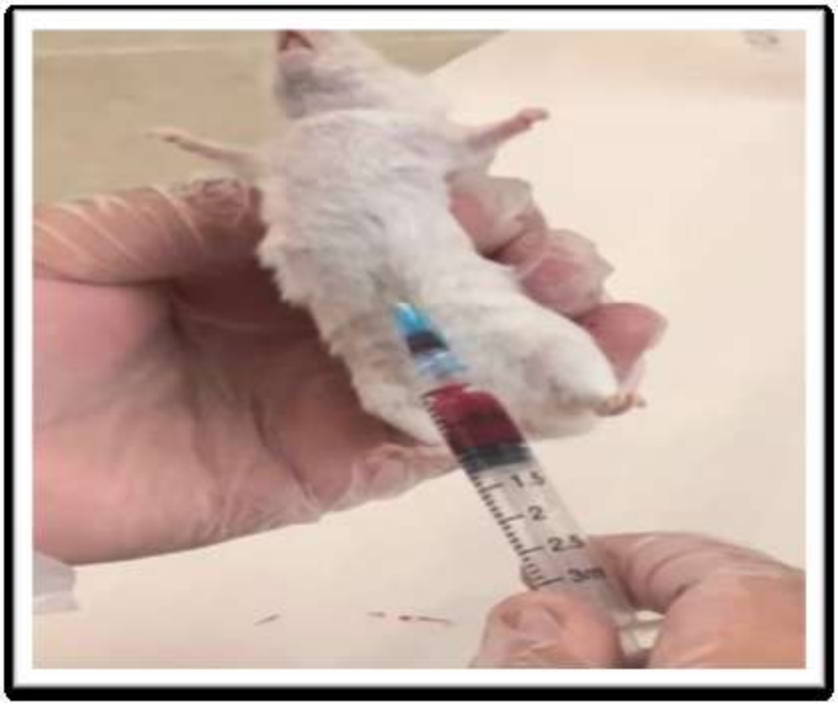


Figure (3-2): The study samples (Euthanasia)

3.5. Histological section preparation

The Histological work was carried out in Histology Laboratory in the College of Science, Misan University.

The process of histological section preparation, based on the instructions of Luna (1968), involved several steps:

1- Fixation:

The study samples were placed in a 10% neutral formalin solution for 48 hours to preserve their structure.

2- Washing:

After fixation, the samples were washed for 3 hours with running tap water to remove any residual fixative.

3- Dehydration:

The samples were dehydrated using a series of ethyl alcohol solutions with increasing concentrations, as follows:

70% ethyl alcohol for 2 hours

80% ethyl alcohol for 1 hour

96% ethyl alcohol for 2 hours (repeated three times)

100% ethyl alcohol for 7 hours (followed by an additional 2 hours)

4- Clearing:

The samples were placed in xylene, first in one stage for 2 hours and then in a second stage for another 2 hours. Xylene is used to remove the alcohol and prepare the samples for embedding.

5- Infiltration and Embedding:

A mixture of xylene and paraffin wax was prepared. The samples were placed in this solution for 1 hour at 60°C in an electric oven. Subsequently, the samples were transferred to pure paraffin wax and kept for 4 hours. The samples were then placed in wax molds for embedding.

6- Trimming and Sectioning:

The wax molds containing the embedded samples were trimmed using a sharp scalpel. The samples were cut longitudinally or transversely into sections with a thickness of 5 microns. A slide was taken, a drop of egg white was placed on it, and the cut tape was transferred to a water bath. The tape was then transferred onto the slide with egg white and placed on a hot plate at 37°C to fix the sample tape on the slide.

7- Staining:

The staining process was performed according to the instructions outlined by Luna (1968):

1- Wax removal: The slide with the samples was placed in xylene for 30 minutes to remove the wax.

2- Hydration: The slide was sequentially placed in decreasing concentrations of ethyl alcohol (100%, 96%, and 70%) for 5 minutes each.

3- Distilled water: The slide was immersed in distilled water for 3 minutes.

4- Hematoxylin staining: The slide was placed in Hematoxylin stain for 12-15 minutes, followed by rinsing with tap water for 8 minutes.

5- Eosin staining: The slide was placed in eosin stain for 5 minutes, followed by rinsing with tap water for 3 minutes.

6- Dehydration: The slide was briefly immersed in increasing concentrations of ethyl alcohol for 5 seconds each, and then transferred to xylene for 15 minutes.

7- Mounting: Canada balsam was applied to a cover slip, which was then placed over the slide. The slide was transferred to a hot plate at 37°C for the balsam to harden.

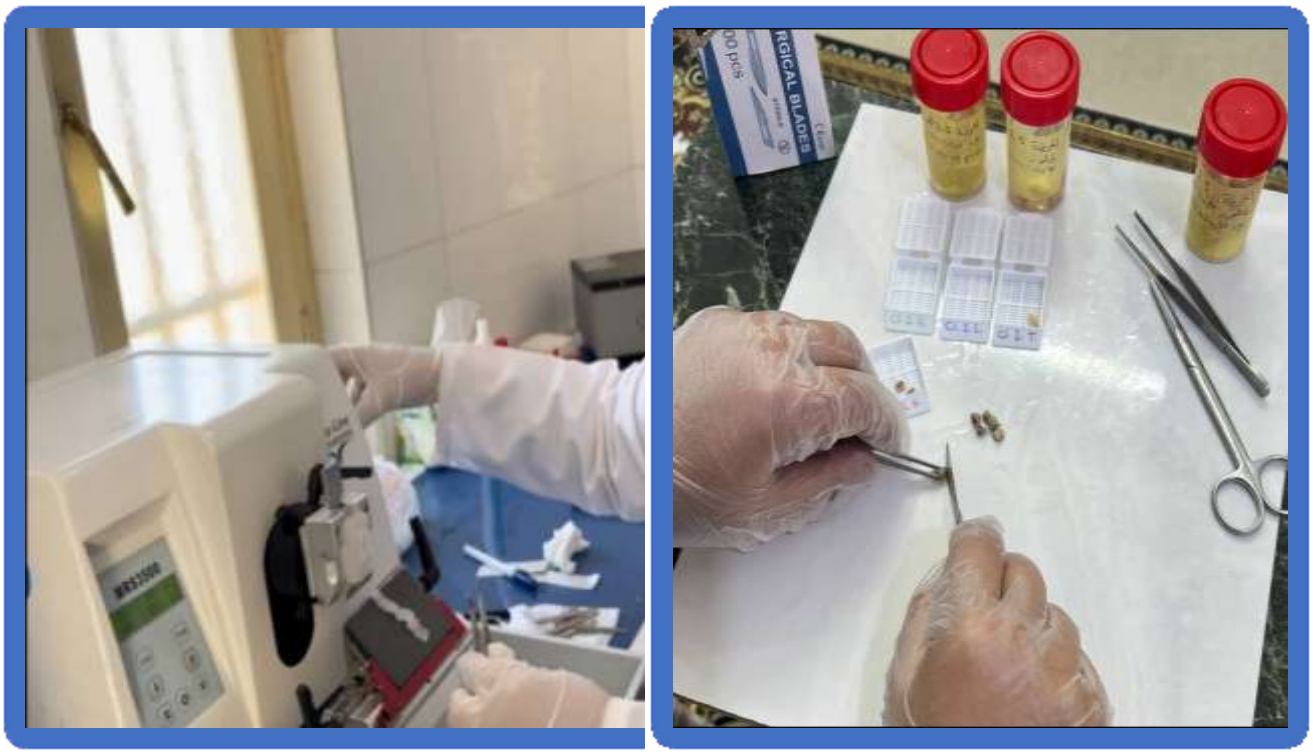


Figure (3-3): Histological preparations

3-6. Genetic study of Blood Samples

The Genetic work was carried out in Laboratory of Al-Ghari center for research, studies and training.

3-6-1. Genomic DNA extraction

DNA was extracted for research purposes from blood samples that were kept inside a special tube (EDTA tube) to prevent blood clotting.

Genomic DNA was extracted from mouse blood using the blood DNA extraction kit (SIM bio lab). The kit contains all of the necessary reagents and buffers for the extraction.

The following steps were followed:

1- 20 µL of Protein kinase(it's function is to enlarge peptide bonds) was added to 200 µL of mouse blood, this sample were stored according to their group inside an EDTA tube.

2- Add 200 micro of lysis buffer (SPL kit),and The mixture was put on the vortex mixer immediately.

3- The mixture was incubated at 57°C for 20 minutes.

4- 200 µL of ethanol in concentration of 96% was added to the mixture, Then, manual mixing was carried out by pipette (pumping and returning) until it dissolved .

5- The supernatant was transferred to a new tube (Filter Tube) for filtering out the DNA impurities .

6- The mixture was vortexed and centrifuged for 1.5 minutes at 13,000 rpm.

7- The general sediment was disposed of at the bottom of the filter tube

after being removed from the centrifuge and the extract was taken in the

top filter tube.

8- 500 µL of the blood DNA extraction kit (Washing buffer1 SBW_1) was added to the supernatant. and was centrifuged for 1 minutes at 13,000 rpm.

9- We Poured the solution from the 2 ml tube into a disposal bottle.

10- 700 µL of the blood DNA extraction kit (Washing buffer2= SBW_2) was added to the supernatant, and centrifuged at 13,000 rpm for 1 min.

11- Step 10 wasrepeated, due to the appearance of impurities in the sample.with the 500ul of Washing buffer2(SBW_2) and centrifuged at 13,000 rpm for 2 min.

12- 150 µL of elution buffer was added to the supernatant(Before adding the solution buffer, it was placed in a water bath in order to accelerate the absorption of the solution through the filter).

13- The mixture was incubated at room temperature for 5 minutes.

14- The mixture was centrifuged for 1minutes at 13,000 rpm.

15- The samples were placed in the refrigerator.

16- The genomic DNA was ready for use.

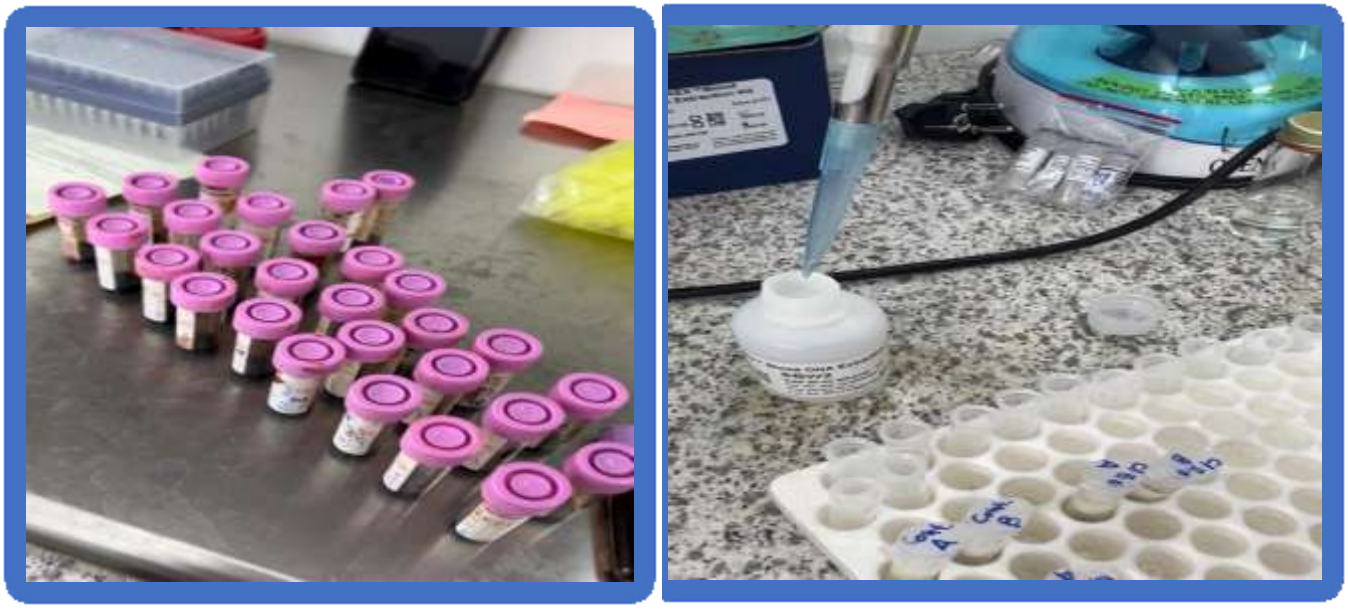


Figure (3-4) Genetic study of Blood Samples.

Table (3-3): The reagents of DNA extraction kit.

Mixture	Volume(μl)
Protein Kinase	20
Lysis (SPL Kit)	200
Mouse Blood	200
Ethanol 96%	200
SBW_1	500
SBW_2	700
Elution buffer	150

3-6-8. Measuring the concentration and purity of DNA

For the purpose of ascertaining the purity and concentration of the extracted DNA, the samples were tested by means of the Nano drop , as 1 microliter of the sample was placed on the designated place in the device, and the results were recorded with a special program installed on the computer. The purity of the DNA can be estimated by recording the optical density (Optic). density O.D) of the sample at wavelength 260 / 280 on the basis that each OD. A260=1 refers to the concentration of DNA equal to: µg/ml pure 50dsDNA and thus finding the concentration of DNA through the following equation:

DNA Concentration (µl/ml) = A260 Reading × dilution factor × 50µg/ml

3-6-3. Primer synthesis

The primers of TNF-∝ and IGF-I were used for genome DNA manufactured by the company of AUGCT DNA SYN Biotechnology /China . The table below show the types of primers and sequences;

Table (3-4) show the types of primers that used in experiment .

Gene	Primer	Sequence (5'—3')	Length(bp)	References
TNF-∝	Forward	GGCTTTCGGAACTCACTGGA	20	Khalilzadeh et al.,(2018)
	Reverse	CCCGTAGGGCGATTACAGTC	20	
IGF-I	Forward	AACACCAGCCCATTCTGATTTG	22	Wang et al.,(2021)
	Reverse	TTCACAGGTTTAGTCCTGGCTTTC	24	
Gapdh control	Forward	AGTGCCAGCCTCGTCTCATA	20	Ramhormozi et al.,(2020)
	Reverse	GATGGTGATGGGTTTCCCGT	20	

3-6-8. PCR (Thermal Cycle)

The samples, which extracted previously by the DNA extraction process, were prepared. The DNA extract used for this process depends on the concentration of the strand, so the quantity is available. It's used a product of Mastermix (Danish origin),It contains nitrogenous bases that will be built, enzymes that link (polymerase tag enzyme), and sodium chloride NaCl, all of which contribute to the multiplication process.

The following steps were followed:

1.10 micron of Mastermix has been added to DNA extract sample (available) in the eppendrof microtube, The sample was then placed on the vortex for 30 seconds.

2.An available amount of distilled water was added to the sample, and it was placed on the vortex for 30 seconds.

3. 1 micron of Forward and Reverse primers (which are manufactured in north Korea) were added to the sample, and it was placed on the vortex for 30 seconds.

4. After that, the samples were placed in a PCR device for DNA replication.

5. Electrophoresis was performed on the samples.

Table(3-5) The conditions of PCR run.

PCR Steps	Temperature (C°)	Time	Cycles
Initial denaturation	95°	4 minutes	1
Denaturation	94°	30 second	35
Annealing	58.5°	30 second	
Extension	72°	35 second	
Final extension	72°	7 minutes	1
Final hold	4°	-------	----

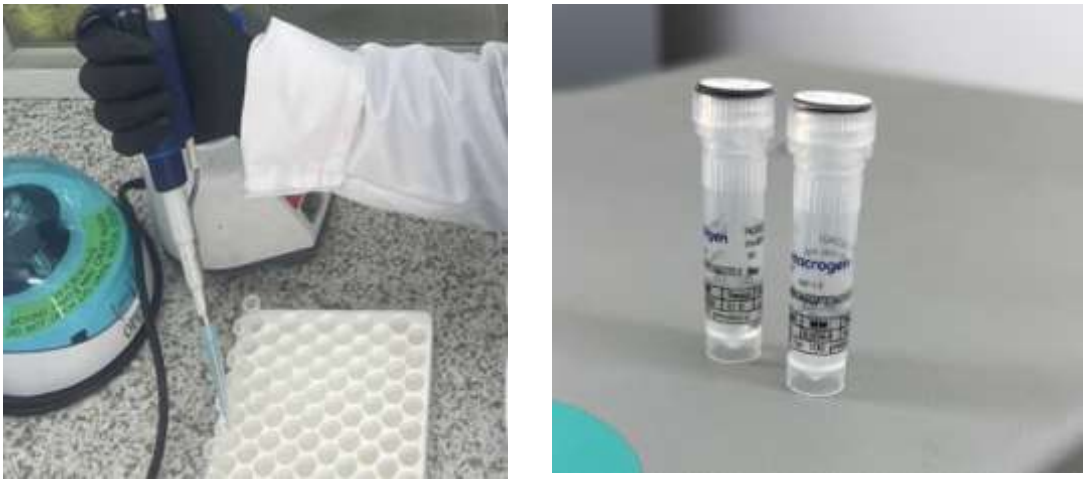


Figure (3-5) Preparation of IGF primers (Foreword and Revers)used in PCR.

Figure (3-6) The Thermcycler ;The screen shows the stages of device opreration , which depends on raising and lowering temperatures for amplify whole DNA of Samples .

3-6-2 Agarose Gel Electrophoresis

The electrophoresis process was carried out for each of the DNA and RNA extracts by using buffer solution TBE (Tris/Borate/EDTA) and ethidium bromide with acarose gel powder. The concentration of the gel used varies according to the amount of extracted DNA, as the volume of the sample increases, the amount of acarose is reduced.

The following steps were followed:

1. In the gel electrophoresis of the whole DNA extract of the sample: a smaller amount of agarose was used than in the DNA sample for PCR, where 300 microns were added using the sensitive balance.

2. Buffer solutionTBE was added in an amount of 30 ml (according to the size of the buffer tank),to the agarose powder in a glass beaker, and shaking by hand.

3. Then the beaker was placed in the microwave for 30 seconds.

4. After removing the beaker from the microwave and leaving it for 3 minutes to cool slightly, 1 micron of Ethidium bromide was added to the mixture and shaken by hand.(Ethidium bromide helps DNA glow under ultraviolet UV light)

5. The mixture was shaken by hand and then poured into Gel casting tray, The comb is installed above the Gel casting tray, The comb was fixed over the Gel casting tray, and the Casting dams was fixed on the sides of the Gel casting tray.

6. The mold was left for half an hour to harden.

7. After we made sure that the gel became coherent, the comb and the Casting dams were lifted slowly .

8. 6 µm of whole DNA extract was added inside the holes of the gel by micropipette at an angle of 45° to prevent rupture in the holes.

9. The Terry gel was placed in a buffer tank and added 300 ml of mixture of buffer and agarose to the buffer tank.

10. the electrodes were attached to the electrophoresis device, with a time set for 30 minutes.

11. After the completion of the electrophoresis process, the gel mold was removed and placed in a fluorescent device to examine the luminous bands under ultraviolet light source with a wavelength 320 nm to ensure the presence of the DNA extract.

Figure (3-7) Gel electrophoresis steps.

3-6-7.DNA sequencing

For determine the sequencing of the nitrogenous bases, samples were sent with an amount of 25 microliters of amplified pieces of genes PCR products to Korean Macrogen Company, And the sequencing process was carried out for the reverse and foreword , according to our request from the company, as the results came in the form of compressed files that can be opened by bioinformatics techniques and algorithms (BLAST), as this tool helped in comparing our results with data belonging to the same genes in the US National Center for Biotechnologies(NCBI). in the figure below, one of these designs that was made in North Korea;

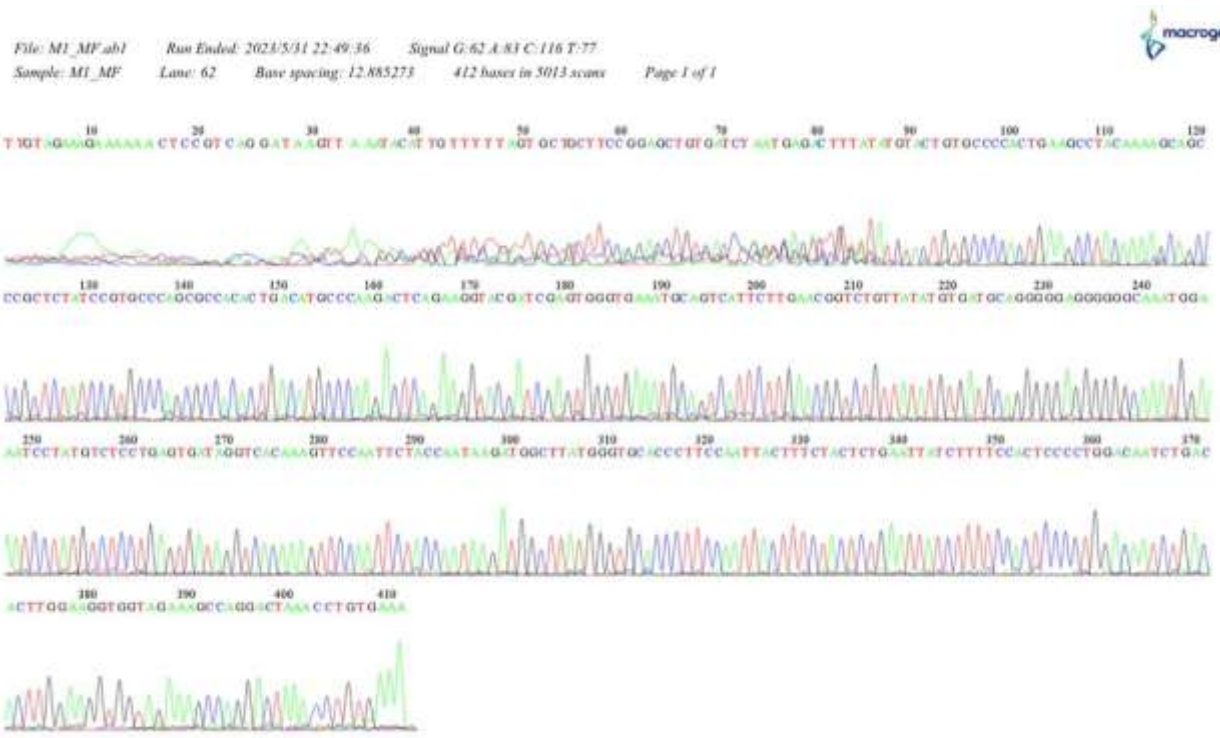


Figure (3-8) One sample of the wavelengths of nucleotides synthesized at macrogene in North Korea.

3-6-3. RNA extraction:

Total RNA was extracted from blood using the Trizol reagent (Life Technologies). The chloroform step was used to separate the RNA from the DNA and proteins, and the isopropanol step was used to precipitate the RNA. The ethanol wash step was used to remove any residual isopropanol, and the DEPC-treated water was used to resuspend the RNA. The RNA was then ready for downstream applications.

The following steps were followed:

1. 500 µL of Trizol reagent was added to 500 µL of blood,and Clumping occurred in the blood sample due to this kit.
2. The mixture was vortexed for 5 minutes, and the mixture was incubated at room temperature for 5 minutes.
3. 100 µL of chloroform was added to the mixture.
4. The mixture was vortexed for 30 seconds,and the mixture was incubated at room temperature for 3 minutes.
5. The mixture was centrifuged for 15 minutes at 10,000 rpm at 2-8° C.

6. The mixture separated into a pink lower organic phase, an interphase, and a colorless upper aqueous phase containing the RNA. The colorless upper phase containing the RNA was transferred to a new tube free of RNA .
7. 250 µL of Isopropanol was added to the aqueous phase.
8. Mix well by inverting the tube, and incubate at room temperature for 10 minutes..
9. The mixture was centrifuged for 10 minutes at 10,000 rpm.
10. Some samples did not come out clearly, so a small amount of Isopropanol 150µL was added again and sent back to the centrifuge for ten minutes.
11. The supernatant was discarded.
12. The pellet was washed with 500 µL of 75% ethanol.
13. The sample was placed on the Vortex mixer for one minute.
14. The pellet was centrifuged for 5 minutes at 7,500 rpm.
15. The supernatant was discarded.
16. The pellet was air-dried for 10 minutes, the lid left open to speed up drying.
17. The pellet was resuspended in 35 µL of injection water.
18. , the samples were kept in the refrigerator at a temperature of -70 degrees, the temperature of the freezer. until use in the RT-PCR process.

