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Field: Pharmacotoxicology

Effect of carbimazole on rabbit central nervous system: histological and immunohistochemical study

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Dedications

To my parents, ***Rachida and Youcef***

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Dedications

First of all, I would like to thank GOD for giving me the strength and courage to complete this modest work.
With all my feelings of respect, with experience of my gratitude, I dedicate my graduation and my joy
To my paradise, to the apple of my eye, to the source of my joy and happiness, my moon and the thread of hope that lights my way, my better half, Mom.
To the one who made me a woman, my source of life, love and affection, to my support who was always by my side to support and encourage me, to my prince daddy.
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To all my family, To all my friends To all those I love and those who love me

MENNAALLAH

Abstract

Carbimazole is a widely used antithyroid drug, but its potential effects on the central nervous system (CNS) remain poorly understood. This study aimed to investigate the histological and immunohistochemical changes induced by repeated exposure to carbimazole in the brain tissue of female rabbits. Ten Female rabbits were divided into control and treated groups. Histological evaluation was performed using Hematoxylin and Eosin (H&E), Masson's Trichrome, and Toluidine Blue stains. Immunohistochemical analysis was conducted using P53, GFAP, and Neurofilament markers to assess neuronal damage, astrocyte reactivity, and axonal integrity. Histological findings in treated animals revealed neuronal degeneration, inflammatory infiltration, and fibrotic changes, particularly in the cerebral cortex and hippocampus. Immunohistochemistry showed upregulation of P53, indicating apoptotic activity, increased GFAP expression, reflecting astrocyte activation, and reduced Neurofilament staining, suggesting axonal damage. Carbimazole administration induced significant neurohistological and immunohistochemical alterations, supporting its potential neurotoxic effects. These results emphasize the need for further research into the neurological safety of long-term carbimazole use.

Keywords: Central nervous system, Carbimazole, Histology, immunohistochemistry, Rabbit.

RÉSUMÉ

Le carbimazole est un antithyroïdien couramment utilisé, mais ses effets potentiels sur le système nerveux central (SNC) restent peu étudiés. Cette étude vise à évaluer les altérations histologiques et immunohistochimiques induites par une exposition répétée au carbimazole chez la lapine. Dix lapines ont été réparties en deux groupes : témoin et traité. L'évaluation histologique a été réalisée à l'aide des colorations hématoxyline-éosine (H&E), trichrome de Masson et bleu de toluidine. L'étude immunohistochimique a utilisé les marqueurs P53, GFAP et Neurofilament pour évaluer respectivement les lésions neuronales, la réactivité astrocytaire et l'intégrité axonale. Les tissus cérébraux des animaux traités ont révélé une dégénérescence neuronale, une infiltration inflammatoire et des modifications fibreuses. L'expression de P53 était augmentée, traduisant une activité apoptotique. Le GFAP était surexprimé, indiquant une activation astrocytaire, tandis que le Neurofilament était réduit, révélant des altérations axonales. L'exposition au carbimazole entraîne des altérations significatives du tissu nerveux central, suggérant un effet neurotoxique potentiel. Des recherches complémentaires sont nécessaires pour mieux comprendre les mécanismes impliqués.

Mots clés : Système nerveux central ; Carbimazole ; Histologie ; Immunohistochimie ; Lapine.

ملخص

الكاربيمازول هو دواء مضاد للغدة الدرقية يُستخدم على نطاق واسع، لكن تأثيره المحتمل على الجهاز العصبي المركزي لم يُدرس بشكل كا ٍف. تهدف هذه الدراسة إلى تقييم التغيرات النسيجية والمناعية الكيميائية التي يسببها التعرض المتكرر للكاربيمازول في دماغ الأرنب الأنثى . تم تقسيم الأرانب إلى مجموعتين: مجموعة ضابطة ومجموعة معالجة. أُجريت الدراسة النسيجية باستخدام تلوينات الهيماتوكسيلين والإيوزين (H&E)، تريكرووم ماسون، وأزرق التوليدين .كما تم استخدام واسمات مناعية كيميائية هيP53 :، GFAPو Neurofilament لتقييم تلف الخلايا العصبية، نشاط الخلايا النجمية، وسلامة الألياف العصبية على التوالي .أظهرت أنسجة الدماغ لدى الأرانب المعالجة تلفاً" عصبي"ا، وتسرب"ا التهابي"ا، وتليف"ا في مناطق القشرة المخية والحُصين. كما أظهر أزرق التوليدين انخفاض"ا في مادة نيسل داخل الخلايا العصبية. وُجدت زيادة في تعبير P53 مما يدل على تحفيز عمليات الاستماتة، وزيادة في GFAP مما يشير إلى نشاط خلوي نجمي، وانخفاض في Neurofilamentمما يدل على تلف في المحاور العصبية .يسبب الكاربيمازول تغيرات نسيجية ومناعية ملحوظة في الجهاز العصبي المركزي، مما يشير إلى احتمالية تأثيره السمي على الدماغ. هناك حاجة إلى مزيد من الدراسات لفهم الآليات الدقيقة لهذه التأثيرات.

الكلمات المفتاحية: الكاربيمازول، الجهاز العصبي المركزي، الأرنب، علم الأنسجة، الكيمياء المناعية النسيجية

Table of contents

List of figures

List of figures

List of tables

List of Abbreviations

ATS	Synthetic antithyroid
CNS	Central nervous system
CSF	Cerebrospinal fluid
CMZ	Carbimazole
DAB	Diaminobenzidine
GFAP	Glial Fibrillary Acidic Protein
HE	Hematoxylin-Eosin
HRP	Horse Radish Peroxidase
IHC	Immunohistochemistry
ITELVL	ivestock Technical Institute
MMI	1-methyl-2-mercaptoimidazole
NF	Neurofilament
P53	Tumor suppressor protein
PTU	Propylthiouracile
TH	Thyroid hormones

INTRODUCTION

The central nervous system (CNS) constitutes a complex and highly specialized network, playing a fundamental role in the regulation of vital functions, motor coordination, cognition, and behavior **(Shokhrukh, 2025).** Any morphological or biochemical alteration of its structures can have major functional consequences. The study of the impact of different substances on the architecture of the CNS, therefore, represents a capital interest, particularly in the context of exposure to drugs with neurotoxic or neuroprotective potential.

Carbimazole, a synthetic antithyroid widely used in the treatment of hyperthyroidism, acts by inhibiting thyroid peroxidase **(Hassan and, 2021).** If its effects on the thyroid gland are well established, its extrapolar effects, notably on the central nervous system, remain little explored. Or, thyroid hormone regulation is closely linked to maturation, myelination, and neuronal function **(Pagnin et *al*., 2021).** A disruption of this balance can induce histopathological or functional changes at the brain level. Histological analysis makes it possible to highlight any structural alterations, while immunohistochemistry allows a more detailed approach, **(Mebratie et *al*., 2024)** revealing the expression of specific markers such as: P53 and GFAP, respectively reflecting phenomena of cellular stress and glial reactivity.

This work is part of this perspective and aims to evaluate the effects of carbimazole on central nervous tissue, based on classical histological analyses and targeted immunohistochemistry techniques. The objective is to better understand the potential interactions between antithyroid treatment and central nervous system health, and to identify possible markers of neuronal disruption or protection. Ultimately, the findings of this study may contribute to a more comprehensive understanding of the safety profile of carbimazole and guide therapeutic strategies that minimize neurological risks.

By integrating these diverse experimental approaches, this work will be structured into three main chapters:

- Chapter one: Literature Review. This section provides a concise synthesis of relevant existing knowledge
- Chapter 2: Materials and Methodology. This chapter provides a detailed description of histological technique
- Chapter 3: Results, Discussion. Presentation, analysis of results obtained, and interpretation of findings, which will be compared with existing literature.

Finally, a conclusion summarizing the key contributions of this research, its limitations, and prospects for future studies.

Chapter one: Literature Review

I. Central nervous system

1- Anatomy

The central nervous system (CNS) is the primary control center of the body, responsible for processing sensory information, coordinating movement, regulating behavior, and maintaining homeostasis. It consists of the brain and spinal cord **(Garcia *et al.*,2024)**.

1.1. Brain

The human brain is the central organ of the nervous system. It consists of the cerebrum, the brainstem, and the cerebellum. (**Thau *et al.*,2022**).

1.1.1 Cerebrum

The largest part, divided into right and left hemispheres, is responsible for higher cognitive functions, voluntary movement, sensory perception, language, and reasoning. The surface is the cerebral cortex, composed of gray matter with characteristic folds (gyri) and grooves (sulci) that increase surface area **(Nowinski, 2011).**

1.1.2. Cerebellum

Located beneath the cerebrum at the back of the head, it coordinates balance, posture, and fine motor skills. It does not initiate movement but refines and regulates. It is divided into three lobes: anterior, posterior, and flocculonodular. In the cerebellum, there is a cerebellar circuit that communicates with other parts of the brain through Purkinje cells and cerebellar peduncles. The cerebellum and cerebral cortex are in constant communication, where the cerebellum processes higher-level instructions about the brain's intentions through the cerebellar cortex and then sends messages to the cerebral motor cortex to cause voluntary muscle contractions. **(Vachha *et al.*,2022)**.

1.1.3. The Brainstem

The brainstem is responsible for connecting the brain and spinal cord. It consists of the pons, medulla, and midbrain. These elements work together to manage and coordinate the messages entering and exiting the brain **(Cucu *et al.*,2021)**.

1.1.4. Deep brain structure

The deep brain structures are a group of nuclei and regions located beneath the cerebral cortex that play critical roles in sensory processing, motor control, memory, emotion, and homeostasis. They include components of the diencephalon, basal ganglia, limbic system **(Fong *et al.*, 2023; Shine, 2023).**

- **Limbic System:** Includes the hippocampus (memory formation), amygdala (emotion processing), thalamus (sensory relay), and hypothalamus (hormonal regulation and homeostasis)
- **Basal Ganglia**: Involved in movement regulation and coordination; includes structures like the caudate nucleus, putamen, and globus pallidus.
- **Ventricular System:** Four interconnected cavities (ventricles) filled with cerebrospinal fluid, which cushions and nourishes the brain.

