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Histological Study on "Intestine, Liver, Kidneys and Thymus" in intoxicated Rabbits by Mercury and Co-treated with Vitamin B6

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Dedication

To my beloved mother

Who gave me the gift of Dreams and the ability to realize them.

To my siblings

"Hadil" "Moncef" "I.Tasnim" "Djinane"

Who have walked every step of this Journey with me.

To my dearest uncle "Amar"

Who never stopped believing in me.

To my soul friend , To the most beautiful things I encountered at university

"Ghada Bennadji"

To the people I love

It 's my pleasure and honor to dedicate this modest work to all of you for your endless love and unconditional support.

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Histological Study on “Intestine, Liver, Kidneys and Thymus” in intoxicated Rabbits by Mercury and Co-treated with Vitamin B6

Abstract

The objective of this study is to investigate the possible protective effect of vitamin B6 against toxicity induced by mercury on Intestine, liver, kidneys and thymus. A total of 24 *Oryctolagus cuniculus* rabbits were equally divided into four groups: the control, the Hg (0.5 g $HgCl_2$/kg in the diet), the VitB6 (5ppm by gavage), and the Hg + VitB6 (0.5 g $HgCl_2$/kg in the diet+VitB6 5ppm by gavage) group. $HgCl_2$ was daily mixed with the standard diet. At the end of treatment, the histological profiles of the intestines, liver, kidneys and thymus were evaluated after 30 days of exposure. The results of this study showed several alterations in organs tissues; Hg group have revealed marked intestinal villus atrophy, sloughing mucosa, loss of brush border, hepatocytes vacuolations, dilatation of the central and portal veins, Glomerular atrophy, tubular necrosis, dilated Bowman's capsule, thymic cortex atrophy and lymphocytes depletion. However, the Hg+VitB6 treated rabbits have demonstrated an improved histological structure of organs. Vitamin B6 has an antioxydant activity that mitigates tissues damage.

Keywords: Mercury, toxicity, vitamin B6, Protective effect, male rabbits.

Etude Histologique des organes des lapins « L'intestin, Le foie, Les reins et Thymus » traités par le mercure et Co-traités avec la Vitamine B6

Résumé

L'objectif de cette étude est d'étudier l'effet protecteur possible de la vitamine B6 contre la toxicité induite par le mercure sur les intestins, le foie, les reins et le thymus. Au total, 24 lapins *Oryctolagus cuniculus* ont été répartis à parts égales en quatre groupes : le témoin, le Hg (0.5 g $HgCl_2$/kg dans l'alimentation), le groupe VitB6 (5ppm par gavage) et le groupe Hg+VitB6 (0.5 g $HgCl_2$/kg dans l'alimentation + VitB6 5ppm par gavage). $HgCl_2$ a été quotidiennement mélangé au régime alimentaire standard. En fin de traitement. Les profils histologiques des intestins, du foie, des reins et du thymus ont été évalués après 30 jours d'exposition. Les résultats de cette étude ont montré plusieurs altérations dans les tissus des organes ; le groupe Hg ont révélé une atrophie marquée des villosités intestinales, une desquamation de la muqueuse, une perte de la bordure en brosse, des vacuolations des hépatocytes, une dilatation de la veine centrale et porte, une atrophie glomérulaire, une nécrose tubulaire, une capsule de Bowman dilatée, une atrophie du cortex thymique et une déplétion des lymphocytes. Les lapins traités par Hg+VitB6 ont démontré une structure histologique améliorée des organes. La vitamine B6 a une activité antioxydant qui atténue les dommages causés aux tissus.

Mots clés : Le mercure, toxicité, vitamine B6, l'effet protecteur, lapins mâles.

دراسة نسيجية لأعضاء أرانب "الأمعاء، الكبد، الكلى والغدة الصعترية" المعاملة بالزئبق والمعالجة بفيتامين B6

الملخص

الهدف من هذه الدراسة هو تقييم التأثير الوقائي المحتمل لفيتامينB6 ضد السمية الناجمة عن الزئبق على الأمعاء، الكبد، الكلى والغدة الصعترية. تم تقسيم 24 فردا من ذكور الأرانب من نوع (*Oryctolagus cuniculus*) بالتساوي إلى أربع أفواج: الفوج الشاهد، فوج الزئبق (0.5 غرام من كلوريد الزئبق/كلغ غذاء)، فوج فيتامين B6 (5 جزء في المليون عن طريق الجرع) وفوج الزئبق+ فيتامين B6 (0.5 غرام من كلوريد الزئبق/كلغ+0.5 جزء في المليون فيتامين B6عن طريق الجرع). كلوريد الزئبق تم خلطه مع الغذاء. في نهاية العلاج تمت الدراسة التشريحية للأمعاء، الكبد، الكلى والغدة الصعترية بعد 30 يوما من التعريض. كشفت نتائج هذه الدراسة تغيرات عديدة في أنسجة الأعضاء؛ كشف فوج الزئبق ضمور ملحوظ في الزغابات المعوية، تقشر الغشاء المخاطي، فقدان حدود فرشاة الخلايا الحدودية للغشاء المخاطي، فراغات خلايا الكبد، توسع الوريد المركزي والبابي الكبدي، ضمور الكبيبات الكلوية، نخر أنبوبي، توسع محفظة بومان، ضمور قشرة الغدة الصعترية واستنزاف الخلايا اللمفاوية. أظهرت الأرانب المعالجة بالزئبق + فيتامين B6 بنية نسيجية محسنة للأعضاء. يحتوي فيتامين B6 على نشاط مضاد للأكسدة مما يخفف من تلف الأنسجة.

الكلمات المفتاحية: الزئبق، التأثير الوقائي، فيتامين B6، السمية، ذكور الأرانب.

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List of abbreviations

1AOX	Aldehyde oxidase 1
4-PA	4-pyridoxic acid
CH_3Hg	Methyl mercury
GPx	Glutathione peroxidase
GSH	Reduced glutathione
Hg	Mercury
Hg^0	Elemental mercury
Hg^{2+}	Mercuric mercury
Hg_2^{2+}	Mercurous mercury
Hg_2Cl_2	Mercurous chloride
Hg_2SO_2	Mercury sulfate
$HgCl_2$	Mercuric chloride
HgO	Mercuric oxide
HgS	Mercuric sulfide
$HgSO_4$	Mercuric sulfate
MDA	Malondialdehyde
MT	Metallothionein
Oat1	Organic anion transport 1
Oat3	Organic anion transport 3
PDX1	Pyridoxine biosynthesis 1
PDX2	Pyridoxine biosynthesis 2
PDXK	Pyridoxal kinase
PL	Pyridoxal
PLP	Pyridoxal 5'-phosphate
PM	Pyridoxamine
PMP	Pyridoaxamin 5'-phosphate

PN	Pyridoxine
PNP	Pyridoxine 5'-phosphate
PNPO	Pyridoxine 5'-phosphate oxidase
RDA	Recommended dietary allowance
ROS	Reactive oxygen species
SH	Sulfhydryl groups
TNSALP	Tissue-non-specific alkaline phosphatase
TrxR	Thioredoxin reductase
USDA	United states department of agriculture

Introduction

Introduction

Nowadays, the world's increasing industrialization has grown to become a source of health concern. Since it is accompanied by increased human exposure to hazardous substances and pollutants generated by these industrial sectors, such as heavy metals **(Boroushaki *et al*., 2014)**, whose toxicity has been demonstrated in several studies. Heavy metals, especially mercury, are ubiquitous in the environment. It exists everywhere in nature as a result of anthropogenic activities or natural sources, and it may enter the human body in many ways.

Historically, mercury was used for medicinal purposes in many countries. Mercury containing preparations and medications has a long history of use in therapeutic practice across countries **(Zhao, 2022)**. Furthermore, Hg has a variety of applications, including electrical equipment, scientific instruments, gold mining, explosives, agriculture, pesticides, batteries, antiseptics, disinfectants, preservatives, dental amalgams, and photographic fixatives **(Zulaikhah & Wibowo, 2018; Rakib, 2021)**.

In Algeria, mercury was refined in the "Mercurial complex of Azzaba", which produces mercury from rocks found as Mercury sulfide (HgS) or cinnabar. Unfortunately, Rock's crushing and grinding activities contaminated the environment and exposed workers to a poisoning danger **(Abdennour *et al*., 2002).**

According to the US Government Agency for Toxic Substances and Disease Registry, mercury is the third-most toxic element on the planet **(Teixeira *et al*., 2018)**. It is considered a serious ecological and manufacturing pollutant **(Rhyaf, 2016)**. Long-term exposure to mercury may damage tissues, thereby altering their structure and functions due to the strong ability of mercury to bind to sulfhydryl groups (SH) found in thiol-containing proteins and low-molecular-weight peptides, reducing antioxidant levels and producing reactive oxygen species (ROS) that lead to oxidative stress **(Elblehi *et al*., 2019; Rojas-Franco *et al*., 2019)**. Mercury's toxicity differs depending on its chemical form, dose, and exposure pathway **(Bernhoft, 2012; Salazar-Flores *et al*., 2019)**.

Mercury is a nephrotoxic and hepatotoxic agent because it accumulates in the kidney and liver. In addition to nephrotoxicity, mercury is associated with immunopathological disorders and histological alterations **(Bracci *et al*., 2008; Uzunhisarcikli *et al*., 2016; Rojas-Franco *et al*., 2019)**.

Several previous studies have shown that vitamins, as antioxidants, protect against mercury damage **(Rao & Sharma, 2001; Huq *et al*., 2013)**. Despite its antioxidant activity,

vitamin B6's preventive benefits against mercury hepato-nephrotoxicity have not been explored, to our knowledge. On the other hand, vitamin B6 alone or in combination with iron has been proven to be an effective antioxidant therapy for heavy metal poisoning (**Islam *et al*., 2017; Salvator *et al*., 2023)**.

Vitamin B6 may indirectly scavenge ROS and hence function as a cofactor in cysteine and glutathione synthesis, increasing antioxidants levels in tissues (**Hsu *et al*., 2015)**. Vitamin B6, which the immune system depends on, plays a role in T-lymphocyte production, and a deficiency of it inhibits T-lymphocyte cell maturation, growth, and proliferation **(Aslam *et al*., 2017)**.

Given the rarity of studies and data on the preventive benefits of vitamin B6 against mercury toxicity, the current study aims to investigate the protective effect of vitamin B6 on mercury-induced histological damage in rabbits. To achieve this aim, this study was divided into two main parts:

- ✓The literature review focuses on the toxicity of mercury and the important function of vitamin B6 in detoxification.
- ✓The experimental review discusses the impacts of mercury and vitamin B6 on histological aspects (some target organs: intestines, liver, kidney, and thymus).

Literature Review

Chapter 01

Overview of Mercury

1. Mercury

1.1. Definition of mercury

Mercury is a highly toxic heavy metal that ubiquitously occurs in nature. It has been recognized as a concern and a global pollutant because it causes potential health problems. Mercury symbol (Hg) is derived from the Greek word *hydrargyrum* «hydra; water, argyros; silver». This non-biodegradable and bioaccumulative metal has a density of 14 and a -38 °C freezing point, as well as a 356.73 °C boiling point. Moreover, it is a shiny silver-white metal slightly soluble in water and has a unique electric configuration and chemical properties **(Jaishankar *et al.*, 2014; Rana *et al.*, 2018; Gupta *et al.*, 2020).**

1.2. Several forms of mercury

Mercury exists mainly in three forms: elemental mercury (Hg^0), inorganic mercury (mercurous Hg^{1+}mercuric Hg^{2+}), organic mercury such methyl mercury (CH_3Hg) **(Balali et al., 2021)**.

