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Protective Role of a Medicinal Plant Against Ibuprofen Overdose-Induced Reproductive Toxicity in Male Rats

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Dedication

Al-Hamdoulilah

OMRANE



Dedication

I dedicate this humble work with great pride and gratitude to:

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Dedication

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Abstract

Ibuprofen (IBU), a widely used non-steroidal anti-inflammatory drug (NSAID), may induce reproductive toxicity through oxidative stress mechanisms. This study investigated the effects of ibuprofen on oxidative stress, reproductive parameters in male Wistar rats, as well as the protective role of green tea extract (GTE). Twenty-eight rats were divided into four groups of seven animals each: control, GTE-treated (200 mg/kg), IBU-treated (200 mg/kg), and IBU+GTE. Treatments were administered orally for 45 consecutive days.

The results showed that ibuprofen exposure induced significant testicular toxicity characterized by decreased testosterone levels, impaired sperm parameters, oxidative stress imbalance in testicular tissue. Oxidative stress was evidenced by reduced glutathione (GSH) levels and increased catalase (CAT) activity, indicating disruption of antioxidant defense mechanisms. Ibuprofen administration also negatively affected sperm concentration, motility, viability, and morphology.

Conversely, co-administration of green tea extract significantly attenuated most of the alterations induced by ibuprofen. Green tea improved antioxidant status, restored reproductive parameters, and reduced histopathological damage in testicular tissue. These protective effects are likely related to the antioxidant properties of green tea polyphenols and catechins, which are capable of scavenging reactive oxygen species and preserving cellular redox balance.

These findings highlight the protective potential of green tea extract against ibuprofen-induced testicular toxicity and oxidative stress, suggesting that it may represent a promising natural therapeutic agent for preventing drug-induced reproductive damage.

Keywords: Ibuprofen toxicity, Testicular toxicity, Reproductive toxicity, Green tea extract, Oxidative stress, Antioxidant activity.

Résumé

L'ibuprofène (IBU), un anti-inflammatoire non stéroïdien (AINS) largement utilisé, peut induire une toxicité reproductive par des mécanismes liés au stress oxydatif. Cette étude a évalué les effets de l'ibuprofène sur le stress oxydatif, les paramètres reproducteurs chez des rats mâles Wistar, ainsi que le rôle protecteur de l'extrait de thé vert (GTE). Vingt-huit rats ont été répartis en quatre groupes de sept animaux chacun : groupe témoin, groupe traité par l'extrait de thé vert (200 mg/kg), groupe traité par l'ibuprofène (200 mg/kg) et groupe IBU+GTE. Les traitements ont été administrés par voie orale pendant 45 jours consécutifs.

Les résultats ont montré que l'exposition à l'ibuprofène induit une toxicité testiculaire significative caractérisée par une diminution du taux de testostérone, une altération des paramètres spermatiques, un déséquilibre du stress oxydatif du tissu testiculaire. Le stress oxydatif s'est manifesté par une diminution du glutathion (GSH) et une augmentation de l'activité de la catalase (CAT), indiquant une perturbation des systèmes de défense antioxydants. L'administration d'ibuprofène a également affecté négativement la concentration, la mobilité, la viabilité et la morphologie des spermatozoïdes.

En revanche, la co-administration de l'extrait de thé vert a considérablement atténué la majorité des altérations induites par l'ibuprofène. Le thé vert a amélioré le statut antioxydant, restauré les paramètres reproducteurs et réduit les lésions histopathologiques du tissu testiculaire. Ces effets protecteurs sont probablement liés aux propriétés antioxydantes des polyphénols et des catéchines du thé vert, capables de neutraliser les espèces réactives de l'oxygène et de préserver l'équilibre rédox cellulaire.

Ces résultats mettent en évidence le potentiel protecteur de l'extrait de thé vert contre la toxicité testiculaire et le stress oxydatif induits par l'ibuprofène, suggérant qu'il pourrait représenter un agent thérapeutique naturel prometteur pour prévenir les dommages reproducteurs induits par les médicaments.

Mots-clés : Toxicité de l'ibuprofène, Toxicité testiculaire, Toxicité reproductive, Extrait de thé vert, Stress oxydatif, Activité antioxydante.

الملخص

يُعد الإيبوبروفين (IBU) من مضادات الالتهاب غير الستيرويدية واسعة الاستعمال، وقد يسبب سمية تناسلية من خلال آليات مرتبطة بالإجهاد التأكسدي. هدفت هذه الدراسة إلى تقييم تأثير الإيبوبروفين على الإجهاد التأكسدي والمعايير التناسلية لدى ذكور جرذان ويست، بالإضافة إلى دراسة الدور الوقائي لمستخلص الشاي الأخضر (GTE) تم تقسيم ثمانية وعشرين جرذًا إلى أربع مجموعات تضم كل مجموعة سبعة جرذان: مجموعة شاهدة، مجموعة معالجة بمستخلص الشاي الأخضر (200 ملغ/كغ)، مجموعة معالجة بالإيبوبروفين (200 ملغ/كغ)، ومجموعة مشتركة (IBU+GTE) أُعطيت جميع المعالجات فمويًا لمدة 45 يومًا متتاليًا.

أظهرت النتائج أن التعرض للإيبوبروفين أدى إلى حدوث سمية خصوية ملحوظة تمثلت في انخفاض مستوى التستوستيرون، واضطراب المعايير المنوية، واختلال مؤشرات الإجهاد التأكسدي في الخصية. وقد تجلّى الإجهاد التأكسدي بانخفاض مستويات الغلوتاثيون (GSH) وارتفاع نشاط إنزيم الكاتالاز (CAT)، مما يدل على اضطراب أنظمة الدفاع المضادة للأكسدة. كما أثر الإيبوبروفين سلبيًا على تركيز الحيوانات المنوية وحركتها وحيويتها وشكلها الطبيعي.

في المقابل، أدى تناول المتزامن لمستخلص الشاي الأخضر إلى التخفيف بشكل ملحوظ من معظم الاضطرابات الناتجة عن الإيبوبروفين. حيث ساهم الشاي الأخضر في تحسين الحالة المضادة للأكسدة واستعادة المعايير التناسلية وتقليل الأضرار النسيجية في الخصية. ويُرجح أن تعود هذه التأثيرات الوقائية إلى الخصائص المضادة للأكسدة للبوليفينولات والكاتيشينات الموجودة في الشاي الأخضر، والتي تمتلك القدرة على معادلة الجذور الحرة والمحافظة على التوازن التأكسدي داخل الخلايا.

تُبرز هذه النتائج الإمكانات الوقائية لمستخلص الشاي الأخضر ضد السمية الخصوية والإجهاد التأكسدي الناتجين عن الإيبوبروفين، مما يشير إلى إمكانية اعتباره عاملًا علاجيًا طبيعيًا واعدًا للوقاية من الأضرار التناسلية الناتجة عن الأدوية.

الكلمات المفتاحية: سمية الإيبوبروفين، السمية الخصوية، السمية التناسلية، مستخلص الشاي الأخضر، الإجهاد التأكسدي، النشاط المضاد للأكسدة،

Abbreviations List

AMH	Anti-Müllerian Hormone
CAT	Catalase
COX	Cyclooxygenase
CYP17A1	Cytochrome P450 17A1
CYP11A1	Cytochrome P450 side-chain cleavage enzyme
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
FSH	Follicle-Stimulating Hormone
GTE	Green Tea Extract
GSH	Glutathione
GPx	Glutathione Peroxidase
H₂O₂	Hydrogen Peroxide
HSD3B2	3 β -Hydroxysteroid Dehydrogenase type 2
IBU	Ibuprofen
LH	Luteinizing Hormone
MDA	Malondialdehyde
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PNAS	Proceedings of the National Academy of Sciences
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
StAR	Steroidogenic Acute Regulatory protein
SOD	Superoxide Dismutase

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INTRODUCTION



INTRODUCTION

Over the past few decades, infertility and subfertility have become more common around the world. This has made male reproductive health a bigger public health issue. The male reproductive system is susceptible to numerous internal and external stressors (**Venkatesh et al., 2011**). Recent global estimates suggest that male factors are involved in approximately 40–50% of infertility cases, highlighting their significant contribution to reproductive disorders (**Agarwal et al., 2021**). There are many reasons why reproductive function may decline, such as environmental pollutants, lifestyle choices, and long-term use of common drugs.

The testis is a very specialized organ that is responsible for both spermatogenesis and steroidogenesis. These processes depend on a carefully controlled interaction between germ cells, Sertoli cells, and Leydig cells (**Walker, 2021; O'Donnell et al., 2022**). Testicular tissue is especially susceptible to biochemical disturbances because it has a high metabolic rate and cells die and are replaced quickly (**Aitken & Baker, 2020; Walker, 2021**). In this regard, oxidative stress has been identified as a principal mechanism underlying testicular dysfunction. It happens when there is too much reactive oxygen species (ROS) and not enough of the body's own antioxidant defense system. Too much ROS production causes lipid peroxidation, DNA damage, protein oxidation, and mitochondrial dysfunction (**Sies et al., 2022**). All of these things lower sperm quality and fertilizing ability. However, healthy levels of ROS are needed for normal sperm functions like capacitation and acrosome response (**Tremellen, 2008**).

There has been a growing interest in the potential reproductive risks of commonly used drugs, as well as environmental toxins. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are some of the most commonly used medicines because they can help with pain, fever, and swelling. Ibuprofen mainly lowers the production of prostaglandins by blocking the cyclooxygenase enzymes (COX-1 and COX-2). Even so, prostaglandins are necessary for keeping the testicles healthy, including communication between cells, steroid production, and controlling blood flow (**Rivière et al., 2017**). Long-term suppression of these systems may disturb testicular homeostasis.

In Algeria, ibuprofen is commonly available without a medical prescription, encouraging widespread self-medication. While this accessibility allows rapid relief of pain and inflammation, it also raises the risk of misuse and prolonged consumption. Unsupervised use of non-steroidal anti-inflammatory drugs (NSAIDs) is often associated with poor awareness of potential side effects and dosing errors (**Hughes et al., 2016**). Furthermore, excessive intake of

ibuprofen may contribute to reproductive disturbances through oxidative stress and hormonal imbalance (**Banihani, 2017**). Therefore, its over-the-counter availability may pose a potential risk to male reproductive health.

Experimental and clinical research indicates that ibuprofen consumption may induce oxidative stress in testicular tissue by enhancing reactive oxygen species (ROS) production and compromising intrinsic antioxidant defenses (**Sharma et al., 2020; Kristensen et al., 2018**). In addition to functional deficits such as reduced sperm count and motility, along with heightened morphological abnormalities, this imbalance may lead to structural alterations, including seminiferous tubule degeneration (**Banihani, 2017**). Ibuprofen has been shown to disrupt the hypothalamic-pituitary-gonadal axis, potentially altering hormonal regulation and testosterone synthesis, both essential for optimal male fertility (**Rivière et al., 2017**). These findings heighten apprehensions regarding the prolonged, unregulated use of ibuprofen and its association with male reproductive disorders.

At the same time, scientists are paying more attention to natural chemicals found in medicinal plants as possible ways to protect against oxidative damage. One of these is green tea (*Camellia sinensis*), which is known for having a lot of polyphenolic compounds, especially flavonoids like catechins, which include epigallocatechin-3-gallate (EGCG) and are very good at fighting free radicals (**Khan & Mukhtar, 2013**). These substances may boost the body's natural defenses against free radicals, get rid of reactive oxygen species, and lower lipid peroxidation. Green tea components possess antioxidant, anti-inflammatory, anti-apoptotic, antibacterial, and anticancer properties, rendering them beneficial in the prevention of various diseases, including metabolic and cardiovascular disorders (**Singh et al., 2011**). Experimental findings suggest that green tea may enhance sperm parameters, maintain testicular integrity, and promote hormonal equilibrium in response to oxidative stress (**Mahmoudi et al., 2018; Azizi & Mehranjani, 2019**).