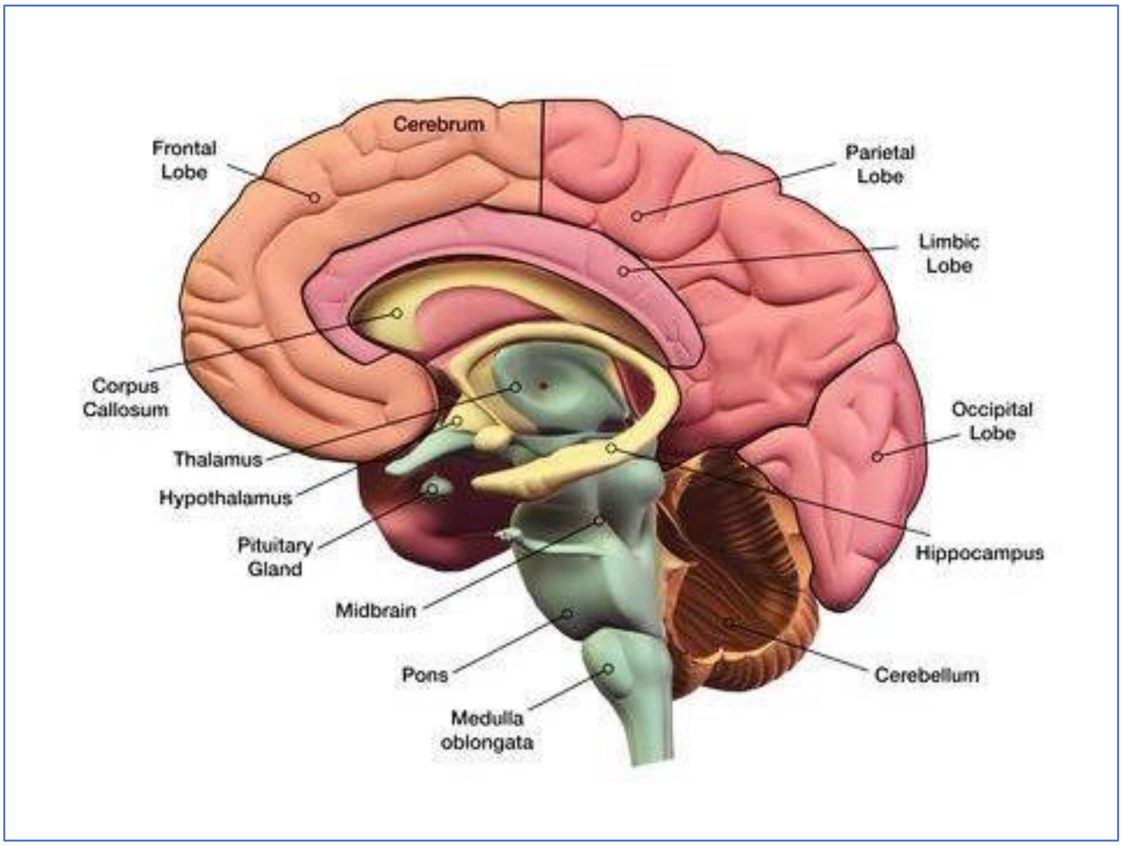

Figure 1 : Anatomy of the human brain **(Klein and Trouville, 2012)**.

1.2. Spinal cord

Extends from the foramen magnum of the skull to the first or second lumbar vertebrae. It creates a 2-way pathway between the brain and the body and divides into 4 regions: cervical, thoracic, lumbar, and sacral. Due to cervical and lumbar enlargements, the spinal cord differs in width throughout its structure.

The white matter is present outside the spinal cord, with gray matter in its core and cerebrospinal fluid in the central canal. The gray commissure and the dorsal, lateral, and ventral horns are all composed of gray matter. The gray commissure surrounds the central canal. The dorsal horns are made of interneurons, while the ventral horns are somatic motor neurons.

Afferent neurons in the dorsal roots carry impulses from the body’s sensory receptors to the spinal cord, where the information begins to be processed. The ventral horns contain efferent motor neurons, which control the body's periphery. The axons of motor neurons are found in the body's skeletal and smooth muscles to regulate involuntary and voluntary reflexes **(Hashmi *et al.*,2022).**

1.3. Protective Structures

- **Meninges:** The meninges are three layers of protective membranes that envelop the brain and spinal cord. From superficial to deep, the three layers are the dura, arachnoid, and pia **(Ghannam and Al Kharazi ,2023).**

- **Cerebrospinal fluid (CSF):** is an ultrafiltrate of plasma contained within the ventricles of the brain and the subarachnoid spaces of the cranium and spine. It performs vital functions, including providing nourishment, waste removal, and protection to the brain **(Telano and Baker S,2023)**.

2. Histology

The CNS is a highly complex organ composed of diverse cell types organized into distinct structural and functional units. Histological examination of brain tissue is essential for understanding normal anatomy, pathological changes, and the effects of injury or disease.

2.1. Neurons

The major function of the nervous system is provided by neurons. Neurons come in a variety of shapes and sizes which are organized into layers and nuclei depending on brain region. **(Boudinet, 2022).** In general, they have the following components:

- **Cell body:** called the soma, contains the nucleus and major organelles.
- **Dendrites:** or dendritic tree, are the branches responsible for receiving information.
- **Axon:** is the part that transmits information.

2.2. Glial Cells

There are a variety of cells which support neuronal function. These include **(Gaussel, 2013)**:

- **Astrocytes:** Cells that supply neurons, remove debris, and participate in wound healing.
- **Oligodendroglia:** They form the myelin sheath (an electrical insulator that facilitates the conduction of nerve impulses).
- **Microglia:** They participate in the defense of brain tissue through their immune functions.
- **Schwann cells:** these cells are responsible for creating the myelin that covers neurons in the peripheral nervous system. Myelin sheaths cover larger neurons and act as insulation for electrical wires. Myelinated axons transmit nerve impulses more efficiently.

2.3. Brain tissue architecture

- **Grey Matter**: Contains neuronal cell bodies, dendrites, unmyelinated axons, glial cells, and capillaries. It forms the cerebral cortex and deep nuclei such as the thalamus and basal ganglia. Grey matter plays the most significant role in allowing humans to function normally daily **(Mercadante *et al.*,2023).**

- **White Matter**: is found in the deeper tissues of the brain (subcortical). It contains nerve fibers (axons), which are extensions of nerve cells (neurons). Many of these nerve fibers are surrounded by a type of sheath or covering called myelin. Myelin gives white matter its color. It also protects the nerve fibers from injury. It improves the speed and transmission of electrical nerve signals along extensions of the nerve cells called axons **(Joseph, 2023).**

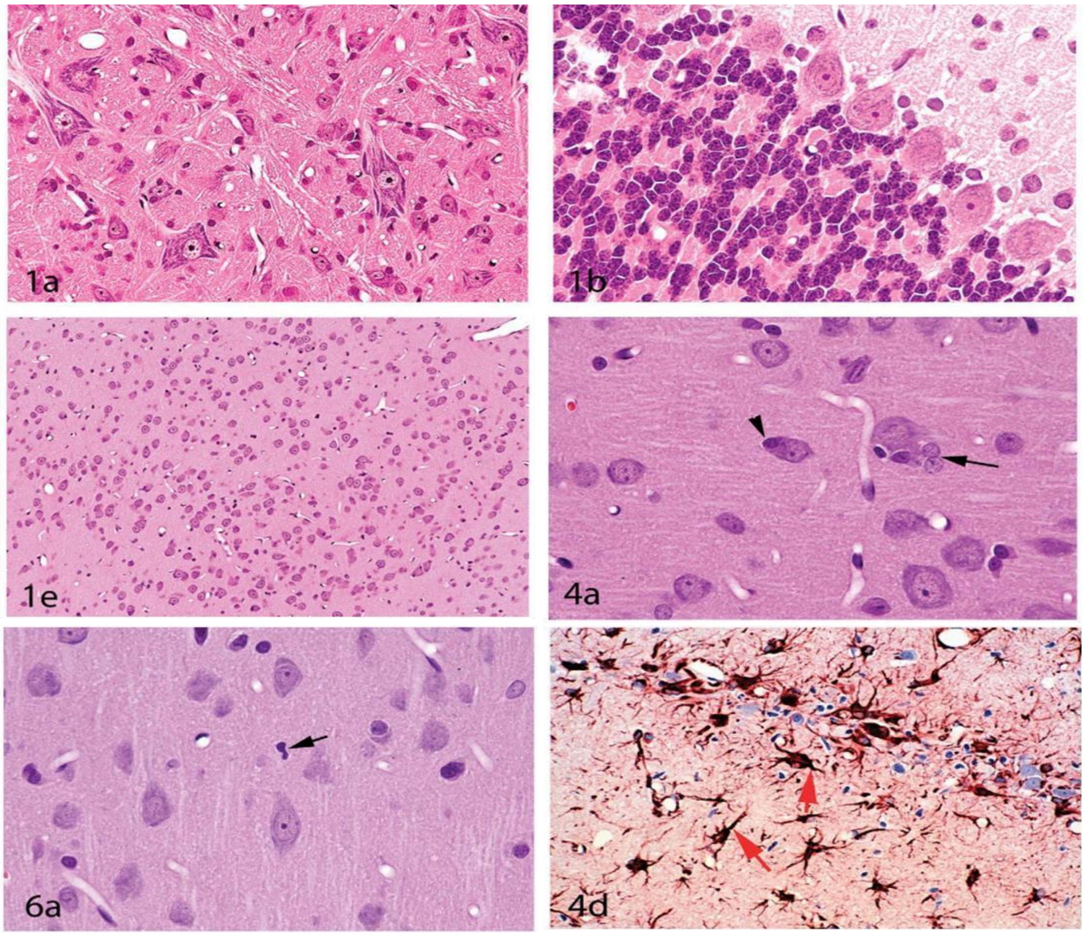

Figure 2: The cellular population found in the different regions of CNS **(Garman, 2011).**
1a: *mixtures of medium- to large-sized neurons;* ***1b:*** *large-sized projection neurons Purkinje neurons for the cerebellum;* ***1e:*** *monomorphic population of medium-sized neurons;* ***4a:*** *a micrograph of cerebral cortex, The arrowhead points to a para-neuronal oligodendrocyte;* ***6a:*** *microglial cell nuclei are observed in relatively small numbers;* ***4d:*** *astrocyte cells red arrow.*

3. CNS and drug injury

Drugs from many different classes, not limited to their primary therapeutic targets, can cause side effects and toxicity in CNS. These off-target or unintended effects may impact brain function, neuronal integrity, and neurological health, manifesting as a broad spectrum of symptoms ranging from mild cognitive disturbances to severe neurotoxicity **(Shi *et al.*,2025)**. Antithyroid drugs, while effective for hyperthyroidism, carry a risk of neurological side effects ranging from mild headaches and sensory changes to rare but serious neuropathies and neuropsychiatric events. These adverse effects underscore the importance of vigilant monitoring during treatment and highlight the complex interplay between thyroid function and nervous system health **(Tahany and Hartano, 2025).**

II. Carbimazole

1. Definition:

Carbimazole (CMZ) is an ethoxycarbonyl derivative of methimazole and has been the main drug used for over 50 years to treat hyperthyroidism. It is viewed as the most prolonged acting the member of thionamide family of drugs. While both carbimazole and methimazole share the same structure (MMI) (1-methyl-2-mercaptoimidazole), they differ due to carbimazole being a pro-drug that rapidly converts to methimazole because of a carbethoxy group that is attached to sulfur at position two of the imidazole ring. Since this drug accumulates in the thyroid gland after administration, it prevents thyroid hormone synthesis **(Bairaji and Mishra, 2020)**.

Table I. Physicochemical proprieties of Carbimazole **(Bairaji and Mishra, 2020)**.

Property	Details
Chemical Name	Ethyl 3-methyl-2-sulfanylidene-2,3-dihydro-1H-imidazole-1-carboxylate
Molecular Formula	$C_7 H_{10} N_2 O_2 S$
Molecular Weight	186.23 g/mol
CAS Number	22232-54-8
Appearance	White or yellowish-white crystalline powder
Melting Point	122–125 °C (252–257 °F)
Water Solubility	Sparingly soluble (<1 mg/mL)
Solubility in Organic Solvents	Ethanol (~12 mg/mL), DMSO (~60 mg/mL)
Chemical Class	Imidazole derivative with ethyl ester and thioxo groups
Storage Conditions	Powder: stable at -20°C for 3 years; solutions: -80°C for 1 year

2. Pharmacodynamics:

Carbimazole is a prodrug that is rapidly converted in the body to its active metabolite, methimazole (also called thiamazole), which exerts its antithyroid effects primarily by inhibiting the enzyme thyroid peroxidase (TPO). TPO catalyzes key steps in thyroid hormone synthesis, including the iodination of tyrosine residues on thyroglobulin and the coupling of iodotyrosines to form the thyroid hormones triiodothyronine (T3) and thyroxine (T4). By blocking TPO, carbimazole prevents the organification of iodide and the coupling reactions necessary for T3 and T4 production, thereby reducing circulating thyroid hormone levels and alleviating hyperthyroid symptoms.