1.2.1. Elemental mercury (Hg^0)

Elemental mercury (Hg^0) or metallic mercury is liquid mercury stands on the sediments' surface and cannot get underground. This liquid is able to be easily converted to colorless and odorless mercury vapor. The level of concentration of this kind of mercury in the general environment is too low to pose a concern to human health; however, concentrations in industries that release mercury vapor will be significantly higher. Around 80% of inhaled vapor is absorbed through the lungs and enters the circulatory system **(Hasrat, 2015).**

1.2.2. Inorganic mercury

Inorganic mercury can be found as mercurous (Hg^{1+}) or mercuric (Hg^{2+}) ions. These ions are often linked to chlorine, sulfur, or oxygen to form mercuric salts and it is poorly absorbed in GI tract **(Bathla & Jain, 2016; Orr & Bridges, 2017).**

- **Mercurous mercury (Hg_2^{2+})**

Chemically, mercurous mercury classified as a cation with two oxidation states: monovalent Hg^{1+} (mercurous cation, stable form Hg_2^{2+}) and divalent Hg^{2+} (mercuric cation, Hg^{2+}). Hg_2 salts, such as mercurous chloride (Hg_2Cl_2) and mercury sulfate (Hg_2SO_2), are not easily soluble in water. Under normal conditions, $Hg_2^{2+}$is unstable and tends to change into Hg^0 and Hg^{2+} through the dismutation process. Furthermore, it can combine with metal ions (chloride, halide, and sulfide) to generate compounds with limited solubility **(Wu *et al.*,**

2024). The mercury salt Hg_2Cl_2 is not easily absorbed by the human intestinal tract due to its low water solubility. However, certain amounts of it may oxidize into more absorbable forms **(Zalups, 2000).**

- **Mercuric mercury (Hg^{2+})**

Mercuric mercury is the most stable form in aqueous solutions. Mercuric sulfide (HgS), mercuric oxide (HgO), mercuric sulfate ($HgSO_4$), and mercuric chloride ($HgCl_2$) are more common inorganic Hg^{2+} compounds. The body absorbs just about 2% of the consumed $HgCl_2$ **(Bernhoft, 2012).** However, long-term exposure to it may increase permeability due to its corrosive action on the intestines.

1.2.3. Organic mercury

Organic mercury refers to compounds with mercury bound to a carbon structure (e.g., methyl, ethyl). The majority of published data on organic mercury compounds focus on methyl mercury, which is the primary source of human mercury exposure. Methyl mercury vapor absorbs with the same effect (80%) as metallic mercury vapor **(Bernhoft, 2012)**. Microorganisms in soil and water mostly methylate inorganic mercuric ions to methyl mercury **(Orr & Bridges, 2017**). The organic mercury compounds are highly lipid-soluble, with 90-100% of an oral dosage absorbed **(Langford & Ferner, 1999)**.

1.3. Exposure sources of mercury

Mercury exposure sources are mostly natural such as emissions from volcanoes or degassing of the earth's crust and anthropogenic **(Langford & Ferner, 1999)**. Natural sources emit 5207 megagrams of mercury per year, however it should be noted that this assume includes re-emissions of previously deposited mercury from both anthropogenic and natural sources. Whereas, anthropogenic sources cover 2320 megagrams of mercury emissions per year **(Figure 01) (Pirrone *et al*., 2010).**

Additionally, it is also important to note that the majority of these air emissions are deposited in soil and water through dry and wet deposition, as well as other chemical transformation processes **(Wu *et al*., 2024).**

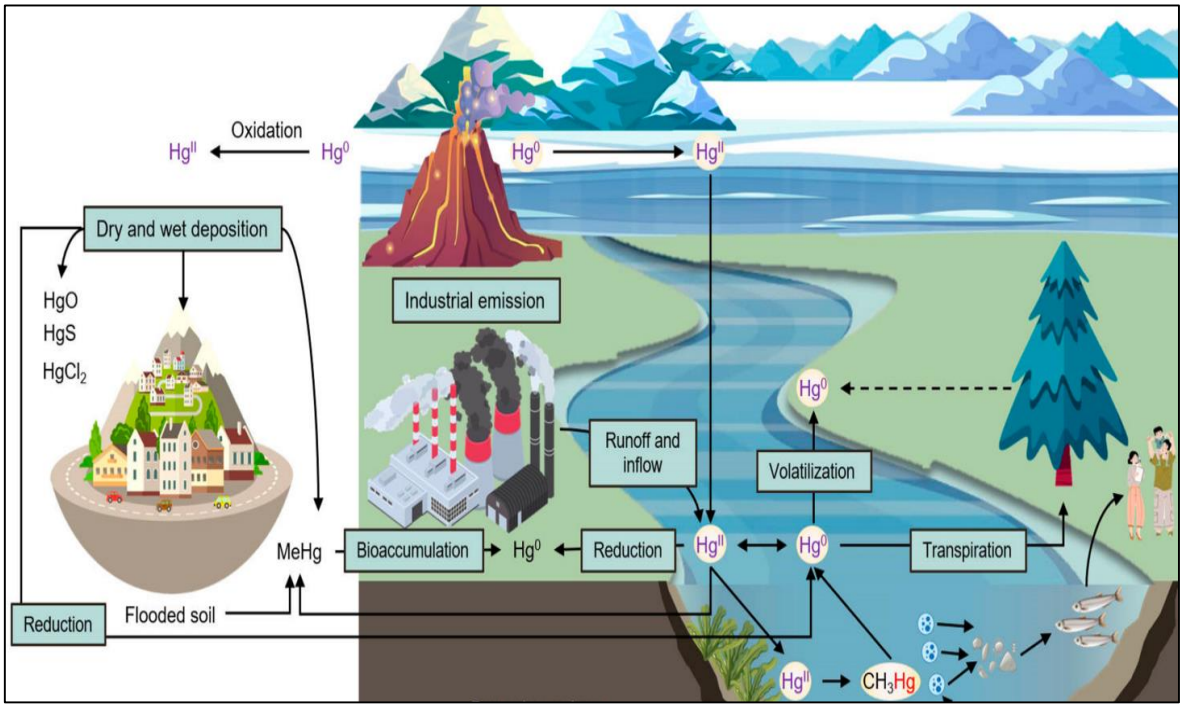

Figure 01: Schematic view of Hg environmental recycling from the atmospheric emission, exposure and bioaccumulation **(Wu *et al*., 2024)**.

According to the World Health Organization (WHO), most human exposure to metallic mercury results from the release of mercury vapor from amalgam fillings at a rate of 2-28 µg per day per side **(Zhao, 2022).**

Table 01: Common sources of mercury exposure.

Forms of mercury	Exposure sources	References
Elemental mercury	Thermometers, Barometers, Diffusion pumps, Lamps, Electrical switches, Paints, Batteries, Catalysts of chlorine, Dental amalgams filling, gold mining.	**(Rana, 2018; Zhao, 2022).**
Inorganic mercury	Explosive detonators, Photographic films, Skin-lightening creams.	**(Bernhoft, 2012; Rana, 2018).**
Organic mercury	Fungicides, Manufacture of plastic, Preservative thimerosal of vaccines, Seafood consumption (fish).	**(Barregard, 2011; Rana, 2018; Zhao, 2022).**

1.4. Toxicokinetics of mercury

1.4.1. Absorption

- **Elemental mercury**

Elemental mercury vapor may be easily inhaled through the lungs, with the body absorbing 75-85% of the dosage. Elemental mercury vapor's strong lipid solubility allows for fast diffusion and dissolution in blood lipids. Liquid metallic mercury is extremely weakly absorbed by the human gastrointestinal system and skin **(Balali *et al*., 2021)**.

- **Inorganic mercury**

Mercury absorption through the gastrointestinal system is estimated at 7-15% in humans. Dermal absorption of mercuric chloride is around 2-3% **(Poupon, 2007)**.

- **Organic mercury**

Methylmercury is easily absorbed through the gastrointestinal system around 95%. While dermal absorption has been around 3-5% **(Bernhoft, 2012; Balali *et al*., 2021)**.

1.4.2. Distribution

- **Elemental mercury**

Because of its lipophilicity, absorbed elemental mercury vapor quickly spreads throughout the body, even penetrating the blood-brain barrier in humans. The distribution of absorbed elemental mercury is limited by its oxidation to the mercuric ion, which has a lower capacity to pass membrane barriers. Thus, it accumulates in the tissues as inorganic mercury **(Poupon, 2007)**.

- **Inorganic mercury**

Mercuric mercury is unable to penetrate the blood-brain or placental barrier. Mercuric ions in the blood bind to sulfhydryl groups in plasma and erythrocytes **(Poupon, 2007)**. Mercuric mercury is initially distributed in the blood to the liver, while the kidneys often have the greatest quantities (90%). The kidney's proximal tubules have the highest amount of mercuric mercury, increasing metallothionein synthesis in the kidneys, leading to mercury accumulation **(Hassett-Sipple *et al*., 1997)**.

- **Organic mercury**

Methylmercury is widely distributed in the body and may easily pass the blood-brain and placental barriers in humans and animals. The transmission of methylmercury into tissues requires the creation of a methylmercury-cysteine complex that enters cells via an amino acid carrier protein. Methylmercury binds to water-soluble compounds like proteins and thiol-containing amino acids due

to its strong affinity for sulfhydryl groups **(Hassett-Sipple *et al*., 1997)**. Complexes of methylmercury with cysteine have been found in the blood, liver, and bile.

1.4.3. Metabolism

- **Elemental mercury**

Elemental mercury dissolved in the blood is rapidly oxidized in red blood cells to mercuric mercury by catalase in the presence of hydrogen peroxide **(Wu *et al*., 2024)**.

- **Inorganic mercury**

Mercurous mercury is unstable in biological fluids and rapidly dissociates to one molecule of elemental mercury and one ion of mercuric mercury. The reduction of mercuric ion to elemental mercury may occur via cytochrome c **(Hassett-Sipple *et al*., 1997)**.

- **Organic mercury**

Methylmercury in the body is relatively stable and slowly demethylates into mercuric mercury (Hg^{2+}). Methylmercury metabolism may contribute to a delayed start of harmful consequences, as seen in epidemiological research **(Orr & Bridges, 2017)**.

1.4.4. Excretion

- **Elemental mercury**

Excretion of mercury after exposure to elemental mercury vapor may occur via exhaled air, urine, feces, sweat and saliva **(Bernhoft, 2012)**.

- **Inorganic mercury:**

Mercury is primarily eliminated in the feces within a few days of oral ingestion due to low absorption. However, the majority of the ingested inorganic mercury is eliminated in the urine **(Wu *et al*., 2024)**. Mercuric mercury may also be excreted in breast milk during lactation.

- **Organic mercury**

Methylmercury has a relatively long half-life of approximately 70-80 days in the human body excretion via the feces, breast milk, and urine **(Poupon, 2007)**.

1.5. Toxicodynamics of mercury

Mercury's effects are widespread and do not affect just one organ system. Mercury's toxicodynamics refers to the disruption of biological processes by binding to SH groups, resulting in enzyme inhibition, defective signaling, oxidative stress, generation of reactive

oxygen species, and calcium dysregulation **(Figure 02).** These effects appear in diverse organ systems, depending on the chemical type, dosage, and route of exposure **(Jan *et al*., 2011)**.

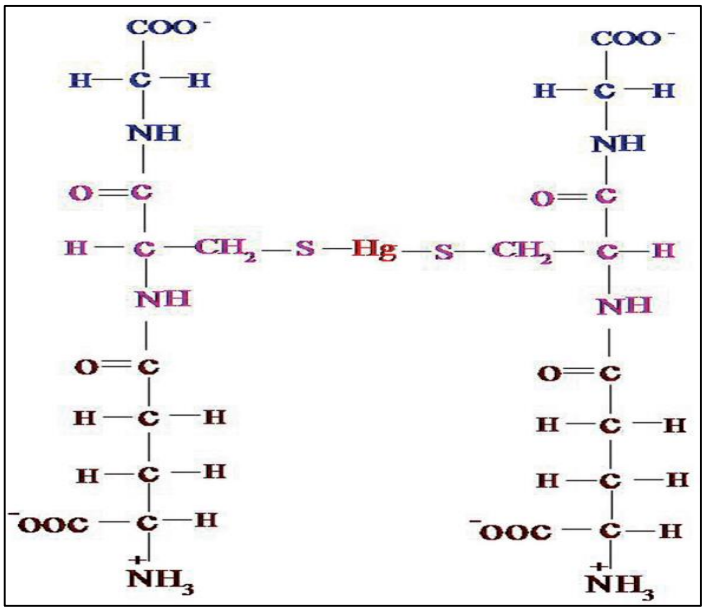

Figure 02: Mercury-glutathione complex **(Jan *et al*., 2011).**

Mercury's biological effects and histological anomalies include renal failure, gastrointestinal and immunological disorders, and liver dysfunction **(Hassett-Sipple *et al*., 1997)**.