The potential correlation between ibuprofen-induced reproductive harm and the role of intrinsic antioxidant defense systems remains insufficiently clarified. The extensive use of ibuprofen and its possible adverse effects on testicular function prompt critical inquiries regarding the extent to which oxidative stress and hormonal disruption contribute to male infertility, as well as the efficacy of natural substances, such as those found in green tea, in alleviating these alterations.

In light of this context, the present study seeks to evaluate the potential protective effects of green tea as a natural antioxidant, while also investigating the impact of ibuprofen on male reproductive function, particularly regarding oxidative stress and endocrine alterations. This method is being used in the study to learn more about how prolonged and unsupervised use of ibuprofen can harm reproduction and to look into other ways to keep men fertile.



Bibliographic part



1. Ibuprofen: general aspects

1.1 Structure and property

Ibuprofen (IBF) is a non-steroidal anti-inflammatory drug (NSAID) that belongs to the class of arylpropionic acid derivatives. It is widely used around the world as an analgesic, antipyretic, and anti-inflammatory drug because it works well and has a good pharmacokinetic profile (Rainsford, 2009).

The chemical name for ibuprofen is 2-(4-isobutylphenyl) propionic acid. The formula for it is $C_{13}H_{18}O_2$ and its molecular weight is about 206.28 g/mol. It has a propionic acid side chain that is very important for its biological activity and a benzene ring that has been replaced by an isobutyl group (Rainsford, 2009).

Ibuprofen is a chiral compound that comes in two forms: R-ibuprofen and S-ibuprofen. The S-enantiomer is the form that works to stop inflammatory mediators from working. Interestingly, a part of the R-enantiomer in the human body is changed into the active S-form through metabolism, which helps the drug work (Rainsford, 2009; Bushra and Aslam, 2010).

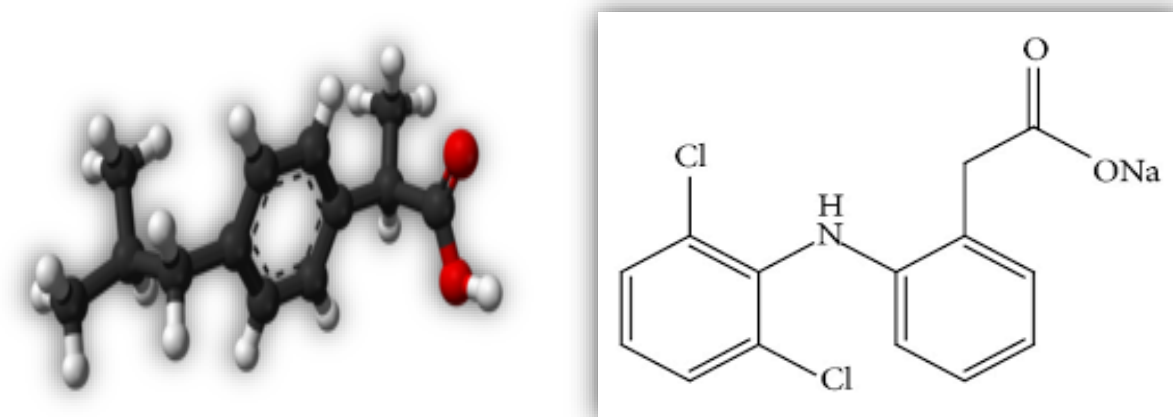


Figure 01. Two-dimensional (2D) and three-dimensional (3D) molecular structures of ibuprofen (PubChem, 2023).

1.2 Mode of action

Ibuprofen exerts its pharmacological effects primarily by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), which are responsible for converting arachidonic acid into prostaglandins. These mediators play a key role in the regulation of inflammation, pain, and

body temperature. By reducing prostaglandin synthesis, ibuprofen effectively lowers fever, alleviates pain, and diminishes inflammation. However, since prostaglandins are also involved in various physiological functions, including gastrointestinal protection, renal regulation, and reproductive processes, their prolonged or excessive inhibition may lead to adverse systemic effects (Vane & Botting, 2003; Rainsford, 2009).

Several experimental and clinical findings indicate that ibuprofen may also affect male reproductive physiology. Exposure to ibuprofen has been linked to modifications in testicular endocrine function, notably influencing Leydig cell activity and testosterone production (Kristensen et al., 2018). These results suggest that ibuprofen may have biological effects beyond its well-known anti-inflammatory properties that could affect reproductive health.

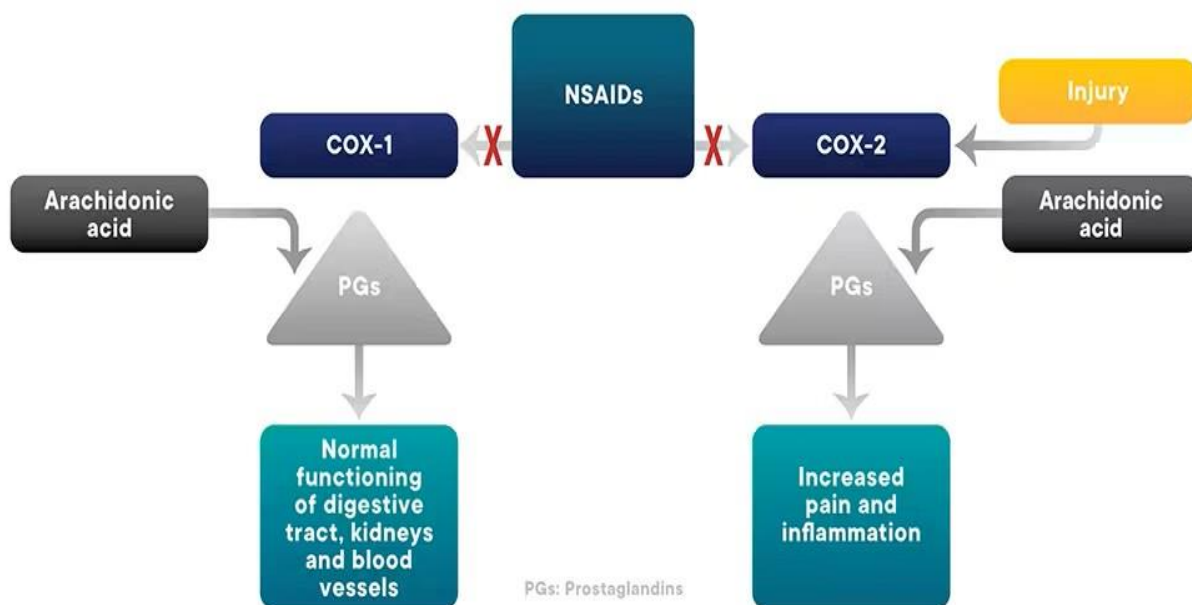


Figure 02. Pharmacological mechanism of ibuprofen involving COX-1 and COX-2 inhibition and decreased prostaglandin production (Vane & Botting, 2003).

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

COX-1: Cyclooxygenase-1

COX-2: Cyclooxygenase-2

PGs: Prostaglandins

2. Potential Reproductive Risks Associated with Ibuprofen and Other NSAIDs

2.1 How It Works: Oxidative Stress, Inflammation, and Hormonal Disruption

2.1.1. Oxidative Stress

A growing body of experimental evidence indicates that ibuprofen may negatively impact the male reproductive system via multiple interconnected mechanisms, notably oxidative stress and endocrine disruption (**Matzkin et al., 2012**).

Oxidative stress transpires when the generation of reactive oxygen species (ROS) surpasses the antioxidant defense capability of cells. In the testes, excessive reactive oxygen species (ROS) production can result in lipid peroxidation, mitochondrial dysfunction, and damage to germ cell membranes, ultimately hindering spermatogenesis (**Agarwal et al., 2014**).

Simultaneously, ibuprofen exposure has been linked to a decrease in the activity of critical antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which typically safeguard cells from oxidative damage (**Agarwal et al., 2014**).

From an endocrine perspective, ibuprofen has been shown to interfere with the normal regulation of the hypothalamic–pituitary–gonadal axis. A regulated clinical trial demonstrated that prolonged exposure to ibuprofen significantly increased circulating LH levels without altering testosterone concentrations, suggesting a functional impairment of Leydig cells (**Kristensen et al., 2018**).

2.1.1. Inflammation

Ibuprofen may change how inflammatory signals work in the tissue of the testicles. Cyclooxygenase enzymes make prostaglandins, which control how cells talk to each other and keep the immune system stable in the testes while also reducing inflammation (**Matzkin et al., 2012**). If the paracrine connections between testicular cells are blocked, the local microenvironment may change.

In normal physiological conditions, a precisely regulated network of cytokines and signaling molecules sustains testicular homeostasis and facilitates spermatogenic development. Ibuprofen's inhibition of cyclooxygenase activity may disturb this equilibrium, leading to aberrant inflammatory mediator expression and diminished cellular coordination in the seminiferous epithelium (**Ricciotti & FitzGerald, 2011**). These alterations may not manifest

as conventional inflammation, but rather as a disruption in immune-related communication essential for reproductive health.

Therefore, prolonged exposure to ibuprofen may contribute to subtle disturbances in testicular immune regulation, which can indirectly affect spermatogenesis and tissue integrity without triggering overt inflammatory responses.

Further molecular analyses suggested that ibuprofen might inhibit steroid hormone synthesis by downregulating the expression of critical steroidogenic enzymes involved in testosterone production (Kristensen et al., 2018).

2.2 Hormonal Disruptions

Hormonal imbalance constitutes a significant mechanism of ibuprofen-induced reproductive toxicity. Research indicates that ibuprofen exposure may lower the testosterone/LH ratio, suggesting diminished efficacy of Leydig cell steroidogenesis (Kristensen et al., 2018). Ibuprofen has been linked to changes in follicle-stimulating hormone (FSH) levels and a reduction in the secretion of anti-Müllerian hormone (AMH), indicating a potential effect on Sertoli cell function (Matzkin et al., 2012). These endocrine disorders may directly affect spermatogenesis and male fertility.

3. Medicinal Plants and Protective Effects on the Reproductive System

3.1 Description of the Plant and Botanical Characteristics of *Camellia sinensis*

Green tea, or *Camellia sinensis*, is a plant that stays green all year round and is a member of the Theaceae family. One of the most common medicinal plants in the world, especially in Asian countries like China, India, and Japan. For hundreds of years, people have used the plant not only as a drink but also for its healing properties in traditional medicine (Musial et al., 2020).

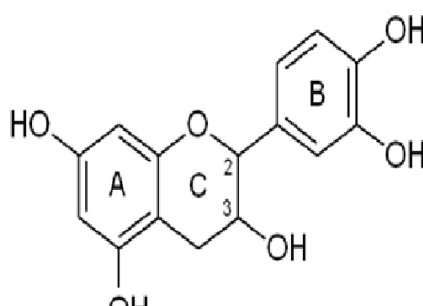


Figure 03. Structure of *Camellia sinensis* (Cabrera et al., 2006 ; Chacko et al., 2010 ; Yang et al., 2018).

Botanically, *Camellia sinensis* has small, dark green leaves, white flowers that smell good, and woody stems. The leaves are the most valuable part of the plant because they have a lot of biologically active compounds in them, especially polyphenols like catechins. The way these leaves are processed determines what kind of tea is made. When it comes to green tea, the leaves don't oxidize much, which keeps their natural chemical composition and antioxidant properties intact (Cabrera et al., 2006; Chacko et al., 2010).

The plant grows best in tropical and subtropical climates, where the soil is acidic and well-drained and the rain is moderate. The chemical composition and quality of green tea can be greatly affected by things like altitude, temperature, and how it is harvested. These differences could change its pharmacological properties, especially its ability to fight free radicals.