Additionally, carbimazole inhibits the enzyme dehalogenase, which interferes with the recycling of organic iodine within the thyroid gland, promoting iodine clearance and increasing the gland's iodine uptake. This dual mechanism contributes to its efficacy in suppressing thyroid hormone synthesis **(Abbara *et al.*, 2020)**.

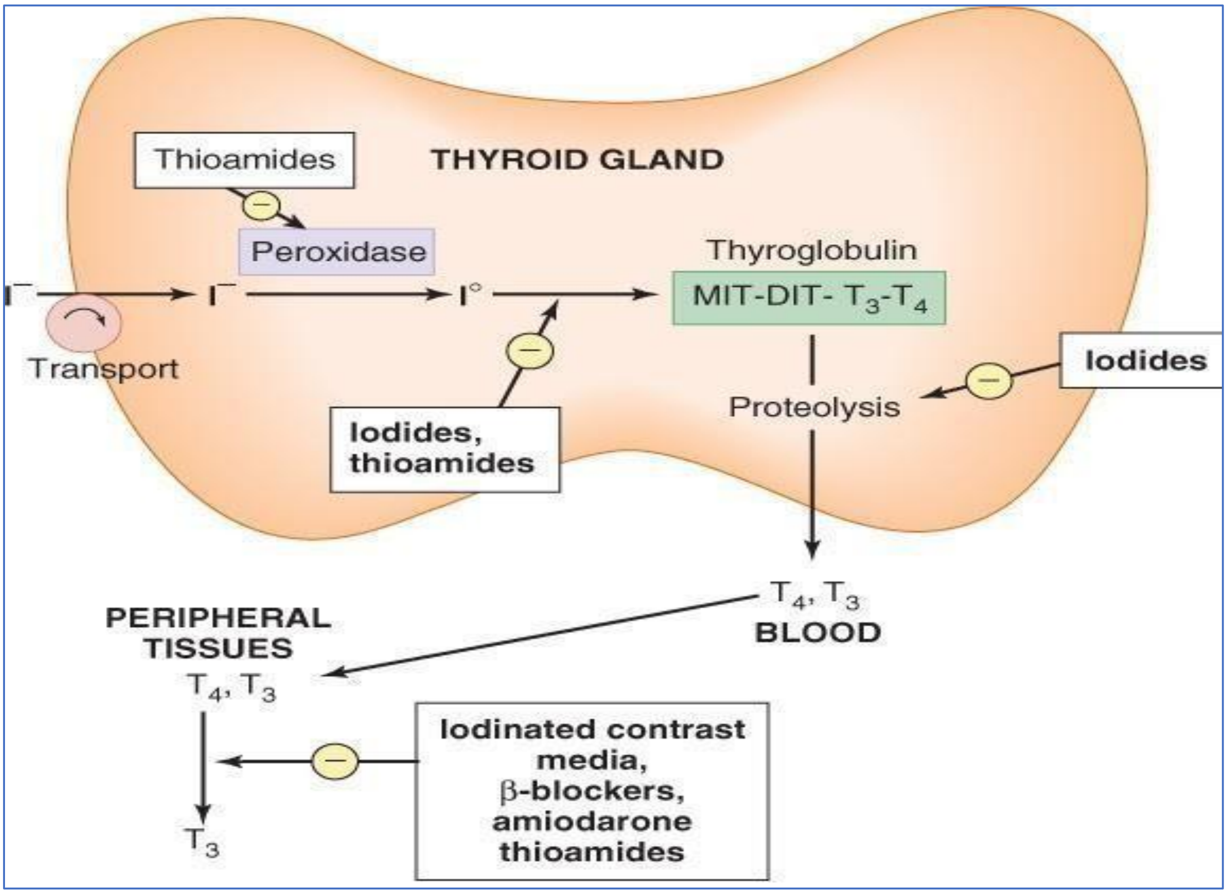

Figure 3: Mechanism of action of Antithyroid drugs **(Abbara *et al.*, 2020).**

3. Pharmacokinetics

- **Absorption**

Carbimazole is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration. It is quickly converted to its active metabolite, methimazole, which reaches peak plasma concentrations within 1 to 2 hours. The oral bioavailability of carbimazole is high, approximately 80–95% **(Shah *et al.*,2024).**

- **Distribution**

Carbimazole concentrates rapidly in the thyroid after administration. It is widely distributed throughout the body, crosses the placenta, and is excreted in milk.

- **Metabolism**

Carbimazole itself is a prodrug and undergoes enzymatic conversion to methimazole, primarily in the liver and bloodstream. Methimazole is the active compound responsible for the antithyroid effect. It is further metabolized hepatically, although the detailed metabolic pathways are not fully characterized.

- **Elimination**

Methimazole has a plasma half-life of approximately 2 to 6 hours and is eliminated mainly via renal excretion of metabolites. Less than 10% of methimazole is excreted unchanged in urine. Its clearance supports once or twice daily dosing without significant accumulation **(Shah *et al.*,2024).**

4. Carbimazole side effects

Carbimazole can cause a range of side effects, most of which occur within the first 8 weeks of treatment and often improve as the body adjusts. Common side effects include nausea, vomiting, stomach upset, headache, dizziness, skin rash or itching, hair thinning, joint and muscle pain, and changes in taste. These symptoms are usually mild and manageable with supportive care, such as taking the medication with food or avoiding rich and spicy meals **(Khan *et al.*,2024)**.

More serious but rare side effects require immediate medical attention. These include signs of agranulocytosis (a dangerous drop in white blood cells) such as high fever, sore throat, mouth ulcers, severe tiredness, or flu-like symptoms, which increase infection risk. Other serious reactions include liver problems indicated by yellowing of the skin or eyes (jaundice), dark urine, muscle pain or weakness **(Haroon *et al.*, 2024)**

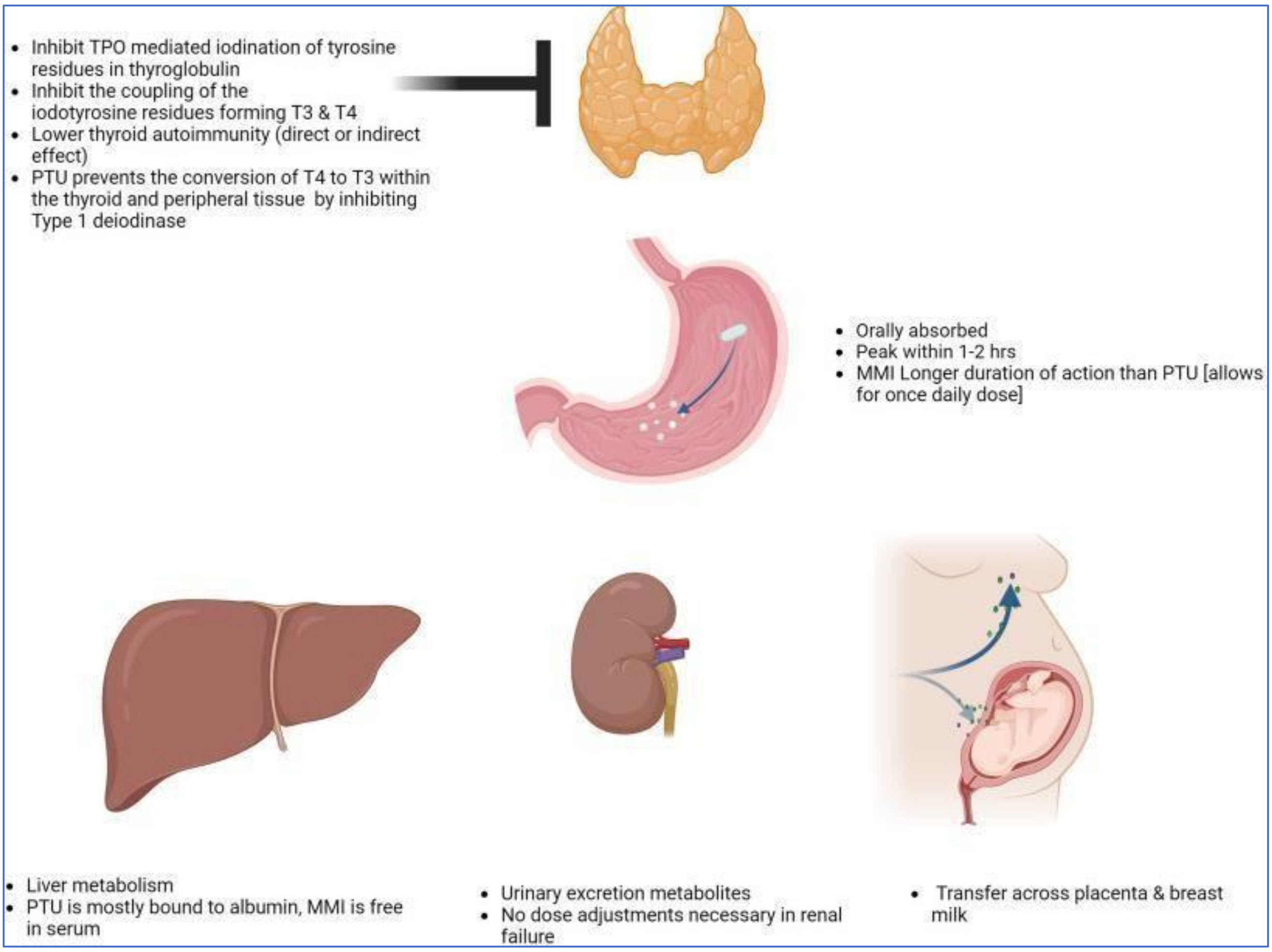

Figure 4: Pharmacokinetics of antithyroid drugs **(Shah *et al.*, 2024)**.
MMI = methimazole; *PTU* = propylthiouracil; *T4* = thyroxine; *TPO* = thyroid peroxidase; *T3* = triiodothyronine.

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Chapter two:

Materials & Methods

Study presentation

Our study focuses on the effect of repeated exposure to an antithyroid drug, carbimazole, on the central nervous system of female rabbits. To achieve this objective, a histological and immunohistochemical investigation was conducted.

The experiment took place between March and June 2025 at the following sites:

- The Experimental Station of Saad Dahleb University – Blida 1, where the animal experimentation was performed.
- The Anatomopathology Laboratory at CHU Mohamed Lamine Debaghine, Bab El Oued, where the histological and immunohistochemical analyses were carried out.

1. Materials

Biological material

Animal model

Ten (10) healthy adult female rabbits (*Oryctolagus cuniculus*), weighing between 2 and 2,5 kg, were obtained from ITELV Baba Ali and to Blida 1 university for the trials. The animals were housed individually in standard cages under controlled environmental conditions (temperature 22 ± 2°C, 12-hour light/dark cycle) with free access to standard rabbit diet and water. All experimental procedures were conducted in accordance with institutional ethical guidelines for animal care and use.