1.5.1. Reactive oxygen species

Reactive oxygen species (ROS) production is a vital process. Oxidative stress is caused by an imbalance between the production and use of ROS. In regularly functioning cells, ROS is produced in the organelles and then transferred to the cytoplasm. An excessive amount of ROS may cause nonreversible cell death. Most mammalian cells are considered to have mitochondria as their primary source of the creation of ROS. During respiration, around 1%to 2% of O_2 acquires an electron and is reduced to superoxide anion (O_2), which produces hydrogen peroxide (H_2O_2). Heavy metals and toxins contribute to the development of this harmful species **(Danielyan & Chailyan, 2020)**.

1.5.2. Toxicodynamics of mercury in kidney

From systemic circulation, Hg is up taken by organic anion transport 1 (Oat1) and organic anion transport 3 (Oat3) into kidney especially into proximal tubule. Here, the cleavage of carbon-mercury bond converts organic to inorganic mercury as metabolites either by the enzymatic or non-enzymatic process. At the same time, Hg deposition is closely related to ROS generation, expression of metallothionein (MT), apoptosis and proximal tubule

damage. Organic anion transport 1 (Oat1) and organic anion transport 3(Oat3) localize mainly in the lysosome of in the proximal tubule, uptake Hg into the kidney. As Hg^{2+} has a greater affinity to bind with thiol-containing enzymes, it inactivates the enzymes with thiol group through irreversible oxidation; results in depletion of total thiol content and oxidative stress **(Rana *et al.*, 2018)**.

Additionally, inactivation of sulfhydryl protein, also affects the cellular integrity interrupting membrane potential and volume of cells as well as cellular organelles. Therefore, its consequences were observed with free radical generation. Absences of detoxifying protein or reduced selenolthiol containing antioxidant activity thioredoxin reductase (TrxR) also facilitate the proximal tubules damage. Mercury also reduces the function of tight junction protein in kidney and perturbs cellular permeability (**figure 03).**

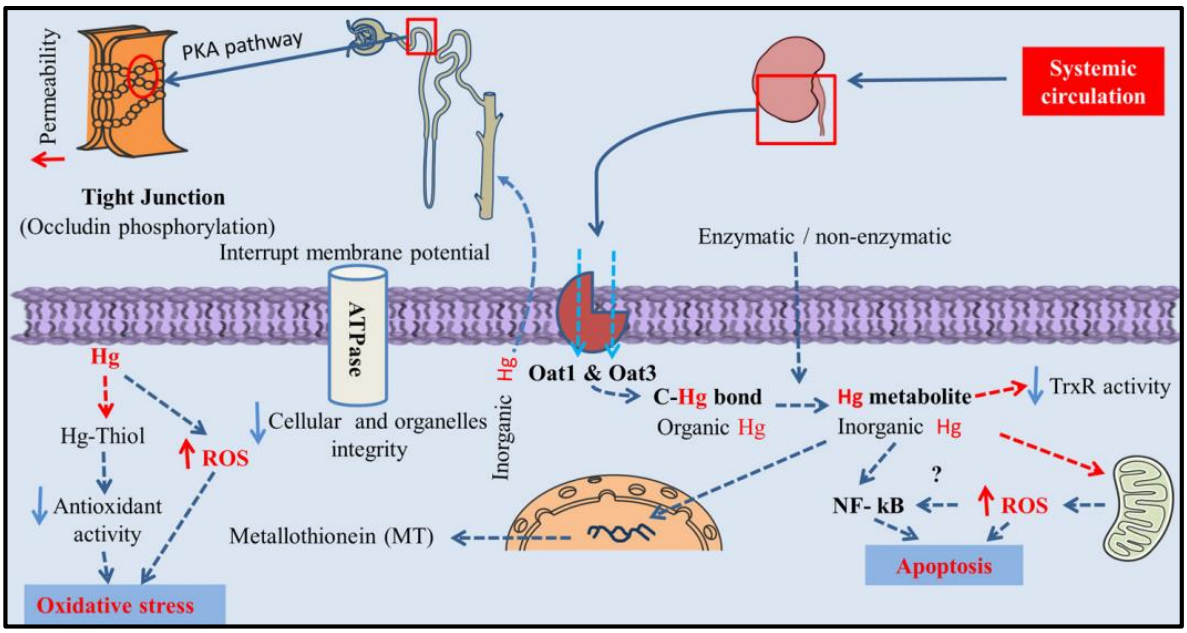

Figure 03: Toxicodynamics of Hg-induced kidney toxicity **(Rana *et al.*, 2018)**.

1.6. Mercury and oxidative stress

Mercury induces oxidative stress, which damages membranes, enzymes, and biomolecules **(Cano-Europa *et al.*, 2010)**. Mercury exposure has been related to decrease catalytic activity of glutathione peroxidase (GPx) and increased generation of hydrogen peroxide H_2O_2 and lipid peroxidation products in renal and mitochondrial membranes. Mercury enhances the synthesis of malondialdehyde (MDA) and advanced protein oxidation products, hence increasing the inflammatory response. Methylmercury disrupts intracellular

calcium cation Ca^{2+} homeostasis, affects protein phosphorylation, promotes thiol group binding, and destroys cellular structure **(figure 04) (Salazar-Flores *et al*., 2019)**.

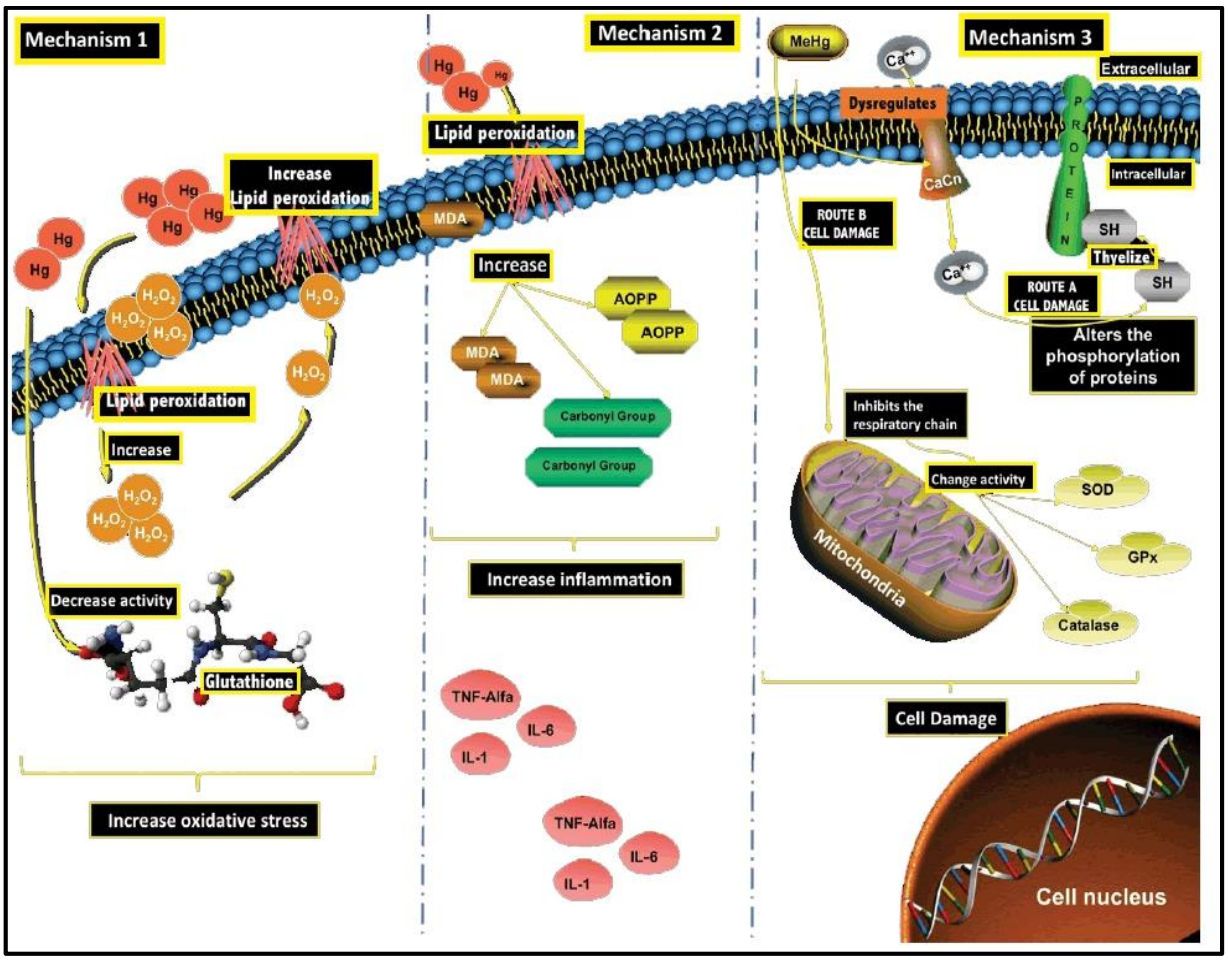

Figure 04: Mecanisms of mercury-induced oxidative stress **(Salazar-Flores *et al*., 2019)**.

1.7. Mercury detoxification

Table 02: Detoxification and action suppression of different forms of mercury in the human body **(Pavlogeorgatos & Kikilias, 2002)**.

Mercury form	Detoxifying compounds
Elemental mercury Hg^0	• Vitamin E is reported to be a protective agent. • Additionally, ethanol reduces the human organism ability for absorption of elemental mercury, possibly by suppressing the activity of the catalase, which oxidizes it to produce bivalent Hg. • Tellurium also appears to have a protective role.
Inorganic mercury compound	• Metaltheionin has a protective role. • Also, various mercury-chelating compounds (e.g. bimercapto-propanol), accelerate its excretion. • Finally, it is worth mentioning that selenium seems to have a protective action by binding Hg to HgSe and limiting its acute action on intestine and kidneys. • Tellurium also has a protective role.
Organic mercury compound	• Vitamin E reduces the toxicity and increases survival chances after an exposure to methyl-mercury. • Glutathione probably catalyzes the rapid dissociation of the Hg-C bond.

Chapter 02

Overview of Vitamin B6

1. Vitamin B6

1.1. Definition of vitamin B6

Vitamin B6 (pyridoxine) is an essential water-soluble vitamin that consists of six different vitamers: the alcohol pyridoxine (PN), the amine pyridoxamine (PM), the aldehyde pyridoxal (PL), and their respective 5'-phosphorylated derivatives: pyridoxine 5'-phosphate (PNP), and pyridoaxamin 5'-phosphate (PMP), pyridoxal 5'-phosphate (PLP). They vary in a variable group found at their fourth position. Furthermore, it is an essential substance required for human health and effective function (**Hellmann & Mooney, 2010; Colinas & Fitzpatrick, 2016)**.

Vitamin B6 is abundant in many foods, as well as added to other foods, and is also used as a nutritional supplement. Fish and red meat, especially beef, are the richest sources of vitamin B6, potatoes and other starchy vegetables and fruits (other than citrus fruits) are also found (**Al-shafie & Alshirifi, 2022)**.

Paluszny & Qiu (2023) mentioned in their study that this bioactive molecule involved in a number of enzymatic processes (around 140 reactions) that are essential for carbohydrate, lipid, and amino acid metabolism. The recommended daily dose of vitamin B6 is between 1.3 and 2.0 mg/day.

1.2. Pyridoxal5'-phosphate (PLP)

Pyridoxal 5'-phosphate (PLP), a physiologically active form of vitamin B6, is a cofactor for enzymes involved in protein, lipid, and carbohydrate metabolism, as well as the synthesis of hemoglobin, neurotransmitters, nucleic acids, one-carbon units, and immunomodulatory metabolites (**Paul *et al*., 2013)**. Additionally, vitamin B6 may play an important part in the antioxidant process. PLP acts as a cofactor in the transsulfuration process from homocysteine to cysteine. This route produces cysteine, which contributes to the production of reduced glutathione (GSH), which is essential for the antioxidant system (**Hsu *et al*., 2015)**.