Due to its rich phytochemical profile and long history of safe consumption, *Camellia sinensis* has gained considerable attention in modern research, especially in studies investigating its protective effects against oxidative stress and reproductive toxicity.

Table 01: Botanical Characteristics of *Camellia sinensis* (Chang., 2015)

Category	Details
Scientific Name	<i>Camellia sinensis</i>
Common Name	Green tea
Family	Theaceae

Plant Type	Evergreen shrub
Geographical Origin	East Asia (China, India, Japan)
Height	1–3 m (cultivated), up to 10–15 m (wild)
Leaves	Dark green, simple, alternate, serrated edges
Flowers	Small, white to pale yellow, fragrant
Fruits	Capsule containing 1–3 seeds
Root System	Deep and well-developed
Climate Requirements	Tropical and subtropical
Soil Type	Acidic, well-drained soil
Harvested Part	Young leaves and buds
Processing Type	Non-fermented (minimal oxidation)
Main Compounds	Catechins (EGCG, EGC, ECG, EC), caffeine, theanine
Pharmacological Properties	Antioxidant, anti-inflammatory, antimicrobial, anticancer

3.2. Geographic repair

Camellia sinensis, which is also known as green tea, is a member of the Theaceae family. It is one of the most widely cultivated commercial and medicinal plants globally. It usually grows best in tropical and subtropical areas where the air is humid, the temperatures are moderate, and the soil is acidic and drains well. Asia is home to a lot of green tea farms, especially in China, which is thought to be the first place to grow it, as well as in India, Japan, Sri Lanka, and Vietnam. Most of the world's green tea comes from these countries. Tea is grown in large regions in the Chinese provinces of Yunnan, Fujian, and Zhejiang. In the Indian states of Assam, Darjeeling, and Nilgiri, there are big tea farms. In addition to Asia, cultivation has spread to Kenya, Rwanda, and Malawi, among other African countries. It has also spread to other South American countries, such as Argentina and Brazil. The global market demand, farming

methods, and climate all affect where *Camellia sinensis* grows. It is one of the most popular non-alcoholic drinks in the world (Chacko et al., 2010).



Figure 04. Distribution Green Tea (FAO, 2022; ITC, 2021).

Legend:

Dark green: Major green tea producing countries.

Grey: Countries with low or no significant green tea production.

3.3 Natural Antioxidants and Male Reproductive Health

Oxidative stress is regarded as a principal contributor to male infertility. Spermatozoa are particularly susceptible to oxidative damage owing to the elevated levels of polyunsaturated fatty acids in their plasma membranes and the restricted ability of their cytoplasm to neutralize reactive oxygen species (ROS). Too much ROS can cause lipid peroxidation, DNA fragmentation, mitochondrial dysfunction, and less sperm motility (Agarwal et al., 2014).

natural antioxidants sourced from medicinal plants have garnered significant interest as protective agents against oxidative reproductive damage. Green tea polyphenols exhibit significant free radical scavenging ability, at times surpassing that of traditional antioxidants like vitamins C and E (Zadkhosh et al., 2016). These substances help balance antioxidants in testicular tissue, lower lipid peroxidation, and neutralize reactive oxygen species.

3.4 Chemical Constituents and Pharmacological Properties of *Camellia sinensis* (Green Tea)

3.4.1 Chemical Constituents

Green tea comes from the leaves of the *Camellia sinensis* plant and is known for having a lot of bioactive chemicals that are good for health in many ways. Catechins and other polyphenols

are the main chemicals that make it work in the body. The main catechins are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG is the most common and biologically active (Cabrera et al., 2006; Chacko et al., 2010). In addition to catechins, green tea has caffeine, theanine, vitamins, and trace minerals. All of these things work together to change how the body works (Khan & Mukhtar, 2013).

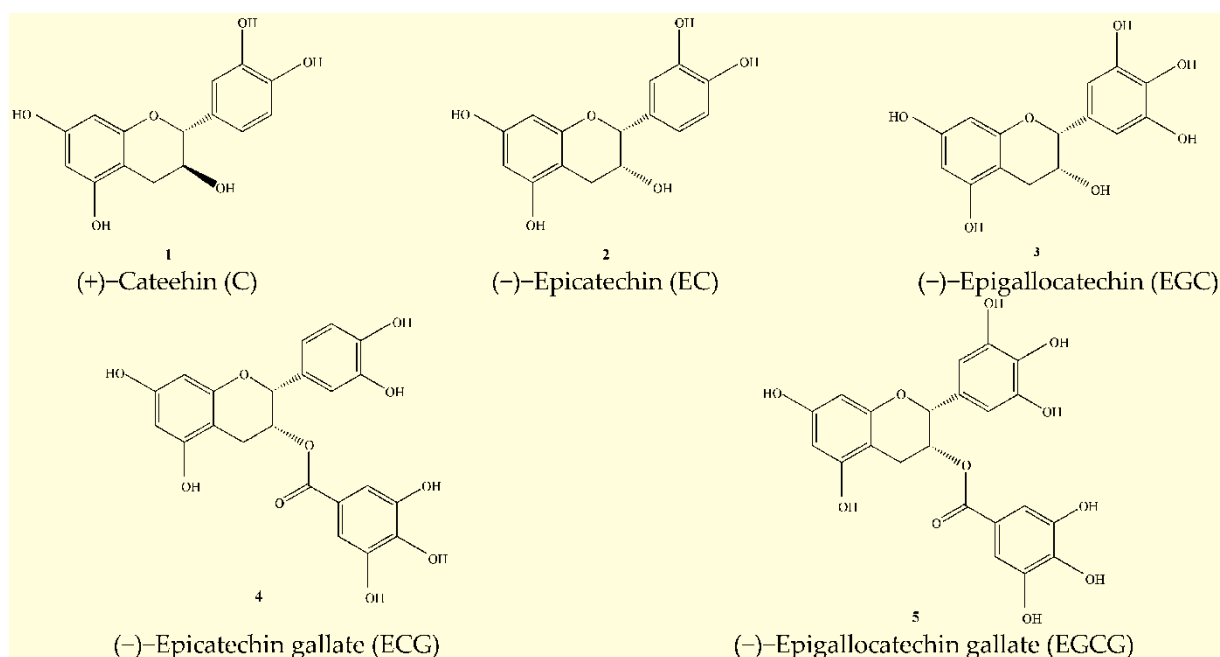


Figure 05. Chemical structures of catechins isolated from green tea (Zhao et al., 2022).

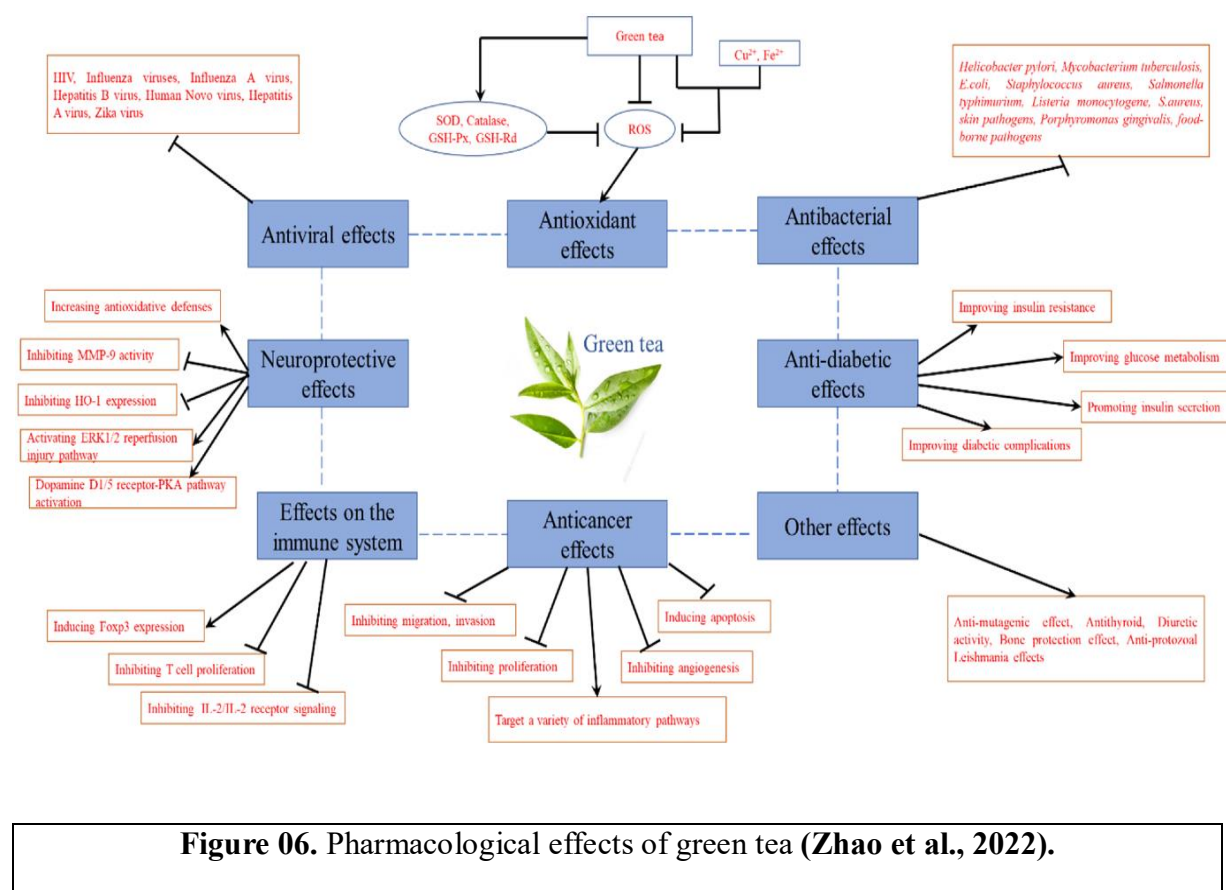
3.4.2. Pharmacological Properties

The pharmacological effects of green tea are diverse. Its polyphenols have significant antioxidant activity, scavenging hydroxyl and peroxy radicals and protecting cellular macromolecules including lipids, proteins, and nucleic acids from oxidative damage (Yassa et al., 2013). This antioxidant activity aids in the maintenance of cellular integrity and is especially crucial in tissues prone to oxidative stress. Beyond their conventional antioxidant Catechins have been demonstrated to function in detoxification processes by competing with cellular proteins for binding to hazardous chemicals, limiting the production of damaging protein adducts and preserving tissue structures at the molecular level (Liu et al., 2024).

Experimental research has demonstrated that green tea possesses preventive properties against reproductive toxicity. In animal models, catechins have demonstrated the ability to preserve the

architecture of seminiferous tubules, enhance sperm count and motility, and sustain testosterone levels following exposure to toxic agents such as cadmium, para-nonylphenol, or deltamethrin (Mahmoudi et al., 2018; Azizi & Mehranjani, 2019; Bagherpour et al., 2019). These benefits are achieved through complementary mechanisms, including the reduction of oxidative stress, the suppression of apoptosis, and, in exceptional cases, direct interaction with harmful molecules.

The evidence indicates that the bioactive compounds in green tea operate via various mechanisms to provide antioxidant, anti-inflammatory, anti-apoptotic, antimicrobial, and anticarcinogenic effects, highlighting its potential as a natural therapeutic agent for preserving tissue integrity and promoting reproductive health (Khan & Mukhtar, 2013; Yang & Wang, 2016; Guimarães-Ervilha et al., 2021).





Experimental Part





Materials and Methods



4. Materiels and Methodes

4.1. Materials

Our study was conducted in the Biochemistry Laboratory of the Department of Applied Biology at the Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, with the objective of evaluating the testicular toxicity induced by ibuprofen in Wistar rats and investigating the potential protective role of green tea (*Camellia sinensis*) extract against drug-induced oxidative damage. To assess ibuprofen toxicity and the protective efficacy of green tea, standardized protocols were applied to analyze key biochemical parameters and oxidative stress markers, including glutathione (GSH) and catalase (CAT).