Table(3-6) Quantities of materials used in RNA extraction

Mixture	**Volume(*µL*)**
Trizol reagent	500
Chloroform	100
Mouse Blood	500
Isopropanol	250
Ethanol 75%	500
injection water	35

3-6-5. qPCR optimization assay and cDNA synthesis

The working principle of qPCR apparatus is cycling parameters. A one-step RT-PCR reaction includes the following materials and procedures:-

1. Cryogenic TNF primers manufactured in North Korea were prepared for the purpose of this study. These primers (forward and reverse) come in the form of a stock (a frozen substance found on the walls and bottom of the tube), therefore 250 ml of distilled water (which is the quantity specified by the company) is added to each tube from the front and back, then it is placed on the vibrator for mixing and placing In the refrigerator.

2. 6 µl of the mRNA sample extracted in a previous step was placed into an Eppendorftube.

3. 1.6 µl of MgCl2 was added to the sample, and the sample was placed on the Vortex mixer for one minute.

4. 10 µl of qPCR was added, and placed on the Vortex mixer for one minute.

5. 0.4 µl of Reverse transcriptase was added , and the sample was placed on the Vortex mixer for one minute.

6. 0.3 µl of CXR was added as well, 3 µl distilled water was added to theEppendorftube, then the tube was centrifuged for 1 min.

7. Then, TNF- primer was added in an amount of 2 µl to each of the receptors and forwards to each test tube, so that the total volume of the sample was 25 µl .

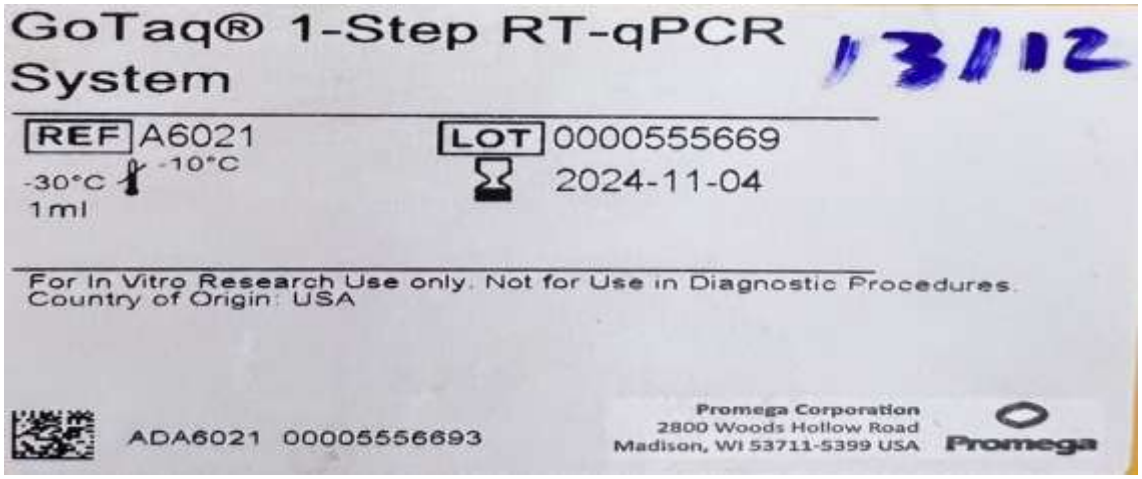
8. The two tubes were placed in the centrifuge for one minute for mixing.

It was placed in an RT-PCR machine for the necessary procedure, with the temperature set at 57 degrees.

Table (3-7) 1-Step RT-qPCR

Regent	Volume (µl)
MgCl2	1.6 µl
qPCR	10 µl
Enzyme(Reverse transcriptase)	0.4 µl
CXR	0.3 µl
Primer(Reverse and Forward)	2 µl each one
Sample of RNA	6-7µl

Injection water	1.7-2.7µl
Total Volume	25 µl



(Figure 3-9) Promega 1-Step

9. The real-time PCR device includes the following steps;

The program for the software that has been updated through the software has been opened, and the samples have been placed in the specified location inside the device.

a_The reverse transcriptase enzyme was incubated at a temperature of 37 degrees for 15 minutes in order to convert the mRNA into cDNA. This step is repeated only once, which targets the entire sample.

b_The temperature was raised to 95 degrees for 10 minutes in order to inactivate Reverse transcriptase enzyme by coagulating the enzyme because it is not needed in subsequent processes.

c_The temperature was raised to 95 for 15 seconds for the denaturation step Initial, where the cDNA is denatured and two single strands are made from it.

d_The temperature of the device decreased to 57 for 30 seconds in order to conduct Annealing process where dNTPS nitrogenous bases are added to the single strand.

e_ The temperature rises to 72 for 30 seconds and is for extension.

The previous three steps are repeated (45 cycles).

f_ Raise the temperature to 95 for 15 sec. This step is used for denaturation DNA double strand

g_ The temperature drops again to 60 for one minute.

h_ The temperature rises to 95 for 30 seconds The aim of the previous three steps is to melt the dimer or prevent their formation.

10.After completing all the steps, it reads the results without need for Gel electrophoresis.

Table(3-8) The real-time PCR steps .

Cycle	Time	Temperature	Temperature
1	15 minutes	37°	Transcription reverse (Incubating the reverse transcriptase)
1	10 minutes	95°	Inhibit the action of the reverse transcriptase enzyme
45	15 second	95°	preliminary denaturation step
45	30 second	57°	Annealing
45	30 second	72°	Extension
1	15 second	95°	Melt carve stage (prevents the formation of dimers)
1	1 minutes	60°	
1	30 second	95°	

11. Real-time Monitoring: The real-time PCR amplification and detection were performed using a compatible instrument with fluorescence detection capabilities. The instrument measured the fluorescence signal emitted by the DNA-binding dye (e.g., SYBR Green) during each amplification cycle. This allowed for the continuous monitoring of the amplification in real-time.

12. Data Analysis: The threshold cycle (Ct) values, which represent the cycle number at which the fluorescence signal crosses a predetermined threshold, were recorded for each sample. The Ct values were then used for further analysis, such

as quantification of gene expression levels and comparative analysis between different experimental groups.

13. Data Interpretation: The Ct values obtained from the qPCR analysis were analyzed using appropriate software or algorithms to determine the relative gene expression levels. This involved normalizing the target gene expression to a reference gene (GAPDH) to account for any variations in the input DNA amount or RNA integrity.

14. Statistical Analysis: The obtained gene expression data were subjected to statistical analysis to evaluate the significance of differences between experimental groups. This could involve using appropriate statistical tests, such as t-tests or ANOVA, to determine the significance of gene expression changes induced by chlorine and cadmium exposure.

The entire rPCR analysis was conducted following established protocols, and the specific reagents and instruments mentioned in the previous response were used accordingly.

Figure (3-10) ; Computer screen for The Real-Time PCR device ,screen shows the stages of the device operations , which depends on raising and lowering temperatures ; First it was transcribed the RNA sample into complementary

DNA (cDNA),which is obtained by reverse transcription of RNA. Second, it was enzymatically amplified specific DNA segments to Several copies were used in this study .

3.7.Data analysis

Quantification of the target mRNA was normalised using reference mRNA. TNF-α, and GAPDH were the most stably expressed reference genes, with similar amplification efficiencies to our target gene,. ΔCq was calculated as the difference between target and the geometric mean of our reference genes. ΔΔCq was obtained by normalising the ΔCq values of the treatments to the ΔCq values of the pre-treatment control.

3.8.Statistical Analysis

The statistical analysis of the data was performed using SPSS software. The mean values and standard deviations of the data were calculated. A one-way analysis of variance (ANOVA) was conducted to assess the statistical differences between groups. Post-hoc analysis was conducted using the least significant difference (LSD) test to determine specific pairwise differences between groups. The significance level was set at $p < 0.05$, indicating that a p-value less than 0.05 was considered statistically significant. The statistical analysis was carried out following the guidelines outlined by Griffith (2007) .

4. The Results

4.1. PCR (Thermal Cycle)

4.1.1. Gel Electrophoresis:

Agarose gel extraction was used to purify appropriate DNA fragments (range PCR product) and also to identify and purify the specific amplified gene *IGF-1* (PCR product).DNA extraction steps from agarose gel were performed according to procedure steps (3-6-2) in Chapter 3.

The locations of the extracted DNA bands were determined using an ultraviolet light source and a wavelength 320 nm, and then the gel images for the purpose of confirming the presence of DNA, Where the length of the sequence was 450 bp by using Ladder.

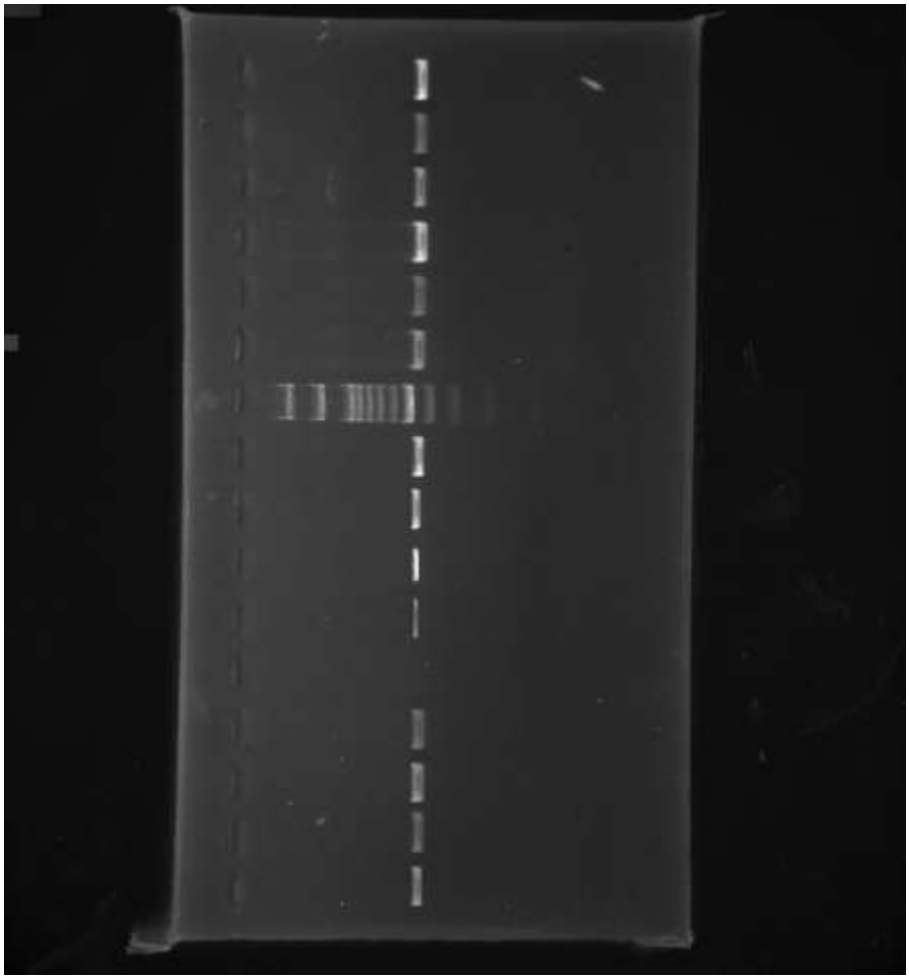


Figure (4-1) Gel Electrophoresis Analysis to identify of *IGF-1* Gene in Chlorine and Cadmium Exposed Mice Subgroups.

4.1.2.Alignments of *IGF-1* genotypes and Histopathological observations for the mice's groups .

4.1.2.1.Alignments of *IGF-1* genotypes and Histopathological observations for the Control mice's groups.

The nucleotide sequence analysis and genotyping of the control mouse *IGF-1* gene using PCR revealed a high sequence similarity with the predicted sequence of (Rattus norvegicus insulin-like growth factor (*IGF*) gene AH002176.2 , The alignment analysis of *IGF-1-F* showed a percent identity of 83.29%. This result indicates that the tested sample shares high sequence homology with the predicted *IGF-1* gene sequence, confirming its similarity to the known variant in Rattus norvegicus . The high percentage of identity suggests that the tested sample likely belongs to the same gene variant, supporting its classification as *IGF-1-F* (Table 4-1).

Table: 4-1: The Alignments of control mouse with the genotypes of *IGF-1 –F*

Dosage type	*Site of SNP*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Control (Cg) 0 Mg/kg B.W.	3260	---	---	---	D=D
	3363	---	---	---	E=E
	3366	---	---	---	C=C
	3369	---	---	---	C=C
	3372	---	---	---	F=F
	3375	---	---	---	R=R
	3378	---	---	---	S=S
	3381	---	---	---	C=C
	3384	---	---	---	D=D
	3387	---	---	---	L=L

Rattus norvegicus insulin-like growth factor (*IGF*) gene.
Sequence ID: AH002176.2
Length: 7074 , Number of Matches: 1
Range 1: 3260 to 3289

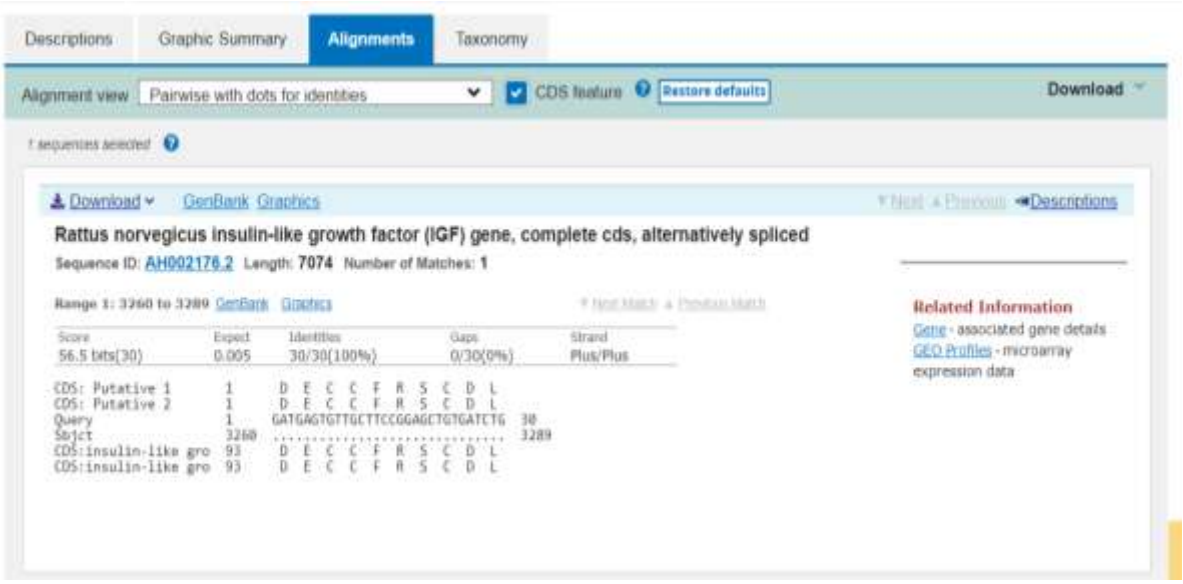


Figure (4-2) : Comparison the sequences of *IGF-1* gen for serial number of albino Mice(Control group) and reference serial number of Rattus norvegicus (AH002176.2).

The Histopathological observation of the renal tissue sections in control group(Cg) revealed normal architecture with normal appearance of glumerular tuft of blood capillaries surrounded Bowmann`s capsule with normal space in the Bowmann`s capsules, also the proxiamal and distal convoluted tubules and lined by cuboidal cells appear in normal size with their nuclei (Figure 4-3).

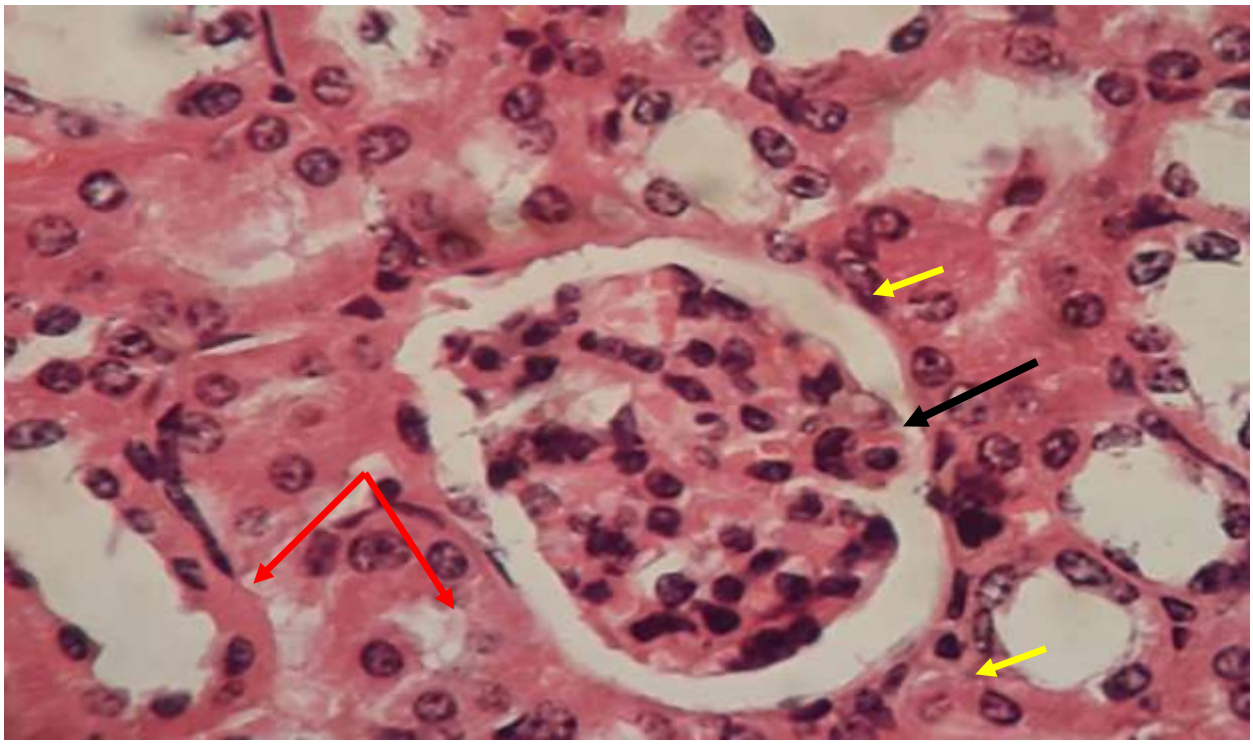


Figure (4-3): Photomicrographs control group (G1) for mice **kidney** tissue sections stained with Haematoxylin & Eosin**(HE.400)** : Observed normal tissue architecture with normal glumerular tuft of blood capillaries ➡ surrounded by Bowmann`s capsule ➡ the proxiamal and distal convoluted tubules and lined by cuboidal cells appear in normal size with their nuclei ➡.

The Histopathological changes in testes.(figure 4-4) which represent testes tissue sections of the control group (Cg) showed normal morphology in the tissues of testes. the cycle of spermatogensis was regulated in normal , sertoli and laydig cells were found in normal structures and in the interstitial space of the testes ,primary spermatocytes ,spermatid and spermatozoa are also noticed in normal structure. All somniferous tubules were Confined with basement membrane and composed of spermatogonia which observed rounded cells .

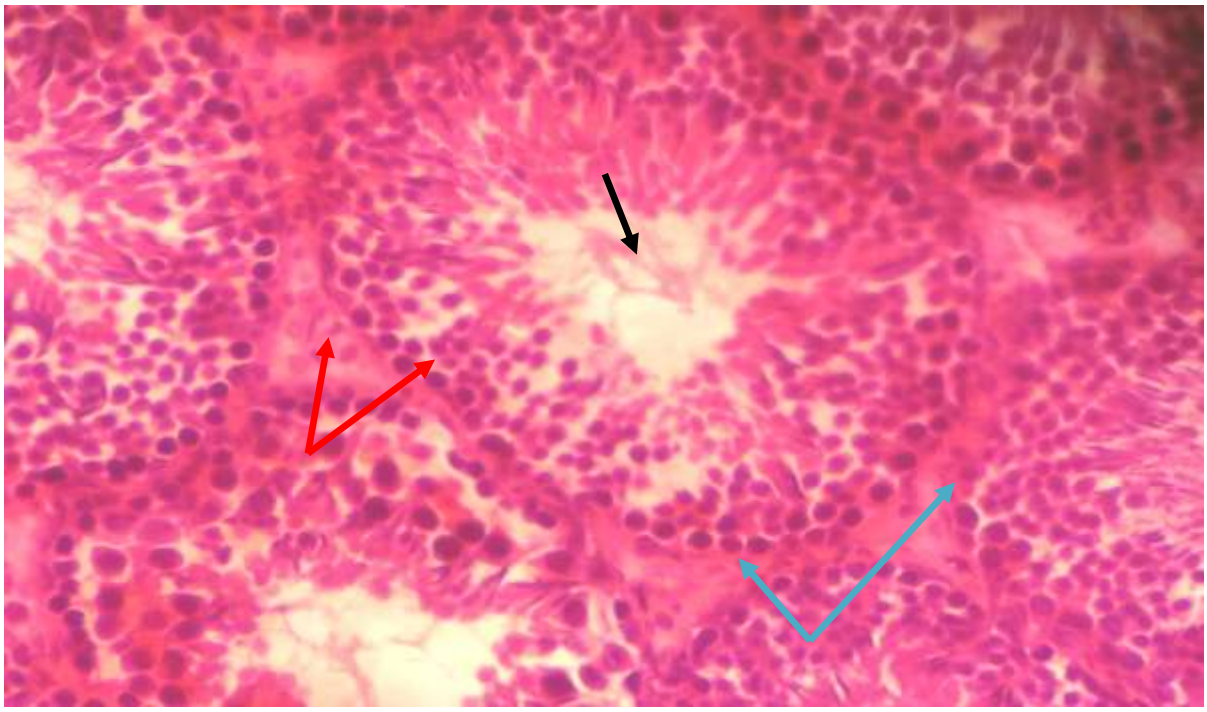


Figure (4-4): Photomicrographs control group (Cg) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed normal morphology in the tissues of testes,normal structures in sertoli and laydig cells → in the interstitial space of the testes,somniferous tubules confined with basement membrane → and composed of spermatogonia which observed rounded cells and the spermatozoa are also noticed in normal structure →.

4.1.2.2.Alignments of IGF-1 genotypes and Histopathological observations for the Cadmium mice's groups.

4.1.2.2.1.Alignments of *IGF-1* genotypes and Histopathological observations for the Cd_1(1.56 mg/kg Bw) group:

The results of the nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the IGF-1 gene ,as the base G changed to A (G3295A), as the base T changed to C (T3313C) and the base G changed to A (G3331A), as the base T changed to G (T3332G), as the base change T to C (T3337C),as the base T

change to C(T3340C), as the base C change to T(C3343T), as the base G change to T(G3349T),and the change in The bases T and G(T3332G) led to a change in the genetic coding, which changed the amino acids in the final protein. These mutations were frameshift mutations. As shown in the table (4-2):

Table-4-2 :The Alignments of Cd_1 with the genotypes of *IGF-1-F,*shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of *IGF-1* gene.