1.3. Pharmacokinetics of vitamin B6

Plants synthesize PLP de novo using two distinct enzymes, pyridoxine biosynthesis 1 (PDX1) and pyridoxine biosynthesis 2 (PDX2). In addition, they have a salvage pathway that produces PLP from any of the other five B6 vitamers. Humans lack a de novo biosynthesis pathway, but they have the necessary salvage pathway enzymes. The vitamin is thus required in the human diet, and any of the six B6 vitamins can be utilized as a PLP source (**Hellmann *et al*., 2021)**.

In the cytoplasm PL, PM, and PN are converted into the 5'-phosphorylatedvitamers through pyridoxal kinase (PDXK), pyridoxine 5'-phosphateoxidase (PNPO) converts PNP and PMP into PLP, as seen in **(Figure 05)** (**Galluzzi *et al*., 2013)**.

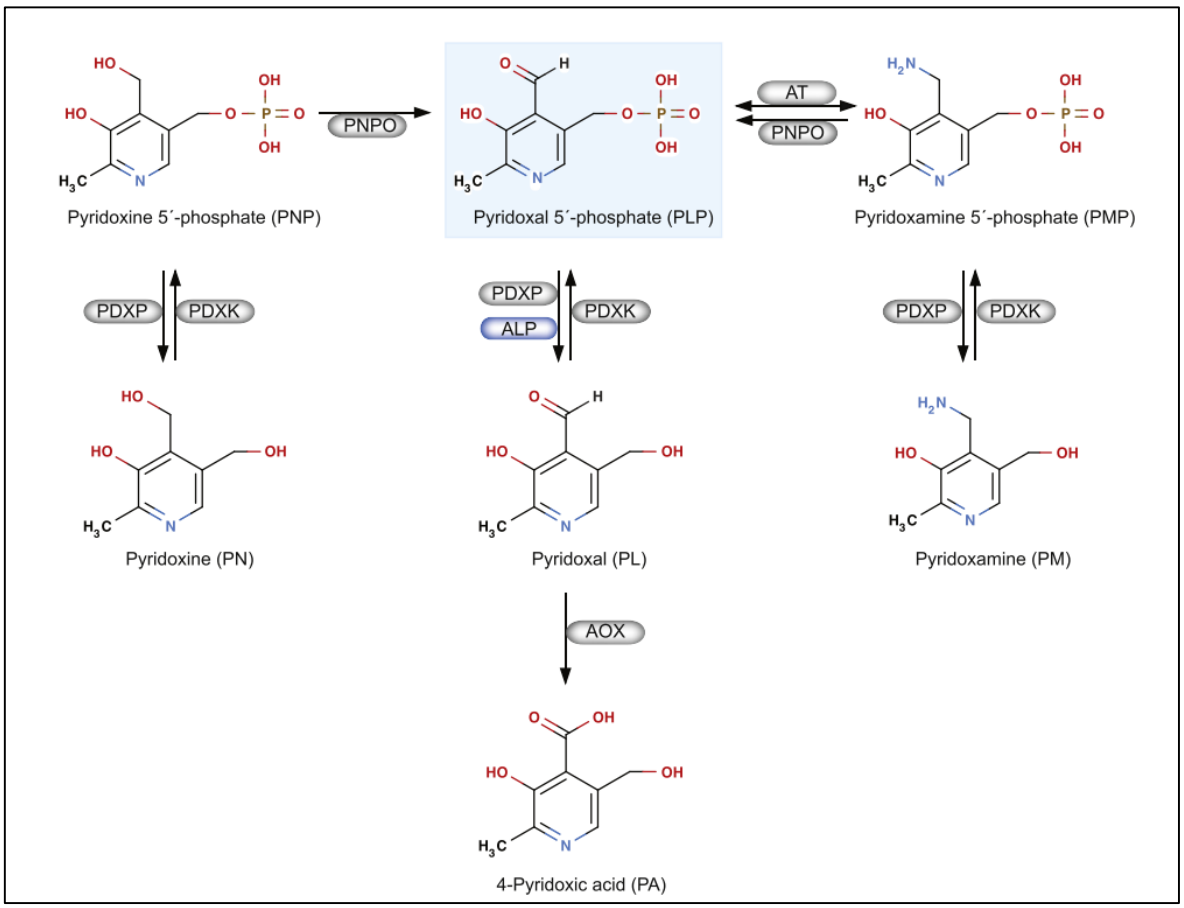

Figure 05: Structure and interconversion of vitamin B6 vitamers **(Ueland *et al*., 2017)**.

1.3.1. Absorption

After ingestion of phosphorylated forms PLP, PNP, and PMP are readily absorbed in the jejunum and ileum via passive diffusion; the intestinal absorption involves dephosphorylation by the tissue-non-specific alkaline phosphatase (TNSALP), which is anchored to the cell membrane. Then, the dephosphorylated forms PM, PN, and PL are absorbed from the upper small intestine by a carrier-mediated system (**Mascolo & Vernì, 2020)**.

1.3.2. Distribution

A specific protein called albumin acts as a carrier for vitamin B6 vitamers (PM, PN, and PL), along with dephosphorylated B6 vitamers (PMP, PNP, and PLP), reach the peripheral tissues through the blood stream (**Parra *et al*., 2018)**.

1.3.3. Metabolism

The dephosphorylated forms PM, PN, and PL are delivered to the liver. Here, they are converted to PLP due to the combined action of PDXK and PNPO (**Galluzzi *et al*., 2013)**.

1.3.4. Excretion

The elimination of PLP mainly proceeds via the aldehyde oxidase 1 (AOX1) mediated production of 4-pyridoxic acid (4-PA), which is excreted in urine **(Figure 06) (Stanulović *et al*., 1976)**.

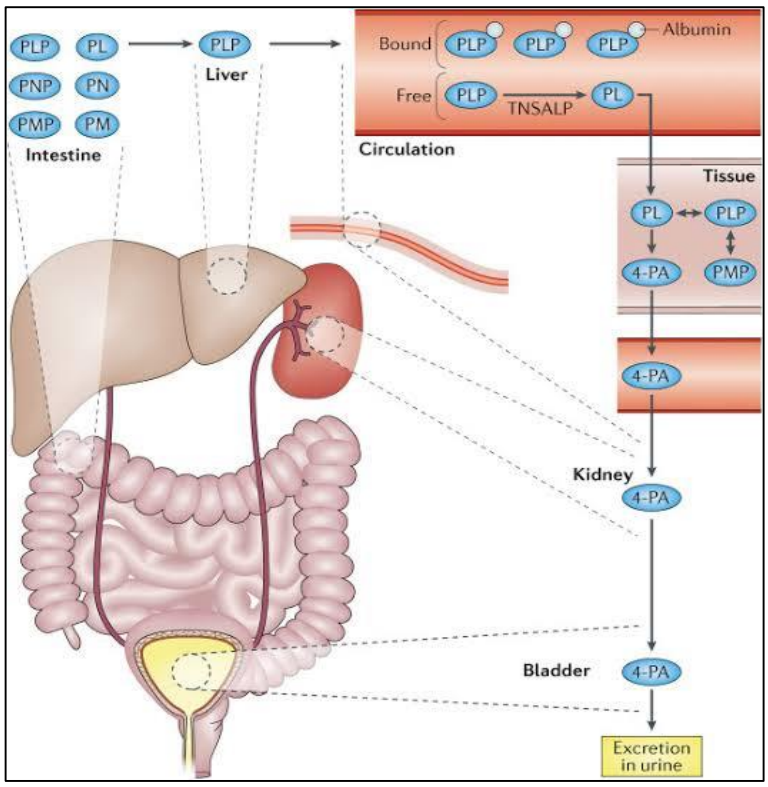

Figure 06: The absorption, distribution, metabolism and excretion of vitamin B6 **(Whyte et al., 2021)**

1.4. Vitamin B6 and oxidative stress

Oxidative stress refers to an imbalance between oxidative mechanisms and antioxidative systems that supports oxidation. The human body's principal oxidant is oxygen. Oxygen has high endogenous activation energy but is quickly activated and decreased by redox-active metal ions. Only 1% of the body's oxygen is transformed into reactive oxygen species (ROS). ROS are extremely reactive, reduced forms of oxygen that cause inflammation. Antioxidative processes inhibit the development of ROS and other oxidative stress products, reducing oxidative damage in several ways. Vitamin B6 is recognized to possess antioxidant effects

(Wit, 2011). Pyridoxamine can scavenge various oxygen radicals, including hydroxyl and superoxide.

Vitamin B6 can directly interact with peroxy radicals, scavenging them and inhibiting lipid peroxidation. While it may indirectly contribute to antioxidant defense by acting as a cofactor in the glutathione system. The GSH-dependent antioxidant system, which includes glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST), is essential for cellular defense against reactive oxygen species and other oxidants. Furthermore, a low vitamin B6 levels can disturb the balance of antioxidants, causing increased oxidative stress and cellular damage (**Hsu *et al*., 2015)**.

Homocysteine is an amino acid that can be harmful in high levels. It is regulated by Vitamin B6, and a deficiency of it can cause high levels of homocysteine. High homocysteine levels are linked to increased oxidative stress **(Papatheodorou & Weiss, 2007; Cappuccilli *et al*., 2020)**.

1.5. Vitamin B6 deficiency

Vitamin B6 is a crucial vitamin that plays a significant role in many bodily functions. Deficient vitamin B6 status might either directly cause higher oxidative stress or might affect cysteine and GSH synthesis and increased malondialdehyde (MDA) levels (**Hsu *et al*., 2015)**. This lack is also able to influence the immune system **(Mahmoudi & Rezaei, 2019)**, and induce cardiovascular diseases, cancer, stroke, and venous thrombosis. Low plasma PLP has also been linked to rheumatoid arthritis **(Ueland *et al*., 2017)**.

1.6. The tolerance upper intake levels for vitamin B6

According to USDA National Nutrient Database recommended intake of vitamin B6 for different age groups are shown in **(Aslam *et al*., 2017) (Table 03)**.

Table 03: Recommended dietary allowance (RDA) for vitamin B6.

Recommended Dietary Intake of Vitamin B6 per day	
Males	**Females**
0.5 mg/day (age 1-3 years)	0.5 mg/day (age 1-3 years)
1 mg/day (age 9-13 years)	1 mg/day (age 9-13 years)
1.3 mg/day (adults)	1.3 mg/day (adults) 1.9 mg/day (pregnant)

Experimental Review

Chapter 01

Materials & Methods

The experimental study was performed at Animal Ecophysiology laboratory of Badji Mokhtar-Annaba University. Microscopic examination of histological slides was performed at the pedagogical laboratory of Abbes Laghrour-Khenchela University.

1. Materials

1.1. Biological materials

The experimental study was carried out on twenty-four male rabbits *Oryctholagus cuniculus* aged from 7 to 8 months and weighting about (2730g ± 130g) were obtained from Pasteur Institute (Algiers). At the "Breeding Center" of University Badji Mokhtar-Annaba Algeria; rabbits were acclimated for two weeks before the experiments. They were housed in cages (50 x 60 x 53cm^3) in a room at ambient temperature with relative humidity of (60°C ± 5%). The cages were illuminated with natural photoperiod (12h light/12dark cycle). Animals were allowed free access to water (*ad libitum*) and fed three times a day with a commercial standard diet **(Annexe 01)**.

Table 04: Scientific classification of *Oryctholagus cuniculus* **(Linnaeus, 1958)**.

Taxonomic Position	
Kingdom	Animalia
Phylum	Chordata
Sub-phylum	Vertebrata
Class	Mammalia
Order	Lagomorpha
Family	Leporidae
Genus	Oryctolagus
Species	*Oryctolagus cuniculus*

1.2. Chemicals

1.2.1. Mercury

Pure and powdered mercuric chloride ($HgCl_2$) was obtained from chemistry laboratory at Badji Mokhtar-Annaba University, Algeria. A concentration of 0.5g/kg was chosen and employed according to literature.

1.2.2. Vitamin B6

Vitamin B6 (Pure encapsulation®) was supplied by a private pharmacy in Khenchela region. A concentration of 5ppm was used following to literature.