Rabbit taxonomy:

According to **Grasse, (1949) and Lebas et al., (1984**), the systematics of the rabbit is as follows:

- Kingdom: Animal
- Phylum: Vertebrates
- Class: Mammals
- Order: Lagomorphs
- Family: Leporidae
- Genus: Oryctolagus
- Species: Oryctolagus cuniculus

Figure 5 : Female rabbit (Original,2024)

Non biological material

During this experiment, the product tested is an anti-thyroid drug from the thionamide family, its commercial name is neomercazole ® (carbimazole). The rest of the non-biological material used, namely: apparatus, reagents and laboratory glassware, etc., is mentioned in the appendix.

2. Methods

2.1. Experimental design

In order to put the rabbits in the best condition, a 15-day acclimatization period was followed. After that, the rabbits were randomly divided into two groups (n=5 per group): a **control group** receiving normal saline and a **carbimazole-treated group** receiving carbimazole orally at a dose of 1 ml every day for 28 consecutive days. The dose was administered via oral gavage once daily in the morning.

N° of batch	Number of rabbits	Treatment
Group 1 : Control	5	1ml of normal saline
Group 2 : Treated	5	1ml of carbimazole

Sacrifice of animals and removal of organs

At the end of the treatment period, all the rabbits were sacrificed by decapitation. The target organs (brain, cerebellum and spinal cord) were carefully removed and then placed in pillboxes containing a fixation liquid (10% formalin) to fix them in a state close to the living state.

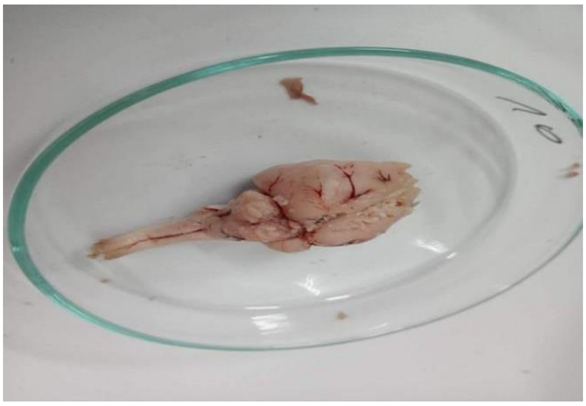

Figure 6*:* Extracted central nervous system (Original,2025)

2.3. Histological examination

- **Fixation**

The goal of this step is to fix the tissue in the closest possible state to its original state and to maintain its morphological structure and chemical integrity. After collection, the organs are fixed in 10% formalin for 48 hours. At the end of the fixation, the organs are placed in plastic cassettes which are marked with a pencil to allow their identification.

- **Dehydration and Clearing**

Dehydration is performed using an automated dehydration system (Figure 6) that immerses the samples in five ethanol baths of increasing concentration (70°C, 95°C, and 100°C), then in four xylene baths. As the tissues are infiltrated by this solvent (xylene), they tend to clear: this step is therefore sometimes called clearing or clarification.

Figure 7*:* Dehydration machine (Original,2025)

- **Impregnation**

At the end of the dehydration stage, the organs are immersed in two successive paraffin baths for 1.5 hours each at 58°C.

- **Sample Embedding**

Embedding is performed according to the following steps:

- Pouring the molten paraffin into metal molds.
- Embedding and orienting the pieces in the paraffin.
- Placing the cassettes.
- Cooling the paraffin blocks on a refrigerated metal plate for approximately 10minutes.

Figure 8 : Paraffin machine (Original,2025***)***

❖ **Sectioning**

3 µm-thick cross-sections are cut from the blocks using a microtome . The ribbons are spread in a water bath (40°C), recovered and glued onto clean slides, then dried in an oven set at 37°C for 24 hours.

❖ **Deparaffinization and Rehydration**

The most commonly used stains in histology are aqueous and therefore cannot act on paraffin-saturated tissue; they must therefore be deparaffinized and rehydrated. Deparaffinization is performed by placing the sections in a xylene bath for 30 minutes. Hydration is then performed by placing them in a 100% alcohol bath for 10 minutes.

❖ **Staining**

2.3. 1/Hematoxylin eosin (HE) staining (Routine staining)

The hematoxylin and eosin (H&E) stain is the most fre-quently used histochemical stain in clinical and research laboratories **(Ortiz-Hidalgo and Pina-Oviedo , 2019) .** This stain has been used for over a centuryto highlight the structures of cytoplasmic and nuclear components in cells and tissues , where hematoxylin stains nuclear and eosin stains cytoplasmic structures.

We follow the following protocol:

Dehydration: Additional dehydration will be performed; the stained slides will be passed through three alcohol baths, then three successive xylene/toluene baths.

- Hematoxylin is used first, as it is a basic stain that binds to acidic components of cells, such as nuclei, for 3 to 5 minutes.

- Rinse with low-flow tap water.
- In eosinitis, an acidic stain binds to basic components of cells, such as the cytoplasm, for 30 seconds to 1 minute.
- Rinse with low-flow tap water.

Figure 9 : Hematoxylin and eosin staining (Original,2025)

2.3. 2 Specific coloring

Masson-trichrome

This method is generally more accurate and informative because Masson's trichrome staining not only maintains intact the overall morphology of the tissues but also has the advantage of utilizing three dyes that allow for the identification of multiple tissue structures.

We follow the following protocol:

-We place the slides in a bath of Mayer's hemalum for 10 minutes.

- We then rinse the slides under running water and let them sit for 5 minutes.
- We then stain the slides with fuchsin-ponceau for 5 minutes.
- We then rinse the slides in acidic water for 1 minute.
- We immerse the slides in Phosphomolybdic acid-Orange G for 3 minutes.

-Without rinsing, we stain the slides light green, which stains the collagen fibers for 5 minutes.

-We then briefly rinse the slides in acidic water

Figure 10 : *Masson's trichrome coloration (Original,2025)*

Toluidine blue

This is a routine, easy, and rapid topographical stain. Toluidine blue is a nonspecific, basic dye and therefore has a good affinity for acidic structures such as nucleic acids. Furthermore, it can stain structures by giving them its own color (orthochromatic dye) or by giving them a different color (metachromatic dye)

We follow the following protocol:

- Staining for 3 second in toluidine blue solution.
- Wash sections with running H2O until all traces of dye have disappeared from the wash bath.
- We put the slices to dry in the air.

Figure 11: toluidine blue staining (Original,2025)

❖ **Mounting**

After the staining step, we place the slides in an oven at 60°C to dry for a few minutes. Then, we begin mounting, which is the final step in slide preparation before microscopic examination. The slides are dried in a denazylene bath. This step consists of mounting the stained slides and slide cover slips using mounting medium and dropping the E-Kitt, removing air bubbles. This results in a diamond slide ready for observation under a light microscope.

▪ **Microscopic Observation**

Microscopic observation of the slides was performed using a Leica light microscope at various magnifications: Gx4, Gx10, and Gx40.

2.4. Immunohistochemical Study

Principle

Immunohistochemistry (IHC) refers to the method of localizing proteins located in tissue cells. It consists of detecting the site of binding of a specific antibody to the protein against which it is directed. IHC can be performed on frozen or fixed and paraffin-embedded tissue sections.

Procedure

In our experiment, we used three types of immunohistochemical markers.

✓ P53 monoclonal antibody, mouse anti-human monoclonal protein, clone DO-7 (ready-to-use).

✓ GFAP monoclonal antibody, rabbit anti-human monoclonal protein, clone EP672Y (ready-to-use).

✓ NF anti-neurofilament antibody, mouse anti-human monoclonal protein, clone 2F11 (ready-to-use).

The preparation of the tissues (fixation, circulation, embedding, microtomy) is identical to that of usual staining, the sections are spread on positively charged silanized slides to ensure tissue adhesion and reduce the risk of the sections detaching, the slides are then placed in the oven at 37C°, for 24 hours.

All the following steps are performed using a VENTANA-group automated system: The BenchMark ULTRA, which is a fully automated staining system for immunohistochemistry and presents multiple features to improve the diagnostic confidence of histopathology laboratories and optimize the workflow with its independent slide drawers.

Figure 12 *:* IHC Benchmark ULTRA (VENTANA) (Original,2025)

Pretreatment

- **Deparaffinization :** to remove remaining traces of paraffin.

- **Antigenic restoration:** This is a key step that involves unmasking antigenic sites to break the methylene bridges formed during fixation under the effect of heat.

- **Inhibition of endogenous molecules:** to block peroxidase sites and prevent false positivity (nonspecific labeling).

- **Immunohistochemical IHC Reaction**

This mainly includes:

- Incubation of a primary antibody specifically directed against the targeted antigenic sites.

- Colorimetric detection of the primary antibody using a secondary antibody conjugated to the enzyme HRP (Horse Radish Peroxidase), which is an oxidoreductase that catalyzes the conversion of the chromogenic substrate DAB (3-3 diaminobenzidine), which forms a brown precipitate that can be visualized under a light microscope. This secondary antibody is used nonspecifically because it binds to the constant portion of the primary antibodies, and its incubation time varies depending on the protocol.

- Counterstaining with hematoxylin to stain the nuclei blue.

- **Slide/coverslip mounting using Eukitt**
- **Observation under a microscope and taking photos**

Chapter Three: Results and Discussion

1. RESULTS

In this work, we performed two topographic histological stains — Hematoxylin and Eosin (H&E) and Masson's Trichrome — as well as a histochemical stain, Toluidine Blue, on nervous tissue samples from both control and treated rabbits. An immunohistochemical study was also conducted using the markers GFAP, Neurofilament, and P53. Microscopic observation of the stained sections at various magnifications enabled us to examine the overall architecture of the nervous tissue and to identify lesions induced by carbimazole

1.1. Histological study

- **Hematoxylin Eosin (H&E)**

▪ **Histology of the cerebral cortex of control rabbits**

The histological study of the control rabbits allows to describe: a normal laminar organization of the cerebral cortex, clearly visible neurons with round nuclei colored in blue, a light eosinophilic cytoplasm and regular distribution of cells without apparent vacuolation (Figure 13).

▪ **Histology of the cerebral cortex of treated rabbits**

The histological examination at low and high magnification shows a disorganization of cortical tissue with less cell density than in control rabbits, presence of vacuoles and pycnotic nuclei (Figure 14).

- **Trichrome of masson**

▪ **Histology of the cerebellum of control rabbits**

In control rabbits the histological study shows a normal structure of the three cerebellar cortical layers: molecular layer (CM), granular (CG) with alignment of Purkinje nerve cells which are well individualized (Figure 15).

▪ **Histology of the cerebellum of treated rabbits**

In the treated rabbit group, while the general structure appeared similar to the control group , we noticed a migration of some Purkinje cells to the inner layer of the cerebellum (Figure 16).

- **Histology of the cerebral cortex of treated rabbits**

In the cerebral cortex, the histological study shows an inflammatory infiltrate (Figure17) .

- **Toluidine blue**

- **Histology of the cerebral cortex of control rabbits**

The histological study of control rabbit allows us to describe healthy brain tissu with normal well-defined neurons and a reduced pericellular space and an average cell density (Figure 18) .