4.1.1. Chemical matériel

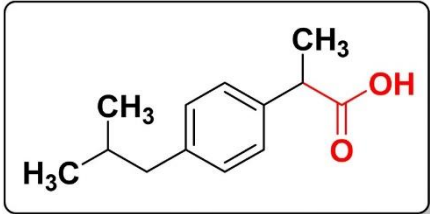
4.1.1.1. Definition and usage

Ibuprofen is a common nonsteroidal anti-inflammatory drug (NSAID) that is well-known for its pain-relieving and fever-reducing effects. It is often used in medicine to help with pain, fever, and inflammatory diseases (Derry et al., 2020; Davies, 2021).

Due to its widespread application and pharmacological properties, ibuprofen is commonly employed in experimental studies to examine its biological effects. It is utilized in toxicological research to examine potential modifications in physiological processes under regulated conditions.

4.1.1.2. Physiochemical properties

Table 02: Physicochemical and Toxicological Characteristics of Ibuprofen (DrugBank., 2023; PubChem., 2024)

Structure	
Molecular Formula	C ₁₃ H ₁₈ O ₂
Molecular Weight	206.28 g/mol
Physical Appearance	White to off-white crystalline powder

Solubility	Practically insoluble in water; soluble in organic solvents (ethanol, methanol)
Melting Point	75–77 °C
pKa	4.9~
Mechanism of Action	Non-selective inhibition of cyclooxygenase enzymes (COX-1 and COX-2)
Half-life (t_{1/2})	Approximately 1.8–2 hours in humans
LD50 (Oral, rat)	636 mg/kg
Thérapeutique Dose (Human)	200–400 mg per dose
Target Organs (Toxicity)	Gastrointestinal tract, kidney, liver, testes
Pharmacological Class	Non-steroidal anti-inflammatory drug (NSAID)

4.1.1.3. Preparation of the solution

Ibuprofen was purchased from a local pharmacy in the form of a commercial formulation (Xydole®). The tablets were finely ground into a homogeneous powder and subsequently dissolved in distilled water. The prepared solution was then administered orally to the rats at a dose of 200 mg/kg body weight using a gavage technique. This dosage was selected based on previously published experimental studies investigating the effects of ibuprofen on male reproductive parameters (Dayyih et al., 2019; Sharma et al., 2020).



Figure 07. Preparation of the ibuprofen solution (personal photos).

4.1.2. Biological materials

4.1.2.1. Plant materials

Our study focuses on green tea, a perennial evergreen plant belonging to the family Theaceae. Green tea is obtained from the leaves of *Camellia sinensis* that undergo minimal processing and no fermentation, allowing the preservation of its bioactive compounds, especially polyphenols.

4.1.2.3. Preparation of the decoction

Green tea (*Camellia sinensis*) was prepared as an aqueous extract by adding the required amount of plant material to boiling distilled water. The mixture was then covered and allowed to infuse for approximately 20 minutes to ensure adequate extraction of bioactive compounds. After cooling, the resulting decoction was used for experimental administration. The administered dose was set at 200 mg/kg body weight and delivered orally by gavage. This

dosage was selected based on previous experimental studies evaluating the protective effects of green tea on reproductive parameters (Azizi & Mehranjani, 2019).



Figure 08. Preparation of decoctions (personal photos).

4.1.3.1. Animal care and experimental design

This study was conducted on 28 male Wistar rats, all healthy and six weeks old, obtained from the Pasteur Institute in Algiers. The animals were housed in the animal breeding facility of the Faculty of Exact Sciences and Natural and Life Sciences at the University of Tebessa, in accordance with approved environmental standards. The rats underwent an adaptation period of 15 days before the experiment began to acclimate to the laboratory environment. During this

period, they were provided with a balanced diet (croquettes) and clean drinking water without restriction. The rats were housed in cages filled with wood shavings, which were replaced daily to maintain a high level of cleanliness and ensure the animals' well-being. Environmental conditions were carefully regulated, maintaining a constant temperature of $(22 \pm 2^{\circ}\text{C})$ along with a regular light cycle of 12 hours of light followed by 12 hours of darkness, ensuring optimal living conditions, and the average weight of the rats before the experiment was about: $310 \pm 10\text{g}$.

Table 03. composition of 1 kg of food.

Quantity in g/kg of food %		
Protein	140-180	14-18
Grains	500-600	50-60
Fats	40-80	4-8
Fiber	80-120	8-12
Minerals & Vitamins	10-30	1-3
Veggies & Herbs	50-100	5-10
Extras	5-10	0.5-1

After an acclimatization period, the rats were divided into four groups (three treated groups and one control group), with each group consisting of seven rats (Table 04), and all treatments were administered for 45 consecutive days. During the experimental period, body weight of the rats was recorded once a week, while daily weighing was carried out only for the purpose of adjusting the administered doses according to individual body weight.

Table 04. The Experimental design of the study.

Experimental Groups	Treatment
Control	Received distilled water by oral gavage.
GTE	Received green tea infusion (200 mg/kg body weight) by oral gavage
IBU	Received ibuprofen (IBU) at a dose of 200 mg/kg body weight by oral gavage.

IBU+GTE	Received ibuprofen (IBU) at a dose of 200 mg/kg body weight, and after 30 minutes Received green tea infusion (200 mg/kg body weight) by oral gavage
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Figure 09. Animal care (personal photos).

4.1.3.2. Blood sampling

Following a 45-day treatment period, the rats were sacrificed by decapitation using a sharp blade. Arterial-venous blood was collected into dry tubes for testosterone measurement, and the tubes were properly labeled according to group identification. The samples were transported to the laboratory in temperature-controlled coolers to preserve their integrity. Blood samples were then centrifuged at 3,000 RPM for 20 minutes to obtain serum, which was subsequently used for testosterone assay



Figure 10. Blood sampling (Personal photos).

4.1.3.3. Tissue sampling (Testicular and Epididymis)

After the sacrifice of the rats, both the testes and epididymides were carefully excised under appropriate experimental conditions. The epididymides were immediately processed for the collection of spermatozoa. One testis from each animal was placed in formalin, while the remaining testicular tissues were rapidly divided and stored at -80°C for further analyses.

All samples were promptly transported to the university laboratory for initial processing, including testicular assessment and spermogram evaluation. Subsequently, sperm samples and tissue specimens were transferred to an external specialized laboratory for additional analyses, including detailed histological examination of testicular tissue and spermocytogram analysis for sperm morphology evaluation.



Figure 11. Tissue sampling (Personal photos).

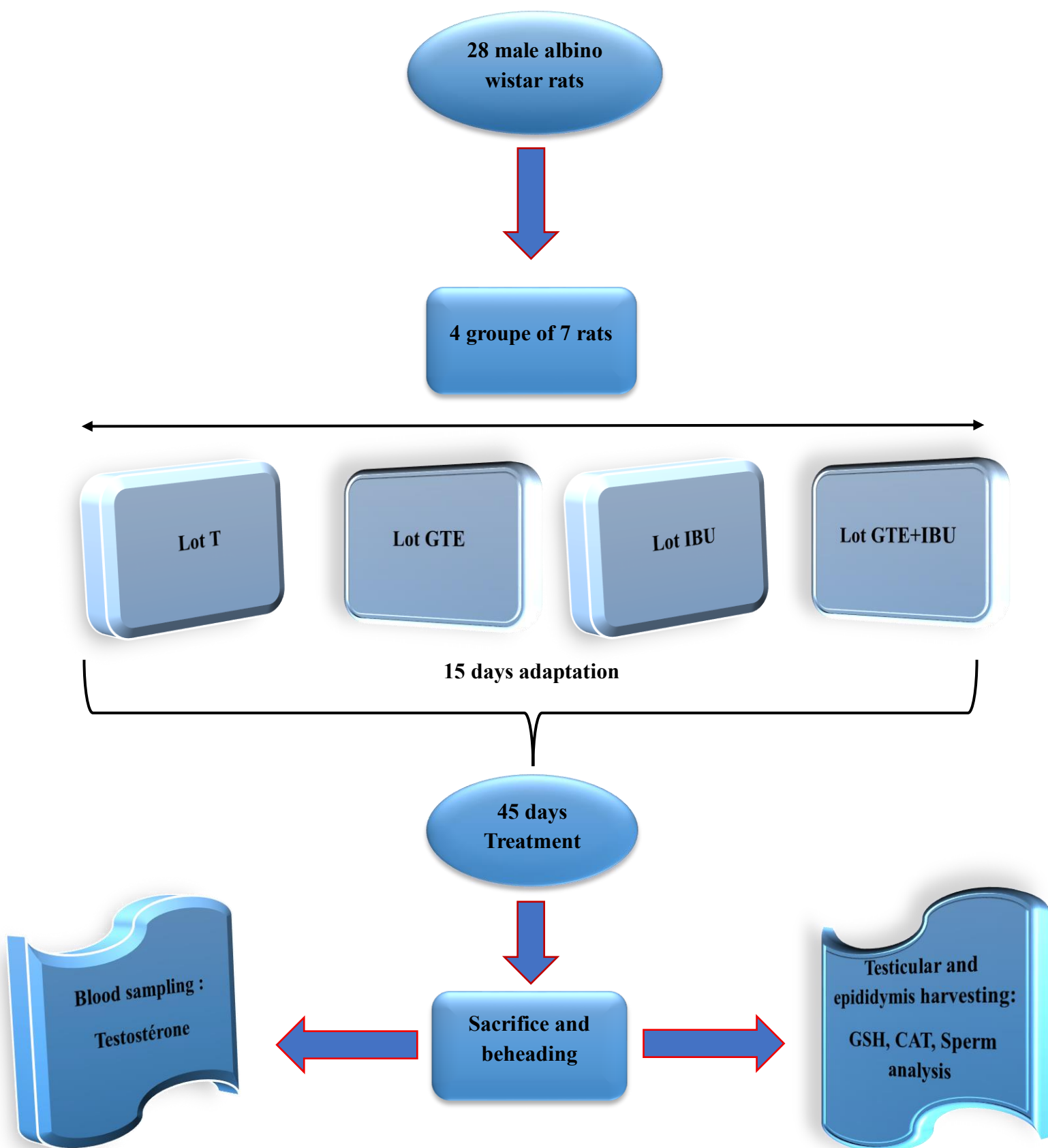


Figure 12. Diagram summarizing the experimental protocol

4.2. Methodes

4.2.1. Biochemical analyses (testosterone assay)

Principe

The MAGLUMI Testosterone kit is based on the competitive chemiluminescence immunoassay (CLIA). It has buffer solution, calibrators, testosterone with ABEI (a chemiluminescent marker), and magnetic microbeads with antibodies that fight testosterone. Throughout the experiment, testosterone in the sample competes with the tagged antigen to bind to certain antibodies. To gather and separate the immune complexes, magnetic microbeads are used. When a chemiluminescent process starts, relative light units (RLU) are used to measure how much light is given off. The amount of testosterone in the sample goes down as the light gets brighter (**Technique CILIA Sur maglumi 800 (Snibe)**).



Figure 13. Testosterone assay (personal photos).

4.2.2. Sperm analysis

Following the isolation of the epididymis, spermatozoa were collected and immediately transported to the university laboratory. A spermogram was performed to evaluate sperm parameters. Subsequently, the samples were transferred to an external laboratory where a spermocytogram was conducted for further analysis of sperm morphology.

Principe

Sperm biology was studied according to the WHO method (1993). After dissection, semen was collected from the head of the epididymis to study sperm concentration, motility, vitality. 16 μL of semen was added to 49 μL of previously prepared 0.9% NaCl physiological saline.