1.3. Experimental procedure

After two weeks of adaptation under the same laboratory conditions, 24 animals were randomly divided into 4 groups of 6 animals (N=6). Rabbits were treated orally **(Figure 07)** for 30 days, as follows:

Group 1: untreated	The control group received potable water and standard diet *ad libitum.*
Group 2: $HgCl_2$	Rabbits received potable water and standard diet contaminated with mercuric chloride **(0.5 g $HgCl_2$/kg diet)** *ad libitum.*
Group 3: VitB6	In addition to potable water and standard diet ad libitum, rabbits received vitamin B6 (5ppm) by gavage.
Group 4: $HgCl_2$ + VitB6	Rabbits received potable water and standard diet contaminated with mercuric chloride **(0.5 g $HgCl_2$/kg diet)** *ad libitum* **+** infusion of vitamin B6 **(5ppm)** by gavage.

Figure 07: Gavage of rabbits (Personal picture).

1.4. Biological samples

At the end of the treatment period, the rabbits are fasted for one hour and sacrificed by decapitation **(Figure 08)**. Intestines, liver, kidneys and thymus were immediately removed then collected and preserved in 10% neutral buffered formalin to achieve the histological profile.

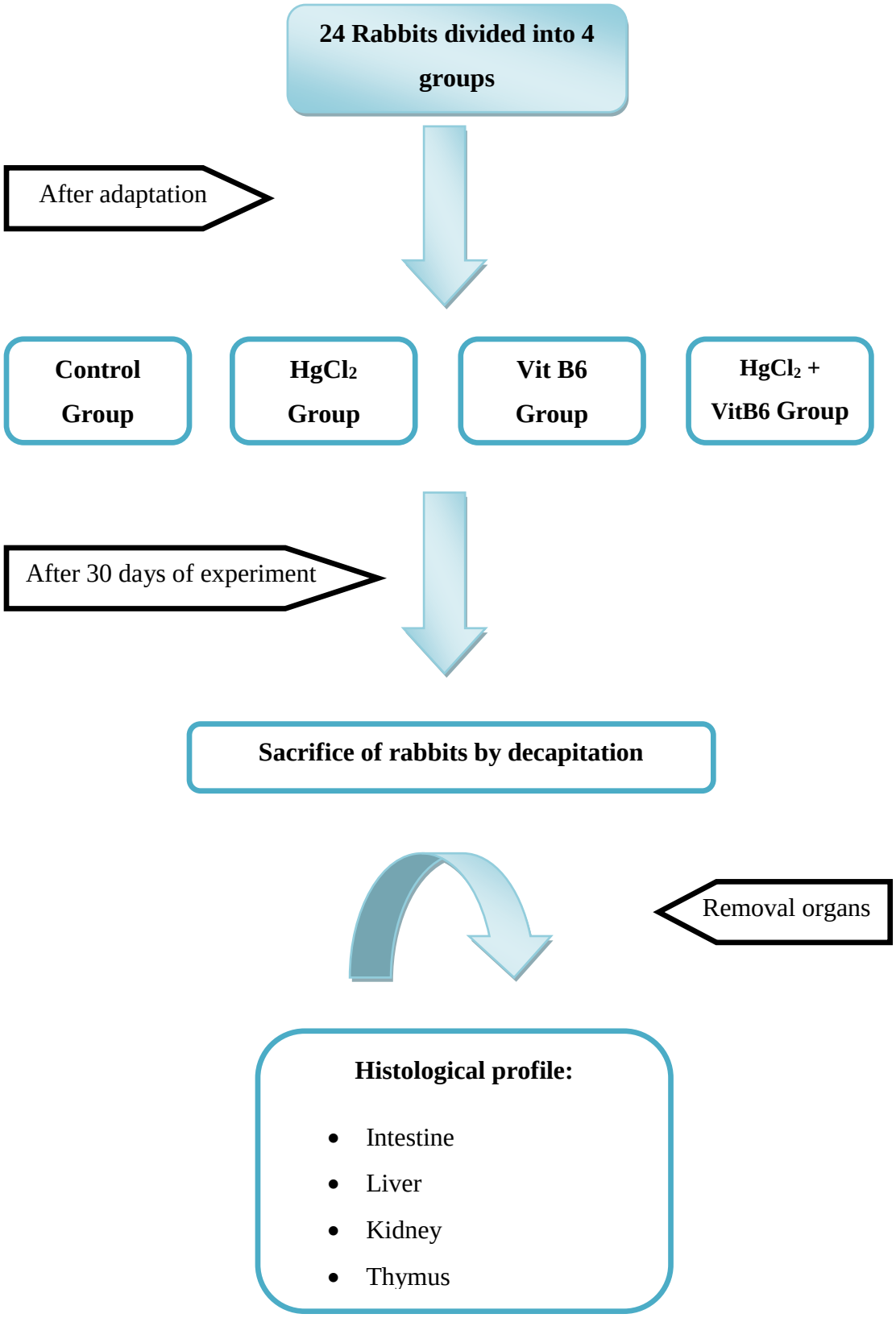

Figure 08: Summary of experimental procedure.

2. Methodes

1.1. Histological profiles

After sacrifice, intestine, liver, kidney and thymus were immediately collected and preserved in 10% buffered formalin, where it examined according to the classical method of **Martoja & Martoja (1967)** according to the following steps **(Figure 09)**:

- **Fixing of the samples**

Fixation is the most crucial phase in histological study, since it allows for structural and microscopic examination of biological specimens (samples). Its primary function is to protect tissues from autolysis and bacterial attack in order to keep them as close to the live condition as possible, allowing scientific data to be obtained exclusively by microscopic examination.

The intestine, liver, kidney, and thymus were cut, and each specimen was put in labeled tubes filled with 10% buffered formalin for 72 hours.

- **For good fixation:**

- The quantity of fixative used should be proportionate to the fragment size.
- The thickness of the fragment to be fixed must allow rapid action of the fixative.
- Fixation must be carried out as early as possible and as quickly as possible.
- The duration of action of the fixative is proportional to the size of the fragment.
- The duration of fixation is a minimum of three days (72 hours).

- **Dehydration**

This stage aims to remove the water contained within the tissues. To achieve this aim, the specimen must be treated with a series of reagents (in increasing alcohol concentration) that replace water with alcohol (dehydration) and toluene (substitution) before impregnating the samples in two liquid paraffin stations.

The paraffin is neither miscible in water nor soluble in alcohol used for the dehydration. Therefore, toluene is an intermediate liquid that is miscible with alcohol and paraffin and has the ability to clearing tissues.

Each of the steps of dehydration is carried out automatically by an instrument equipped with twelve stations; ten of which are made of glass for the reagents and two of heated stainless steel for the paraffin, as well as a basket for inserting the cassettes containing specimens. This instrument, known as an automatic tissue processor.

The specimens were placed in cassettes to be passed through the ascending concentrations mentioned in the procedure carried out for the dehydration of the samples, as follows:

- Diluted formalin for one hour;
- Alcohol at 70°for one hour and 30 minutes;
- Alcohol at 80° for one hour and 30 minutes;
- Alcohol at 90° for one hour and 30 minutes;
- Alcohol at 100° for one hour;
- Alcohol at 100° for one hour;
- Alcohol at 100° for one hour;
- Diluted toluene for one hour and 30 minutes;
- Diluted toluene for one hour and 30 minutes;
- Diluted paraffin for two hours;
- Diluted paraffin for two hours.

➢ **Inclusion (paraffin embedding)**

The purpose of this step is to treat the samples with liquid paraffin, which may penetrate the tissues. An inclusion machine may perform all of the processes involved in obtaining paraffin blocks containing the examined specimen, resulting in a paraffin block ready for microtomization.

This automated system is a compact and programmable unit for embedding biological tissues in paraffin. Their objective is to allow for the final assembly and hardening of biological tissues saturated in paraffin before being sliced using a microtome. This system has a metal bath heated to 60 °C to preserve the samples during assembly. The working platform is divided into two surfaces: tempered which keeps the paraffin liquid and allows for sample handling and orientation, and refrigerated, which solidifies the paraffin. The paraffin reservoir, molds, and dispenser are all tempered to make embedding easy.

- A little liquid paraffin was poured into stainless steel molds;
- With heated forceps, the specimen was immersed in the molds containing paraffin;
- The bottom of the metal molds was filled, previously warmed with paraffin, and kept on the tempered surface;
- The cassette containing the sample was moved from the hot paraffin bath and quickly transferred to the mold;

- The mold was placed on the cold surface (plate), thereby fixing the sample in the center (hardening of the paraffin by the cold);
- The upper part of the cassette was placed on the mold, and the level of paraffin was topped up until it covered the bottom;
- The mold was then placed on a cold surface for 30 minutes to harden the paraffin.

➢ **Sectioning**

This step seeks to obtain extremely thin histological slices that allow light from the optical microscope to flow through them, allowing us to view the various tissue and cellular components.

An instrument consisting of a knife holder and a block holder (specimen holder) used to carry out this work is called the microtome. This category of tools is characterized by an advancement mechanism that uses a crank to make a rotary movement of the block to be cut towards the knife, which remains fixed. That is to say, the biological tissue is cut by moving the specimen towards the knife.

The following steps were followed to obtain the visible sections under the optical microscope:

- The pre-cut and prepared paraffin block was mounted in the microtome specimen holder;
- The knife was adjusted to create a clean cutting surface (thick cut);
- The final cut thickness is set (3-5 µm);
- The making of the cutting ribbons has started;
- A little egg-albumin solution was poured onto the dried slides, allowing good spreading of the sections, then the cutting ribbons was placed on these slides using a forceps;
- The whole slides were placed on a hot plate for a few seconds to stabilize and stick the cutting ribbons;
- The slides were filtered and stored in slice baskets and placed in the oven at a temperature of 72 degrees Celsius for ten minutes.

➢ **Staining**

Staining is an essential step in histological procedures because it allows for the preparation of specimens that are easy to interpret under the microscope. Hematoxylin-eosin is one of the staining techniques available, allowing the nucleus to be colored blue and the cytoplasm to be pink.

The concept behind this stain is based on chemical factors that are commonly involved. Hematein (NH^+) binds to the phosphoric acids of the nucleus, whereas eosin (CO^-) binds to

the positive groups of cytoplasmic proteins. However, since paraffin is hydrophobic and stains are hydrophilic, sections must be deparaffinized and rehydrated before staining. This stage is guaranteed by a series of baths.

This step was assured by passing the slides through a series of baths:

- The slides were placed in a solvent to eliminate paraffin (xylene) from the part for 24 hours, then two xylene baths of 5 minutes each;
- The slides were passed in a bath of a mixture of alcohol and xylene (1/3 alcohol and 2/3 xylene) for 3 minutes; Then a pure alcohol bath for 3 minutes;
- The slides were agitated in a tap water bath for final rehydration; the sections with decreased concentrations of ethanol (100°,100°,90°,80°,70°) for 2 minutes and washing with distilled water;
- After rehydration, the slides were stained and first immersed in hematoxylin for 45 seconds, then rinsed with moderate shaking in a water bath;
- The sections were then placed in eosin for 1.5 minutes and then rinsed with running water;
- Then the staining process was carried out, which is the reverse process of that carried out at the beginning, before assembly could be carried out.
- The slides were stained by immersing them successively in a bath containing 70° alcohol, two baths of pure alcohol for 3 seconds each, and a bath of a mixture of alcohol and xylene (1/3 d alcohol and 2/3 xylene) for 3 seconds each, followed by four seconds of moderate stirring in two solvent baths (xylene).

- **Mounting**

During this stage, 3 to 4 drops of Eukit were applied to each blade, and a coverslip was put to ensure that the Eukit covered the whole cut. Eukit polymerized in around twenty minutes, but the process may be accelerated by heating the slides in an oven at 72°C for ten minutes.

Finally, the microscopic preparation was ready for observation. The slides were examined at low magnification to obtain a good look at the tissue, then at high magnification to view details. The pictures below were taken using an optical microscope.