- **Histology of the cerebral cortex of treated rabbits**

The histological study of treated rabbits shows altered neurons, sometimes vacuolated (neuronal damage) with an enlarged pericellular space and a higher cell density but with anomaly (Figure 19) .

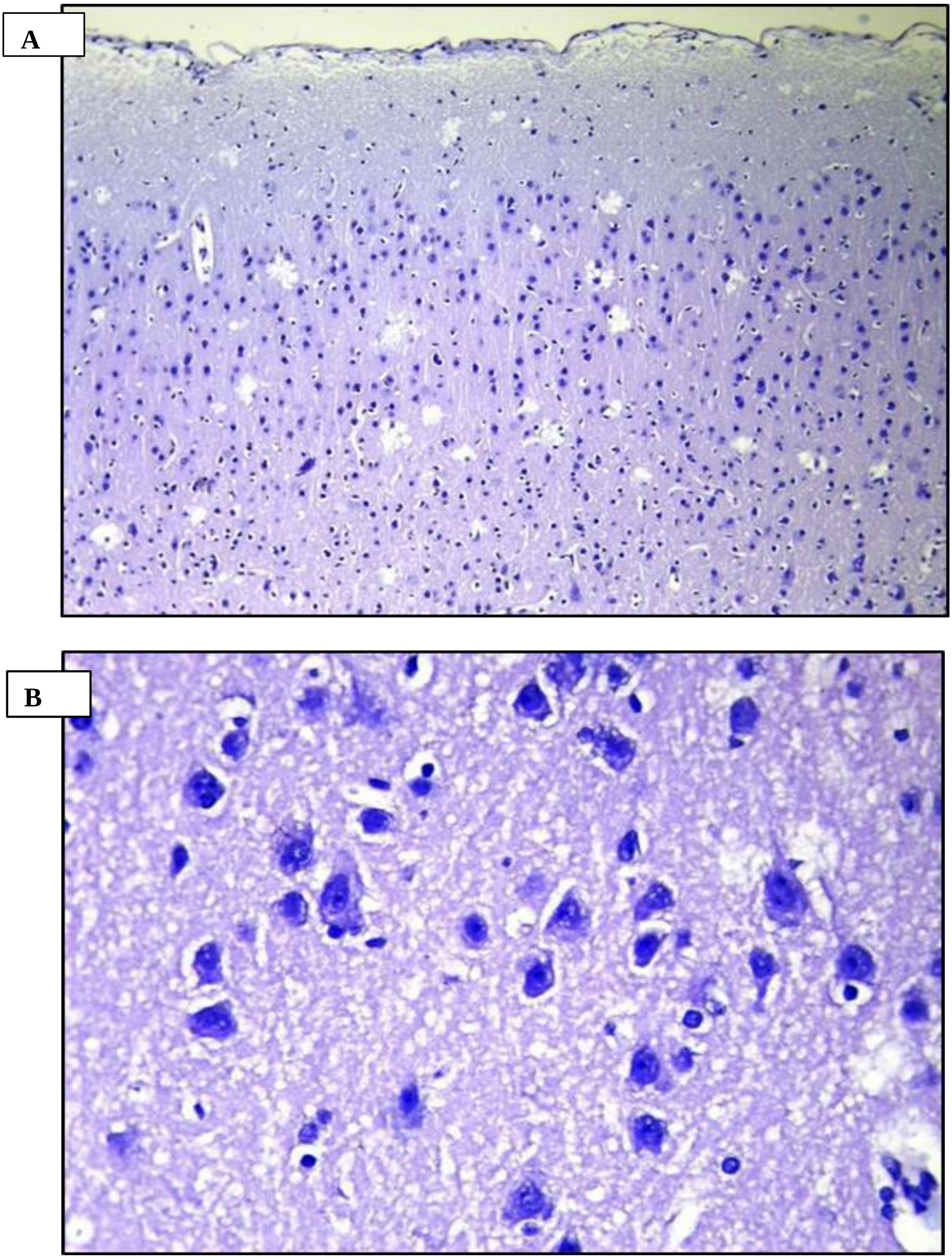

Figure 13: (A and B) Histological structure of the cerebral cortex in the control rabbit

HE staining; Magnification: x10 (A), x40 (B)

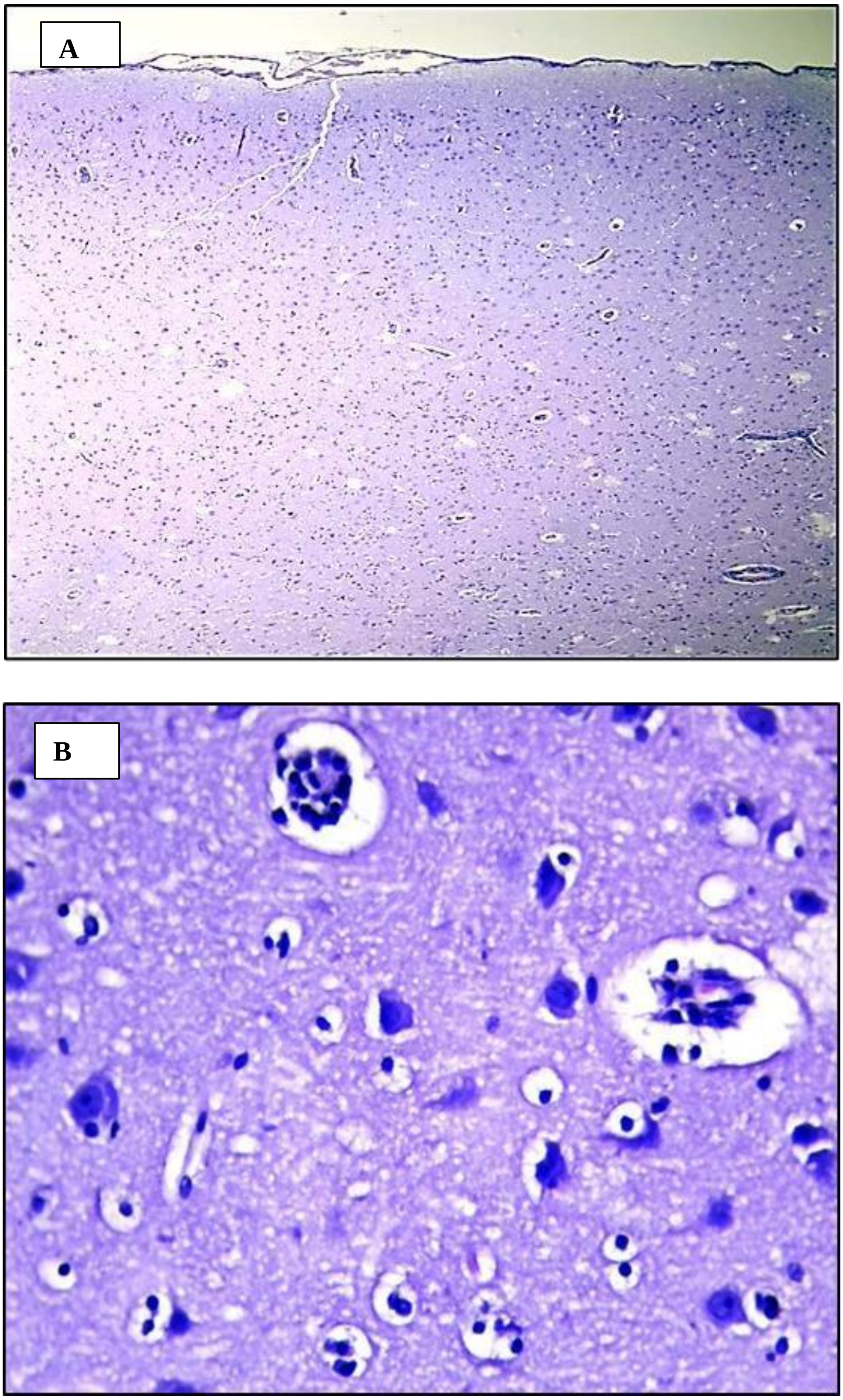

Figure 14 : (A and B) Histological structure of the cerebral cortex in treated rabbits

HE staining; Magnification: x10 (A), x40 (B)