4.2.2.1. Sperm concentration

Sperm concentration was measured using a Malassez counting chamber. Spermatozoa were counted in five squares at 40 $\times$ magnification with a closed diaphragm. A drop of diluted semen was mixed with a drop of 1% methylene blue solution, and then a drop of the mixture was placed into the counting chamber. The sperm concentration was then calculated using the following equation:

$$\text{Concentration (sperm} \times 10^6 / \text{mL)} = (\text{D} \times \text{V} \times \text{n}) / \text{N}$$

(sperm $\times 10^6$ /mL) D: Dilution coefficient 1/4

V: Volume of the Malassez counting chamber

n: Number of sperm counted in 5 cells

N: Number of small cells (100)

4.2.2.2. Sperm motility

A drop of semen is placed on a slide and then covered with a coverslip. Observation is performed under a microscope at 40 $\times$ magnification with a closed diaphragm. The field of view is divided into 3 sections, and the percentage of motile sperm is calculated per 100 sperm.

4.2.2.3. Sperm viability

Vitality staining: This study uses a staining technique based on the principle that dead cells with damaged plasma membranes allow certain stains, such as 1% eosin, to penetrate. Mix a drop of

diluted semen with a drop of 1% eosin solution on a slide and then cover the preparation with a coverslip. After one to two minutes, the observation is made under an optical microscope at 100×magnification with an open diaphragm. We count 100 spermatozoa: live (unstained) and dead (stained). The percentage of live spermatozoa is then calculated.

4.2.2.4. sperm morphology (Spermocytogram)

a. Smear preparation

A drop of semen is placed on a clean, degreased slide and spread into a thin layer using a second inclined slide to obtain an even smear. The smear is then air-dried.

b. Fixation and staining

The smear is fixed using a fixative (usually methanol or ethyl alcohol), then stained using a method suitable for morphological examination, such as:

Fix Ral 555 staining

This staining allows visualization of sperm structures (head, midpiece, and flagellum).

c. Microscopic examination

Observation is performed using a light microscope with oil immersion (×100 objective). Between 100 and 200 spermatozoa are analyzed to assess their morphology. They are classified into:

Normal forms

Abnormal forms (defects of the head, midpiece, or flagellum)

d. Morphological analysis

The evaluation is based on the strict criteria of the WHO (World Health Organization).

Abnormalities may include:

Head: elongated, round, double, or vacuolated

Midpiece: thickened, bent, or irregular

Flagellum: short, multiple, coiled, or broken

The final result is expressed as the percentage of morphologically normal spermatozoa.

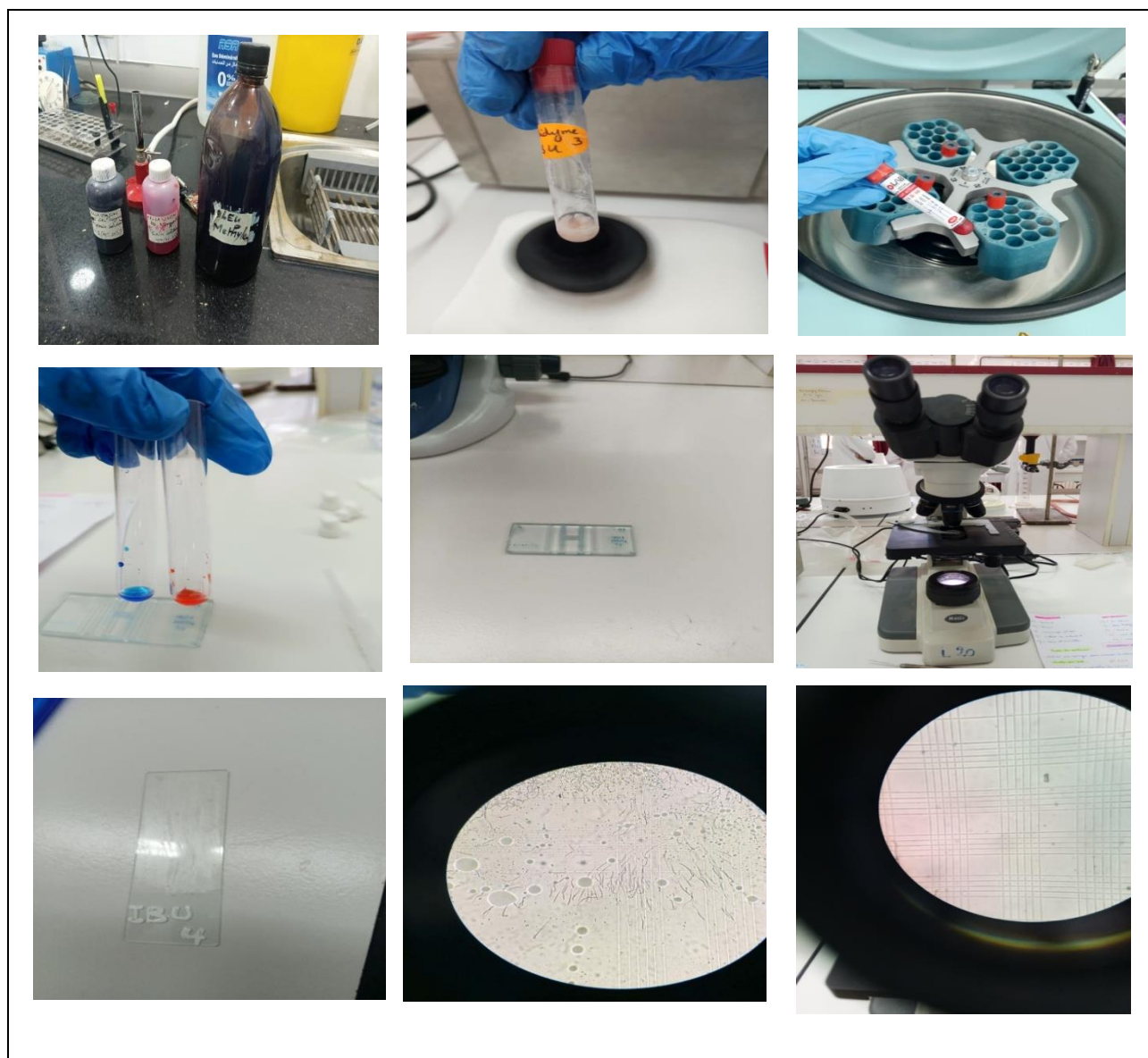


Figure 14. Sperm assay (personal photos).

4.2.3. Oxidative stress analyses

4.2.3.1. Assay of Catalase enzymatic activity

Preparation of the homogenate

100 mg of tissue + 1ml phosphate buffer (0.1M pH 7)



Cold grinding



Homogenate / Centrifugation 9000 rpm for 15 min

Principle

The method used was that of **Aebi (1984)**. Catalase activity was measured at 240 nm using a spectrophotometer, by observing the change in optical density following the decrease of hydrogen peroxide (H₂O₂) at 25°C in the presence of an enzyme source in the cytosolic fraction. The absorption reading was taken after a 15-second delay and during a 60-second measurement period.

Operating mode

Table 05. The dosage of the Catalase enzymatic activity.

The reagents used	Blanc (μl)	Sample (μl)
Phosphate buffer (0.1 M ,PH 7)	800	780
H ₂ O ₂ (0.5 M)	200	200
Supernatant	/	20

Calcula: the catalase activity is calculated according to the following equation.

CAT Activity (mmol H₂O₂ / min/mg protein) =	$\frac{\Delta DO \times 10}{\epsilon \times L \times X \times Fd}$
--	--

ΔDO : Variation in optical densite typer minute.

ε: Extinction coefficient ofH₂O₂ = 0.04 Mml. Cm1

L: Tank length= 1 Cm

X: Protein quantity in mg/ml

Fd: H₂O₂ dilution factor in buffer (0.02)

Preparation of the calibration curve

BSA (bovine serum albumin) stock solution

1 mg of BSA + 1 ml of distilled water> shake

Procedure

Prepare 6 tubes of different compositions. After shaking, read the optical density at 595 nm:

Table 06. Preparation of BSA concentrations in BBC medium

Tube	Composition
1	4ml BBC + 100 µl distilled water + 0 µl BSA
2	4ml BBC + 80 µl distilled water + 20 µl BSA
3	4ml BBC + 60 µl distilled water + 40 µl BSA
4	4ml BBC + 40 µl distilled water + 60 µl BSA
5	4ml BBC + 20 µl distilled water + 80 µl BSA
6	4ml BBC + 0 µl distilled water + 100 µl BSA

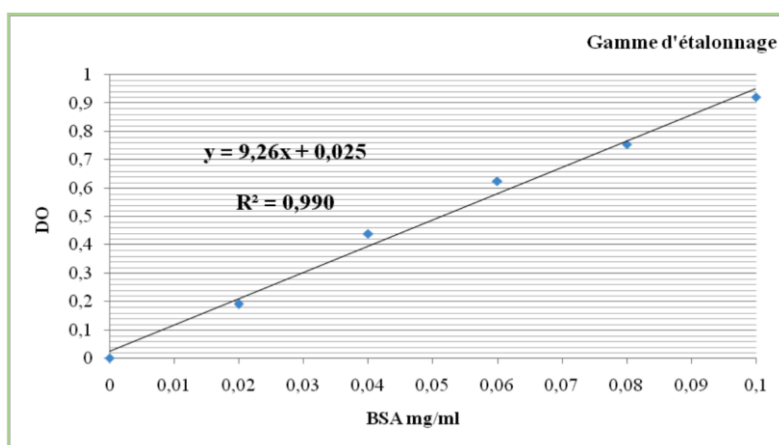


Figure 15. Calibration curve of bovine serum albumin



Figure 16. Assay of Catalase enzymatic activity (personal photos).

4.2.3.3. Assay of glutathione reduces GSH

Principle

The principle of this assay is based on measuring the optical absorbance of 2-nitro-5-mercapturic acid, which results from the reduction of 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) by the (-SH) groups of glutathione. This is achieved by deproteinizing the tissue to retain only the (-SH) groups specific to glutathione.

Preparation of the homogenate:

100 mg of tissue + 4 mL EDTA (0.02 M) →→→→→ cold grinding →→→→→ homogenate.

Procedure

- Take 0.8 mL of the homogenate.
- Deproteinize by adding 0.2 mL of 0.25% sulfosalicylic acid (SAA) solution.
- Shake the mixture and leave it in an ice bath for 15 minutes.
- Centrifuge at 1000 rpm for 5 minutes.
- Take 0.5 mL of the supernatant.
- Add 1 mL of Tris buffer + EDTA (0.02 M EDTA), pH 9.6.
- Mix and add 0.025 mL of 0.01 M 5,5-dithio-bis-(2-nitrobenzoic acid) DTNB.
- Leave at room temperature for 5 minutes to stabilize the color, which develops instantly.
- Read the optical densities at 412 nm against white.



Figure 17. GSH assay (personal photos).

Protein assay in a sample according to Bradford, (1976)

Principle

This method uses Coomassie blue as a reagent that reacts under acidic conditions with arginine, and to a lesser extent, with histidine, lysine, and aromatic amino acids. Coomassie blue binds to proteins through non-covalent interactions (hydrogen bonds, hydrophobic interactions, and ionic interactions). Its maximum absorption length increases from 465 nm (red) to 595 nm (blue).

Procedure

- Take 0.1 ml (100 μ l) of the sample (supernatant).
- Add 4 ml of Bradford reagent.
- Shake and let stand for 5 minutes to stabilize the color.
- Read the optical density at 595 nm against a blank (4 ml of BBC + 0.1 ml distilled water).
- The obtained optical density is plotted on a previously drawn calibration curve. The protein concentration is determined by comparison to a bovine serum albumin (BSA) standard curve (1 mg/ml) obtained under the same conditions.