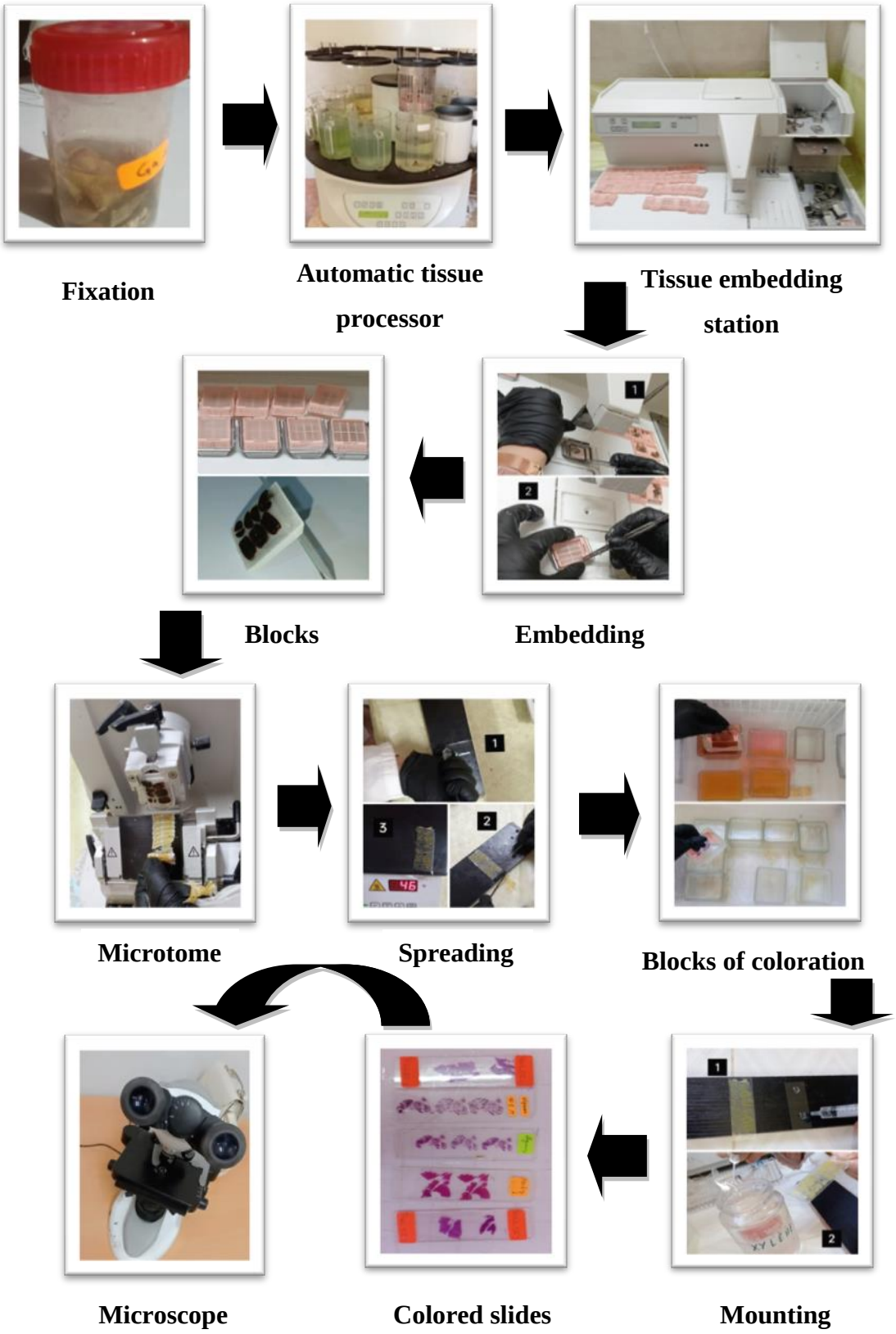

Figure 09: Materials and steps of slides preparation for histological study.

*Chapter*02

Results & Discussion

1. Results

1.1. Histological profiles

1.1.1. Intestine

Histological studies on intestinal tissues following rabbit's administration of mercuric chloride for 30 days showed remarkable structural changes in the intestinal mucosa characterized by an atrophy of villi, sloughing mucosa and submucosa, and degeneration of brush border cells **(Figure10)**. Additionally, enterocytes disappeared due to mucosa desquamation in certain endroits; dissimilar to the control group, where normal mucosa, submucosa, and even normal villi were observed. These severe alterations affect intestine function and can contribute to absorption disorders in this organ.

Treatment with vitamin B6 alone or in combination with $HgCl_2$ lowered the injury in the intestine tissues. Therefore, the nearly normal structure of chloride-mercury combined with vitamin B6 in treated tissues. This study proved that vitamin B6 protects the intestines and increases the antioxidant defense system in rabbits intoxicated with $HgCl_2$.

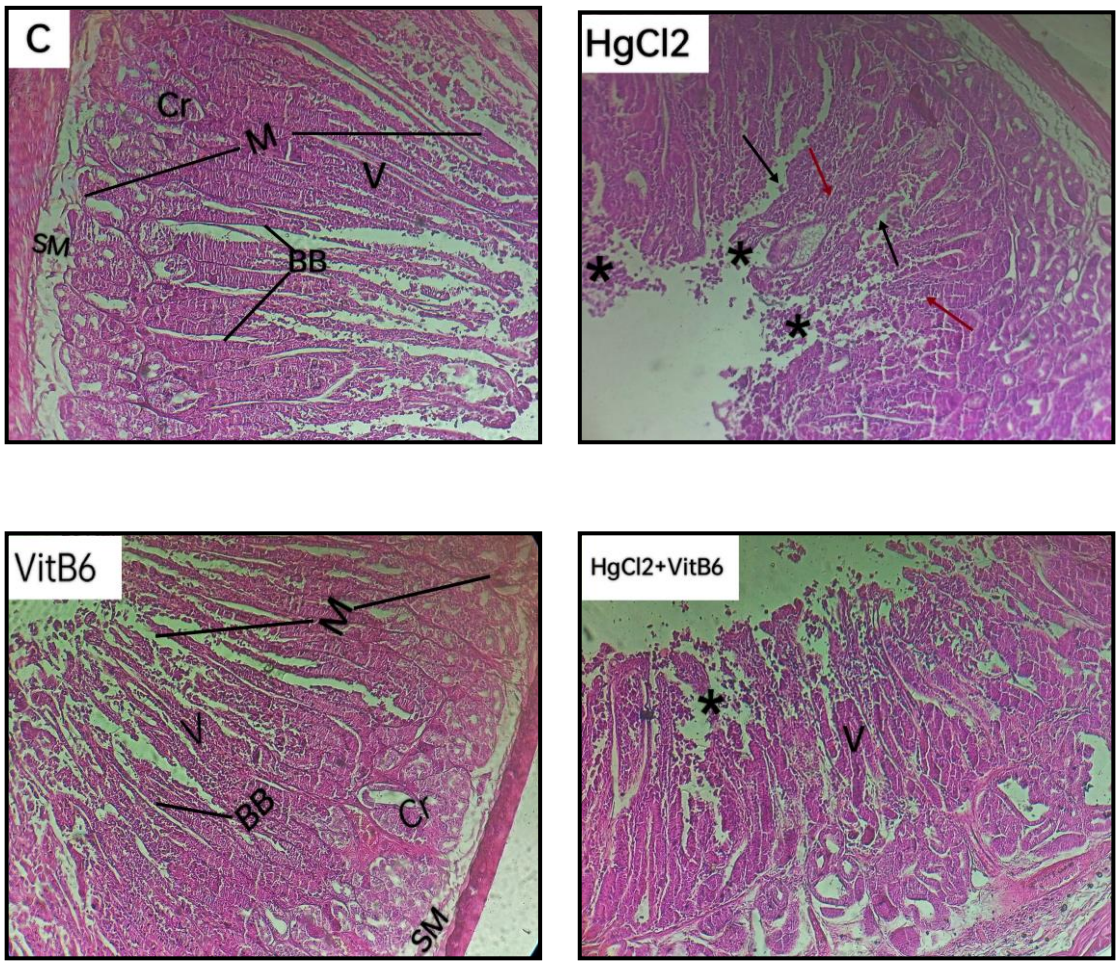

Figure 10: Histological profiles of rabbit's intestine showing the control, the $HgCl_2$, the VitB6 and the $HgCl_2$+VitB6 groups after 30 days treatments **(H & E x. 400)**.

M: Mucosa. **SM:** Submucosa. **V:** Villi. **BB:** Brush border. **Cr:** Crypts of Luberkuhn.

(Asterisk) Villus atrophy. (Black arrows) sloughing mucosa. (Red arrows) Loss of brush border cells.

1.1.2. Liver

The representative pictures under the microscopic light of histo-pathological examination in the liver tissues are shown in **(Figure 11)**. Liver sections from the control group and vitamin B6-treated group revealed no damage and normal hepatic architecture of the periportal triad; normal bile duct, and hepatic artery. However, abnormalities and significant alterations in the liver of mercuric chloride-treated rabbits were detected in hepatic tissues compared to the control. The main characteristic findings were the appearance of vacuolization, swelling of hepatocytes, and dilatation of the central vein.

However, the co-administration of Vitamin B6 with $HgCl_2$ showed marked improvement in the histological structure of the liver, regardless of the dilated portal vein, in comparison to the $HgCl_2$-treated rabbits only.

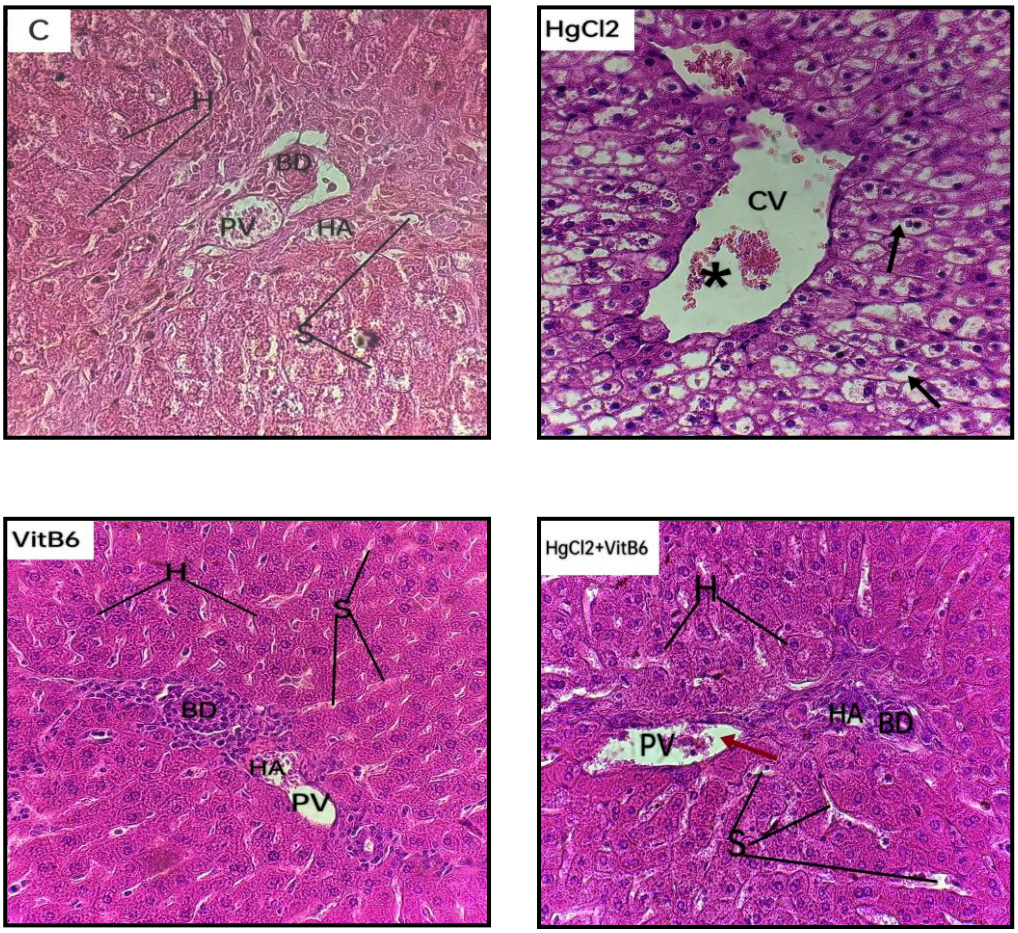

Figure 11: Histological profiles of rabbit's liver showing the control, the $HgCl_2$, VitB6 and $HgCl_2$+ VitB6 groups after 30 days treatments **(H & E x. 400)**.

PV: Portal vein. **CV:** Central vein. **BD:** Bile duct. **HA:** Hepatic artery. **H:** Hepatocytes. **S:** Sinusoid.

(Asterisk) Centrilobular dilated vein. (Black arrows) Hepatocytes vacuolations.