Dosage type	*Site of SNP/InDel polymorphism*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cd 1 1.56 Mg/kg B.W.	3295	G➞A	Transition	AGG➞AGA	R=R
	3313	T➞C	Transition	GCT➞GCC	A=A
	3331	G➞A	Transition	AAG➞AAA	K=K
	3332	T➞G	Transversion	TCA➞GCA	A➞S
	3337	T➞C	Transition	GCT➞GCC	A=A
	3340	T➞C	Transition	CGT➞CGC	R=R
	3343	C➞T	Transition	TCC➞TCT	S=S
	3349	G➞T	Transversion	CGG➞CGT	R=R

Rattus norvegicus insulin-like growth factor (IGF) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length: 7074
Range : 3256 to 3604

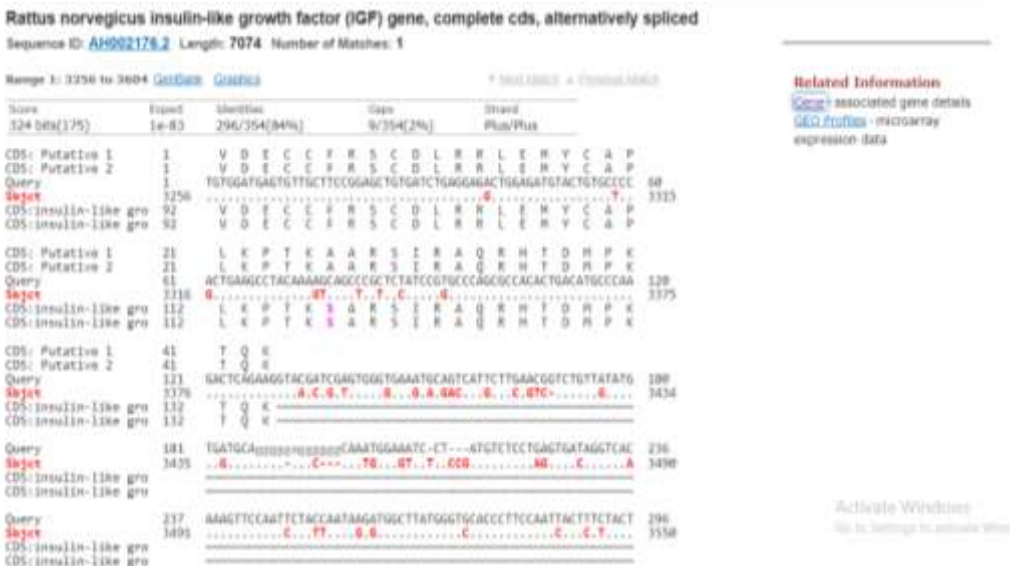


Figure (4-5): Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cd_1) and reference serial number of Rattus norvegicus (AH002176.2), in NCBI.

4.1.2.2.2.Alignments of IGF-1 genotypes and Histopathological observations for the Cd_2(1.56 mg/kg Bw) group:

The results of the nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the *IGF-1* gene ,as the base G changed to A (G3295A), as the base T changed to C (T3313C) , the base G changed to A (G3316A), the base G changed to A (G3331A), as the base T changed to G (T3332G), as the base change T to C (T3336C), as the base T change to C(T3339C), as the base C change to T(C3342T), as the base G change to T(G3348T), and the change in The bases T and G(T3332G) led to a change in the genetic coding, which changed the amino acids in the final protein. These mutations were frameshift mutations. As shown in the table (4-3):

Table-4-3 : The Alignments of Cd_2 with the genotypes of *IGF-1-F* gene , shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of *IGF-1* gene.

Dosage type	*Site of SNP*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cd_2 2.52 Mg/kg B.W.	3295	G➞A	Transition	AGG➞AGA	R=R
	3313	T➞C	Transition	GCT➞GCC	A=A
	3316	G➞A	Transition	CCG➞CCA	P=P
	3331	G➞A	Transition	AAG➞AAA	K=K
	3332	T➞G	Transversion	TCA➞GCA	A➞S
	3339	T➞C	Transition	CGT➞CGC	R=R
	3342	C➞T	Transition	TCC➞TCT	S=S
	3348	G➞T	Transversion	CGG➞CGT	R=R

Rattus norvegicus insulin-like growth factor (IGF) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length: 7074
Range 1: 3263 to 3585

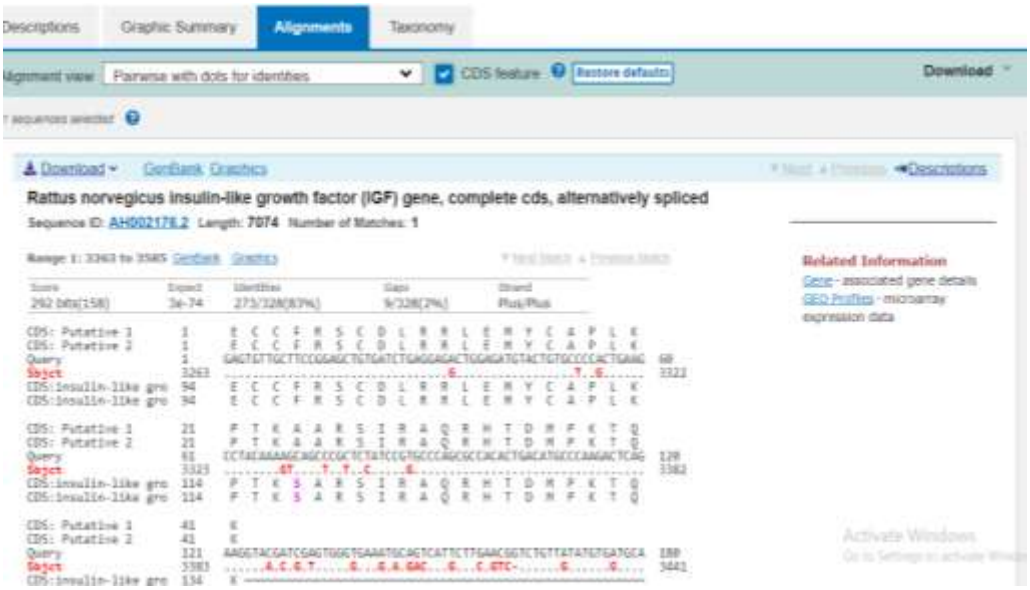


Figure 4-6 Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cd_2) and reference serial number of Rattus norvegicus (AH002176.2), in NCBI.

The Histopathological observation of the renal tissue sections in The groups of mice (Cd_1 & Cd_2) that treated with cadmium in doses (1.56mg/Kg B.W.),(2.52mg/Kg B.W.) respectively exhibited mild to moderate histopathological focal interstitial inflammation with mild abnormalities and atrophy in the glumerulous ,the renal tubules observe mild to moderate abnormal changes in their architectures with cytoplasm degeneration of cells in some of renal tubules, moderate dilatation and congestion in the renal blood vessels in kidney tissues sections in (Cd_2) of mice, (Figures 4-7,4-8).

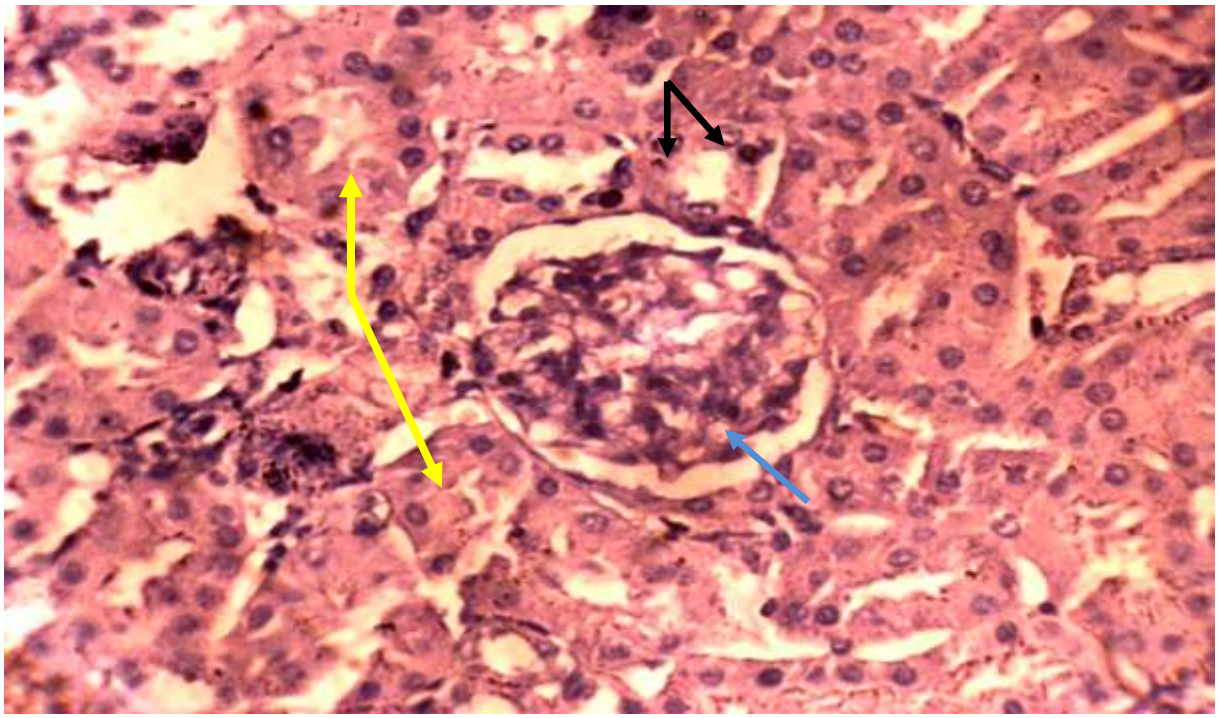


Figure (4-7): Photomicrographs (Cd_1) for mice **kidney** tissue sections stained with Haematoxylin & Eosin**(HE.400)** : Observed mild focal inflammation and atrophy → in the glumerulous ,mild abnormal changes in renal tubules with decrease in lumen with → cytoplasm degeneration of cells in some of renal tubules →

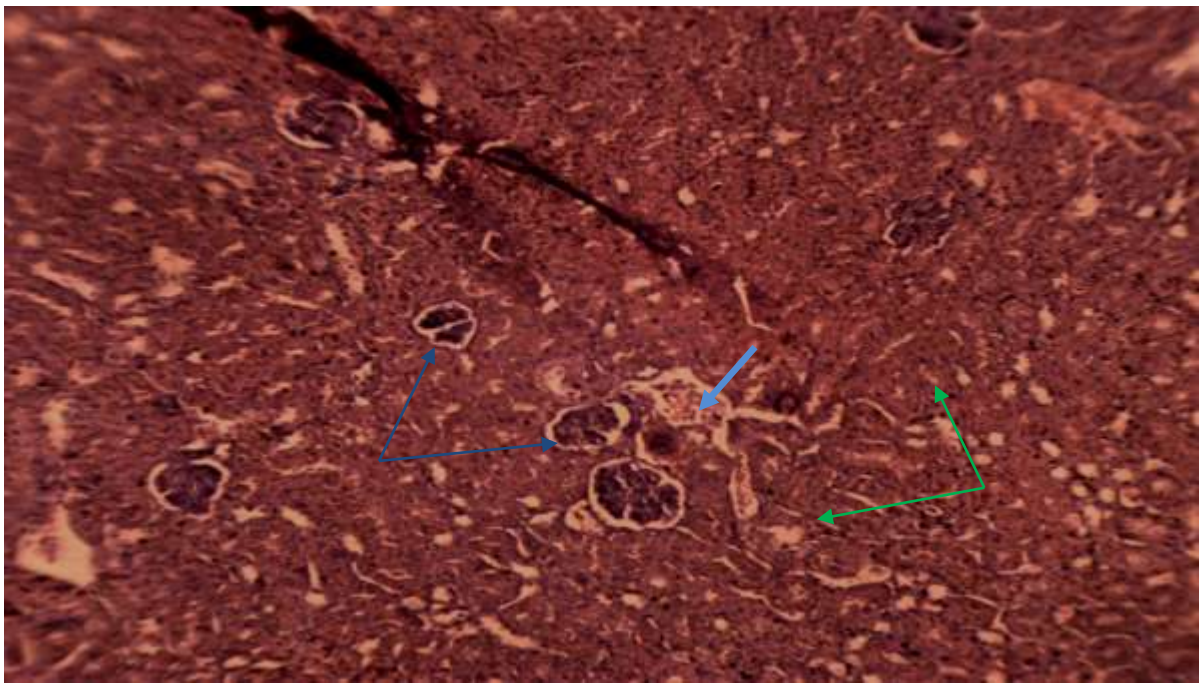


Figure (4-8): Photomicrographs (Cd_2) for mice **kidney** tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed moderate abnormal changes in the architectures and coagulate necrosis in the renal tubules , glumerular atrophy and moderate dilatation and congestion in the renal blood vessels .

The Histopathological observation of testes that obtained from groups (Cd_1 & Cd_2) which were treated with cadmium in doses(1.56mg/Kg B.W.),(2.52mg/Kg B.W) respectively showed mild to moderate various damages in the testis with sloughing and degeneration of the spermatocytes and filled the tubular lumen with desquamated immature cells with reduce germ cells production .Atrophy with mild to moderate necrosis of the spermatogonia and spermatid with infiltration few of the inflammatory cells and moderate congestion and dilatation of the blood vessels. (Figure 4-9,4-10) :

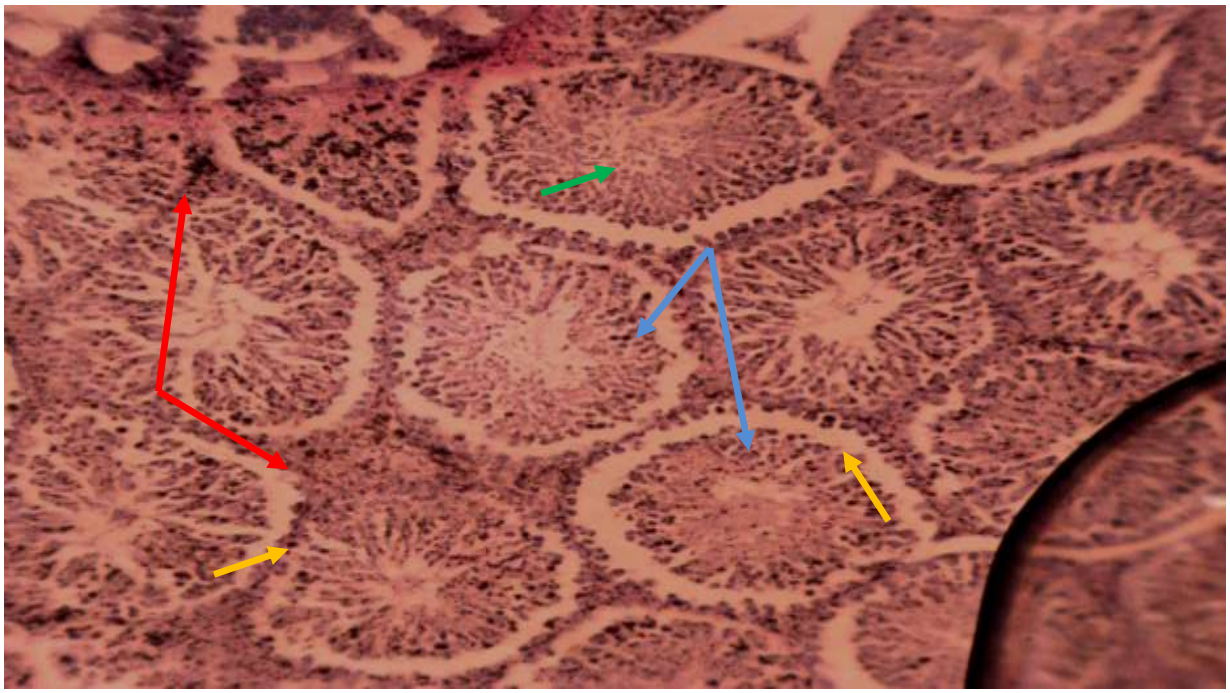


Figure (4-9): Photomicrographs (Cd_1) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed mild sloughing and degeneration of the spermatocytes and filled the tubular lumen with desquamated immature cells, atrophy with mild to moderate necrosis of the spermatogonia and spermatid with infiltration few of the inflammatory cells .

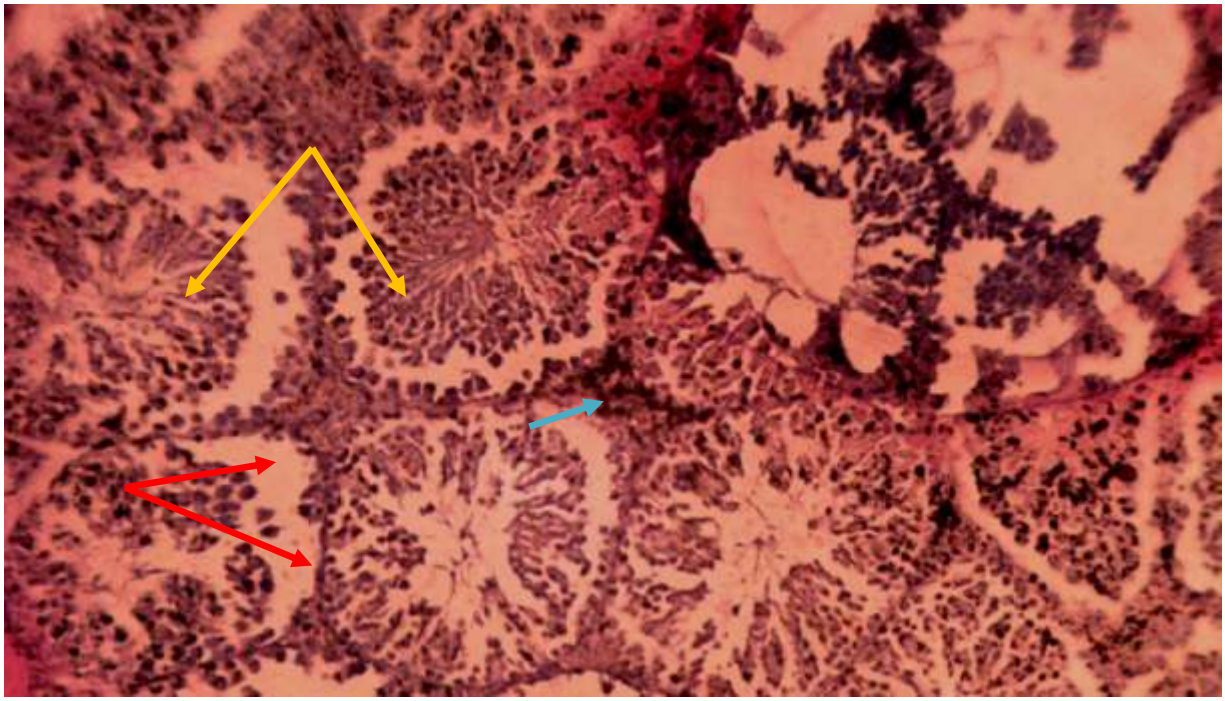


Figure (4-10): Photomicrographs (Cd_2) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed moderate degeneration ⟶ of the spermatocytes and filled the tubular lumen ⟶ with desquamated immature cells, atrophy with moderate necrosis of the spermatogonia and spermatid with infiltration few of the inflammatory cells ⟶.

4.1.2.2.3.Alignments of *IGF-1* genotypes and Histopathological observations for the Cd_3 (8.645 mg/kg Bw) group:

The results of the nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the *IGF-1* gene ,as the base T changed to C (T3268C), as the base G changed to A (G3289A) , the base G changed to T (G3291T), the base G changed to A (G3295A), as the base G changed to T (G3298T), as the G change to A(G3331A), as the base T change to G(T3337G), as the base T change to G(T3340G). Despite of the change in the genetic codes, the amino acids did not change because the amino acids have more than one cod.But the change in The bases G to T(G3291T)led tochange the amino acid from the Mithionine to Arginine, and the change in The bases G to T(G3299T,G3301 respectively) led to a change in the genetic coding, which changed the amino acid from Tyrosine to Glutamic acid and the change in The bases T to G (T3332G) led to a change in the genetic coding, which changed the amino acid from Alanine to Serine. These mutations were frameshift mutations. As shown in the table (4-4):

Table 4-4 : The Alignments of Cd_3 with the genotypes of *IGF-1-F* gene , shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of *IGF-1* gene.

Dosage type	*Site of SNP/InDel polymorphism*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cd_3 8.645 Mg/kg B.W.	3268	T⟶C	Transition	TCT⟶TGC	C=C
	3289	G⟶A	Transversion	CTG⟶CTA	L=L
	3291	G⟶T	Transition	AGG⟶ATG	M⟶R
	3295	G⟶A	Transition	AGG⟶AGA	R=R
	3298	G⟶T	Transversion	CTG⟶CTT	L=L
	3299 3301	G⟶T G⟶T	Transversion	GAG⟶TAT	Y⟶E
	3313	T⟶C	Transition	GCT⟶GCC	A=A

	3316	G→A	Transition	CCG→CCA	P=P
	3331	G→A	Transition	AAG→AAA	K=K
	3332	T→G	Transversion	TCA→GCA	A→S
	3337	T→C	Transition	GCT→GCC	A=A
	3340	T→C	Transition	CGT→CGC	R=R

Rattus norvegicus insulin-like growth factor (IGF) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length:7074
Range 1: 3266 to 3605

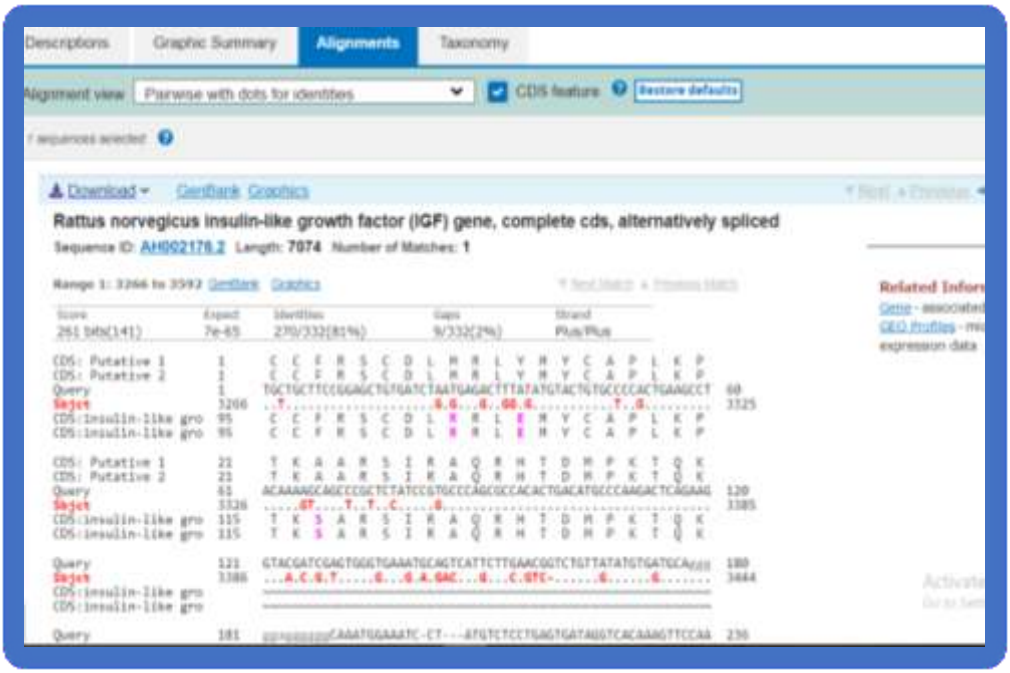


Figure 4-23 Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cd_3) and reference serial number of Rattus norvegicus (AH002176.2),in NCBI.

The Histopathological observation of Kidney in group (Cd_3) the mice treated with high doses of cadmium (8.645 mg/Kg B.W.) the renal tissue sections observed sever degeneration and damage of the glumerulous with sever coaggulative necrosis in the cells of the proximal and distal convoluted renal tubules with infiltration of the inflammatory cells , multiple foci of hemorrhage , dilatation and congestion of the blood vessels with RBCs (Figures 4-12,4-13).