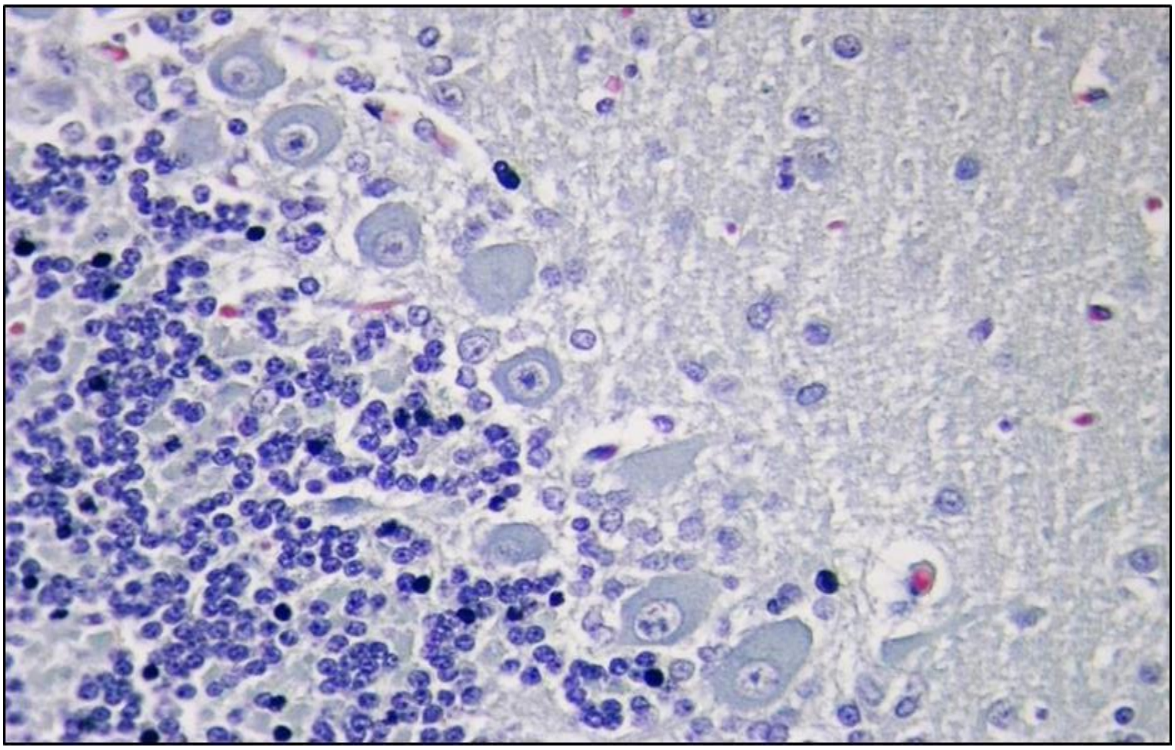

Figure 15 : Histological structure of the cerebellum in the control rabbit

Masson's trichrome staining ; Mg: x40

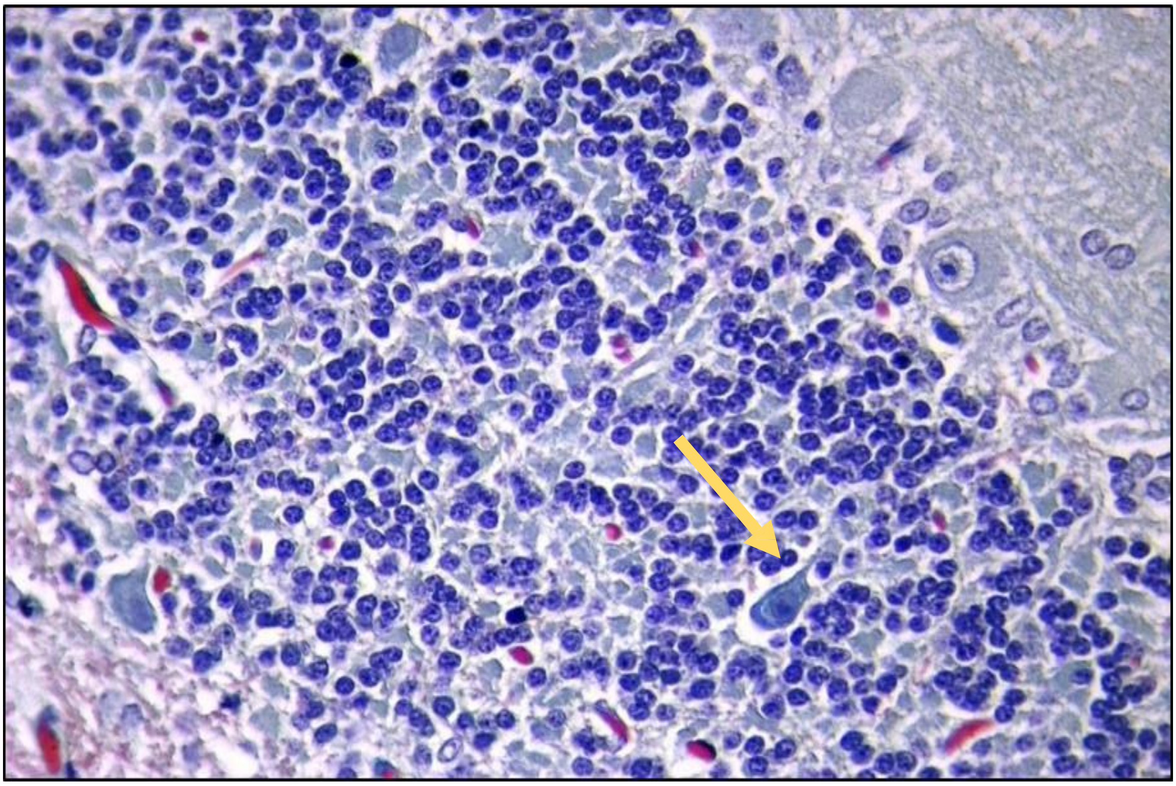

Figure 16 : Histological structure of the cerebellum in the treated rabbit

Masson's trichrome staining ; Magnification: x40

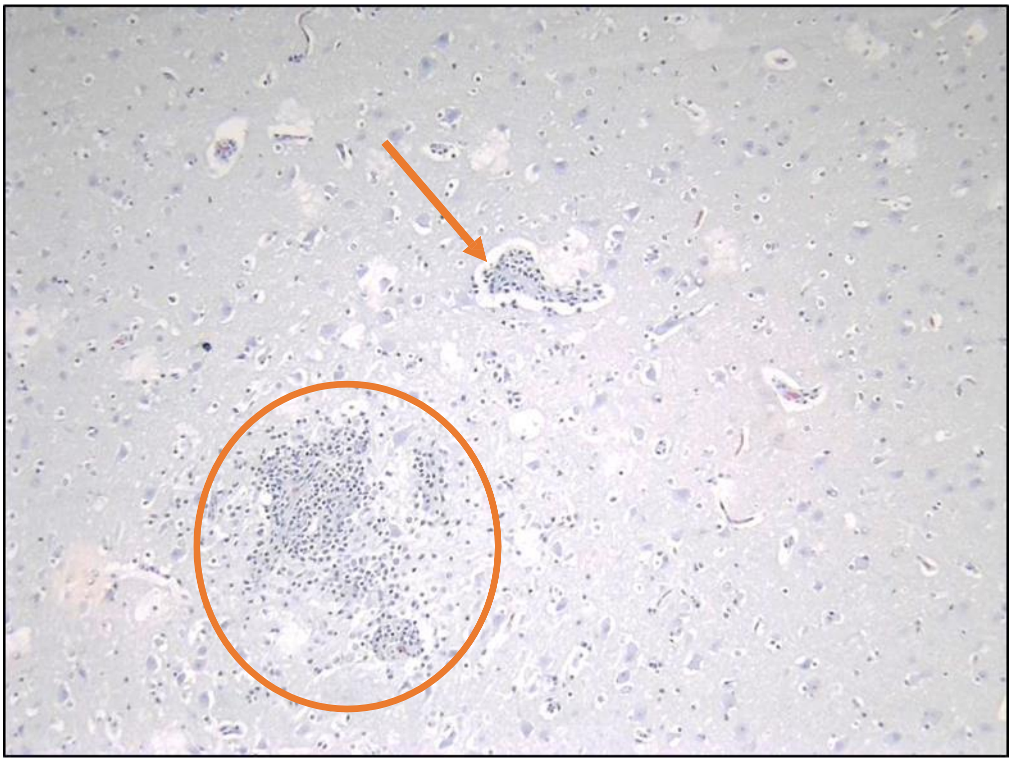

Figure 17 : Histological structure of the cerebral cortex in treated rabbits

Masson's trichrome staining; Magnification: x10

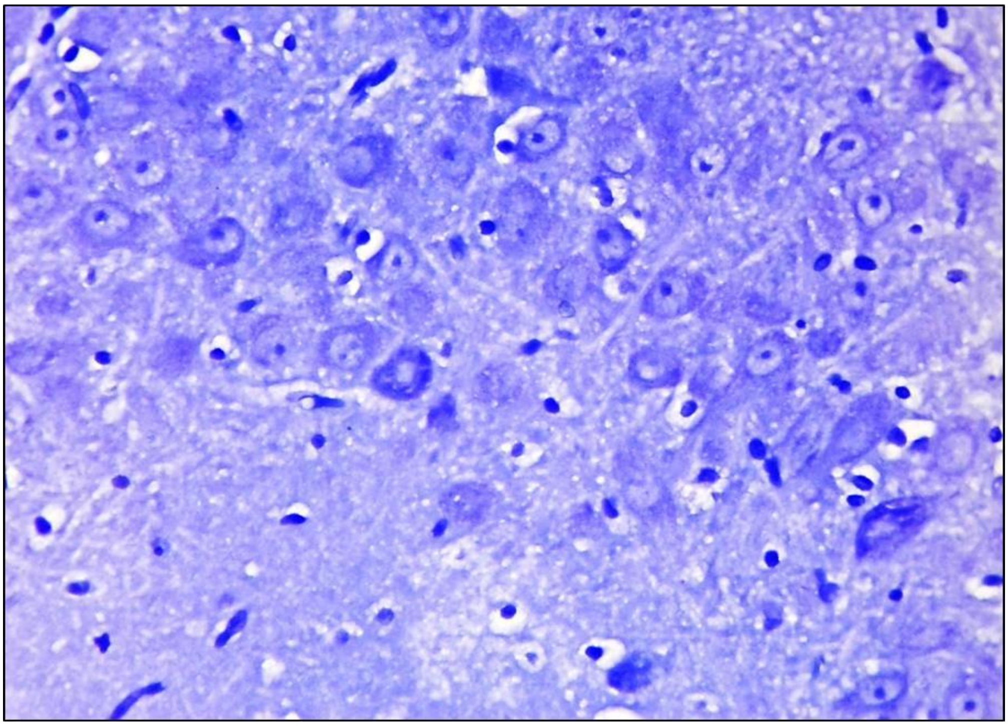

Figure 18 : Histological structure of the cerebral cortex in the control rabbit

Toluidine blue staining ; Magnification: x40

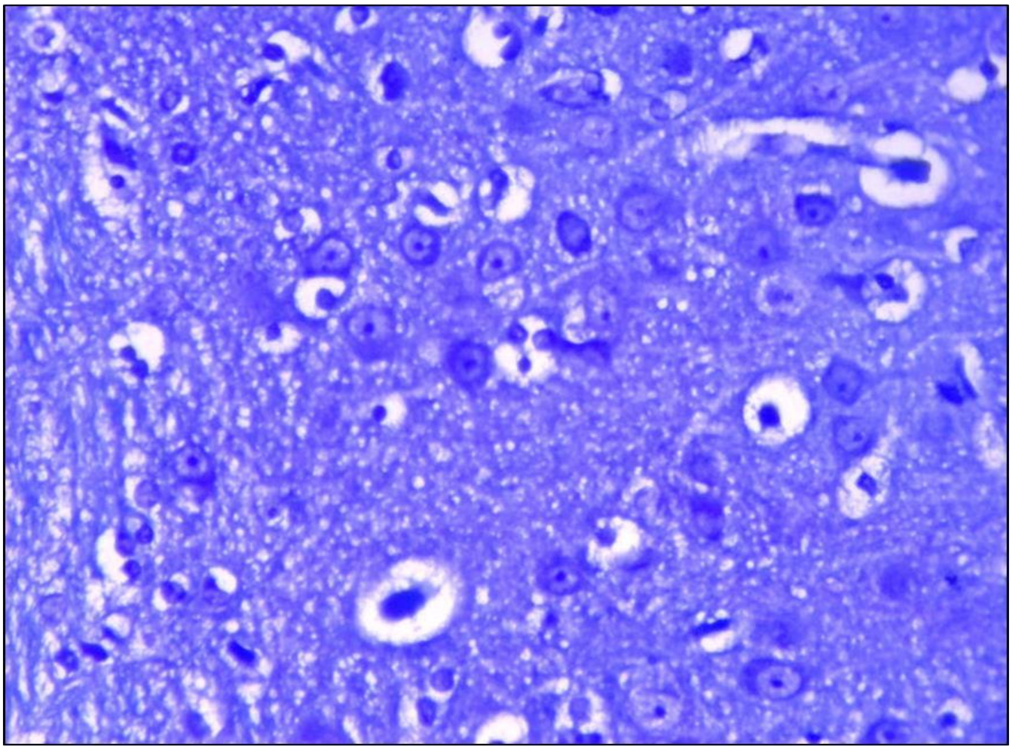

Figure 19 ***:*** Histological structure of the cerebral cortex in the treated rabbit

Toluidine blue staining ; Magnification: x40

1.2. Immunohistochemical study

- **P53 marker:**

Immunohistochemical analysis of P53 expression revealed notable differences between the control and carbimazole-treated groups.

In the control group, P53 immunoreactivity was either absent or very weak, with only a few scattered cells in the cerebral cortex and hippocampus showing faint nuclear staining. This low level of expression is consistent with normal, non-stressed neural tissue (Figure 20) .

In contrast, in the treated group, there was a marked increase in P53-positive nuclei, particularly in the neurons of the cerebral cortex. The staining was predominantly nuclear, indicating activation of the P53 pathway (Figure 21) .

- **GFAP marker**

In the control group, GFAP expression was observed as fine, delicate astrocytic processes with low to moderate staining intensity, mainly distributed in the cerebral cortex. The astrocytes showed typical morphology with thin processes and limited proliferation, consistent with a normal, non-inflamed neural environment (Figure 22) .