(Red arrow) Dilatation of portal vein.

1.1.3. Kidney

Histological profiles of kidney tissues from different groups are shown in **(figure 13)**. Since the microscopic assessment of the renal system in the control group showed a normal intact histological structure of glomeruli, and Bowman's capsule also showed well-defined renal tubules. While mercuric chloride intoxication in rabbits caused kidney injury and histological changes leading to renal failure. As it is shown, necrotic renal tubules are accompanied by tubular bruh border loss and glumular atrophy with dilated Bowman's capsule.

The alterations were reduced remarkably in the kidneys of rabbits supplemented with mercuric chloride and vitamin B6 which showed a nearly normal morphology, close to the control group. Vitamin B6 co-treated animals revealed minimal tubular damage and enhanced kidney functions.

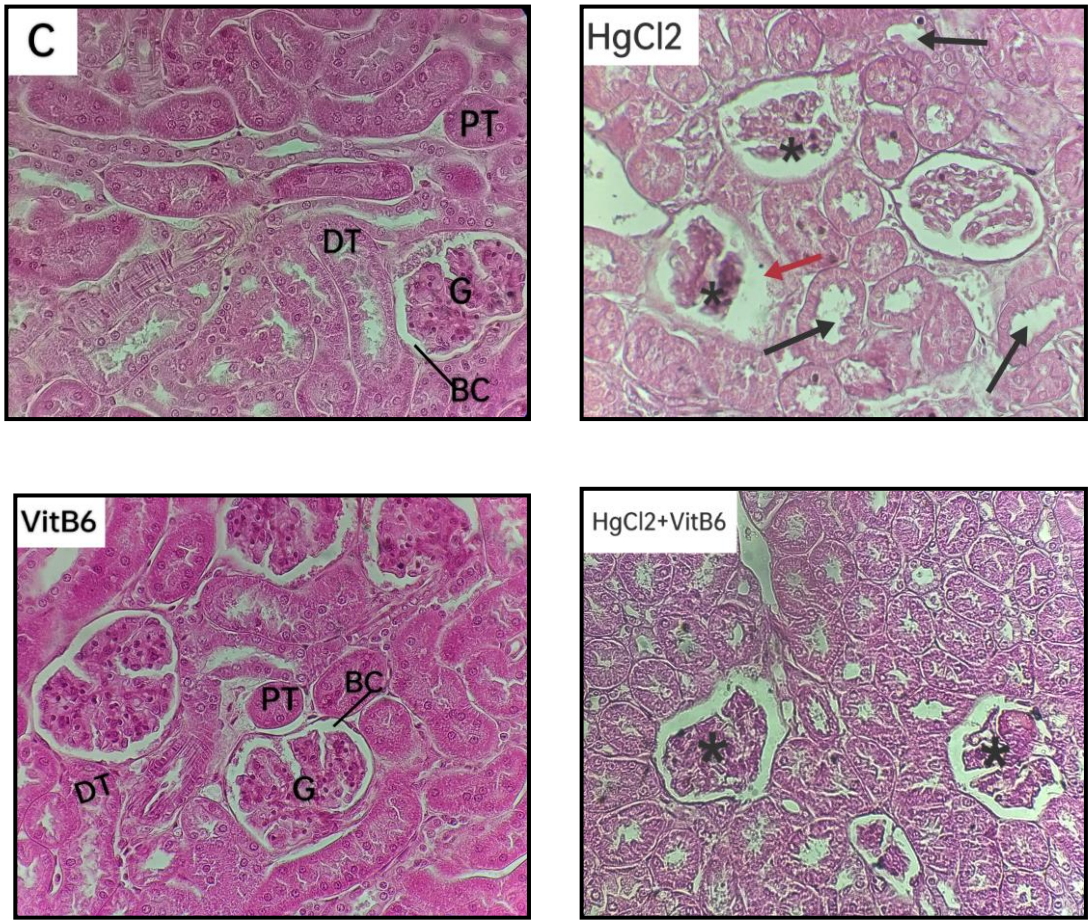

Figure 12: Histological profiles of rabbit's kidneys showing the control, the $HgCl_2$, the VitB6 and $HgCl_2$+VitB6 groups after 30 days treatments **(H & E x. 400)**.

G: Glomeruli. **PT**: Proximal tubule. **DT:** Distal tubule. **BC:** Bowman's capsule.

(Asterisk) Glomular atrophy. (Black arrows) Tubular necrosis. (Red arrow) Dilatation of Bowman's capsule.

1.1.4. Thymus

A light microscopic evaluation of the thymus gland tissues of the mercuric chloride-treated group demonstrated significant histological changes in both cortical and medullary areas **(Figure 13)**. Atrophy of the thymic cortex and lymphocyte depletion accompanied by necrosis were clearly observed. In addition, there is a loss of delineation between the cortex and medulla compared to the control and vitamin B6-treated groups, which showed normal thymus lobe architecture and densely packed cortical lymphocytes.

Despite the less atrophy revealed in $HgCl_2$ and vitamin B6-treated rabbits, histological thymic improvement and nearly normal Histological structure.

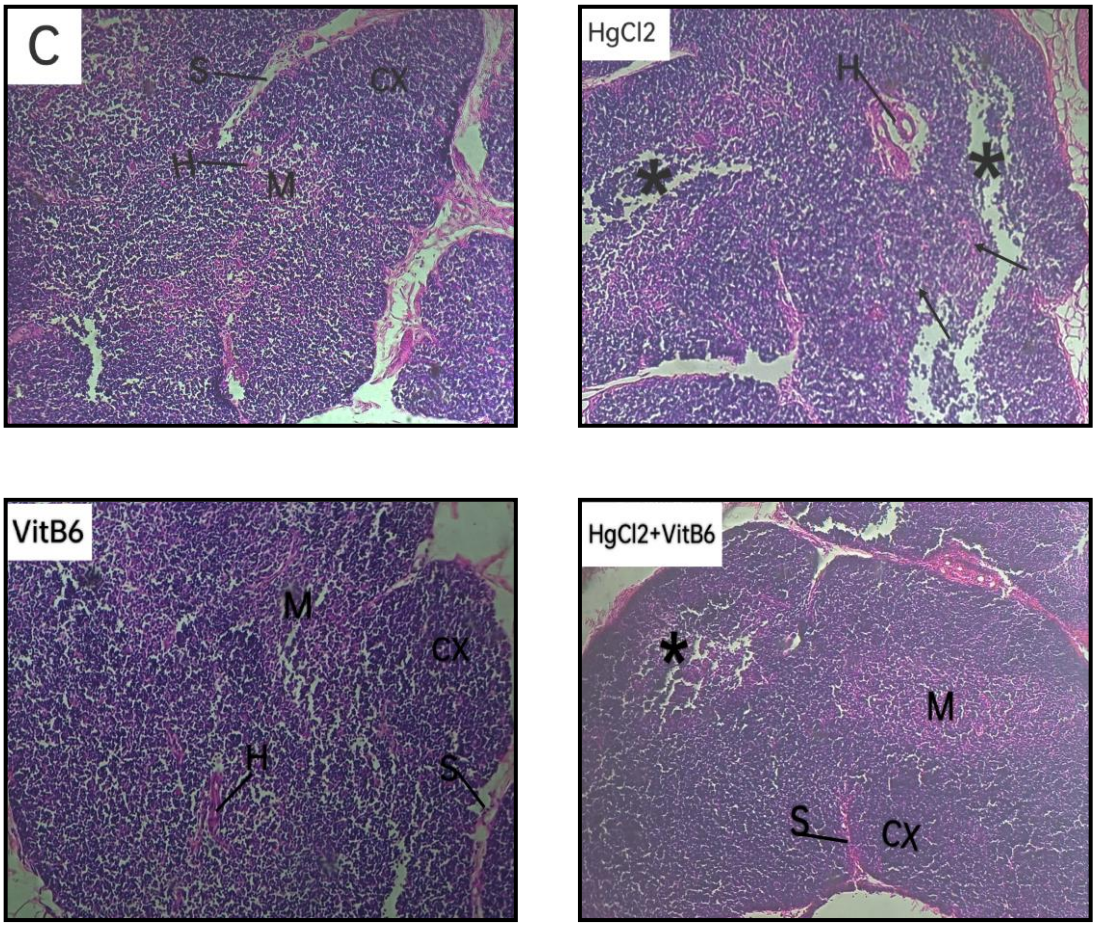

Figure 13: Histological profiles of rabbit's thymus showing the control, the $HgCl_2$, VitB6 and $HgCl_2$+VitB6 groups after 30 days treatments **(H & E x. 400)**.

M: Modula. **CX:** Cortex. **H:** Hassall's corpuscle. **S:** Septum.

(Asterisk) Atrophy of thymic cortex. (arrows) Lymphocytes depletion.

2. Discussion

Mercury is an extremely harmful heavy metal, and it has been demonstrated that it has toxicological effects on a wide variety of organs. It is recognized as a free radical that will bind thiol or sulfhydryl groups present in antioxidant proteins, therefore the antioxidant activity will decrease, leading to histological anomalies describe microscopic changes in tissues caused by mercury exposure **(Zulaikhah & Wibowo, 2018)**. Mercury exposure has been associated with histological damage in a variety of critical organs, including the intestines, liver, kidney, and thymus **(Kosuda *et al*., 1996; Jan *et al*., 2011)**. The present study's findings about mercury toxicity are in accordance with all of these previous studies.

Exposure of mercury is unavoidable since it occurs everywhere. For this reason, numerous studies have shown that bioactive substances and antioxidants such as vitamins can prevent tissue damage (**Rao & Sharma, 2001; Raeeszadeh *et al*., 2021)**.

The results demonstrate significant histological damage caused by $HgCl_2$ in the intestines, liver, kidneys, and thymus of the treated rabbits. Additionally, the administration of vitamin B6 showed promising protective and restorative effects on these tissues.

Concerning the histological study of intestine. The microscope examination of rabbits intestines treated with mercury reveals significant intestinal alterations and substantial structural changes following $HgCl_2$ administration, compared to other groups where it characterized by an atrophy of villi, sloughing mucosa, submucosa and degeneration of brush border cells. These alterations of intestinal histo-architecture could mainly due to the toxic effect of mercury. The results obtained are in accordance with **Yasmina (2017)** who recorded histological damage on rabbits intestinal tissues exposed orally to mercuric chloride for 30 days. This damage in the form desquamation of the intestinal flora or mucosa and structural changes in the mucosa and submucosa, a reduction in the number of Brunner's glands. Also, the enterocytes disappear due to the degeneration of cell membranes; necroses consisting of clusters of dead cells, goblet cells are more numerous which increases mucous secretions. Additionally, certain regions of the intestine lose their striated plate. These variations were attributed to the uniform distribution of the noxious effect of mercuric chloride in the intestinal epithelium, whereas organic mercury was found in specific locations on the intestinal epithelial surface **(De Oliveira Ribeiro *et al*., 2002)**.

Mercury destroys epithelial cells in the intestines, producing severe lesions due to its ability to attach to the SH groups of cell membranes alters the permeability of the intestinal mucosa **(Boadi *et al*., 1992; Stejskal & Stejskal, 1999)**. Moreover, Hg interacts to the thiol

groups of membrane proteins, which causes cellular degeneration in the villi **(Farmanfarmaian *et al.*, 1989)**. Villus end rupture, and localized enterocyte necrosis were induced by mercury **(Naidu *et al.*, 1983)**.

However, co-administration of $HgCl_2$ with vitamin B6 showed that histological changes were almost mitigated these adverse effects, maintaining nearly normal intestinal structures compared with the $HgCl_2$ group. This can be related to the ability of vitamin B6 to participate in the elimination of mercury-induced ROS mechanisms, protecting the intestinal tissues against oxidative stress induced by mercury **(Dalto & Matte, 2017)**.

The treatment with vitamin B6 minimized the harmful effects of mercury, maintaining nearly normal intestinal structures. This suggests that vitamin B6 plays a crucial role in enhancing the antioxidant defense mechanism, thereby protecting the intestinal mucosa from $HgCl_2$-induced oxidative stress.