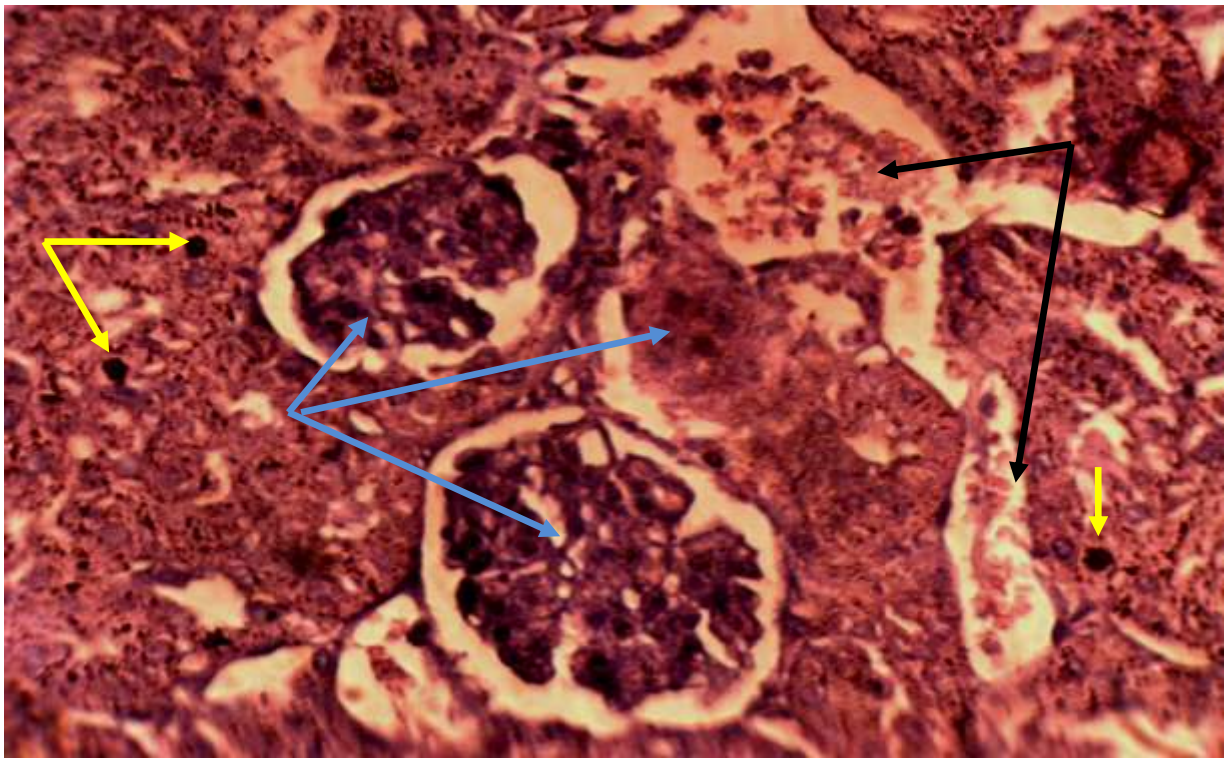


Figure (4-12): Photomicrographs (Cd_3) for mice kidney tissue sections stained with Haematoxylin & Eosin**(HE.400)** : Observed sever degeneration of the glumerulous and necrosis in the renal tubules →, dilatation and congestion in the renal blood vessels →,infiltration of the inflammatory cells →

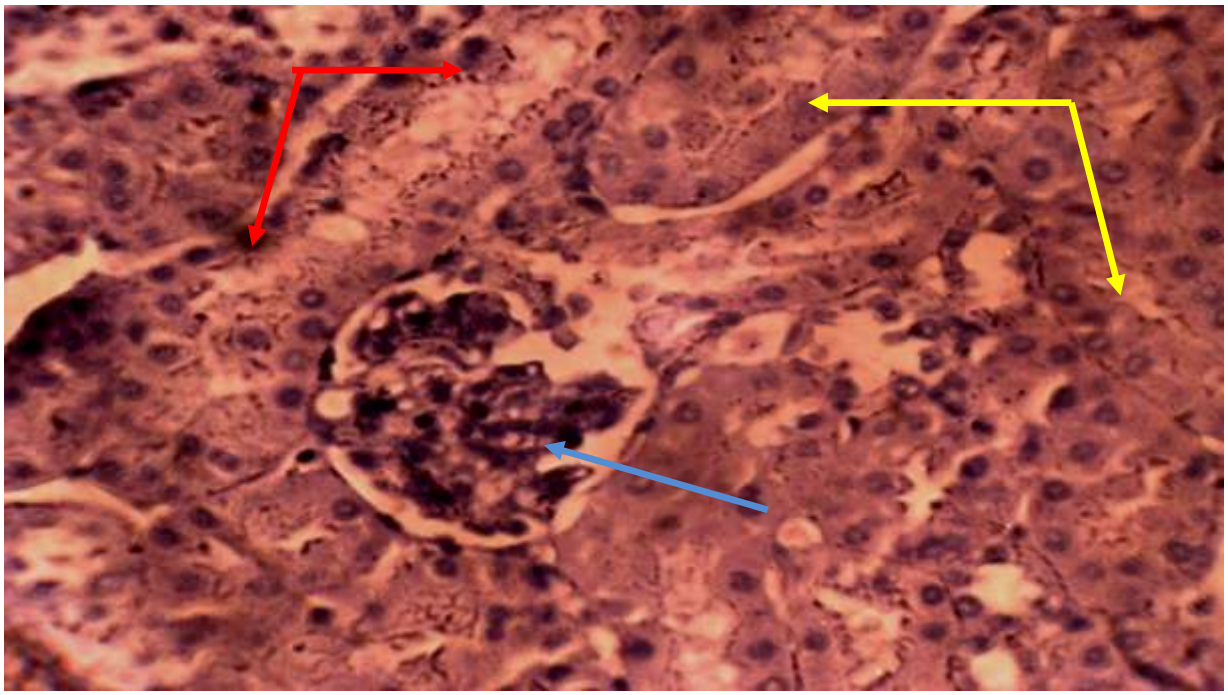


Figure (4-13): Photomicrographs (Cd_3) for mice kidney tissue sections stained with Haematoxylin & Eosin**(HE.400)** : Observed sever degeneration and damage of the glumerulous → and necrosis in the renal tubules → , infiltration of the inflammatory cells →.

The Histopathological observation of testes Tissue sections that obtained from mice treats with cadmium in dose (8.645mg/Kg B.W.) in group (Cd3) showed sever damage to the some of somniferous tubules with complete degeneration and necrosis of spermatogenic cells, pyknosis nuclei of spermatocytes with reduction the spermatozoa in the lumen of somniferous tubules in compared with control, sever dilatation and congestion in the blood vessels ,Figure (4-14).

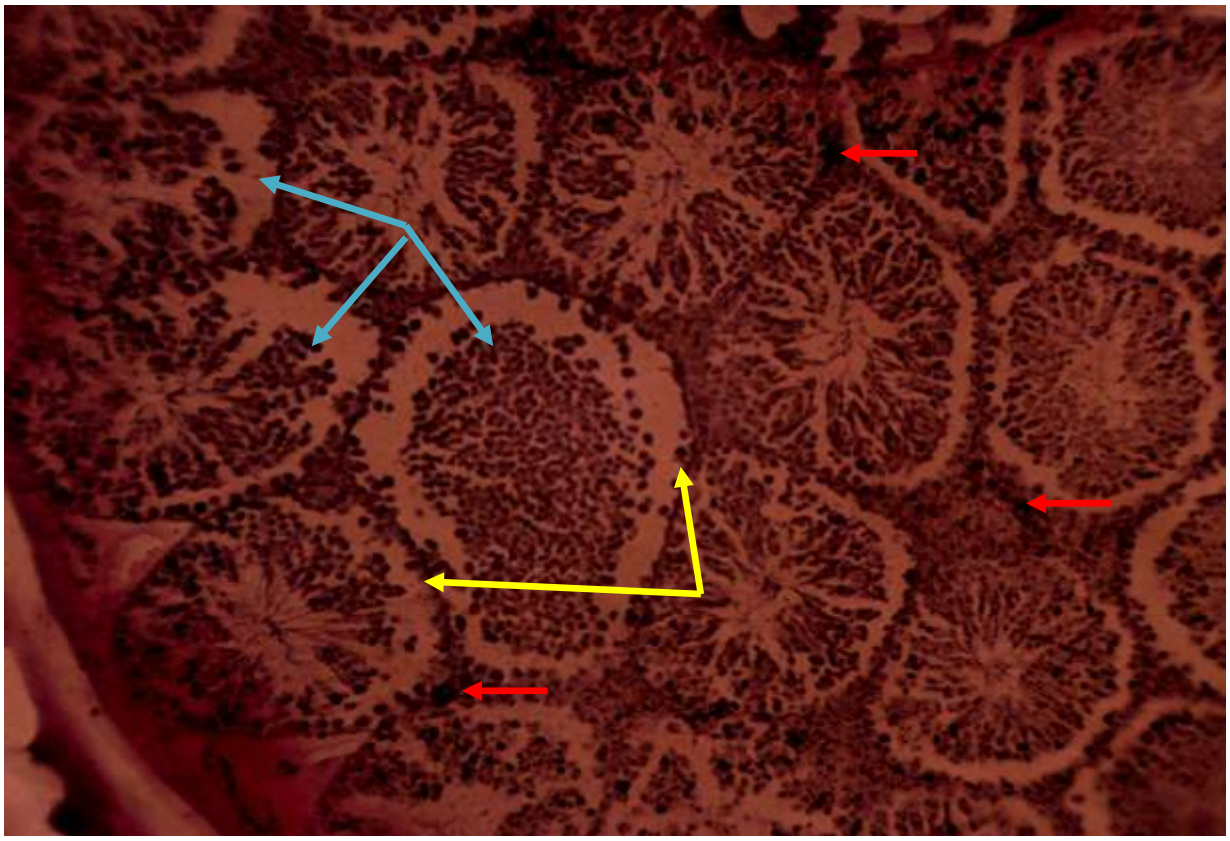


Figure (4-14): Photomicrographs (G4) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed sever damage to the some of somniferous tubules with complete degeneration and necrosis of the spermatocytes, sever necrosis of the spermatogonia and spermatid with infiltration few of the inflammatory cells

4.1.2.3.Alignments of *IGF-1* genotypes and Histopathological observations for the Chlorine mice's groups.

4.1.2.3.1.Alignments of *IGF-1* genotypes and Histopathological observations in Cl_1(0.0016 mg/kg Bw) group:

The results of the Alignments of Cl_1 (0.0016 mg/kg Bw) nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the *IGF-1* gene ,as the base G changed to A (G3295A), as the base T changed to C (T3313C) , the base G changed to A (G3316A), the base G changed to A (G3331A), as the base change T to C (T3337C), as the base T change to C(T3340C), as the base G change to T(G3349T), All this change in the codes did not cause a change in the amino acids because it has more than one code ,But the change in The bases T and G(T3332G) led to a change in the genetic coding, which changed the amino acids in the final protein. These mutations were frameshift mutations. As shown in the table (4-5):

Table:4-5 Alignments of Chlorine of Cl_1, with the genotypes of *IGF-1-F* gene , shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of IGF-1 gene.

Dosage type	*Site of SNP*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cl_1 0.0016 Mg/kg B.W.	3295	G➞A	Transition	AGG➞AGA	R=R
	3313	T➞C	Transition	GCT➞GCC	A=A
	3316	G➞A	Transition	CCG➞CCA	P=P
	3331	G➞A	Transition	AAG➞AAA	K=K
	3332	T➞G	Transversion	TCA➞GCA	A➞S
	3337	T➞C	Transition	GCT➞CGC	R=R
	3340	T➞C	Transition	TCC➞TCT	S=S
	3349	G➞T	Transversion	CGG➞CGT	R=R

Rattus norvegicus insulin-like growth factor (*IGF*) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length:7074
Range 1: 3265 to 3595

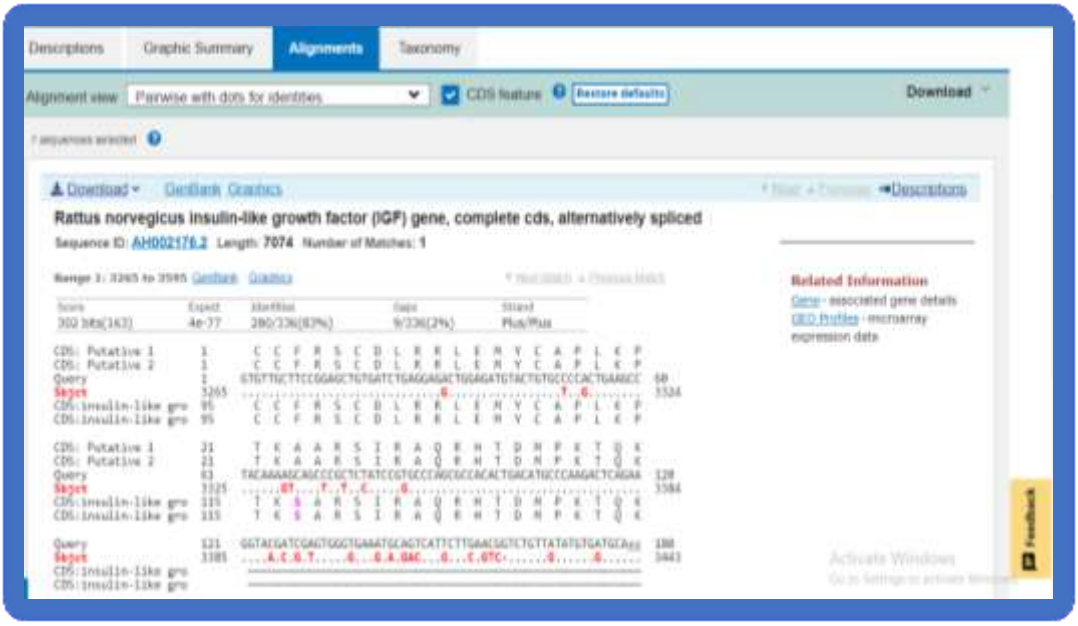


Figure 4-14 Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cl_1) and reference serial number of Rattus norvegicus (AH002176.2), in NCBI

4.1.2.3.2.Alignments of *IGF-1* genotypes and Histopathological observations in Cl_2(0.0016 mg/kg Bw) group:

The results of Alignments of Cl_2 (0.0033 mg/kg Bw) in nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the *IGF-1* gene ,as the base G changed to A (G3295A), as the base T changed to C (T3313C) , the base G changed to A (G3316A), the base G changed to A (G3331A), as the base change T to C (T3337C), as the base T change to C(T3340C), as the base G change to T(G3349T), All this change in the codes did not cause a change in the amino acids because it has more than one code ,While the change in The bases T and G(T3332G) led to a change in the genetic coding, which changed the amino acid from Alanine to serine. As shown in the table (4-6):

Table:4-6 Alignments of Chlorine of Cl_2, with the genotypes of *IGF-1-F* gene , shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of *IGF-1* gene.

Dosage type	*Site of SNP/InDel polymorphism*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cl_2 0.0033 Mg/kg B.W.	3295	G➞A	Transition	AGG➞AGA	R=R
	3313	T➞C	Transition	GCT➞GCC	A=A
	3316	G➞A	Transition	CCG➞CCA	P=P
	3331	G➞A	Transition	AAG➞AAA	K=K
	3332	T➞G	Transversion	TCA➞GCA	A➞S
	3337	T➞C	Transition	GCT➞GCC	A=A
	3340	T➞C	Transition	CGT➞CGC	R=R
	3349	G➞T	Transversion	CGG➞CGT	R=R

Rattus norvegicus insulin-like growth factor (IGF) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length:7074
Range 1: 3283 to 3581

Figure (4-15) Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cl_2) and reference serial number of Rattus norvegicus (AH002176.2), in NCBI.

The Histopathological observations for the kidney's mice which treated with Chlorine in (Cl_1 & Cl_2) groups, that treated with chlorine in doses (0.0016mg/Kg B.W) , (0.0033mg/Kg B.W) respectively exhibited abnormal histopathological changes in the cortex of the kidney , mild to moderate dilation of the renal tubules , focal interstitial inflammation with mild abnormalities and atrophy in the glumerulous , cytoplasm degeneration of cells in some of renal tubules, moderate

dilatation and congestion in the renal blood vessels with infiltration of the inflammatory cells in kidney tissues that treated with dose (0.0033mg/Kg B.W), (Figure 4-16):

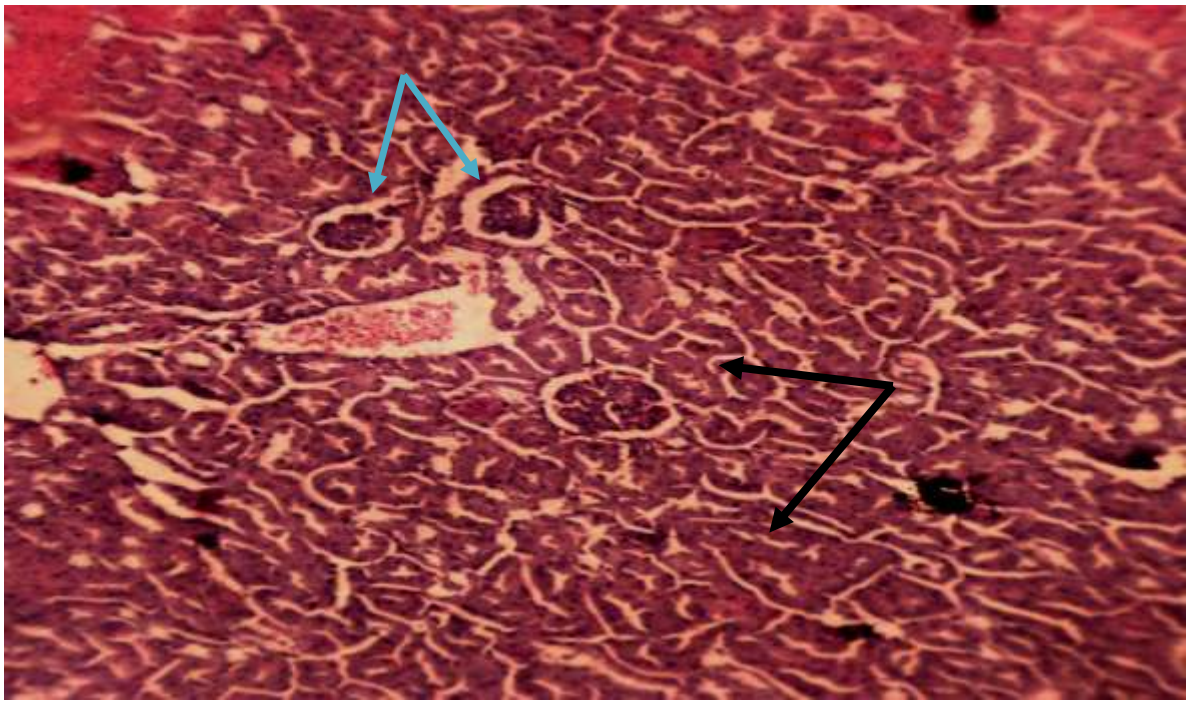


Figure (4-16): Photomicrographs (Cl_1) for mice kidney tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed mild focal inflammation and atrophy in the glumerulous mild abnormal changes in renal tubules → with decrease in lumen with cytoplasm degeneration of some tubular cells . →

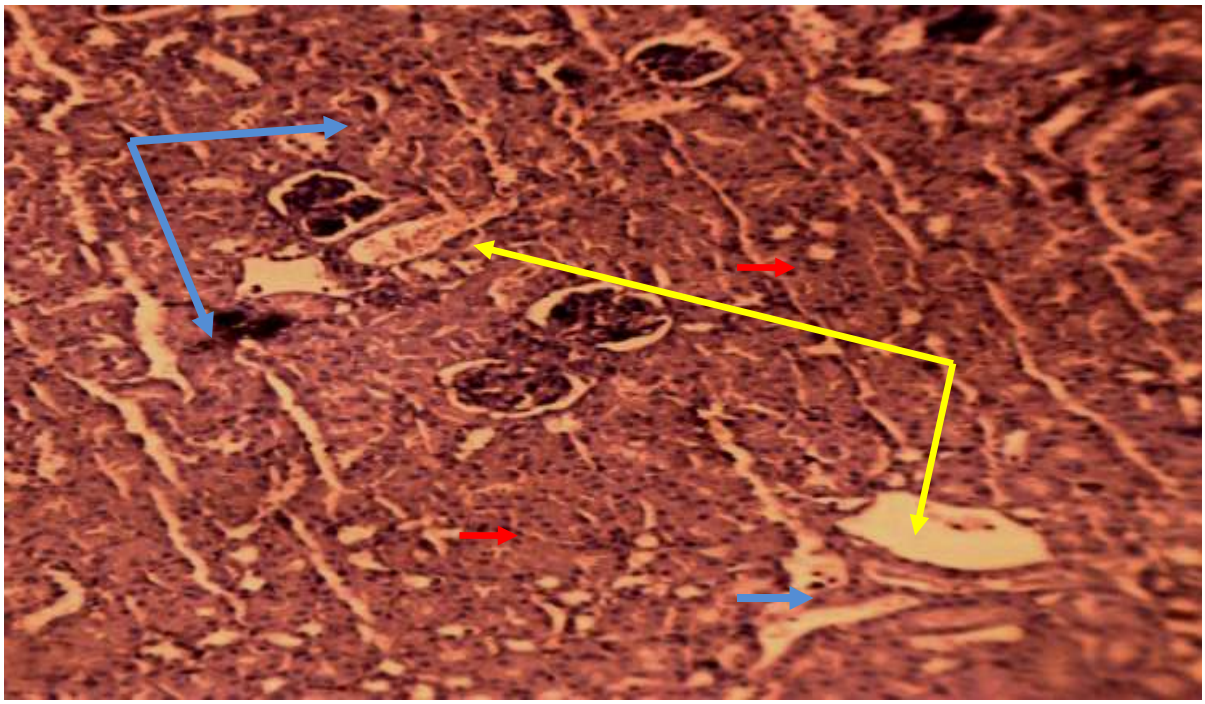


Figure (4-17): Photomicrographs (Cl_2) for mice kidney tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed degeneration of cells in some of renal tubules moderate dilatation and congestion in the renal blood vessels with infiltration of the inflammatory cells .