4.2.5. Comparative Statistical Evaluation of Experimental Groups

A statistical analysis was performed to compare the effects of the different treatments among the experimental groups: control (C), green tea-treated group (GTE), ibuprofen-treated group (IBU), and the co-treated group (IBU+GTE). Data were expressed as mean $\pm$ standard deviation (SD), and one-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance. Differences were considered statistically significant at $p < 0.05$. This analysis revealed significant variations between groups, highlighting the potential effects of ibuprofen and the possible protective role of green tea.



Results and Discussion



5. Results and discussion

5.1. Physiological parameters

5.1.1. Effect of treatments on body weight, behavioral responses, and physical appearance

Depending on the type of therapy, the weight change data clearly demonstrate disparities between the experimental groups. Over the course of the trial, the control group (C) saw a typical weight rise. The weight rise in the green tea extract group (GTE) was similar or somewhat better, indicating a favorable impact on growth performance. The ibuprofen group (IBU), on the other hand, had a significant drop in body weight that was followed by a long period of stagnation. The ability of green tea extract to support recovery from ibuprofen-induced weight loss was demonstrated by the group that received the combination therapy (IBU + GTE), which exhibited a little initial decline followed by a gradual rebound to values around control levels.

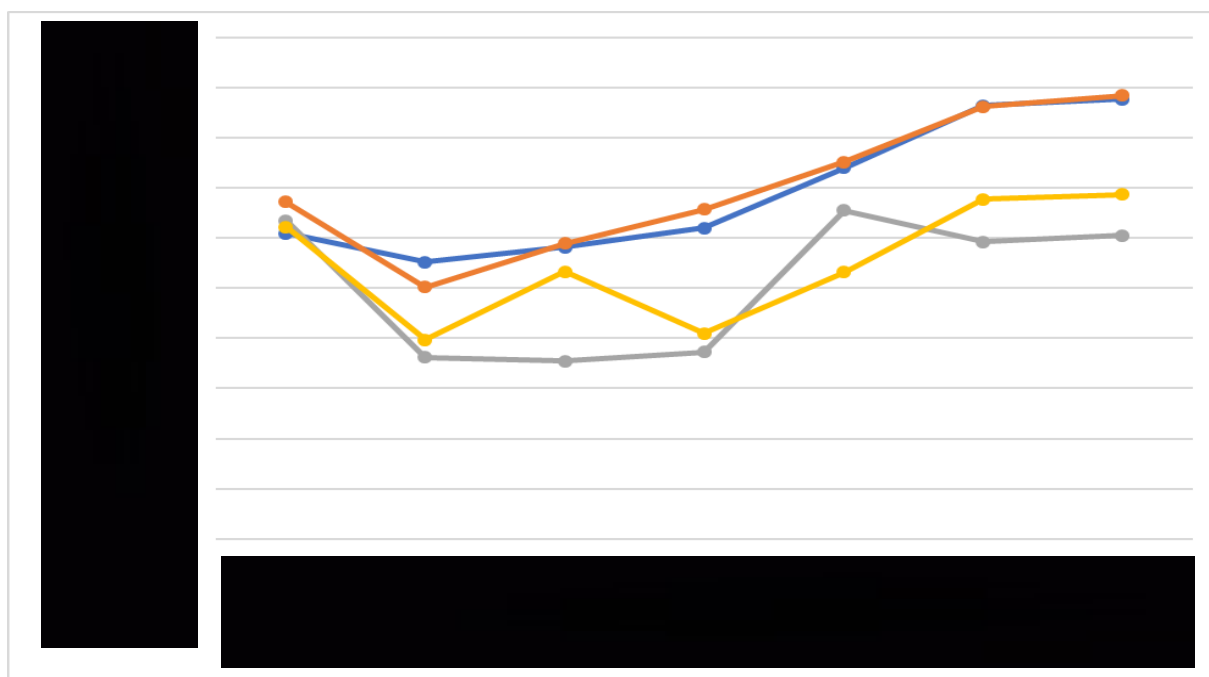


Figure 18. Effect of green tea extract and/or Ibuprofen treatment on body weight gain after 45 days of experience.

The dietary consumption of the animals remained consistent during the treatment phase in both the control group and the group administered with green tea extract. In contrast, the group subjected solely to ibuprofen exhibited a decline in both food intake and appetite compared to

the control group. However, the group that received the combined treatment (IBU + GTE) showed improved food consumption compared to the IBU group, though still slightly below control levels.

Daily physical and behavioral observations were made during the animals' observation period. The rats in the IBU treated group fought and behaved aggressively with each other so much that they even cannibalized (ate) each other, which could have been extreme stress related reactions to ibuprofen poisoning. In addition, some of rats showed obvious testicular abnormalities and testicular descent (prolapse), which suggested possible problems in reproductive system. Also, there was a noticeable hair loss, primarily in the dorsal and ventral areas, that is suggestive of poor general health and nutritional status.

Aggressive behavior was also present in the co-treatment group (IBU + GTE), but the frequency and intensity were significantly reduced compared to the IBU group and cannibalistic behavior was observed only rarely and much less than in the IBU group. Testicular prolapse and hair loss were, however, much less pronounced, with most animals having almost normal testicular placement and mostly intact fur coverage.

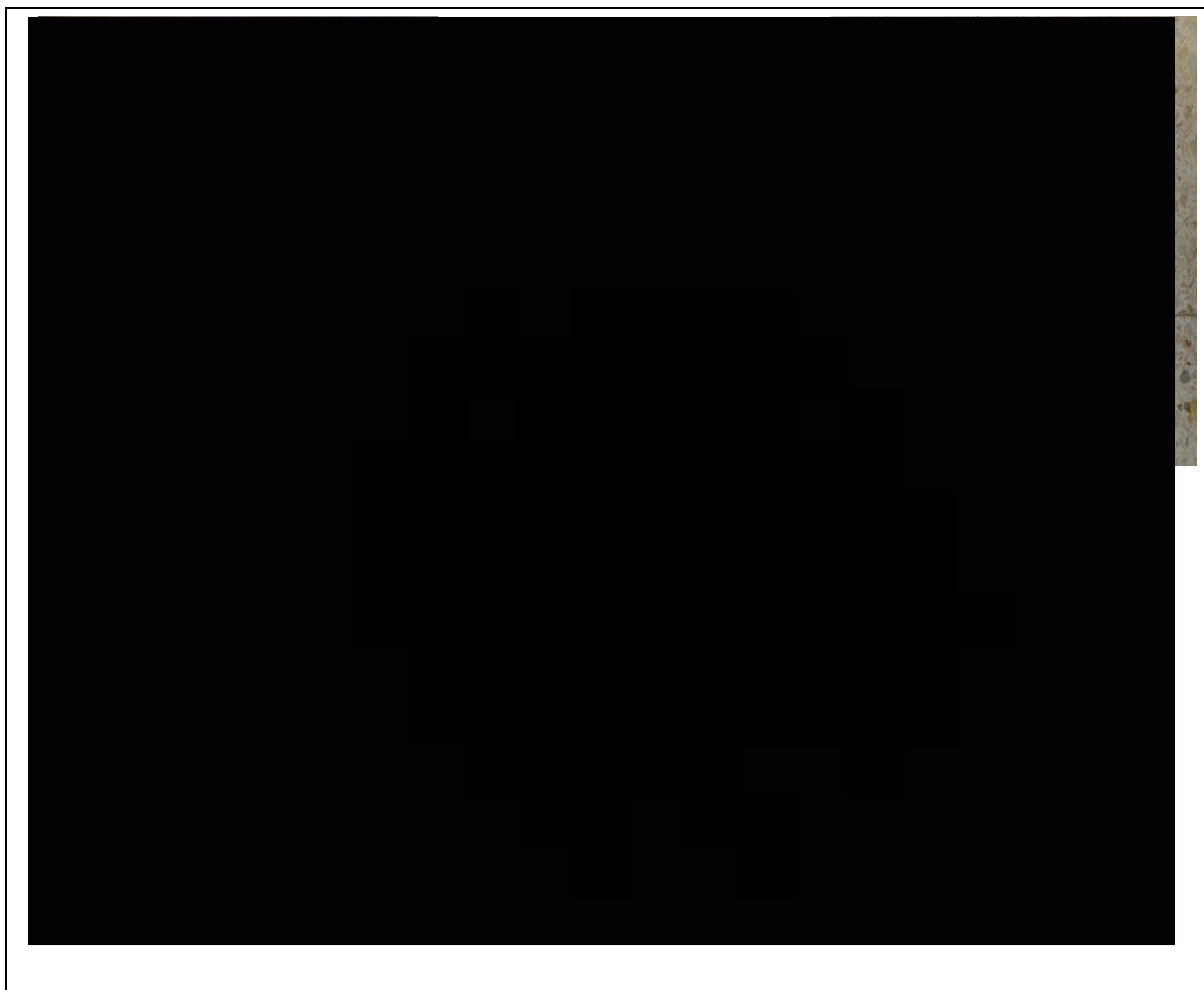


Figure 19. Physical manifestations of ibuprofen toxicity in experimental rats.

Ibuprofen is one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) in the world. However, it has been demonstrated that chronic administration of ibuprofen causes severe testicular toxicity and oxidative stress (**Rainsford, 2012; Barbosa et al., 2020**).

Ibuprofen-treated rats in the current study showed a significant decrease in body weight gain, along with serious behavioral abnormalities such as cannibalism, inter-animal aggressiveness, testicular prolapse, and loss of hair. Similar toxic manifestations have been associated with oxidative stress and metabolic disturbances induced by NSAIDs and other toxic agents. **Sharma et al. (2020)**, reported that oxidative stress severely altered normal physiological and behavioral functions in experimental rats, while **Tiwari et al. (2020)**, demonstrated that ibuprofen exposure disrupted energy metabolism and protein regulation

pathways. In addition, **Sharma et al. (2020)**, reported that chronic oxidative stress conditions may compromise overall health status and normal biological functions.

The significant loss of body weight in the IBU group may be due to decreased food intake and poor nutrient absorption resulting from the damage of the gastric mucosa and systemic inflammation caused by ibuprofen. These effects were also demonstrated in previous research with **Wallace (2008) and Rainsford (2009)**, showing that NSAIDs such as ibuprofen inhibit prostaglandin production that compromise the integrity of the stomach mucosa and causes gastrointestinal irritation and ulceration. IBU+GTE group had better weight gain and significantly reduced physical abnormalities and aggressive behavior suggesting that co-administration of green tea extract GTE significantly reduced these negative effects. The protective effect may be due to the powerful anti-inflammatory and antioxidant activity of green tea polyphenols, especially epigallocatechin-3-gallate EGCG, that has been shown by **Hassan et al. (2019) and Pérez-Aguirre et al. (2025)**, to reduce oxidative damage induced by drugs and maintain the integrity of the tissue.

We observed testicular prolapse in the IBU group. In this study, it indicates a significant change in the testicular position and is a sign of serious reproductive toxicity. These findings are in agreement with previous studies by **Kristensen et al (2018)**, who showed that ibuprofen exposure may alter testicular endocrine function and lead to changes in luteinizing hormone and testosterone levels, indicating a state of compensated hypogonadism in human subjects. The physical abnormalities observed in the IBU group may be due to the metabolic perturbation and systemic physiological stress caused by ibuprofen. The administration of green tea extract (GTE) was effective in preventing reproductive and systemic toxicity of ibuprofen (IBU) by modulation of inflammatory cytokines and scavenging of reactive oxygen species (ROS), as shown by the reduction of these adverse effects in the IBU+GTE group (**Bagherpour et al., 2019**).