In the carbimazole-treated group, there was a notable increase in GFAP expression, particularly in the cortical. Astrocytes appeared hypertrophic, with thicker and more numerous processes, and a higher density of GFAP-positive cells was recorded (Figure 23) .

- **Neurofilament marker**

Neurofilament immunostaining was used to evaluate neuronal integrity and axonal structure in both control and carbimazole-treated rabbits.

In the control group, neurofilament expression was strong and uniformly distributed, highlighting well-organized axonal fibers and intact neuronal processes in the cerebral cortex and hippocampus. The staining pattern showed continuous, filamentous labeling consistent with a healthy neuronal network (Figure 24) .

In the carbimazole-treated group, a marked reduction in neurofilament immunoreactivity was observed. Axonal fibers appeared fragmented, swollen, or disorganized, especially in cortical regions. Some areas showed loss of staining continuity, indicating neuronal damage or axonal degeneration. The overall staining intensity was also significantly lower compared to controls (Figure 25).

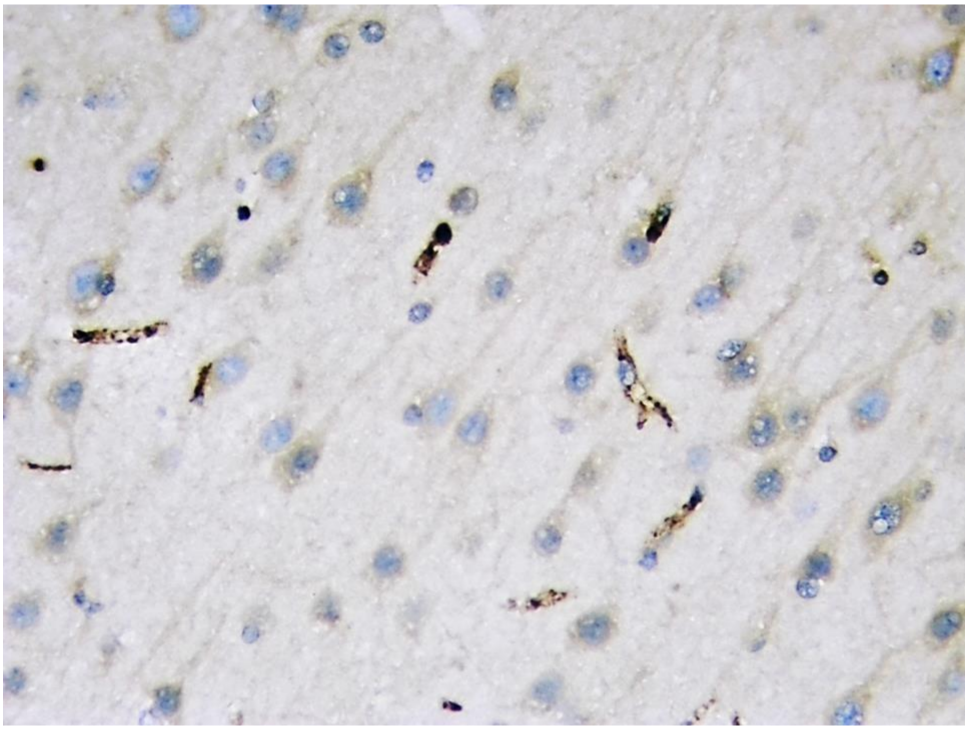

Figure 20 : Immunohistochemical analysis of P53 in the cerebral cortex of control rabbits showing a negative immune reaction for P53; **Magnification: x40**

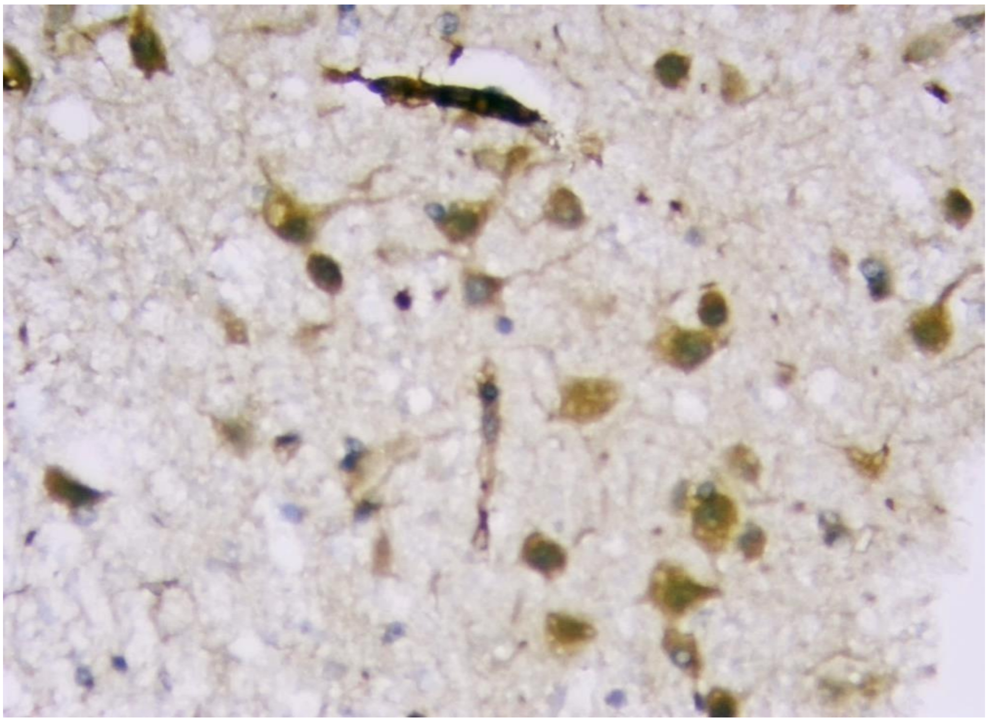

Figure 21 : Immunohistochemical analysis of P53 in the cerebral cortex of treated rabbits showing a positive immune reaction for P53; **Magnification: x40**

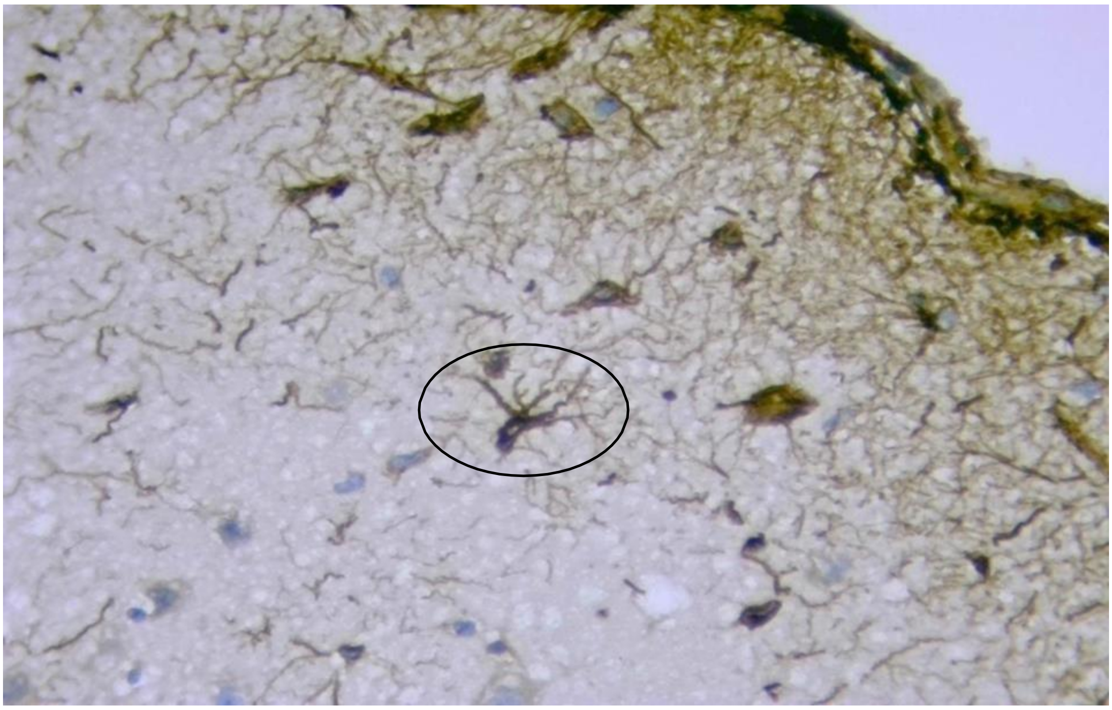

Figure 22 : Immunohistochemical analysis of GFAP in the cerebral cortex of control rabbits showing a moderate positive immune reaction astrocytes (circle) ; **Magnification: x 40**

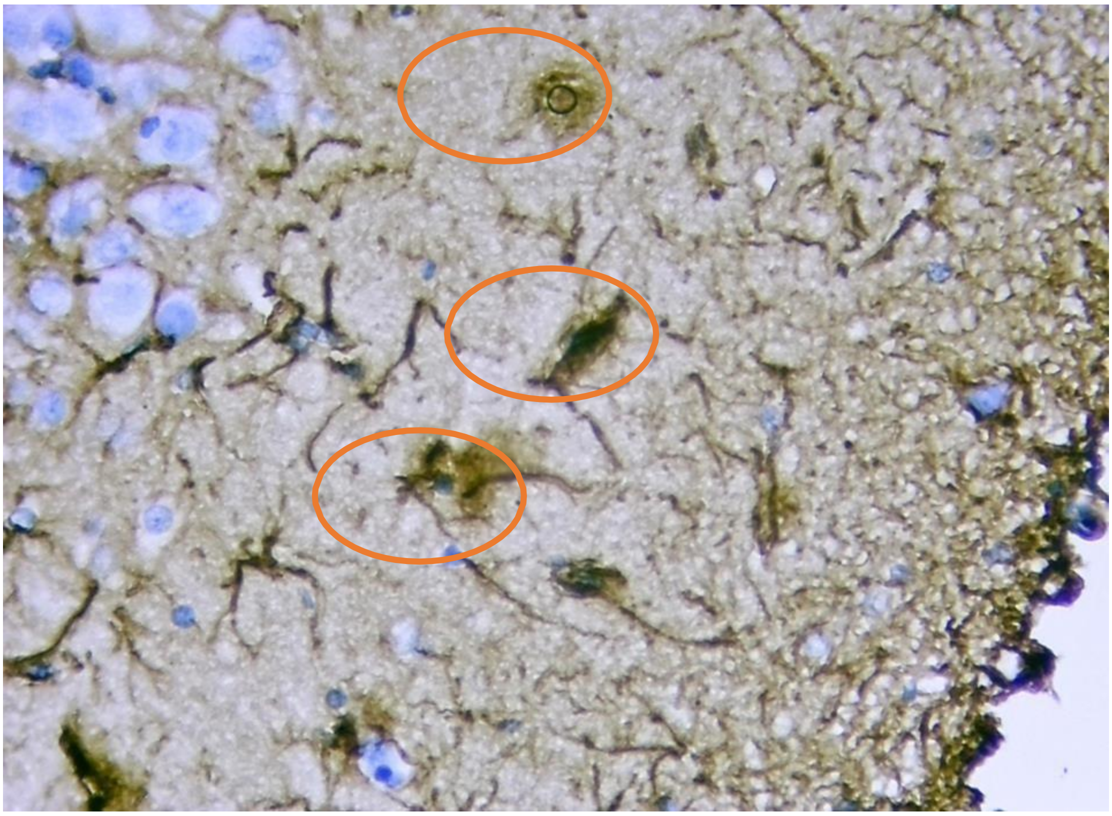

Figure 23 : Immunohistochemical analysis of GFAP in the cerebral cortex of treated rabbits showing an increase of the positive immune staining of astrocytes; **Magnification: x 40**