The Histopathological observations for the kidney's mice which treated with Chlorine (Cl_1) which were treated with chlorine in dose (0.0016mg/Kg B.W) showed abnormal alteration in the testes with mild sloughing and degeneration of the spermatocytes which filled the tubular lumen with desquamated immature cells with mild reduce germ cells production, atrophy with mild necrosis of the spermatogonia and spermatid , figure (4-18) :

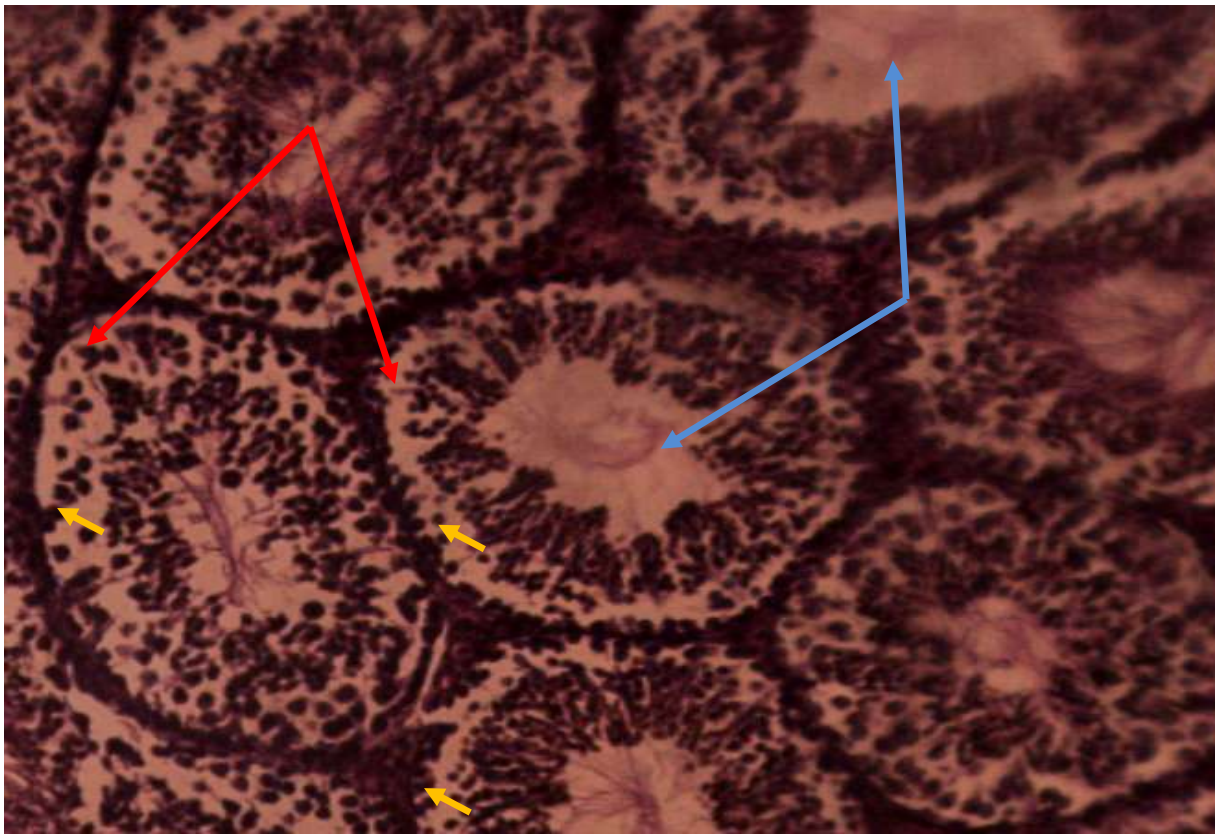


Figure (4-18): Photomicrographs (Cl_1) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed mild sloughing and degeneration of the spermatocytes → and filled the tubular lumen, mild reduce germ cells production → mild necrosis of the spermatogonia and spermatid →.

Tissue sections of testes that obtained from groups (Cl_2) which were treated with chlorine in dose (0.0033mg/Kg B.W) showed moderate damages in the testes with sloughing of the spermatogonia and degeneration of the spermatocytes and accumulated in the lumen with reduce germ cells production , atrophy and moderate necrosis of the spermatogonia , laydig cells and spermatid with infiltration few of the inflammatory cells, figure (4-19) :

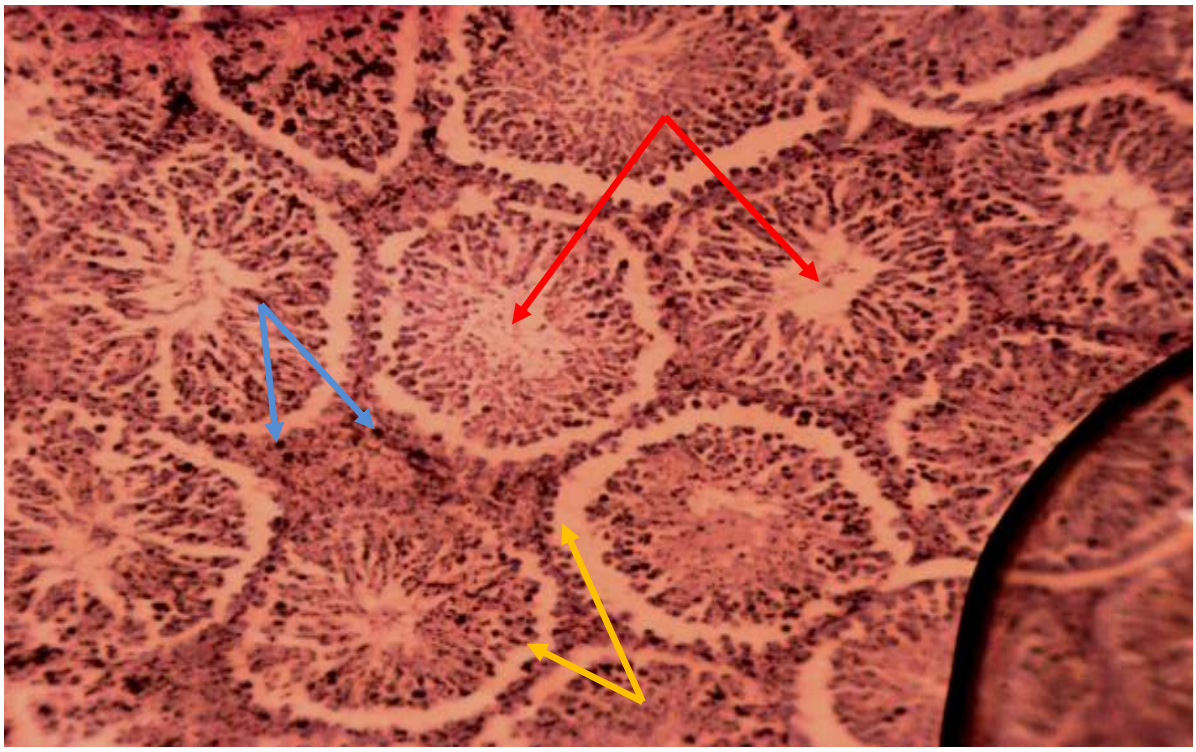


Figure (4-19): Photomicrographs (Cl_2) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : Observed damages in the testes with sloughing of the spermatogonia and sertoli cells →, degeneration of the spermatocytes and accumulated in the lumen →, infiltration few of the inflammatory cells →.

4.1.2.3.3.Alignments of *IGF-1* genotypes and Histopathological observations in Cl_3(0.0062 mg/kg Bw) group:

The results of Alignments in the nucleotide base sequence analysis show the presence of alterations in the nitrogenous bases, specifically in the accession number (AH002176.2) Of the *IGF-1* gene ,as the base C was changed to A (C3327A) lead to change the amino acid from Threonine to Alanine, as the base T deleted (T3332) , the base C was changed to G (C3336G) and base T was changed to C (T3331C) respictivly lead to a change in the genetic coding, which changed the amino acid from Alanine to Glycine, as the base change T to C (T3340C), as the base c change to g(c3343g), as the base G change to T(G3349T),. As shown in the table (4-7):

Table:4-7 Alignments of Chlorine of Cl_3, with the genotypes of *IGF-1-F* gene , shown the Nucleotide changes and type of mutations, the resulting amino acid changes, and their impact in the translation process of *IGF-1* gene.

Dosage type	*Site of SNP/InDel polymorphism*	*Nucleotide SNP*	*Type of Mutation*	*Triple code*	*Amino acid*
Cl_3 0.0062 Mg/kg B.W.	3313	T➞C	Transition	CTC➞CCC	A=A
	3316	G➞ A	Transition	CGC➞CAC	P=P
	3331	G➞ A	Transition	AAG➞AAA	K=K
	3332	T➞G	Transversion	TCA➞GCA	A➞S
	3337	T➞C	Transition	GTC➞GCC	R=R
	3340	T➞C	Transition	CGT➞CGC	R=R
	3343	C➞T	Transition	TCC➞TCT	S=S
	3349	G➞T	Transversion	CGG➞CGT	R=R

Rattus norvegicus insulin-like growth factor (*IGF-1*) gene.
Sequence ID: AH002176.2
Number of Matches: 1
Length:7074
Range 1: 3300 to 3675

Figure (4-20) Comparison the sequences of *IGF-1* gen for serial number of Albino Mice(Cl_3) and reference serial number of Rattus norvegicus (AH002176.2), in NCBI

The kidney tissue sections that obtained from mice in group (Cl_3) treated with chlorine in dose (0.0062mg/Kg B.W.) exhibited shrinking and degeneration of the glumerular blood vessels tuft , necrosis in the proximal and distal convoluted renal tubules ,multiple foci of hemorrhage with dilatation and congestion of the blood vessels with infiltration of inflammatory cells , (Figure 4-21,4-22).

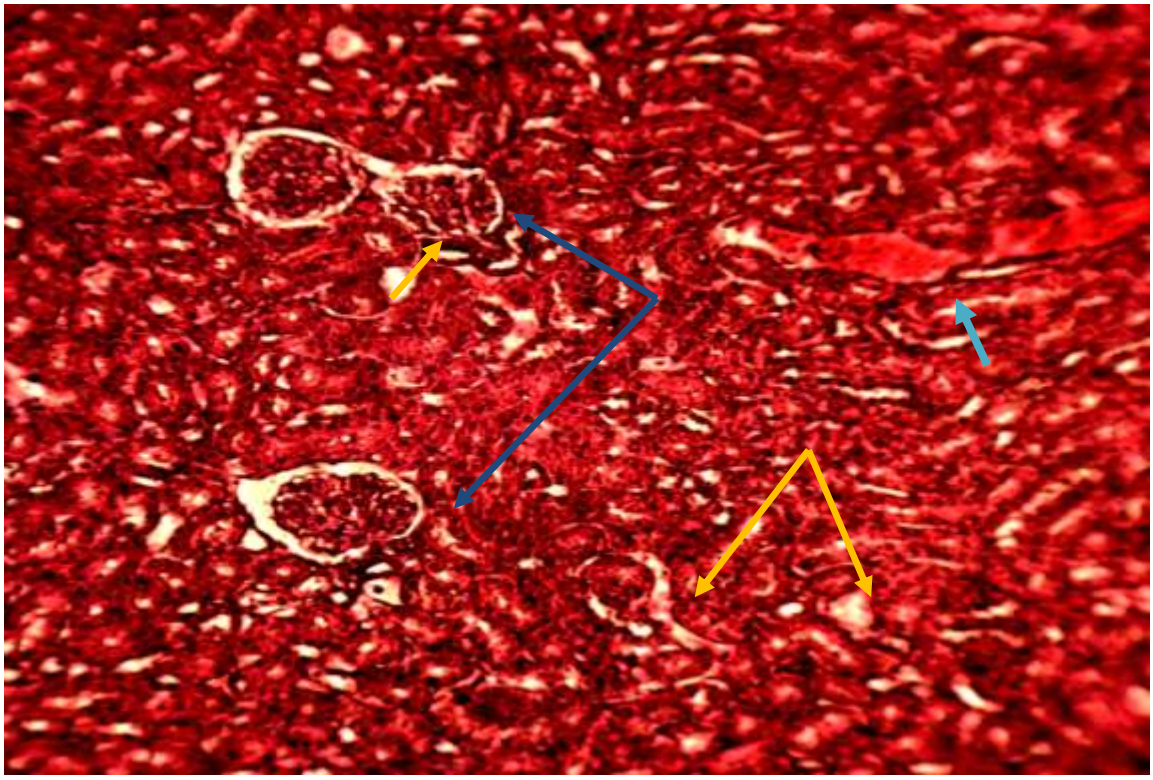


Figure (4-21): Photomicrographs (Cl_3) for mice kidney tissue sections stained with Haematoxylin & Eosin **(HE.100)** : Observed shrinking and degeneration of the glumerulous , necrosis in the proximal and distal convoluted renal tubules multiple foci of hemorrhage with dilatation and congestion of the blood vessels

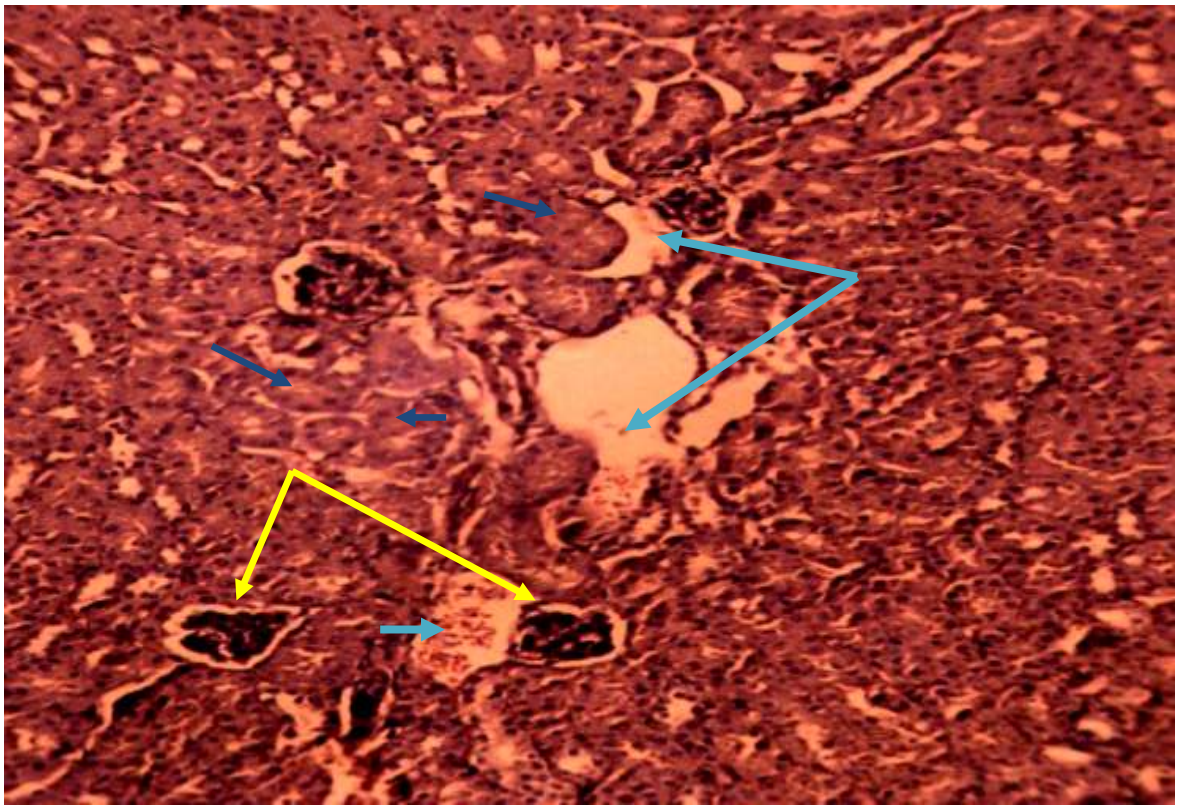


Figure (4-22): Photomicrographs (Cl_3) for mice kidney tissue sections stained with Haematoxylin & Eosin **(HE.100)**: observed sever degeneration and damage of the glumerulous coaggulative necrosis in the renal tubules , dilatation and congestion of the blood vessels

The Histopathological observation of testes Tissue sections that obtained from mice treats with chlorine in dose (0.0062mg/Kg B.W.) in group (Cl_3) showed sever damage with irregular shaped of the somniferous tubules with increase distances between the somniferous tubules , sever coaggulative necrosis of spermatogenic cells, pyknosis nuclei of spermatocytes with reduction the spermatozoa in the lumen of somniferous tubules ,sever damage in the lydig cells sever necrosis of the spermatogonia and spermatid, Figure (4-23).

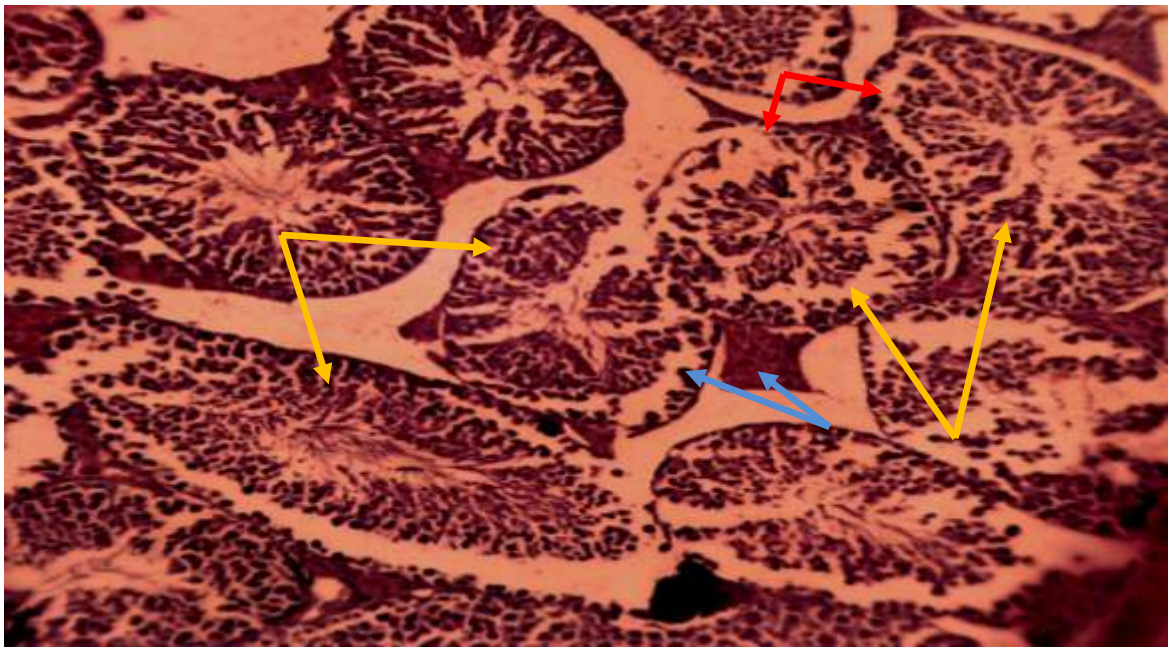


Figure (4-24): Photomicrographs (Cl_3) for mice testes tissue sections stained with Haematoxylin & Eosin**(HE.100)** : showed sever damage with irregular shaped of the somniferous tubules → sever coaggulative necrosis of spermatogenic cells → sever damage in the laydig cells necrosis of the spermatogonia →.

4..2.Gene expression

Real time PCR

The present study aimed to investigate the impact of chlorine (Cl) and cadmium (Cd) exposure on the gene expression levels of TNF-α in male mice. The mice were divided into different subgroups based on the concentration of Cl or Cd to which they were exposed. The Cl exposure subgroups included Cl_1 (2.5 mg/l), Cl_2 (5 mg/l), and Cl_3 (10 mg/l), while the Cd exposure subgroups included Cd_1 (3 ppm), Cd_2 (6 ppm), and Cd_3 (13 ppm). A control group was also included for comparison.

The *TNF-α* gene expression analysis results revealed significant differences among the subgroups. In the Cl exposure group, the highest *TNF-α* expression level was observed in Cl_3 (10 mg/l) with a mean value of (2.92 ± 0.28). This was followed by Cl_2 (5 mg/l) with a mean value of 0.083 ± 0.08, and the lowest expression level

was found in Cl_1 (2.5 mg/l) with a mean value of 0.015 ± 0.08. On the other hand, in the Cd exposure group, the highest *TNF-α* expression level was observed in Cd1 (1.56 mg/kg Bw) with a mean value of 6.19 ± 0.77, followed by Cd_2 (2.52 mg/kg Bw) with a mean value of 4.89 ± 0.55, and the lowest expression level was found in Cd3 (8.645 mg/kg Bw) with a mean value of 1.4 ± 0.46. The control group exhibited a *TNF-α* expression level of 2.17 ± 0.044.

Table 8 and 9 results indicate that both Cl and Cd exposure can lead to alterations in *TNF-α* gene expression in male mice. In the Cl exposure group, there was a dose-dependent increase in *TNF-α* expression, with the highest expression observed at the highest concentration of Cl (Cl_3). This suggests that Cl exposure may induce an inflammatory response mediated by *TNF-α*. On the other hand, in the Cd exposure group, there was an inverted U-shaped relationship between Cd concentration and *TNF-α* expression, with the highest expression observed at intermediate Cd concentrations (Cd_1 and Cd_2). This finding implies that Cd exposure may trigger an inflammatory response, but higher concentrations might lead to a reduced *TNF-α* expression, possibly due to cytotoxic effects on cells.

A statistical analysis was conducted to compare the significance of differences between the subgroups. The significance levels were determined using appropriate statistical tests (ANOVA or t-tests), and the results were represented using alphabetic notations (a, b, c) to indicate significant differences. The respective tables for Cd and Cl exposure groups present the specific statistical analysis and significant differences.

Table 4-8: *TNF-α* Gene Expression in Cadmium (Cd) Exposure Subgroups.

#	Group	Dose	*TNF-α*	Significant litter
1	Cd_1	1.56 mg/kg Bw	**6.19 ± 0.77**	**A**
2	Cd_2	2.52 mg/kg Bw	**4.89 ± 0.55**	B
3	Cd_3	8.645 mg/kg Bw,	**١.٤ ± 0.٤٦**	**C**
4	Control	**0** mg/kg Bw	**2.17 ± 0.044**	**D**

Different lowercase letters (a, b, c, d) denote significant differences ($p < 0.05$) among the groups based on post-hoc analysis.

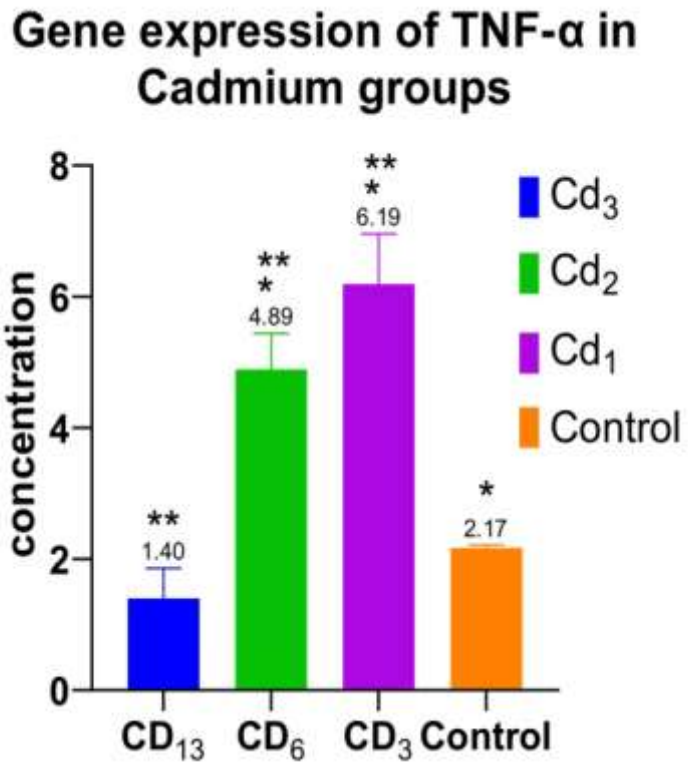


Diagram (4-1) Gene expression of *TNF*-∝ in Cadmium groups .

Table 4-9: *TNF-α* Gene Expression in Chlorine (Cl) Exposure Subgroups.

	Group	Dose	*TNF-α*	Significant litter
1	Cl_1	0.0016 mg/kg Bw	**0.015 ± 0.08**	**B**
2	Cl_2	0.0033 mg/kg Bw	**0.083 ± 0.08**	B
3	Cl_3	0.0062 mg/kg Bw,	**2.92 ± 0.28**	**A**
4	Control	**0** mg/kg Bw	**2.17 ± 0.044**	**A**

Different lowercase letters (a, b, c, d) denote significant differences ($p < 0.05$) among the groups based on post-hoc analysis.