Liver histology in this investigation of $HgCl_2$-treated rabbits has showed histological changes and significant alterations, including hepatocytes vacuolization, swelling, and central vein dilatation. Such histopathological changes are indicative of hepatic injury. The present findings are agreement with the study of **Oda & El-Ashmawy (2012)** who found after 7^{th} days of orally treatments of rats with Hg a considerable hepatocytes vacuolation, notably in the periportal zones, hepatic sinusoids dilated, and serum ALT, AST, and ALP levels increased significantly ($P < 0.05$), leading to hepatic cord atrophy and focal areas of hepatic necrosis. These finding are Similar changes were also reported by **Rakib *et al.* (2021)** in liver tissues of mercury-exposed mice for 7^{th} days showed degeneration of the cytoplasm, pyknotic nuclei, presence of vacuoles and dilatation of sinusoidal spaces. Several previous results show severe histopathological changes in the liver characterized by loss of readily arrangement of hepatic architecture also there is congestion of central vein of white rats which treated with mercury chloride **(Rhyaf, 2016)**. Also, there was degeneration, vaculation of hepatocytes and necrosis. The liver's role in detoxification makes it particularly vulnerable to toxic insults, which explains the observed damage.

Furthermore, the structure damage mercury exposure was established showed mild degeneration in the cytoplasm, loss of nuclei as well as the occasional presence of vacuoles and dilatation of sinusoidal spaces **(Agarwal *et al.*, 2010)**. Rat livers treated with $HgCl_2$ exhibited extensive cytoplasmic vacuolation of hepatocytes, mostly of hydropic and lipid kinds. Periportal and mid-zonal hepatocellular necrosis with mononuclear cell infiltration were consistent results. There were also reports of multifocal zones of lytic necrosis, when

hepatocytes were replaced by red blood cells (RBCs) or mononuclear cells. Hepatic sinusoids widened, Kupffer cells hyperactivated, hepatic cords atrophied, and significant vascular congestion was seen. The portal areas expanded significantly and were heavily infiltrated with lymphocytes **(Elblehi *et al*., 2019)**.

Conversely, the treatment with $HgCl_2$ and vitamin B6 both showed a marked improvement in liver histology, reducing the extent of hepatocytes vacuolization. This protective effect can be attributed to the antioxidant properties of vitamin B6, which likely reduced oxidative damage. For this reason, it is predictable to be protective in hepatic injury. To establish the validity of these results the protective function of vitamin B6 was demonstrated in **Zulaikhah & Wibowo (2018)** study where "Tender coconut water" contains antioxidants such as vitamin C, vitamin B1, vitamin B6 that are very useful for protecting body cells from free radical attacks and preventing effects caused by mercury exposure. Other similar study about *Medicago sativa L. (Alfalfa)* plant is a well-known traditional medicinal herb that is used to treat and prevent a variety of diseases. Alfalfa extract's nutritional and antioxidative properties can reduce mercury poisoning and enhance liver function due to its high vitamin content, including B6 **(Raeeszadeh *et al*., 2021)**.

A recent study has reported that treatment with vitamin E in mercury-exposed animals was also effective in reducing mercury accumulation in liver tissue. Vitamin E provides complete protection from mercury-induced hepatotoxicity both pre- and post-treatment; this may be due to the antioxidant properties of vitamin E after the antioxidative process **(Agarwal *et al*., 2010)**. The effectiveness of the vitamin against mercury toxicity in the liver was also demonstrated in **Hounkpatin *et al*. (2017)** histological evaluation on liver tissue after exposure to mercury. In rats intoxicated and treated with vitamin C, it was observed a slight vacuolar modification of periportal hepatocytes and a moderate congestion of the hepatic veins. The comparison of these results shows the protective role played by vitamin C used as a dietary supplement. In other similar study; vitamin C can stabilize the cell membrane in hepatic insufficiency induced by mercury and cadmium in rabbit liver **(Mumtaz *et al*., 2019)**.

Kidney Histological study can confirm the nephrotoxic impact of mercury by detecting the development of reactive oxygen species (ROS), membrane lipid peroxidation, and protein denaturation. In this present research, kidneys Mercury-treated rats after 30 days of Hg exposure exhibited tubular necrosis, glomerular atrophy, and dilatation of Bowman's capsule. These results are consistent with the nephrotoxic effects of mercury, evaluated by **Rakib *et al*. (2021)** whereas the degeneration of tubular cells with denaturation of the proximal tubule,

atrophy of the glomerulus and enlarged Bowman's capsule were observed in kidneys of $HgCl_2$ exposed mice. Mercuric chloride is a well-known nephrotoxic agent because it produces greater renal damage compared to others **(Rojas-Franco *et al.*, 2019).** Therefore, $HgCl_2$ accumulates in the proximal tubule of the nephron as a target site, making the kidney the most targeted organ **(Ali *et al.*, 2019)**. Glomerular atrophy and tubular degeneration were observed in the kidney tissues of the mercuric chloride-treated rats **(Aslanturk *et al.*, 2014)**. Particularly, the pars recta of the proximal tubule appear to be the most sensitive to the toxic effects of mercury and is usually the first segment of the nephron affected by exposure to mercuric compounds. The pars convoluta and distal segments of the nephron are not usually affected by exposure to low doses of mercury, but exposure to higher doses can lead to injury and necrosis in these segments **(Orr & Bridges, 2017)**. Additionally, the results of current study is agree with who demonstrated kidney damage including; atrophy of glomerulus and dilatations were seen in Bowman's capsule, whereas the tubules showed dilatation, degeneration. Tubular necrosis and atrophy of renal tissue were observed. Also, severe congestion and hemorrhage also there was degeneration and destruction of epithelial cells in rats exposed to chloride mercury (**Boroushaki *et al.*, 2014; Rhyaf, 2016; Francis *et al.*, 2020)**.

In contrast, the treatments of $HgCl_2$ and vitamin B6 treatment significantly ameliorated these effects, resulting in a histological appearance close to that of the control group. Vitamin B6's ability to restore glomerular hemodynamics and renal vasodilator metabolism corresponds to the accelerated formation of renal pyridoxal 5′-phosphate (PLP), which inhibits nephrotoxicity's oxidative responses. This is likely the primary mechanism by which the vitamin achieves its nephroprotective action. Vitamin B6 supplementation enhances SOD enzyme activity and kidney filtration through direct antioxidant actions by PLP in renal tissues. This helps neutralize the negative effects of superoxide anions on glomeruli **(Rastegar *et al.*, 2014)**. It has also been mentioned that this plant has great effectiveness against mercury toxicity because it contains antioxidants such as vitamin B6. It could also prevent the accumulation of mercury and the production of free radicals in the kidneys **(Raeeszadeh *et al.*, 2021)**. This further proves the validity of the current study. Post-treatment with vitamin E reduced mercury accumulation in the kidneys of mercuric chloride-intoxicated rats, but had no protective effect on histopathological alterations in kidney tissue. Post-treatment with vitamin E reduced mercury induced nephrotoxicity, while its pre-treatment was not effective. It may be due to the fact that in pre-treatment with vitamin E, the amount of vitamin E administered may be used for other normal body functions **(Agarwal *et al.*, 2010)**.

Compared to the results of the current study, vitamin B6 played an effective role in improving liver tissue damage.

The histological examinations of thymus gland have demonstrated significant histological changes upon $HgCl_2$ exposure. These included cortical atrophy, lymphocyte depletion, and loss of cortical-medullary delineation compared to control group. These structural alterations are indicative thymus injury and have been consistently documented in include the histological analysis of **Ficek (1994)** who revealed numerous thymocytes in both the cortical and medullar areas of thymus cultured in $HgCl_2$. Heavy metal salts disturbed the distribution of thymocytes in the cortex and medulla. In all preparations there were a significant number of areas in which cell damage caused by cytolysis was observed. Additionally, the thymic architecture of $HgCl_2$-treated BN rats was changed in mercury-treated BN thymus whereas substantial cortical atrophy after 15 days has been selected. Furthermore, the cortical and medullary areas were separated, and thymic atrophy and stromal epithelial disorder were also demonstrated in $HgCl_2$-treated BN rats after 28 days **(Kosuda *et al.*, 1996)**. The thymus is crucial for immune system development, especially in the differentiation and maturation of T cells. Mercury ions ($Hg2^+$) exposure can cause a variety of immunopathological disorders leading to impaired thymus function, which is one of the target organs. Interestingly, $Hg2^+$ inhibits active thymulin synthesis, through accumulation of mercury in thymic epithelial cells which Secret thymulin **(Bracci *et al.*, 2008)**. Mercury may disturb cell membrane permeability and by causing functional abnormalities of T-lymphocytes **(Jan et al., 2011).**

Remarkably, co-administration of $HgCl_2$ with vitamin B6 showed a marked improvement in thymus characterized by less thymic atrophy and lymphocyte depletion, indicating its protective effect. Vitamin B6 deficiency affects thymic epithelial cells capacity to induce T-cell precursor development, potentially through accumulation in thymic epithelial cells impairs thymulin formation. In rats fed a vitamin B6 deficient diet, lymphocytes were depleted in the thymus, and differentiation between the thymic cortex and medulla was reduced **(De Wit, 2011; Kumrungsee *et al.*, 2020).**

The effective role of vitamin B6 is also proven in another study against another heavy metal, “lead acetate," on rats, which showed significant damage to the organs of rabbits. **Ali *et al.* (2010)** demonstrated in his study gross pathological lesions in liver, kidney, and intestinal muscle, while vitamin B6 treatment revealed Slight congestion in the kidneys and mild hemorrhages in the liver.

Lastly, the toxic effect of mercuric chloride alone is clear; however, it is possible to recover the effect of mercury toxicity by using vitamin B6 as a protective agent. Vitamin B6's protective effects observed in this study can be attributed to several mechanisms; primarily, its role as a coenzyme in numerous enzymatic reactions that maintain cellular metabolism and function is well-established. Vitamin B6 is essential for the synthesis of neurotransmitters, hemoglobin, and nucleic acids, which are crucial for cellular health and function. Moreover, its antioxidant properties help scavenge free radicals, reducing oxidative stress and preventing cellular damage **(Hsu *et al*., 2017)**. These properties likely underpin the observed histological improvements in the intestines, liver, kidneys, and thymus.

Additionally, vitamin B6 has anti-inflammatory properties, which can mitigate the inflammatory response induced by $HgCl_2$ toxicity. By reducing inflammation, vitamin B6 helps preserve tissue architecture and function, facilitating recovery and preventing further damage.

Conclusion

Conclusion

The objective of this research is to investigate, on the one hand, the tissue damage induced by the hazardous heavy metal “mercury” and, on the other hand, to evaluate the beneficial effects of Vitamin B6 in tissue protection against this heavy metal, as well as its ability to enhance this damage.

This study highlights the severe histological damage induced by mercuric chloride in various vital organs of *Orycthola*gus *cuniculus* rabbits; including the intestines, liver, kidneys, and thymus. Besides the role of vitamin B6 in improving these damages and maintaining the function of each organ.

Interestedly the results are significant as they demonstrate that vitamin B6 can substantially mitigate these toxic effects, preserving the structural integrity and function of these organs. The protective mechanisms of vitamin B6 are likely due to its antioxidant and anti-inflammatory properties, which help maintain cellular health under oxidative stress.

The implications of this research are considerable, suggesting that vitamin B6 supplementation could be a viable strategy to protect against heavy metal toxicity. This could be particularly relevant in clinical settings where exposure to heavy metals is a risk, or in environmental health strategies aimed at populations exposed to these toxicants. Future research should focus on elucidating the precise molecular mechanisms by which vitamin B6 exerts its protective effects, and on evaluating its efficacy in other models of toxicity.

In conclusion, the administration of vitamin B6 offers a promising approach to mitigating the detrimental effects of heavy metal exposure, underscoring the importance of antioxidant therapy in toxicology and environmental health. The findings of this study advocate for the inclusion of vitamin B6 in diet of people exposed to mercury toxicity. Particularly, therapeutic protocols aimed at protecting vital organs from toxic insults, thereby enhancing overall health and resilience against environmental toxins.

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Bibliographic References

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Annexes

Annexe 01: Ingredients and chemical composition of commercial standard diet.

Ingredients	Percentage (%)
Curde proteins	15%
Curde fat	2.5%
Curde Ash	10%
Curde cellulose	12%
Calcium	1.48%
Phosphorus	0.80%
Vitamins (A, E, D3)	\
Salts (NaCl)	\