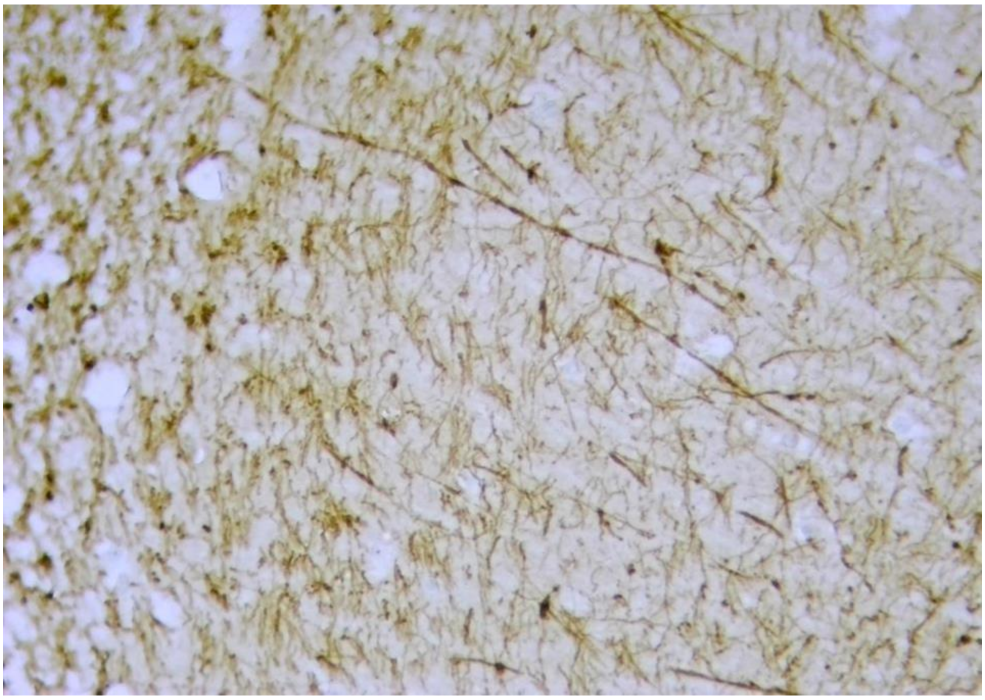

Figure 24 : Immunohistochemical staining of NF in the cerebral cortex of control rabbits showing a positive immune reaction ; **Magnification: x 40**

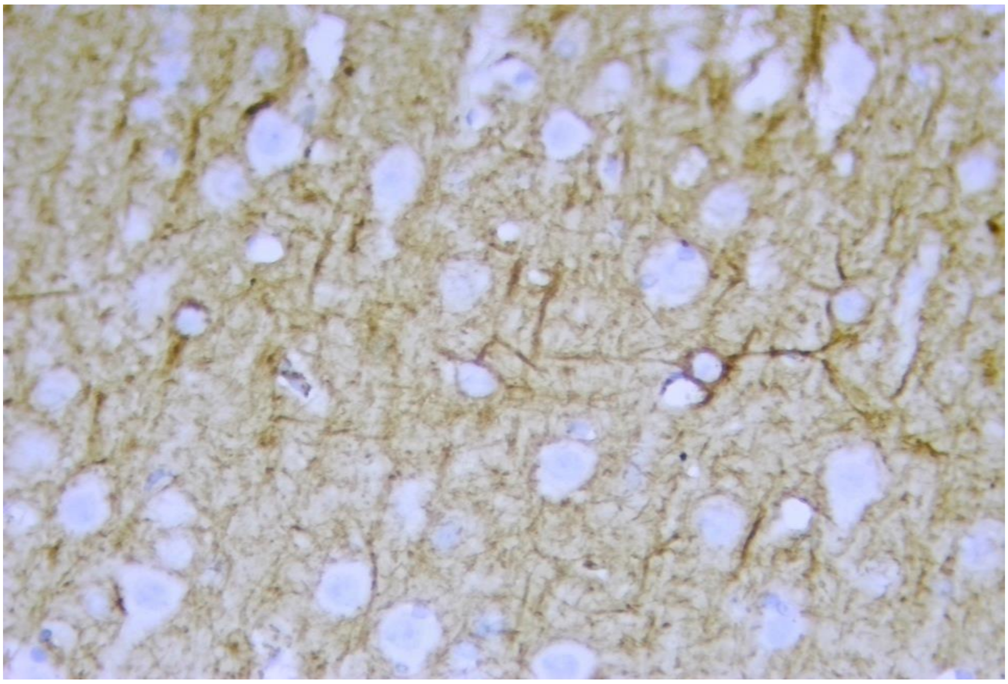

Figure 25 : Immunohistochemical analysis of NF in the cerebral cortex of treated rabbits showing a positive immune reaction for NF ; **Magnification: x 40**

DISCUSSION

This study aimed to investigate the effects of repeated exposure to carbimazole, an antithyroid drug, on the CNS of female rabbits through a combination of histological and immune-histochemical techniques.

Histological analysis of the brain and cerebellum in carbimazole-treated rabbits revealed notable morphological alterations compared to the control group.

Hematoxylin-eosin staining showed disorganization of the cortical structure, characterized by vacuolation of the nervous tissue and condensation of neuronal nuclei.

These signs are indicative of cellular stress or a degenerative process, probably related to the antithyroid effect of carbimazole **(Farag *et al.*, 2023).** These changes are consistent with previous reports of CNS alterations in hypothyroid or antithyroid drug models, where reduced thyroid hormone levels disrupt neuronal metabolism and survival **(El kholy *et al.*, 2024)**.

Toluidine blue, which highlights acidic cellular components such as nuclei and cytoplasmic granules, revealed a decrease in cell density and morphological changes in nerve cells. These alterations could be associated with a slowdown in neuronal activity, linked to treatment-induced hypothyroidism. This is in agreement with the literature, where toluidine blue is used to assess neuronal integrity and detect subtle changes in cell populations **(Ghnenis *et al.*, 2018; Rao *et al.*, 2025).**

Furthermore, Masson's trichrome staining revealed an increase in lymphoid cell in certain regions of the brain parenchyma. This accumulation suggests activation of the inflammation pathway a possible chronic inflammatory reaction, already observed in other models of hypothyroidism **(Sarlak *et al.*, 2013; Zhang *et al.*, 2025).**

These results indicate that carbimazole, by inducing a thyroid hormone deficiency, can alter the structure of the central nervous system, particularly at the level of the cerebral and cerebellar cortex. These alterations can affect neurological function, particularly in developing subjects or those sensitive to endocrine disruption **(Salas-Lucia, 2024)**.

Immunostaining for p53, a marker of cellular stress and apoptosis, showed increased nuclear positivity in neurons and glial cells of carbimazole-treated rabbits. This suggests that carbimazole-induced hypothyroidism may trigger apoptotic pathways in the CNS, possibly through p53-mediated mechanisms.

Elevated p53 expression has been associated with neuronal cell death in various models of CNS injury and neurodegeneration, supporting its role as a marker of drug-induced neurotoxicity **(Lei *et al.*, 2023 ; Xu *et al.*, 2025).**

Immunohistochemical study with the GFAP marker revealed a marked increase in the expression of reactive astrocytes in the cerebral cortex and cerebellum. This astrogliosis, characteristic of glial activation, is often observed in response to neurotoxic stress, inflammation, or neuronal degeneration.

The increase in GFAP expression indicates that carbimazole treatment induced a significant glial response, probably related to an alteration of neuronal homeostasis secondary to hypothyroidism **(Sarlak *et al.*, 2013; Li *et al.*, 2025)**. Furthermore, the observed gliosis is consistent with previous studies showing that GFAP upregulation is a sensitive indicator of astrocytic response to injury, hypoxia, or toxic insults in the brain **(Zhang *et al.*, 2017; Sacco *et al.*,2024)**.

Immunohistochemical analysis of neurofilament, a marker of axonal integrity, revealed decreased expression in carbimazole-treated regions. This suggests nerve fiber damage and a weakening of the neuronal cytoskeleton, corroborating the lesions observed histologically.

Neurofilaments are essential for the stability and conduction of neuronal signals; their reduction is often associated with neurodegeneration **(Chang *et al.*, 2016; Akaba *et al.*, 2025)**.

CONCLUSION

The objective of this study was to evaluate the impact of carbimazole on the histological architecture and the expression of certain cellular markers in the central nervous system. Through a combined approach of histological analysis and immunohistochemistry, this study provided a better understanding of the indirect effects of this antithyroid drug on brain tissue.

This study demonstrated that repeated exposure to carbimazole induces significant histological and immunohistochemical alterations in the central nervous system of female rabbits. Structural changes such as neuronal degeneration, inflammatory infiltration, and fibrosis were observed using H&E, Masson's Trichrome, and Toluidine Blue staining.

Immunohistochemical analysis further revealed increased expression of P53, indicating cellular stress and potential apoptosis; elevated GFAP, reflecting astrocyte activation and gliosis; and reduced Neurofilament expression, suggesting axonal damage.

These findings suggest that carbimazole may have neurotoxic effects and highlight the importance of monitoring its impact on the nervous system, particularly with prolonged use.

Further investigations are recommended to clarify the mechanisms involved and to evaluate possible protective strategies.

Thus, it would be relevant to :

- Supplement this study with a behavioral analysis (memory, motor skills, anxiety).
- Evaluate long-term effects or effects in cases of co-administration with other substances.
- Explore other cellular markers (Bcl-2, caspase-3, etc.).

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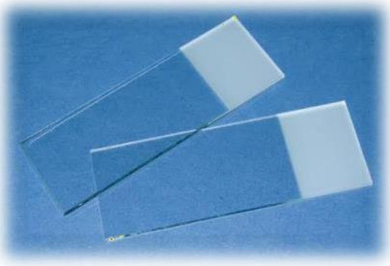
Appendix

- Main materials used during the study

Mold	
Paraffine	
Lamella	
Blade	
Eu kit	

Hematoxylin and eosin	
xylene	
Drying oven	
Toluidine blue	
Masson’s trichrome staining kit	

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
SAAD DAHLAB UNIVERSITY-BLIDA 1

Faculty of Natural and Life Sciences
Department of Biology

A Dissertation Submitted in Fulfillment of the Requirements for the Degree of Master in Biological Sciences

Field: Pharmacotoxicology

Effect of carbimazole on rabbit central nervous system: histological and immunohistochemical study

Presented by:

Ms. BOULBENE Chourouk

Ms. ELNEGIRY Mennaallah

In front of a jury composed of:

Dr. MAKHLOUF C	MCB at University of Blida 1	President
Dr. BOULESNAM L.S.	MAB at University of Blida 1	Examiner
Dr. SETTAR A.	MAB at University of Blida 1	Supervisor
Pr DJENNANE N.	Professor at CHU BEO	Co-supervisor
Pr. KHALDOUN H.	Professor at University of Blida	Co-supervisor

A/ Favorable
Dr. Boulesnam S.L

Academic year 2024/2025