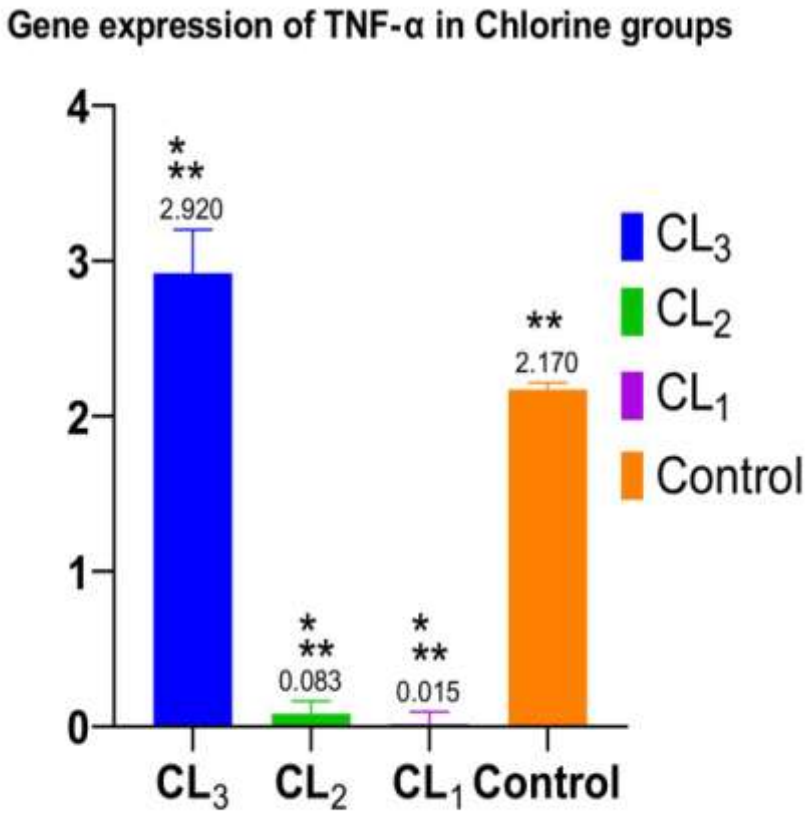


Diagram (4-2) TNF-∝ Gene expression levels of Chlorine groups .

4.3. The concentration of chlorine and cadmium in Iraqi water

In this study, the concentrations of chlorine and cadmium were highlighted in some water areas in Iraq, specifically in Misan Governorate. Samples were collected from the well water, the water of Shatt al-Amarah and the water of Shatt al-Kahla. In addition, samples were taken from the chlorinated water at the al-Munajjid water station, and tap water that reaches the houses, and the results were as follows:

4.3.1 Tigris river in Misan

Information provided by the Department of Environment in Misan province indicates in May 2023, that the percentage of cadmium is zero ppm in the river and 0.02 mg/l in the well water.

According to the information provided by the Environment Department in Misan Governorate, according to the analyzes conducted in May 2023, the percentage of chlorine in the water of Shatt al-Amarah has reached 360 parts per million, and in the water of Shatt al-Kahla is 480 mg / liter, while the percentage of chlorine in the water of the well has reached 1925 mg / liter. The percentage of river water is relatively high compared to the natural level specified by the Ministry of Environment, which ranges from 0 to 600 milligrams per liter, while the percentage of chlorine in well water is very high.

4.3.2 Al-Munajjid water station in the city of Amarah

The information provided by the Environment Department in Maysan Governorate, according to the analyzes conducted in June 2023, indicates that chlorine levels in the Al-Munajjid water station in the city of Amarah amount to 375 parts per million when taken directly from the station, and 3 parts per million in tap water that is delivered to the houses. Chlorine is commonly used as a disinfectant in water treatment processes to kill harmful microorganisms and ensure safe drinking water. The chlorine levels of 375 ppm in Al-Munjid water station and tap water appear to be relatively high, respectively. It is important to note that acceptable chlorine levels in drinking water can vary according to regional regulations and guidelines.

4.3.3. Marshes of Misan

The Information provided by the Department of Environment in Misan province indicates in June 2023, that the percentage of cadmium is zero or Nil ppm in Al-Khair sub-district.

Through the analysis that was conducted in the Maysan Environment Department in June 2023, the concentration of chlorine in the Al-Amarah marshes, specifically in the Al-Khair marshes, reached 1100 parts per million, which exceeds the maximum normal rate of 200 parts per million. This high chlorine concentration indicates a potential environmental concern in the marshes.

Table (4-10) The concentration of cadmium in Iraqi water/Misan .

Regions In Misan governorate	Amount in Parts per million(ppm)	Amount in milligrams per liter(Mg/l)
Al-Munajjid Water Station (treated)	--------	---------
Tap water in the houses(treated)	---------	-------
The well (untreated)	0.02	0.019977
Tigris River /Shatt al-Amarah (untreated)	Nil	------
Tigris River/Shatt al-alkahla (untreated)	Nil	---------
The marshes /the marsh of Al-khair sub-district	Nil	---------

According to the standards of the Iraqi Ministry of Environment, the concentration of cadmium in water should be between 0-0.005 ppm.

Table (4-11) The concentration of chlorine in Iraqi water/Misan .

Regions In Misan governorate	Amount in Parts per million(ppm)	Amount in milligrams per liter(Mg/l)
Al-Munajjid Water Station (treated)	375	359.58
Tap water in the houses(treated)	3	22.9
The well (untreated)	1925	1922.8
Tigris River /Shatt al-Amarah (untreated)	360	374.57
Tigris River/Shatt al-alkahla (untreated)	480	479.45
The marshes /the marsh of Al-khair sub-district	1100	1098.74

According to the guidelines of the World Health Organization, the concentration of the amount of “optional free chlorine” in drinking water should be between 0.2 to 0.5 mg / liter.

And the concentration of the amount of “optional free chlorine” in drinking water should be between 0-200 ppm(Mg/l) ,and the concentration of the chlorine in the rever water should be between 0-600 ppm , According to the standards of the Iraqi Ministry of Environment.

5. Discussion

5.1. Histopathological observations

5.1.1 Histopathological observations in the mice treated with Cadmium.

5.1.1.1 Histopathological changes in kidney.

The renal tissue sections in control group revealed normal architecture with normal appearance of glumerular tuft of blood capillaries surrounded Bowman's capsule with normal space in the bowman's capsules, also the proxiamal and distal convoluted tubules and lined by cuboidal cells appear in normal size with their nuclei. The kidney has two distinct regions, the outer cortex, and an inner medulla. The cortex consists of Malpighian corpuscles and both proximal and distal convoluted tubules, while the medulla consists mainly of Henle's loop's descending and ascending limbs. However, the collection tubules are located in both the cortical and medullary regions. The Malpighian corpuscles consist of a tuft of blood capillaries, the glomerulus, and Bowman’s capsule. The latter is a double-walled cup formed of two layers of simple squamous epithelium, an outer parietal layer, and an inner visceral layer or glomerular epithelium consisting of a single layer of epithelial cells, which closely invest the glomerulus and dips down the lobules of the glomerular capillaries. In addition, the glomerular capillaries possess certain cells defined as mesangial cells (Mescheral ,2016).

The results of this study observed the treated with cadmium in doses (1.56mg/Kg B.W.) , (2.52mg/Kg B.W.) respectively exhibited mild to moderate histopathological focal interstitial inflammation with mild abnormalities and

atrophy in the glumerulous ,the renal tubules observe mild to moderate abnormal changes in their architectures, while the mice treated with high dose of cadmium (8.645 mg/kg Bw) observed sever degeneration and damage of the glumerulous with sever coaggulative necrosis in the cells of the renal tubules, multiple foci of hemorrhage , dilatation and congestion of the blood vessels, multiple foci of hemorrhage , dilatation and congestion of the blood vessels and infiltration of the inflammatory cells. Gaseous chlorine is poisonous and classified as pulmonary irritant ,it has intermediate water solubility with the capability of causing acute damage to the upper and lower respiratory tract. Toxicity to chlorine gas depends on the dose and duration of exposure, where at concentrations of 1 to 3 ppm, chlorine gas acts as an eye and oral mucous membrane irritant, while at 15 ppm, there is an onset of pulmonary symptoms, and it can be fatal at 430 ppm within 30 minutes(Morim and Guldner,2022). In study that performed by Daniel et al. (1990) by exposed groups of Sprague-Dawley rats (10 male and 10 female) to chlorine dioxide in drinking water for (90 days) at concentrations of 0, 25, 50, 100, or 200 mg/L caused significant reductions in the body weights and body weight gain (26%–29% lower than controls). Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, fertilizer industries, and cigarette smoke. Cadmium is dangerous because humans consume both plants and animals that absorb cadmium efficiently and concentrate it within their tissues (Stohs and Bagchi, 1995).

Cadmium exposure can stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals. Once absorbed the cadmium rapidly cleared from the blood and concentrates in various tissues mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic

and histological changes, membrane damage, altered gene expression and apoptosis (Casalino et al, 2002, Waisberg et al, 2003).

Genchi et al (2020) reported when exposure to the cadmium can enters the body and transport into the bloodstream via erythrocytes and albumin and is then accumulated in the kidneys and excretion from the body is slow and occurs via the kidneys, urine, saliva, and milk during lactation. In humans the exposure to the cadmium can cause a variety of adverse effects, such as renal and hepatic dysfunction, pulmonary edema, testicular damage, osteomalacia, and damage to the adrenals and hemopoietic system.

When the cadmium acetate is administered to the rat in varying concentration, there is interaction between the Cd2+ and the enzyme molecule which inhibits the activity of superoxide dismutase (SOD) to increase the lipid peroxidation in liver and kidney. It is indicated that Cd-induced elevation in lipid peroxidation is not only due to the inhibition of the activity of the superoxide dismutase (SOD) but also due to the direct action of Cd2+ on the peroxidation reaction (Hussain et al,1987).

Siddiqui (2010) reported that a regular oral intake of CdCl2 solution (in drinking water)to the rats in daily dose (0.6mg /kg B.W) for 30 days causes severe damage to the kidneys, and the cortex region is most affected. Cytosolic damaged observed in the renal tubular epithelium. Chen et al (2011) detected that exposed to (1.5 mg Cd/kg) observed glomerulus had shrink and the nuclei were irregular, renal tubule damage, denaturalization and necrosis of the cells. The microvilli of proximal tubule epithelial cells were shortened.

Capaldo et al(2016) were reported the administration of the cadmium in highest dose (178 nM/L of Cd) for three months effected on the kidney and caused

glomerular expansion, reduction of Bowman's space, degeneration in the epithelial cells with karyolysis and karyorrhexis of the neucli.

Yan et al (2021) found cadmium exposure has been tightly associated with renal dysfunction and kidney damage, causing polyuria and proteinuria . The proximal tubule is the major site of cadmium deposition, accumulation, and damage because of the development of proximal tubular epithelial cell hypertrophy with occurrence of polyuria and proteinuria. Therefore, it is important to counteract cadmium-induced kidney injury to safeguard kidney function.

Cadmium can induce kidney injury by ROS production and culminate in oxidative stress which suggests that oxidative damage is a unifying mechanism of cadmium induced renal toxicity and injury and the major sources of ROS causing oxidative damage in this context are mitochondria and NADPH oxidase(Yan et al ,2021).

5.1.1.2 Histopathological changes in testes.

The control group showed normal morphology in the tissues of testes. the cycle of spermatogensis was regulated in normal , sertoli and laydig cells were found in normal structures and in the interstitial space of the testes ,primary spermatocytes ,spermatid and spermatozoa are also noticed in normal structure. All somniferous tubules were Confined with basement membrane and composed of spermatogonia which observed rounded cells .

The testicle appeared covered by a capsule of connective tissue (the tunica albuginea) and consisted of somniferous tubules which appeared rounded or oval with regular contour. The interstitial spaces in-between the tubules contain a delicate loose C. T and Leydig cells, the somniferous tubules were enclosed by a

basement membrane encircling myoid cells and lined by stratified spermatogenic cells and supporting Sertoli cells, spermatogonia were seen close to the basement membrane with their dark nuclei, primary spermatocytes were the largest cells, the spermatids appeared smaller than primary spermatocytes and lying near the lumen. The lumen enclosed large sperms, and some of these sperms were attached to the apex of Sertoli cells(Hasanin et al ,2018).

Tissue sections of testes that obtained from mice in group (Cd_1) treated with cadmium in dose (1.56 mg/Kg B.W.) , also form mice in group (Cd_2) treated with cadmium in dose (2.52 mg/Kg B.W.) showed mild to moderate various damages in the testes with sloughing and degeneration of the spermatocytes and filled the tubular lumen with desquamated immature cells with reduce germ cells production .Atrophy with mild to moderate necrosis of the spermatogonia and spermatid with infiltration few of the inflammatory cells and moderate congestion and dilatation of the blood vessels. The mice treats with cadmium in dose (8.645 mg/kg Bw) in group (Cd_3) showed sever damage to the some of somniferous tubules with complete degeneration and necrosis of spermatogenic cells, pyknosis nuclei of spermatocytes with reduction the spermatozoa in the lumen of somniferous tubules in compared with control, sever dilatation and congestion in the blood vessels.

Cadmium can causes cell damage through depleting the antioxidant content, such as glutathione and protein-bound sulfa hydral groups that results in the production of reactive oxygen species (ROS), including superoxide ions and hydroxyl radicals (Zamani et al ,2021). Some of studies have detected that cadmium could induce irreversible detrimental effects on mammalian testis by disrupting the vascular system and impacts on the somniferous epithelium result in necrosis and testicular ischemia (Shojaeepour et al ,2021).

Several researches have shown that the cadmium could diminish semen quality such as motility, viability, sperm count, and morphology in addition, cadmium can impose toxicity through activating a number of signal transduction pathways, namely MAPK/phosphoinositide 3-kinase (PI3K)/c-Jun Nterminal kinase (c-JNK) signaling pathway that in turn disrupts blood-testis barrier and cell junction in the somniferous epithelium (Saeed,2013).

Ali et al (2022) reported that the administration of a single high dose cadmium can decreased testicle weight, sperm count and testosterone production by perturbation the cell cycle, DNA repair system, and cell proliferation. Moreover, the deterioration of sperm count results from decrease testosterone levels.

Environmental exposure to the single dose of cadmium induced apoptosis in the testes and reduced serum testosterone level which may contribute reduce human male sperm concentration , sperm motility , induce testicular and epididymal damage which may contribute to male infertility by reducing sperm quality in both humans and rodents (Predes et al ,2010; Roychoudhury et al ,2010).

Genchi et al (2020) reported that the cadmium induces alteration of steroidogenesis, disorders of the menstrual cycle and reproductive hormones, delay in puberty and menarche, pregnancy loss, premature birth, and reduced birth weight , also have negative role bone mineral density in postmenopausal women.

Women have a higher cadmium body burden than men, reflecting a higher concentration of cadmium in the blood, urine, and kidneys and intestinal cadmium absorption increases with a depleted body iron store and with overt iron deficiency, conditions that are prevalent in women of fertile age (Kippler et al ,2007).

Cadmium has a negative effect on sperm metabolism through the inhibition of glycogen phosphorylase, magnesium-dependent ATPase, glucose-6-phosphatase, and succinate dehydrogenase as well as reduced viability and motility of sperm (Ali et al ,2022)

5.1.2 Histopathological observations for the mice treated with Chlorine

5.1.2.1 Histopathological changes in kidney.

The groups of mice (Cl_1 & Cl_2) that treated with chlorine exhibited abnormal histopathological changes in the cortex of the kidney with mild to moderate dilation of the renal tubules , mild abnormalities and atrophy in the glumerulous , cytoplasm degeneration of cells in some of renal tubules, moderate dilatation and congestion in the renal blood vessels with infiltration of the inflammatory cells .While the kidney tissue sections that obtained from mice in group (Cl_3) treated with chlorine in dose (0.0062mg/Kg B.W.)observed shrinking and degeneration of the glumerular blood vessels tuft , necrosis in the proximal and distal convoluted renal tubules ,multiple foci of hemorrhage with dilatation of the blood vessels with increase infiltration of inflammatory cells.

Gaseous chlorine is poisonous and classified as pulmonary irritant ,it has intermediate water solubility with the capability of causing acute damage to the upper and lower respiratory tract. Toxicity to chlorine gas depends on the dose and duration of exposure, where at concentrations of 1 to 3 ppm, chlorine gas acts as an eye and oral mucous membrane irritant, while at 15 ppm, there is an onset of pulmonary symptoms, and it can be fatal at 430 ppm within 30 minutes(Morim and Guldner,2022).

In study that performed by Daniel et al. (1990) by exposed groups of Sprague-Dawley rats (10 male and 10 female) to chlorine dioxide in drinking water for (90 days) at concentrations of 0, 25, 50, 100, or 200 mg/L caused significant reductions in the body weights and body weight gain (26%–29% lower than controls).

Harrington et al (1995) were reported the administered doses of sodium chlorite to the rats (80 mg/kg-day) orally via gavages for 13 weeks exhibited salivation, significantly decreased erythrocyte counts, and decreased total serum protein levels. decreased hematocrit and hemoglobin levels and increased methemoglobin which have oxidative effects and lead to morphological changes in erythrocytes ,also increases in relative liver and kidney weights in the females.

Nabil , et al (2020) reported that the mercury chloride $HgCl_2$ is the most toxic salts of mercury and metabolized primarily in the liver then accumulated in the kidneys which considered the most affected in the liver and kidneys . Administration of initiates the formation of highly reactive substances such as reactive oxygen species in addition to the stimulation of oxidative stress and lipid peroxidation level increased while, the antioxidant enzymes activities decreased.

Animals exposed to chlorine concentrations of 100 mg/L exhibited renal pathology, which characterized by distention of the glomerular capsule and appearance of a pale pinkish staining material in the renal tubules , concluded that the renal pathology was a nonspecific salt effect, but this observation does not alter the observation that concentrations of 100 mg/L or higher led to adverse effects (EPA,2000).

Mild exposure Chlorine gas may cause mucosal membrane irritation,while severe exposure will induce edema of both the upper airway and the lung parenchyma and acute exposure can induce wheezing, cough, and dyspnea. Acute lung injury adult respiratory distress syndrome (ARDS) can also be seen in some severe cases ,while chlorine gas is primarily reactive only at a local level, thus absorbed systemic effects are not commonly observed(Morim and Guldner,2022).

Vajner and Lung (2013) were detected that exposed to low concentration chlorine gas (up to 2 ppm)can cause mucous membrane irritation ,while the higher concentration exposures (between 9 ppm and 50 ppm) may lead to chemical pneumonitis and bronchiolitis obliterans and leads to extensive bronchial constriction.

5.1.2.2 Histopathological changes in testes.

Tissue sections of testes that obtained from groups (Cl_1) which were treated with chlorine in dose (0.0016mg/Kg B.W.) showed abnormal alteration in the testes with mild sloughing and degeneration of the spermatocytes with mild reduce germ cells production and atrophy with mild necrosis of the spermatogonia and spermatid, while the tissue sections of testes for the mice in groups (Cl_2) were treated with chlorine in dose (0.0033 mg/Kg B.W.) showed moderate damages in the testes with sloughing of the spermatogonia and degeneration of the spermatocytes with reduce germ cells production , atrophy and moderate necrosis of the spermatogonia , laydig cells and spermatid with infiltration few of the inflammatory cells. In group (Cl_3) the tissue sections of testes that obtained from mice treats with cadmium in dose (7.92 mg/Kg B.W.) observed sever damage with irregular shaped of the somniferous tubules with increase distances between the

somniferous tubules , sever coaggulative necrosis of spermatogenic cells, pyknosis nuclei of spermatocytes with reduction the spermatozoa and sever damage in the lydig cells .

Chlorine treatment considered as main way of disinfection drinking water in Iraq, it is inexpensive, safe, simple in used, easily stored, not make the water unpalatable, chlorine in does (2 to 3ppm) concentration effective against most pathogenic bacteria and when combined with filtration became an excellent way for elimination all viruses, cysts, or worms (National Academy of Sciences, Yang).

El-Naggar et al (2022) were found histopathological changes testicular tissue of the rats after exposed to the chlorine which showed irregular in seminiferous tubules with their lumina containing scanty spermatids and spermatozoa. Moreover, the number of spermatocytes lining the tubules was decreased with increasing dose, several spermatocytic giant cells were observed and the interstitial space and vacuolated and necrotic interstitial cells of Leydig.

Chapin and Creasy(2012) Reported that exposed to chlorine (100 and 200 mg/kg) revealed altered structure in the seminiferous tubules with vacuolated and necrotic the interstitial cells of Leydig , also documented changes in testosterone level or decrease in the activity of the androgenic receptors could alter the histological structure of testicles and epididymis, this can occur due to a central depression of GnRH, direct depression of Leydig cell function or through increased elimination of testosterone.

5.2. Genetic analysis

5.2.1. Nucleotide Sequence and identify genotypes of *IGF-1*

Regarding the genotypic variations associated with *IGF-1*, the nucleotide sequence analysis and genotyping revealed a high sequence similarity between the control mouse group and the predicted sequence of the *IGF-1 gene*. This finding indicates that the control group shares a high degree of sequence homology with the known variant in Rattus norvegicus insulin-like growth factor (*IGF*) gene,which that high percentage of identity that suggests the control group likely belongs to the same gene variant. Similar studies have reported the association of specific genotypes with different phenotypic traits and susceptibility to environmental toxins (Smith et al., 2007). The current study contributes to understanding genotypic variations in the *IGF-1* gene and their potential implications for the response to chlorine and cadmium exposure.

The mentioned tables (4-1) alignments of the control mouse group with the genotypes of the *IGF-1* gene, specifically the *IGF-1-F* sequences, revealing the similarities and differences between the tested sample and the predicted sequences. Based on these alignments, it is possible to discuss the mechanism of the detected defect and how the references support these findings. The alignment analysis of the *IGF-1-F* sequence in Table (4-1) shows a high percent identity of 84.92% between the tested sample and the predicted sequence. This indicates a high degree of sequence homology, suggesting that the tested sample shares similarities with Mus musculus's known variant of the *IGF-1* gene. The reference sequence serves as a benchmark for comparison, and the high percent identity supports the classification of the tested sample as *IGF-1-F*. Similarly, Table 4-1 reveals a percent identity of 84.92% between the tested sample and the predicted sequence, indicating a moderate level of sequence similarity. which there are some differences or variations between the tested sample and the reference sequence. The defect that was detected,can be attributed to genetic variations or mutations within the *IGF-1* gene since it suggest the presence of specific nucleotide substitutions or deletions within the gene. These genetic variations may alter the structure or function of the *IGF-1* protein, potentially affecting its biological activity or signaling pathways (Wang et al., 2021; Yang et al., 2023). Although they may not specifically address the identified genetic variations but depending to the references mentioned provide support for the mechanism of the detected defect in the *IGF-1* gene of the tested sample, they contribute to the understanding of genetic mutations and their potential consequences (Forbes et al., 2020; Zhao et al., 2023).

The provided tables present the alignments of the cadmium mouse group with the genotypes of the *IGF-1* gene, specifically the *IGF-1-F* sequences, for the sample Cd_1 (1.56 mg/kg Bw). The alignments demonstrate the similarities and differences between the tested sample and the reference sequence and provide insights into the mechanism of the detected defect. The earlier references do not directly address the specific genetic variations identified in the cadmium-exposed sample. However, they provide a broader understanding of the implications of genetic mutations and their potential consequences.

Based on the alignment analysis presented in (Table 4-2) the cadmium-exposed sample Cd_1 shows a high percent identity with the reference sequence Rattus norvegicus insulin-like growth factor (*IGF*) gene,. *IGF-1-F* exhibits identity of 84.90%, suggests that high percentages identity a close match indicating a high sequence homology. The absence of mutations or significant sequence differences suggests that the cadmium exposure in this sample may not have caused substantial alterations in the *IGF-1* gene sequence which contradicts the expectations of a potential defect resulting from cadmium exposure (Murakami, 2006). However, it is important to note that the references cited earlier do not specifically address the identified genetic variations in the *IGF-1* gene of the cadmium-exposed sample which can affect on various cellular processes, including DNA damage, oxidative stress, and epigenetic modifications (Mahrous et al., 2015; Manigandan et al., 2015; Waalkes, 2003) . Finally absence of detectable mutations contradicts the expectation of a defect resulting from cadmium exposure.