5.1.2. Effect of treatment on testicular weight

ibuprofen administration resulted in a significant decrease in relative testicular weight compared with the control group, indicating testicular toxicity and atrophy. However, no significant difference was observed in absolute testicular weight between the ibuprofen-treated and control groups. Green tea extract alone did not significantly affect either absolute or relative testicular weights. In contrast, co-administration of green tea extract with ibuprofen improved relative

testicular weight to values close to those of the control group, while absolute testicular weight remained unchanged. These findings suggest that green tea extract may attenuate ibuprofen-induced testicular damage and exert a protective effect against reproductive toxicity.

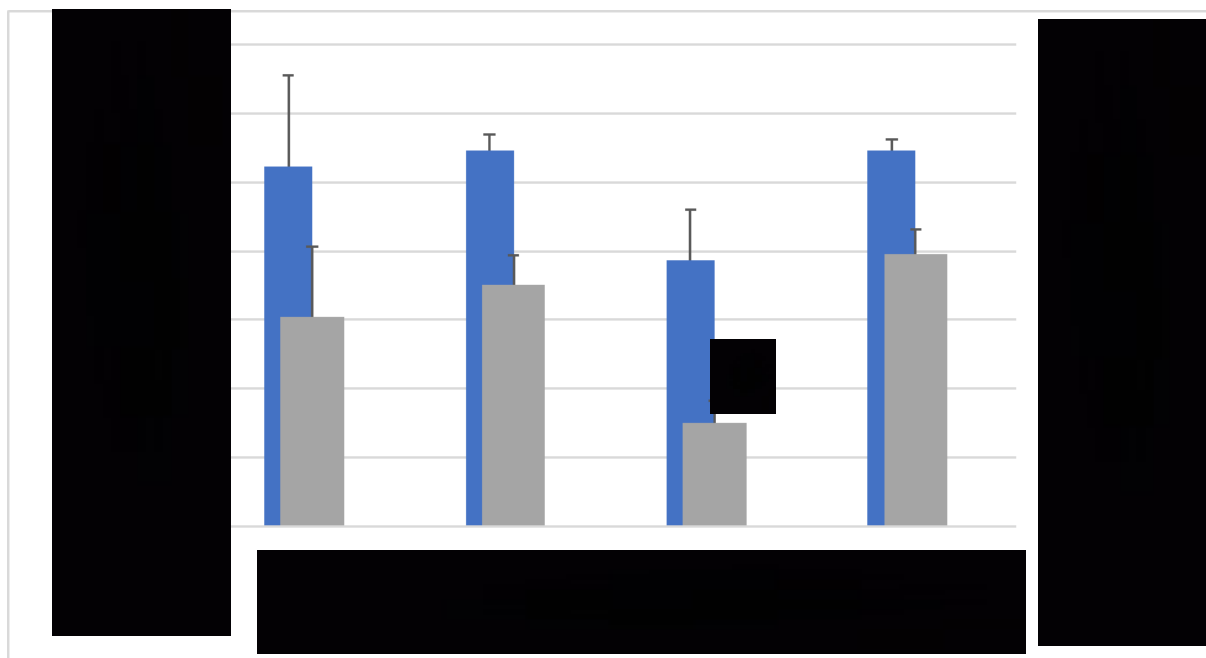


Figure 20. Effect of green tea extract and/or ibuprofen treatment on testicular weight evolution after 45 days of experience.

Taking ibuprofen significantly reduced relative testicular weight compared to the control group, suggesting possible alterations in testicular development or body-to-organ weight balance. However, absolute testicular weight showed no statistically significant difference. Such changes may reflect oxidative stress-mediated peroxidative damage and subtle structural modifications in testicular tissue. **Barbosa et al. (2020)**, reported that ibuprofen-treated rats exhibit reduced testicular weight, which may be associated with impaired steroidogenic function and decreased prostaglandin synthesis. Similarly, **Sharma et al. (2020)**, demonstrated that ibuprofen-induced redox imbalance is characterized by increased lipid peroxidation (MDA levels) and reduced antioxidant defenses, which can contribute to altered organ homeostasis.

The application of ibuprofen at high doses has been shown to alter testicular physiology by suppressing luteinizing hormone (LH)-stimulated testosterone production, as demonstrated by **Kristensen et al. (2018)**, in human testis explants. This hormonal imbalance creates a state of compensated hypogonadism, characterized by increased LH levels in response to reduced

testosterone feedback, ultimately leading to testicular tissue wasting and weight loss. Furthermore, ibuprofen exposure has been associated with decreased daily sperm production and reduced Leydig cell number, both of which contribute to the observed reduction in testicular mass (**Barbosa et al., 2020**).

On the other hand, green tea extract alone did not produce any change in the testes weight indicating that it does not possess any intrinsic modulatory activity on the testes under normal physiological conditions. However, with the combination with ibuprofen, green tea extract showed a wonderful moderating effect, reaching almost the same testicular weights as the control group. The potent anti-inflammatory and antioxidant properties of the main polyphenol in green tea, epigallocatechin-3-gallate (EGCG), may explain this protective role. EGCG effectively scavenges reactive oxygen species (ROS) and reduces lipid peroxidation in testicular tissue (**Hassan et al., 2019; Pérez-Aguirre et al., 2025**).

The protective efficacy of green tea extract against ibuprofen-induced testicular atrophy is further supported by the findings of **Bagherpour et al. (2019)**, who demonstrated that green tea extract restored testicular histopathological architecture and improved sperm parameters in deltamethrin-exposed mice. Similarly, **Pérez-Aguirre et al. (2025)**, reported that EGCG enhanced antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) and preserved histological integrity of testes and epididymides in cadmium-exposed prepubertal rats. These mechanistic insights corroborate our findings and underscore the multifaceted protective potential of green tea polyphenols against drug-induced testicular toxicity.

5.2. Reproductive parameters

5.2.1. Serum testosterone level

Significant alterations in serum testosterone levels were observed in the IBU group in comparison with the control group (marked decrease). Furthermore, the IBU+GTE co-treatment group exhibited significant improvements in hormonal profiles, with testosterone levels restored to near-control values, suggesting potential protective and ameliorative effects of green tea polyphenols against ibuprofen-induced testicular endocrine disruption and Leydig cell dysfunction.

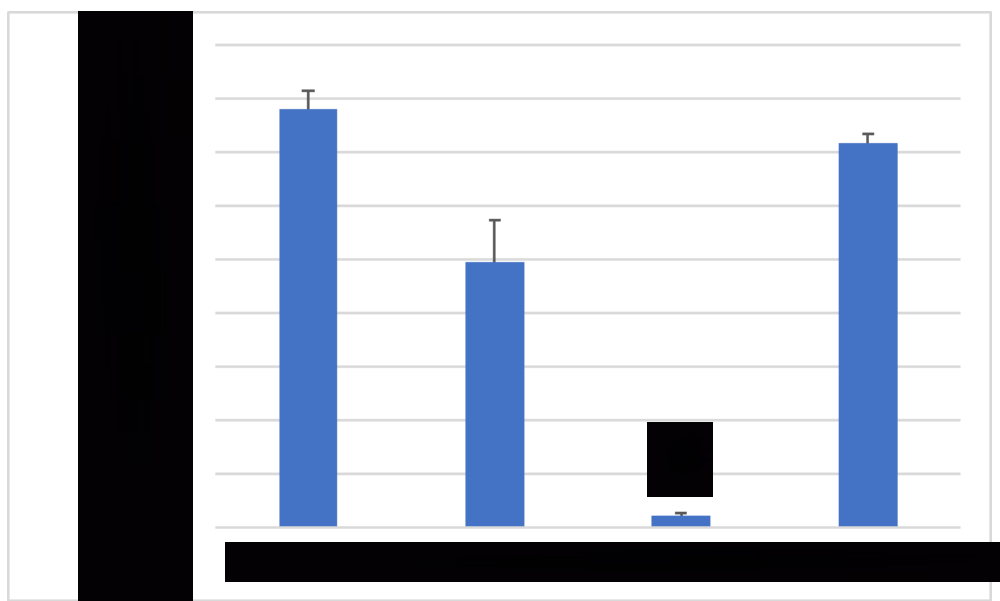


Figure 21. Effect of green tea extract and/or ibuprofen treatment on serum testosterone level after 45 days of experience.

Animals taking ibuprofen had a shocking decrease in circulating testosterone in their hormonal profile, suggesting serious damage to Leydig cells and interference with steroidogenesis. These findings are in line with earlier data from **Kristensen et al. (2018)**, showing that ibuprofen suppressed testosterone production in human testicular cultures by downregulating important steroidogenic transcripts. More recently **Salehi et al. (2025)**, have demonstrated that natural antioxidants could successfully combat this suppression by increased catalase activity and decreased lipid peroxidation. They have confirmed that rats exposed to ibuprofen show a marked drop in testosterone along with architectural damage to the seminiferous tubules.

Interestingly, the testosterone levels in the green tea only group decreased a little but not significantly. This could be due to slight aromatase regulation or changes in feedback along the hypothalamic-pituitary-gonadal axis under relaxed conditions. But when ibuprofen was taken with green tea, testosterone levels returned to near normal. This important improvement is in accordance with new clinical findings by **Onuora et al. (2025)**, who found that 12 weeks of green tea drinking increased testosterone in obese men with gonadal dysfunction while decreasing oxidative stress indicators (MDA) and strengthening antioxidant defenses (CAT, GPx).

The mechanism may involve the ability of EGCG to preserve cholesterol side chain cleavage enzyme activity, to inhibit lipid peroxidation, and to preserve mitochondrial integrity in steroidogenic cells. protecting the cascade of synthesis of testosterone. EGCG persistently elevates testosterone levels and semen quality in various toxicant models by suppressing testicular inflammation and scavenging ROS, as recently highlighted by **Roy et al. (2025)**, in a comprehensive analysis. Similarly, **Wang et al. (2025)**, presented a molecular road map to maintain testicular endocrine function by green tea polyphenols, linking the protection of EGCG to the maintenance of blood–testis barrier via AMPK/AKT/mTOR signaling.

Collectively, these data position green tea extract not merely as a passive adjuvant, but as an active hormonal protector capable of countering ibuprofen-driven testicular endocrine disruption.

5.2.2. Seminal parameters (pH, concentration, motility, viability)

Seminal pH analysis revealed noticeable differences among the experimental groups according to the administered treatments. The control group (C) maintained stable alkaline pH values, indicating normal seminal conditions required for optimal sperm function. Animals treated with green tea extract alone (GTE) showed pH values similar to or slightly higher than those of the control group, reflecting preserved seminal fluid balance. In contrast, the ibuprofen-treated group (IBU) displayed a pronounced reduction in seminal pH, indicating an abnormal acidic shift in the seminal environment. However, co-administration of green tea extract with ibuprofen (IBU + GTE) resulted in a progressive normalization of seminal pH, with values approaching those observed in the control group.

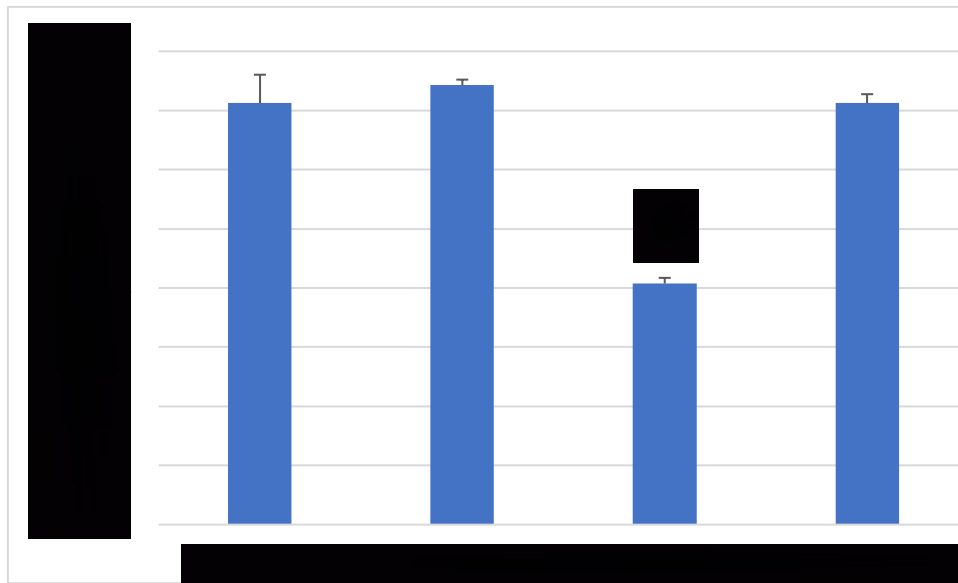


Figure 22. Effect of green tea extract and/or ibuprofen treatment on Seminal pH in rats after 45 days of experience.