Based on the provided alignments of the cadmium-exposed sample Cd_2 (2.52 mg/kg Bw) with the genotypes of the IGF-1 gene, the following mechanism of the defect can be inferred: The alignment analysis, as shown in Table 4-3, indicates a high sequence similarity between the tested sample Cd2 and the reference sequence (Rattus norvegicus insulin-like growth factor (*IGF*) gene,. The forward primer (*IGF-1-F*) exhibits a percent identity of 84.26%, compared to the reference sequence. These high percentages of identity between the primer and the reference sequence suggest a strong sequence match, indicating that the *IGF-1-F* primer specifically amplified regions of the Igf1 gene that correspond to the Rattus norvegicus insulin-like growth factor (*IGF*) gene. This finding supports the presence of the no mutant allele in the cadmium-exposed sample Cd_2 which absence of detectable mutations or significant sequence differences between the tested sample and the reference sequence suggests that the cadmium exposure in sample Cd_2 did not result in substantial alterations in the *IGF-1* gene sequence(Forbes et al., 2020). However, it is important to note that the provided references do not directly address the specific genetic variations identified in the

IGF-1 gene of the cadmium-exposed sample Cd_2(Manigandan et al., 2015; Waalkes, 2003). purify alignment analysis indicates a high sequence similarity between the cadmium-exposed sample Cd_2 and the reference sequence, suggesting the presence of the no mutant allele. The absence of detectable mutations contradicts the expectation of a defect resulting from cadmium exposure.

Based on the provided alignments of the cadmium-exposed sample Cd_3 (8.645 mg/kg Bw) with the genotypes of the *IGF-1* gene, the following mechanism of the defect can be inferred:

The alignment analysis, as shown in Table 4-4, indicates a high sequence similarity between the tested sample Cd_3 and the reference sequence Rattus norvegicus insulin-like growth factor (*IGF*) gene, complete cds, alternatively spliced AH002176.2. The forward primer (*IGF-1-F*) exhibits a percent identity of 84.34%, compared to the reference sequence. These high percentages of identity between the primer and the reference sequence suggest a strong sequence match, indicating that the *IGF-1-F* primer specifically amplified regions of the *IGF-1* gene that correspond to the Rattus norvegicus insulin-like growth factor (IGF) gene.This finding supports the presence of the mutant allele in the cadmium-exposed sample Cd_3. The detection of the mutant allele in sample Cd_3 suggests a potential genetic variation in the *IGF-1* gene that may be associated with cadmium exposure. However, it is important to note that the specific genetic variation or defect associated with this mutant allele is not provided in the given information. To fully understand the mechanism of the defect, additional research and relevant references focused on *IGF-1* gene mutations and their association with cadmium exposure are needed. In summary, the alignment analysis indicates a high sequence similarity between the cadmium-exposed sample Cd_3 and the reference sequence, suggesting the presence of a mutant allele. However, a detailed defect mechanism cannot be provided without further information on the specific genetic variation or defect associated with this mutant allele.

Based on the provided alignments of the chlorine-exposed samples Cl_1 (0.0016 mg/kg Bw) and Cl_2 (0.0033 mg/kg Bw) with the genotypes of the *IGF-1* gene, the following mechanism of the defect can be inferred:

The alignment analysis, as shown in Table 4-5, indicates a high sequence similarity between the Cl_1 sample and the Rattus norvegicus insulin-like growth factor (IGF) gene, which is a transgenic strain. The forward primer (*IGF-1-F*) exhibits a percent identity of 84.92%, when compared to the reference sequence.

These high percentages of identity between the primers and the reference sequence confirm a close sequence match, suggesting that both IGF-1-F and *IGF-1-R* primers successfully amplified specific regions of the Rattus norvegicus insulin-like growth factor (IGF) gene. These findings support the absence of a mutant allele in the Cl_1 sample.

The alignment analysis, as shown in Table 4-6, indicates a significant sequence similarity between the Cl_2 sample and the Rattus norvegicus insulin-like growth factor (*IGF*) gene, which is a transgenic strain. The forward primer (*IGF-1-F*) exhibits a percent identity of 84.64 ,when compared to the reference sequence. These relatively high percentages of identity between the primers and the reference sequence suggest a close sequence match, supporting the absence of a mutant allele in the Cl_2 sample. However, it is important to note that there may be a potential genetic alteration in the *IGF-1* gene of the Cl_2 sample, as indicated by detecting a mutant allele. The specific nature and implications of this genetic alteration require further investigation.

In summary, the alignment analysis of the chlorine-exposed samples Cl_1 and Cl_2 suggests the absence of a mutant allele in Cl_1 and the potential presence of a genetic alteration in the *IGF-1* gene of Cl_2. However, the specific details of the genetic alteration and its implications are not provided in the given information. Further research and relevant references focused on *IGF-1* gene mutations and their association with chlorine exposure are necessary to fully understand the defect's mechanism in these samples.

Based on the provided alignment of the chlorine-exposed sample Cl_3 (0.0062 mg/kg Bw) with the genotypes of the *IGF-1* gene, the following mechanism of the defect can be inferred:

The alignment analysis, as shown in Table 4-15 and Table 4-16, indicates significant sequence similarities between the Cl_3 sample and the Rattus norvegicus insulin-like growth factor (IGF) gene,which is a transgenic strain. The forward primer (*IGF-1-F*) exhibits a percent identity of 84.94%, when compared to the reference sequence. These findings suggest that the *IGF-1-F* primer successfully amplified specific regions of the *IGF-1* gene corresponding to the Rattus norvegicus insulin-like growth factor (IGF) gene. The relatively high percent identities between the primers and the reference sequence indicate a close sequence match, supporting the presence of a mutant allele in the Cl_3 sample. The detection of this mutant allele in the Cl_3 sample suggests a potential genetic alteration in the Igf1 gene. However, the specific details of this genetic alteration and its functional

implications are not provided in the given information (Gao et al., 2022; Lembo et al., 1996; Ting et al., 2021).

In summary, the alignment analysis of the chlorine-exposed sample Cl_3 suggests the presence of a mutant allele in the IGF-1 gene. However, without additional information or references explaining the functional consequences of this genetic alteration, it is challenging to determine the exact mechanism of the defect. Further research and relevant references focused on *IGF-1* gene mutations, transgenic strains, and their association with chlorine exposure are necessary to fully understand the implications of this mutant allele in the context of chlorine-induced responses.

5.2.2. Gene expression

The present study investigated the impact of chlorine and cadmium exposure on genetic instability and histological changes in male mice. The results revealed significant alterations in gene expression and provided insights into the potential genotypic variations associated with exposure to these toxic substances. This discussion will compare and contrast the current study's findings with previous research, highlighting the significance of the new findings and their implications for understanding the effects of chlorine and cadmium on male mice.

To begin with, the gene expression analysis in this study showed that chlorine and cadmium exposure led to changes in *TNF-α* gene expression levels in male mice which the chlorine exposure group showed a dose-dependent increase in *TNF-α* expression, with the highest expression observed at the highest chlorine concentration (Cl_3). This finding aligns with previous studies that have reported increased *TNF-α* expression in response to chlorine exposure (Hossein-Khannazer *et al.*, 2020), furthermore have suggested that chlorine exposure can induce an inflammatory response mediated by *TNF-α*. In additionally, in terms of other tissues, previously published papers also showed that cadmium exposure can cause damage to the intestine, with decreased mucus layer thickness and increased (*TNF-α*) levels in the colon (Liu *et al.*, 2014), while the current study further supports this notion and provides additional evidence for the dose-dependent effect of chlorine on *TNF-α* gene expression.

In contrast, the cadmium exposure group exhibited an inverted U-shaped relationship between cadmium concentration and *TNF-α* expression. The highest expression level was observed at intermediate cadmium concentrations (Cd_1 and Cd_2), while higher concentrations (Cd_3) reduced *TNF-α* expression. This finding

suggests that cadmium exposure may trigger an inflammatory response mediated by *TNF-α,* but higher concentrations might lead to cytotoxic effects and a subsequent decrease in *TNF-α* expression. Similar observations have been reported in previous studies investigating the effects of cadmium on *TNF-α* expression (Derfus *et al.*, 2004).

Some studies have suggested that cadmium can induce an inflammatory response (Genchi *et al.*, 2020), but excessive exposure may disrupt cellular functions and lead to decreased *TNF-α* expression (Zhou *et al.*, 1998). The current study adds to this body of knowledge and highlights the complex relationship between cadmium exposure and *TNF-α* gene expression as shown in previous studies (Zhang *et al.*, 2009). Furthermore, the study investigates the effect of cadmium on cytokine production by different T-helper subsets, which can modulate cytokine production, including *TNF-α*, by T-helper cells. The findings indicate that cadmium exposure can lead to altered immune responses, including changes in *TNF-α* expression (Baumann *et al.*, 2015).

5.3 The concentration of chlorine and cadmium in Iraq

5.3.1 Tigris river in Misan

The provided information in table (4-10) states that the percentage of cadmium is 0 ppm in the river and 0.02 mg/l in the water well of Dijlah in Iraq. Comparing these values, it indicates that the cadmium levels in both the river and water well are relatively low. A concentration of 0 ppm in the river suggests the absence or negligible presence of cadmium, while a concentration of 0.02 mg/l in the water well indicates a very low level of cadmium. Cadmium is a toxic heavy metal that can have adverse effects on human health and the environment. The World Health Organization (WHO) has established guidelines for safe levels of cadmium in drinking water, with a maximum acceptable concentration of 0.003 mg/l (3 µg/l). In the case of the water well in Dijlah, the concentration of cadmium (0.02 mg/l) is slightly higher than the WHO guideline, but still relatively low. It is important to continue monitoring the cadmium levels in the water and take appropriate actions if the concentration increases beyond acceptable limits,because The city of Amarah is one of the oil cities in the world in terms of oil extraction, as industrial activity is a major source of pollution with heavy metals in the environment,it are sources of metal pollution, including petroleum industries and oil refineries (Majed *et. al*, 2002; Rashed,2001;Papagiannis *et. al*, 2004).

The provided information in table (4-11) states that the percentage of chlorine in the water of Shatt al-Amarah has reached 360 ppm, and in the water of Shatt al-

Kahla is 480, These results are considered relatively high according to the concentrations set by the Iraqi Ministry of Environment, which range from zero to 600 ppm. This occurs as a result of throwing industrial waste and sewage water into residential areas near the Tigris River, especially Shatt al-Kahla (figures 5-1,5-2,5-3), this is indicated by the study conducted by Al-Mashkoor (2006), where it was observed that the predominance of chloride and calcium ions in the waters of the Tigris River in Maysan Governorate.

(Apple iPhone 13 Pro Max ,Telephoto Camera - 77 mm f2.8 ,12 MP • 4032 × 3024 • 2.4 MB)

Wednesday, 6 September 2023 at 6:07 PM, shatt al-kahla

(Figure 5-1) A sewage water drainage pipe has been extended from the residential areas adjacent to Shatt al-Kahla. It drains the water at a rate of every half hour and according to the population activity.

Apple iPhone 13 Pro Max,Telephoto Camera - 77 mm f2.8 ,12 MP • 4032 × 3024 • 3.6 MB

Wednesday • 6 Sep 2023 • 18:06, shatt al-kahla

(Figure 5-2) Note the change in the color of the river water as a result of the discharge of bilge sewage.

Apple iPhone 13 Pro Max,Wide Camera - 26 mm f1.5,12 MP • 3024 × 4032 • 3 MB

Wednesday • 6 Sep 2023 • 5:36 PM shatt al-kahla,

(Figure 5-3) Note the accumulation of heavy water at the drain area .

5.3.2 Al-Munajjid water station in the city of Amarah

No analysis was conducted for cadmium in the station's water or the tap water, because the results of the analysis of the Shatt al-Amara water near the station were free of the presence of cadmium in the water. this what Jazza et al. (2022) referred to in his study when they found that the water samples from the water treated that are supplied to residential areas for drinking purposes are not polluted with heavy metals.

The provided information states that the chlorine levels in Al-Munajjid water station in the city of Amarah are 375 ppm when directly taken from the station, and 3 ppm in tap water. Chlorine is commonly used as a disinfectant in water treatment processes to kill harmful microorganisms and ensure the safety of drinking water. The concentration of chlorine in water is typically measured in parts per million (ppm).The chlorine levels of 360 ppm and 375 ppm in Al-Munajjid water station and tap water appear relatively high, respectively. It is important to note that the acceptable chlorine levels in drinking water can vary depending on regional regulations and guidelines.

Apple iPhone 13 Pro Max , No lens information.
1080p • 1080 × 1920 • 113.3 MB
Sunday • 23 Oct 2022 • 4:17 PM ,Degla water station .

Apple iPhone 13 Pro Max, HEVC ,No lens information
1080p • 1080 × 1920 • 23.9 MB
Tuesday • 18 Oct 2022 • 4:47 PM, Al-Munajjid station .

Figure (5-4) Collection basins, sedimentation basins and filters in the Al-Munajjid and Degla water stations in Amarah city .

5.3.3. Marshes of Iraq

The concentration of Cadmium in the marshes in Iraq,specilly in the marsh of Al-khair sub-district,as mentioned in table 4-10.is nil, This indicates that there is no environmental pollution with cadmium in this area ,while The results of the study conducted by Lazim (2019) showed that the concentrations of heavy metals in water for cadmium were 0.112 µg/L as the entry of heavy elements to the aquatic environment changes over time, the main factor affecting this change is what the water receives from Untreated agricultural, industrial and household wastes.(Taghizadeh et al.,2018). Khudhair and his team (2021) also concluded that the concentrations of heavy metals in water at the particle stage are higher In the dissolved phase, while the sediment and concentrations higher than the concentrations in some of the studied plants, as well as the concentrations of heavy metals differed in Selected stations, seasons and plants.

The concentration of chlorine in the marshes in Iraq, as mentioned in table (4-11), is 1100 ppm, which exceeds the maximum normal rate of 600 ppm. This elevated concentration of chlorine suggests a potential environmental concern in the marshes. Al-Mashkoor foundin in his study 2006 that the chloride values in the Tigris River increased in an upstream direction, and ranged between 99-981 mg/L. This is due to the quality of the soil, the low water level of the river and the movement of groundwater towards its section during the study period, In addition to the influence of Hor Al-Shweija before the city of Sheikh Saad. also he found that the chloride values were more than that, starting from the north of the city of Amarah to the estuary.

High levels of chlorine in natural water bodies can have detrimental effects on aquatic ecosystems and biodiversity. Exposure to high levels of chlorine can lead

to various negative impacts. Chlorine is known to be toxic to aquatic organisms, including fish, amphibians, and invertebrates. It can disrupt their respiratory system, damage their tissues, and impair their overall health and reproduction. Furthermore, chlorine can react with organic matter present in water to form disinfection by-products, such as trihalomethanes (THMs), which have been associated with potential health risks, including carcinogenic effects in humans.

6. Conclusions and Recommendation

6.1. Conclusions:

This study provides valuable insights into the impact of chlorine and cadmium exposure on genetic instability and histological changes in male mice. The findings reveal that both pollutants adversely affect biological systems and highlight the importance of understanding the potential risks associated with environmental exposure. The dose-dependent effects of chlorine exposure on *TNF-α* gene expression indicate that higher concentrations of chlorine lead to increased inflammatory responses in male mice. On the other hand, the inverted U-shaped relationship observed in cadmium exposure suggests that intermediate concentrations of cadmium induce the highest *TNF-α* expression levels.

The histopathological analysis further confirms the detrimental effects of chlorine and cadmium on the tissue structure and morphology of the reproductive and renal organs in male mice. Severe damage, degeneration, necrosis, and inflammatory cell infiltration were observed in the higher concentrations of both pollutants.

These findings underscore the need for effective pollution control measures and human health protection. Understanding the adverse effects of chlorine and cadmium on genetic stability and tissue health is crucial for assessing the potential risks associated with environmental exposure.

Further research is warranted to elucidate chlorine and cadmium exposure's underlying molecular mechanisms and long-term consequences. By gaining a deeper understanding of these mechanisms, scientists and policymakers can develop strategies to mitigate the negative impacts of environmental pollutants and safeguard human health.

6.2 Recommendation

Based on the findings of this study, several recommendations can be made:

1- Environmental Regulations: Strengthening and enforcing regulations on using and releasing chlorine and cadmium in industrial processes and waste disposal is crucial. This can help reduce the overall exposure of both humans and wildlife to these pollutants.

2- Water Treatment: Improving water treatment processes and technologies to minimize chlorine levels in drinking water is important. This can help reduce the potential health risks associated with chlorine exposure.

3- Industrial Emission Control: Implementing effective measures to control and reduce cadmium emissions from industrial sources is essential. This can involve adopting cleaner production technologies and promoting responsible waste management practices.

4- Soil Remediation: Developing and implementing strategies for the remediation of cadmium-contaminated soil can help mitigate the risks of exposure to this heavy metal. This can involve soil washing, phytoremediation, and biochar application.

5- Awareness and Education: Increasing public awareness about the potential risks associated with chlorine and cadmium exposure is crucial. Educational campaigns and outreach programs can help inform individuals about the sources of these pollutants and the measures they can take to minimize their exposure.

6- Further Research: Additional research is necessary to investigate the underlying molecular mechanisms and long-term consequences of chlorine and cadmium exposure. This can provide a more comprehensive

understanding of the risks posed by these pollutants and help guide the development of effective strategies for pollution control and human health protection.

7- By implementing these recommendations, policymakers, industries, and individuals can work together to minimize the adverse effects of chlorine and cadmium pollutants and safeguard the environment and human well-being.

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University of Misan
College of Science
Managerial Section
No:
Date: / /

بسم الله الرحمن الرحيم

جامعة ميسان
كلية العلوم
الشعبة الإدارية
العدد: ٩٦٠
التاريخ: ٢٠٢٢/١٠/٨

الى/ السيطرات الثابتة والمتحركة ونقاط التفتيش كافة
م/ تسهيل مهمة

نهديكم أطيب تحياتنا ...

يرجى تفضلكم بتسهيل مهمة الاستاذ المساعد الدكتور(ميثم عبدالكاظم دراغ) رئيس قسم علوم الحياة في كليتنا لغرض جلب فئران مختبرية عدد(١٠٠) لحاجتنا الماسة اليها، علماً ان عملية نقلها بواسطة السيارة (كيا/ ريو) والمرقمة(٧٥٩٦٣/ميسان) لمالكها السيد(عباس ماجد كباشي)

شاكرين تعاونكم معنا.....مع التقدير ...

أ.د صبيح جاسم كاطع
العميد/وكالة
٢٠٢٢/١٠/٨

نسخه منه إلى :-
- مكتب السيد العميد المحترم / ..مع التقدير.
- مكتب السيد معاون العميد للشؤون الادارية المحترم/ ...مع التقدير.
- قسم علوم الحياة/....مع التقدير.
- الشعبة الإدارية/وحدة الموارد البشرية مع الاوليات...مع التقدير.
- الصادرة..

Misan-Amara- 110 College complex-Amara-Baghdad Road
sci.col@uomisan.edu.iq
ميسان - العمارة - مجمع الكليات ١١٠ - طريق العمارة - بغداد

Appendix No.1 Task facilitation letter to the Checkpoints .


University of Misan
College of Science
Scientific Affairs and postgraduate Section
No:
Date: / /

جامعة ميسان
كلية العلوم
شعبة الشؤون العلمية والدراسات العليا
العدد: ١٨١
التاريخ: ٢٠٢٣/٥/١٦

العدد: ٦٦٠
مديرية ماء محافظة ميسان
التاريخ: ٢٠٢٣/٥/١٦

الى / مديرية ماء ميسان
م/ تسهيل مهمة

تحيـــة طيبـــة ....
يرجى التفضل بالموافقة على تسهيل مهمة طالبة الماجستير (خولة صادق بهجت)
من اجل اكمال البحث ومتطلبات رسالة الماجستير الموسومة .
(تأثير التلوث بالكلور والكادميوم على عدم الاستقرار الجيني والتغيرات النسيجية في ذكور الفئران)

مع التقدير

أ.د. احمد هاشم عبود
المعاون العلمي للشؤون العلمية والدراسات العليا
٢٠٢٣/٥/١٦

نسخة منه الى:-
- مكتب السيد العميد ..... للتفضل بالاطلاع مع التقدير.
- مكتب المعاون العلمي ..... للتفضل بالاطلاع مع التقدير.
- الصادرة

Misan-Amara- 110 College complex-Amara-Baghdad Road
sci.col@uomisan.edu.iq

Appendix No.2 Task facilitation letter to Maysan Water Directorate.


University of Misan
College of Science
Scientific Affairs and postgraduate Section
No:
Date: / /

جامعة ميسان
كلية العلوم
شعبة الشؤون العلمية والدراسات العليا
العدد: ١٤٢
التاريخ: ٢٠٢٣/٤/٢٤

الى/ محافظة النجف – مركز الغري للدراسات والبحوث
م/ تسهيل مهمة

تحيـــة طيبـــة ....
يرجى التفضل بالموافقة على تسهيل مهمة طالبة الماجستير (خولة صادق بهجت) من اجل أكمال الجانب العملي لبحثها من رسالة الماجستير.

مع التقدير ....

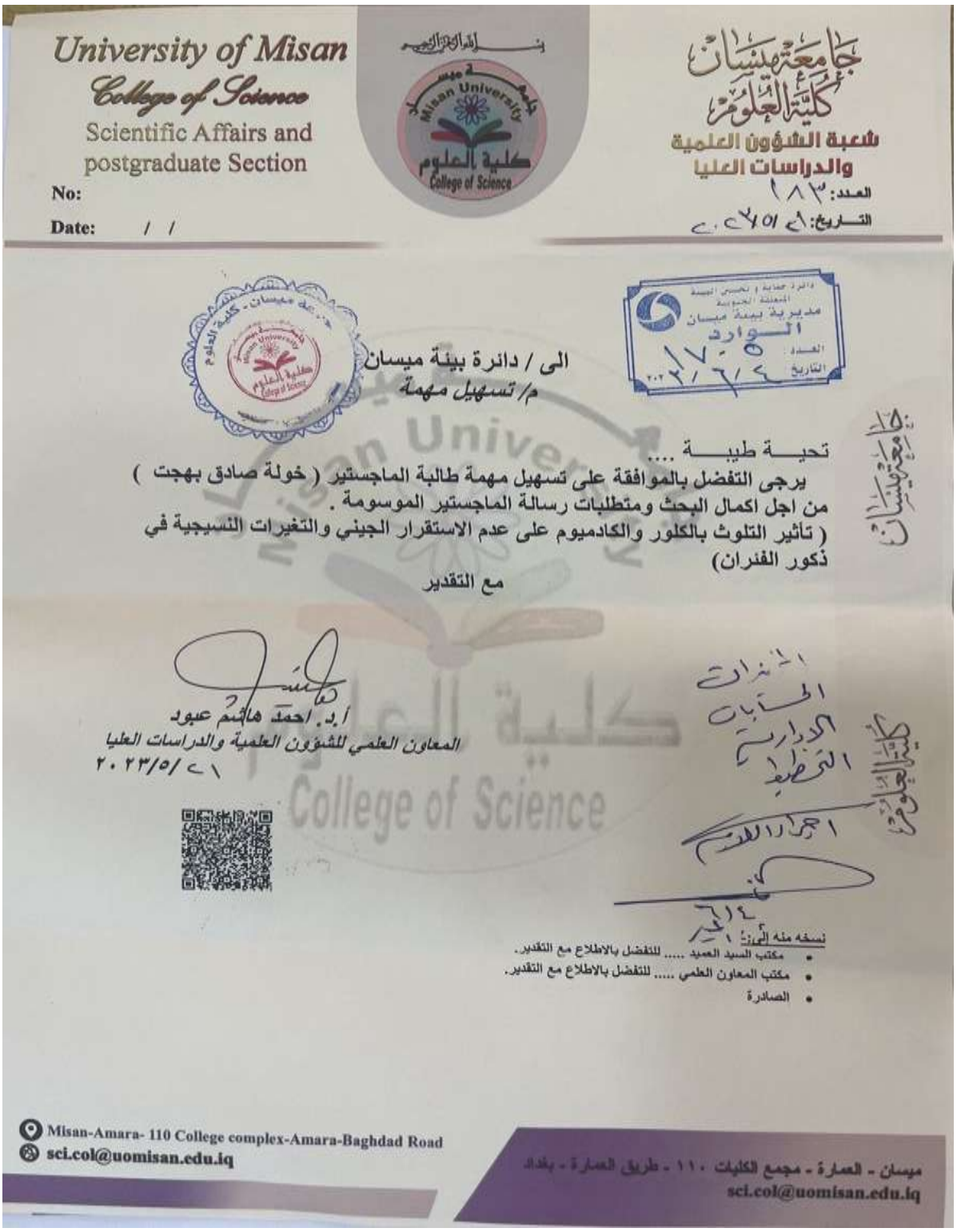
أ.د.احمد هاشم عبود
معاون العميد للشؤون العلمية والدراسات العليا
٢٠٢٣/٤/ ٢٠

نسخة منه إلى:-
- مكتب السيد العميد ... للتفضل بالاطلاع مع التقدير.
- مكتب المعاون العلمي ... للتفضل بالاطلاع مع التقدير.
- الصادرة

Misan-Amara- 110 College complex-Amara-Baghdad Road
sci.col@uomisan.edu.iq

ميسان - العمارة - مجمع الكليات
sci.col@uomisan.edu.iq

Appendix No.3 Task facilitation letter to Al-Ghari center for research, studies and training.



University of Misan
College of Science
Scientific Affairs and postgraduate Section
No:
Date: / /

جامعة ميسان
كلية العلوم
شعبة الشؤون العلمية والدراسات العليا
العدد: ١٨٣
التاريخ: ٢٠٢٣/٥/١٠

دائرة حماية وتحسين البيئة
المعلنة الجوية
مديرية بيئة ميسان
الوارد
العدد: ٥
التاريخ: ٢٠٢٣/٥/٢

الى / دائرة بيئة ميسان
م/ تسهيل مهمة

تحيـــة طيبـــة ....
يرجى التفضل بالموافقة على تسهيل مهمة طالبة الماجستير (خولة صادق بهجت)
من اجل اكمال البحث ومتطلبات رسالة الماجستير الموسومة .
(تأثير التلوث بالكلور والكادميوم على عدم الاستقرار الجيني والتغيرات النسيجية في ذكور الفئران)

مع التقدير

أ.د. احمد هاشم عبود
المعاون العلمي للشؤون العلمية والدراسات العليا
٢٠٢٣/٥/١٠

نسخه منه إلى:
- مكتب السيد العميد ..... للتفضل بالاطلاع مع التقدير.
- مكتب المعاون العلمي ..... للتفضل بالاطلاع مع التقدير.
- الصادرة

Misan-Amara- 110 College complex-Amara-Baghdad Road
sci.col@uomisan.edu.iq

ميسان - العمارة - مجمع الكليات ١١٠ - طريق العمارة - بغداد
sci.col@uomisan.edu.iq

Appendix No.4 Task facilitation letter to the Department of Environment in Maysan.

REPUBLIC OF IRAQ
Ministry of Industry and Minerals
State Company For Mining Industries
ASSOCIATE FACTORY OF IBN SINA

جمهورية العراق
وزارة الصناعة والمعادن
الشركة العامة للصناعات التعدينية
معاونية مصانع ابن سينا

العـــدد : ٣٨٧
التاريـخ : ٢٠٢١/١٢/٢٩

الى / مديرية ماء محافظة ميسان/شعبة التشغيل

الموضوع / تحليل نماذج

تحية طيبة ....

إشارة الى كتابكم ذي العدد ٥٢٨٤ في ٢٠٢١/١٢/٢١ ، ادناه نتيجة تحليل نماذج عدد (٢) والمرسلة من قبلكم بصحبة مخولكم السـيد (صافي هاشم فالح) راجين تسديد مبلغ ١٧٥٠٠٠ مائة وخمسة وسبعون الف دينار لاغير واستلام النتيجة .

مع التقدير...

اسم النموذج	أوكسيد الالمنيوم / AL_2O_3
مسحوق الشب/إيراني المنشا	18.43%

اسم النموذج	الكلور الفعال المتاح
مسحوق القاصر/إيراني المنشأ	75.5%

المهندس
صبار عثمان حمادي
معاون المدير العام
٢٠٢١/١٢/٢٩

نسخة منه الى :-

- مكتب السيد المدير العام المحترم ... مع التقدير
- مكتب السيد معاون المدير العام ... مع التقدير
- مكتب السيد مدير المصنع ... مع التقدير
- شعبة الإدارية . للعلم مع التقدير ..
- قسم المختبرات والسيطرة النوعية

hdad- Iraq- Al-Waziraya- Behind Assabah newspaper
ıail : ibn_sina_1999@yahoo.com

العراق – بغداد – الوزيرية – خلف جريدة الصباح

Appendix No.5 Analysis of models of available effective chlorine used in Maysan water stations.

جمهورية العراق / وزارة البيئة

دائرة حماية وتحسين البيئة / المنطقة الجنوبية/ مديرية بيئة ميسان

تقرير نتائج الفحوصات الكيميائية

الى / شعبة البيئة الحضرية

رقم التوثيق: ١٧

نوع العينة : بئر +نهر

اسم جامع العينة : طالبة الدراسة خولة صادق

اسم مسلم العينة : طالبة الدراسة خولة صادق

اسم مستلم العينة : مصطفى هيثم جبار

اسم العينة او رقمها: بئر + نهر

الموقع : العمارة /ميسان

تاريخ التوثيق : ٢٠٢٣/٥/١٨

تاريخ جمع العينة :

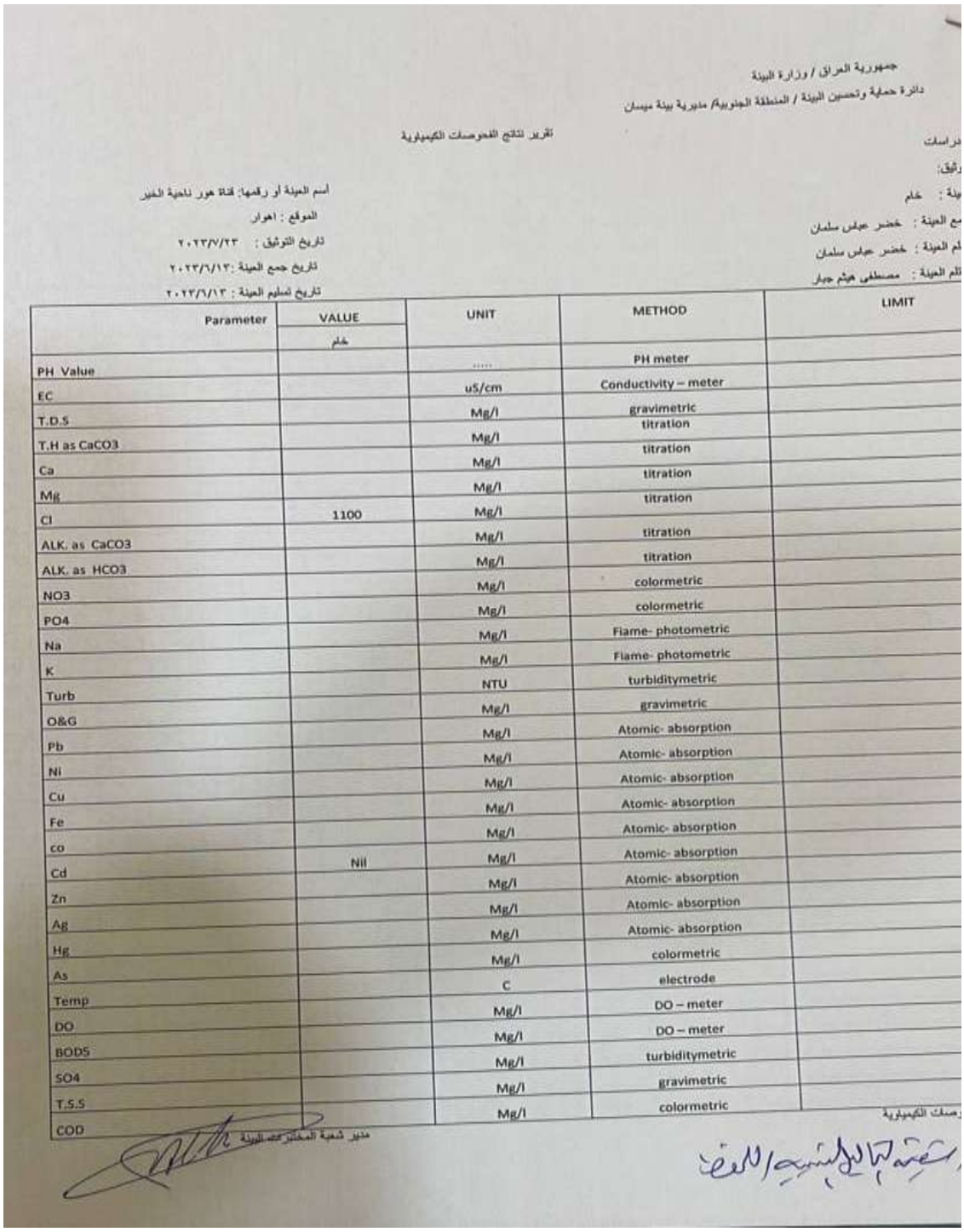
تاريخ تسليم العينة : ٢٠٢٣/٥/١٨

Parameter	VALUE			UNIT	METHOD	LIMIT
	نهر	بئر				
PH Value				.....	PH meter	6.5-----8.5
EC				uS/cm	Conductivity – meter	-
T.D.S				Mg/l	gravimetric	-
T.S.S				Mg/l	gravimetric	-
ALK. as CaCO3				Mg/l	titration	-
ALK. as HCO3				Mg/l	titration	-
BOD5				Mg/l	DO – meter	0-5
COD				Mg/l	colormetric	-
T.H as CaCO3				Mg/l	titration	-
Ca				Mg/l	titration	-
Mg				Mg/l	titration	-
Cl	480	1925		Mg/l	titration	0—200
SO4				Mg/l	turbiditymetric	0—200
NO3				Mg/l	colormetric	0—15
Na				Mg/l	Flame- photometric	-
K				Mg/l	Flame- photometric	-
Fe				Mg/l	Atomic- absorption	-
Ni				Mg/l	Atomic- absorption	0—0.1
Cu				Mg/l	Atomic- absorption	0-0.05
Cr				Mg/l	Atomic- absorption	0-0.05
Cd	nil	0.02		Mg/l	Atomic- absorption	0-0.005
Pb				Mg/l	Atomic- absorption	0-0.05
Zn				Mg/l	Atomic- absorption	-
Al				Mg/l	Atomic- absorption	0-0.1
O&G				Mg/l	gravimetric	-
Hg				Mg/l	Atomic- absorption	0-0.001
CN				Mg/l	colormetric	0-0.02
Turb				NTU	turbiditymetric	-
Temp				C	electrode	-
DO				Mg/l	DO – meter	-
PO4				Mg/l	colormetric	0-0.4

مدير شعبة المختبرات البيئة

مدير وحدة الفحوصات الكيميائية

Appendix No.6 Analysis of Chlorine and Cadmium concentrations in Shatt Al-kahla and the well .



جمهورية العراق / وزارة البيئة

دائرة حماية وتحسين البيئة / المنطقة الجنوبية/ مديرية بيئة ميسان

تقرير نتائج الفحوصات الكيميائية

دراسات

وثيق:

نة : خام

مع العينة : خضر عباس سلمان

م العينة : خضر عباس سلمان

لم العينة : مصطفى هيثم جبار

اسم العينة أو رقمها: قناة هور ناحية الخير

الموقع : اهوار

تاريخ التوثيق : ٢٠٢٣/٧/٢٣

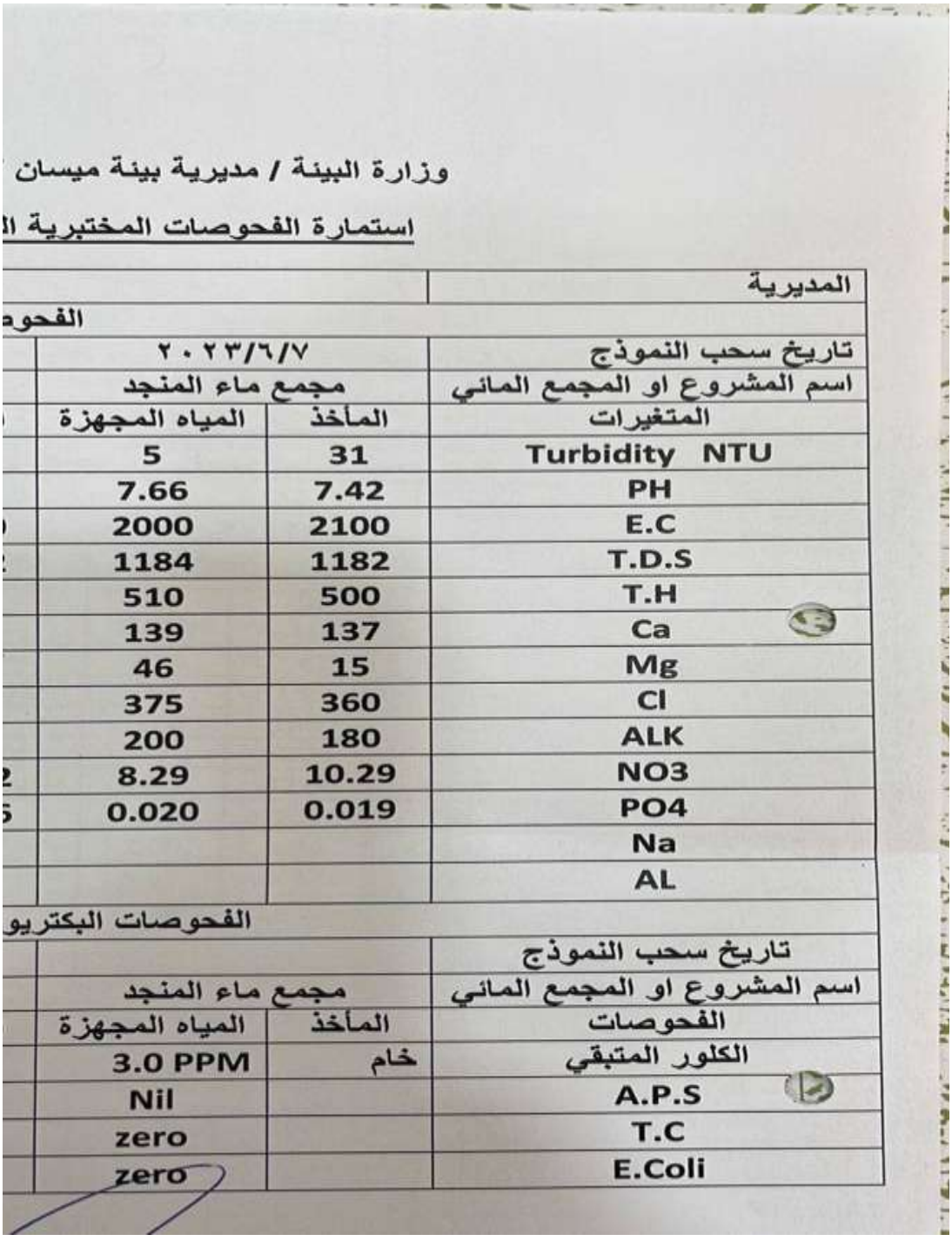
تاريخ جمع العينة :٢٠٢٣/٦/١٣

تاريخ تسليم العينة : ٢٠٢٣/٦/١٣

Parameter	VALUE	UNIT	METHOD	LIMIT
	خام			
PH Value		.....	PH meter	
EC		u5/cm	Conductivity – meter	
T.D.S		Mg/l	gravimetric	
T.H as CaCO3		Mg/l	titration	
Ca		Mg/l	titration	
Mg		Mg/l	titration	
Cl	1100	Mg/l	titration	
ALK. as CaCO3		Mg/l	titration	
ALK. as HCO3		Mg/l	titration	
NO3		Mg/l	colormetric	
PO4		Mg/l	colormetric	
Na		Mg/l	Flame- photometric	
K		Mg/l	Flame- photometric	
Turb		NTU	turbiditymetric	
O&G		Mg/l	gravimetric	
Pb		Mg/l	Atomic- absorption	
Ni		Mg/l	Atomic- absorption	
Cu		Mg/l	Atomic- absorption	
Fe		Mg/l	Atomic- absorption	
co		Mg/l	Atomic- absorption	
Cd	Nil	Mg/l	Atomic- absorption	
Zn		Mg/l	Atomic- absorption	
Ag		Mg/l	Atomic- absorption	
Hg		Mg/l	Atomic- absorption	
As		Mg/l	colormetric	
Temp		C	electrode	
DO		Mg/l	DO – meter	
BOD5		Mg/l	DO – meter	
SO4		Mg/l	turbiditymetric	
T.S.S		Mg/l	gravimetric	
COD		Mg/l	colormetric	

مديرة شعبة المختبر/ صفات الكيميائية

Appendix No.7 Analysis of Chlorine and Cadmium concentrations in Maysan Marshes(marsh of Al-khair sub-district) .



وزارة البيئة / مديرية بيئة ميسان

استمارة الفحوصات المختبرية ال

المديرية		
الفحو		
تاريخ سحب النموذج	٢٠٢٣/٦/٧	
اسم المشروع او المجمع المائي	مجمع ماء المنجد	
المتغيرات	المأخذ	المياه المجهزة
Turbidity NTU	31	5
PH	7.42	7.66
E.C	2100	2000
T.D.S	1182	1184
T.H	500	510
Ca	137	139
Mg	15	46
Cl	360	375
ALK	180	200
NO3	10.29	8.29
PO4	0.019	0.020
Na		
AL		
الفحوصات البكتريو		
تاريخ سحب النموذج		
اسم المشروع او المجمع المائي	مجمع ماء المنجد	
الفحوصات	المأخذ	المياه المجهزة
الكلور المتبقي	خام	3.0 PPM
A.P.S		Nil
T.C		zero
E.Coli		zero

Appendix No.8 Analysis of Chlorine and Cadmium concentrations in Al-Munajjid water station.

الملخص

الملوثات البيئية تهدد الكائنات الحية بشكل كبير، بما في ذلك الإنسان والحياة البرية . الكلورين والكادميوم هما من الملوثات التي درست بشكل واسع لتأثيراتهما الضارة على مختلف الأنظمة البيولوجية. يتم استخدام الكلورين على نطاق واسع في معالجة المياه والعمليات الصناعية والتطهير، بينما يعتبر الكادميوم معدناً ثقيلاً ساماً يوجد عادة في انبعاثات الصناعات والبطاريات والتربة الملوثة . يمكن أن يؤدي التعرض لهذه الملوثات إلى عدم الاستقرار الوراثي وتغيرات هستولوجية، وهو ما يمكن أن يكون له آثار عميقة على الصحة والرفاهية العامة . في هذه الدراسة، هدفنا هو التحقيق في تأثير الكلورين والكادميوم على عدم الاستقرار الوراثي والتغيرات الهستولوجية في الفئران الذكور، مما يوفر رؤى قيمة حول المخاطر المحتملة المرتبطة بالتعرض البيئي لهذه الملوثات.

في هذه الدر اسة، تم استخدام سبعون فأراً ذكر اً تم الحصول عليها من مركز الرازي في بغداد كحيوانات تجريبية. تم اختيار الفئران كنموذج حيواني بسبب ملاءمتها لأهداف البحث. كانت الفئران تزن في المتوسط ٣١ جراماً وتبلغ أعمارها من ١٠ إلى ١٢ أسبوعاً. تم تقسيم الفئران إلى سبع مجموعات، بما في ذلك مجموعات للتعرض للكلورين والكادميوم ومجموعة ضابطة . تم توضيب الحيوانات في أقفاص تحت ظروف بيئية مضبوطة وتوفير طعام قياسي للقوارض وماء الصنبور بشكل حر . تم اتباع الاعتبارات الأخلاقية وتوفير الرعاية السليمة لضمان رفاهية الحيوانات ومعاملتها بشكل أخلاقي. تم تقسيم الفئران إلى سبع مجموعات: ثلاثة مجموعات فرعية للتعرض للكلورين (Cl_1 , Cl_2 , Cl_3) ، ثلاثة مجموعات فرعية للتعرض للكادميوم(Cd_1, Cd_2, Cd_3) ، ومجموعة ضابطة (Cg) لم تتعرض للكلورين أو الكادميوم . تم إعطاء التعرض للكلورين بتراكيز ٢.٥ ملغ/لتر و ٥ ملغ/لتر و ١٠ ملغ/لتر، في حين تم إجراء التعرض للكادميوم بتراكيز ٣ جزء في المليون و ٦ جزء في المليون و ١٢ جزء في المليون. استمرت مدة التعرض شهرًا، خلاله تم إعطاء الماء الملوث بالكلورين أو الكادميوم للفئران للشرب.

تم جمع عينات الأ نسجة من الخصيتين والكلى للتشخيصات المرضية النسيجية المتبعة وتقييم تعبير جين $TNF\text{-}\alpha$. تم تعديل تراكيز التعرض بناءً على وزن الجسم للفئران لضمان الجرعة الدقيقة وإنشاء مستويات تعرض مناسبة. تم تحليل مستويات التعبير لجين $TNF\text{-}\alpha$ ، وهو سايتوكين مضاد للالتهاب، لتقييم تأثير التعرض للكلورين والكادميوم على عدم الاستقرار الوراثي . كشف تحليل التعبير الجيني عن اختلافات كبيرة بين المجموعات الفرعية. في مجموعة التعرض للكلورين، لوحظ زيادة تعتمد على الجرعة في تعبير $TNF\text{-}\alpha$، مع أعلى تعبير في تركيز الكلورين الأعلى (Cl_3). لوحظت علاقة عكسية مع شكل حرف "يو" في

مجموعة التعرض للكادميوم، حيث أظهرت التراكيز المتوسطة من الكادميوم (Cd_1 , Cd_2) أعلى مستويات تعبير *TNF-α* . تشير هذه النتائج إلى أن التعرض للكلورين والكادميوم يمكن أن يؤثر على تعبير جين *TNF-α* في الفئران الذكور، مما يشير إلى تحفيز استجابات التهابية. تم إجراء تحليل الهستولوجي للخصيتين والكلى لتقييم التغيرات الهستولوجية التي يسببها التعرض للكلورين والكادميوم . أظهرت النتائج تغيرات كبيرة في هيكل الأنسجة والمورفولوجيا بالمقارنة مع المجموعة الضابطة . في مجموعة التعرض للكلورين، تسبب تركيزات الكلورين الأعلى (Cl_2 و Cl_3) أضرارًا شديدة، بما في ذلك التدهور والنخر واختراق الخلايا الالتهابية. بالمثل، في مجموعة التعرض للكادميوم، تسببت تركيزات الكادميوم الأعلى (Cd_2 و Cd_3) في أضرار واسعة للأنسجة، بما في ذلك التدهور الأنابيبي والالتهاب البين ي والتليف. تشير هذه النتائج إلى أن التعرض للكلورين والكادميوم يمكن أن يؤدي إلى تغيرات هستولوجية في أعضاء التناسل والكلى لدى الفئران الذكور.

تقدم هذه الدراسة إسهامات مهمة في فهم تأثير التعرض للكلورين والكادميوم على عدم الاستقرار الوراثي والتغيرات الهستولوجية في الفئران الذكور . تسلط النتائج الضوء على التأثيرات التي تعتمد على الجرعة للتعرض للكلورين على تعبير جين *TNF-α* والعلاقة العكسية المشبوهة التي لوحظت في حالة التعرض للكادميوم . يؤكد التحليل الهستولوجي التأثيرات الضارة لكل من الملوثين على هيكل الأنسجة والمورفولوجيا. فهم التأثيرات الضارة للكلورين والكادميوم على استقرار الجينات وصحة الأنسجة أمر بالغ الأهمية لتقييم المخاطر المحتملة المرتبطة بالتعرض البيئي.

وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم علوم الحياة

تأثير الكلور و الكادميوم على الاستقرار الجيني و التغيرات النسيجية في ذكور الفئران

رسالة مقدمة
الى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

خولة صادق بهجت

بكلوريوس علوم حياة (٢٠٠٤)

باشراف

أ.م.د ميثم عبد الكاظم دراغ

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