The control group (C) exhibited high sperm viability with minimal mortality, reflecting normal spermatogenic function. The green tea extract group (GTE) displayed comparable viability rates, suggesting that the extract does not adversely affect gamete survival under physiological conditions. In contrast, the ibuprofen group (IBU) demonstrated a marked decline in sperm viability accompanied by elevated mortality, indicating severe cytotoxic insult to germinal epithelium. The combined treatment group (IBU + GTE) showed restored sperm viability and reduced mortality compared to the IBU group, demonstrating the protective capacity of green tea polyphenols against ibuprofen-induced spermatotoxicity.

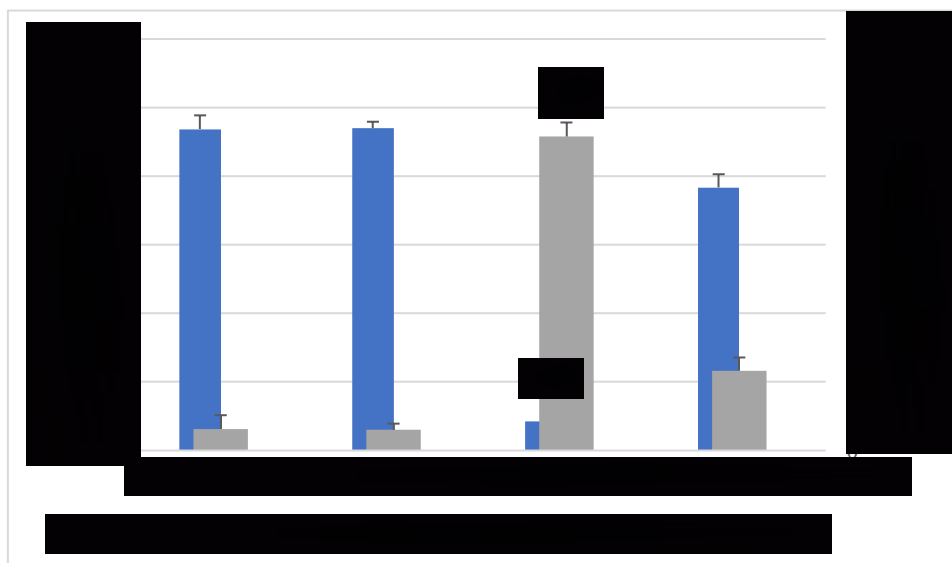


Figure 23. Effect of green tea extract and/or ibuprofen treatment on Sperm viability and mortality rate in treated rats after 45 days of experience.

Significant flagellar metabolic disruption was indicated by the ibuprofen group's (IBU) marked decline in both total and progressive motility as well as a sharp increase in immotile gametes. The green tea extract group (GTE), on the other hand, did not differ from the controls. Although results were still below control levels, the combined therapy group (IBU + GTE) demonstrated a significant improvement in motility metrics as compared to the IBU group.

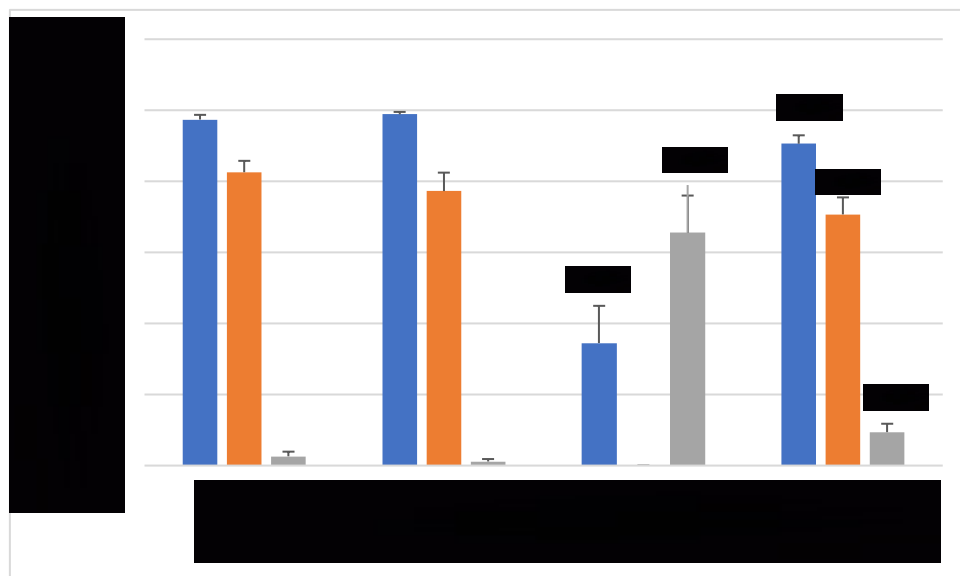


Figure 24. Effect of green tea extract and/or ibuprofen treatment on Sperm motility parameters in treated rats after 45 days of experience.

In the ibuprofen group (IBU), sperm concentrations fell sharply to extremely low levels. This implies serious damage to sperm production. Green tea extract may restore ibuprofen-damaged sperm production, as seen by the combination treatment group (IBU + GTE) regaining sperm concentration around control values.

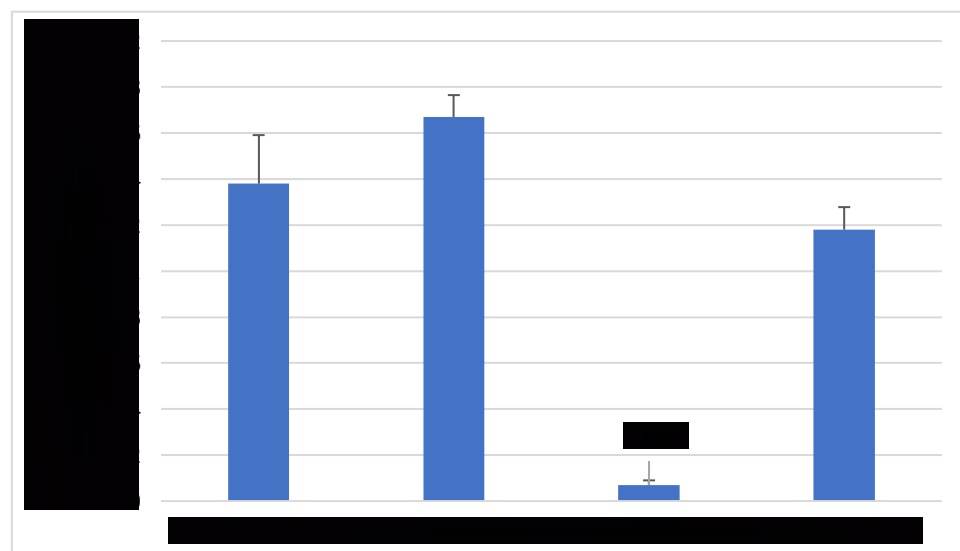


Figure 25. Effect of green tea extract and/or ibuprofen treatment on Sperm concentration in treated rats after 45 days of experience.



Figure 26. Microscopic appearance of spermatozoa in the Control (C), Green Tea Extract (GTE), Ibuprofen (IBU), and Ibuprofen + Green Tea Extract (IBU+GTE) groups, showing improved sperm density and distribution in the IBU+GTE group compared with the ibuprofen-treated group.

A: Control

B: GTE

C: IBU

D: IBU+GTE

Spermogram (semen analysis) is a fundamental diagnostic tool in andrology, providing comprehensive assessment of male reproductive potential through quantitative and qualitative evaluation of semen characteristics. Standard spermogram parameters include seminal pH, sperm concentration, motility (total and progressive), viability, and morphology. These indices collectively reflect the functional integrity of the testes, epididymides, and accessory glands, serving as sensitive biomarkers for toxicant-induced reproductive dysfunction (**World Health Organization, 2010**).

Ibuprofen exposure caused catastrophic deterioration in all assessed seminal parameters in the current investigation. Sperm could not survive in the unfavorable microenvironment created by the sharp change in seminal pH from alkaline to severely acidic levels. This acidification is probably caused by ibuprofen-induced oxidative damage to the epididymal epithelium and malfunction of the accessory glands, which impairs seminal plasma's ability to act as a buffer (**Sharma et al., 2020**). At the same time, sperm concentration fell to almost undetectable levels, suggesting that spermatogenesis and/or epididymal sperm storage were

severely disrupted. In a similar vein, **Barbosa et al. (2020)**, found that ibuprofen decreases daily sperm production in prepubertal mice by damaging the germinal epithelium and depleting Leydig cells.

Sperm motility and kinetic characteristics exhibited equally severe impairment in ibuprofen-treated animals. Total motility, progressive motility, and velocity parameters collapsed, while immotile sperm populations surged dramatically. These findings align with the work of **Roodbari et al. (2015)**, who demonstrated that ibuprofen exposure decreases sperm motility and viability in mice through mitochondrial dysfunction and ATP depletion. The flagellum, being highly dependent on oxidative phosphorylation for energy supply, appears particularly vulnerable to NSAID-induced metabolic disruption (**Kristensen et al., 2018**).

Sperm viability mirrored the motility profile, with ibuprofen-treated animals showing massive gamete mortality. This cytotoxic effect likely reflects lipid peroxidation of sperm membranes, DNA fragmentation, and activation of apoptotic pathways triggered by excessive reactive oxygen species generation (**Salehi et al., 2025**). The blood-testis barrier, which normally shields germ cells from systemic toxins, may also be compromised by chronic ibuprofen exposure, allowing direct toxicant access to the adluminal compartment (**Wang et al., 2025**).

Remarkably, green tea extracts co-administration produced substantial restoration across all seminal parameters. Seminal pH recovered to near-control alkaline values, sperm concentration rebounded significantly, motility parameters improved markedly, and viability rates increased concomitantly with reduced mortality. These protective effects likely operate through multiple synergistic mechanisms. Epigallocatechin-3-gallate (EGCG), the principal catechin in green tea, scavenges free radicals, chelates transition metal ions, and upregulates endogenous antioxidant defenses including superoxide dismutase, catalase, and glutathione peroxidase (**Hassan et al., 2019; Roy et al., 2025**).

Furthermore, EGCG preserves mitochondrial membrane potential and ATP synthase activity, ensuring adequate energy supply for flagellar beating and sperm motility (**Pérez-Aguirre et al., 2025**). The anti-inflammatory properties of green tea polyphenols also mitigate ibuprofen-induced prostaglandin inhibition and cytokine dysregulation, thereby protecting epididymal function and seminal fluid composition (**Bagherpour et al., 2019**). Additionally, EGCG has been shown to maintain blood-testis barrier integrity through modulation of tight junction proteins and suppression of autophagic cell death (**Wang et al., 2025**).

5.2.3. Spermocytogram analysis (Sperm morphology)

5.2.3.1. Total head abnormalities

The ibuprofen group (IBU) exhibited a dramatic increase in sperm head abnormalities compared to the control group (Figure 27), indicating severe teratogenic effects on spermatogenesis. In contrast, the green tea extract group (GTE) maintained normal levels of morphological integrity comparable to controls. The combined treatment group (IBU + GTE) showed restored sperm head morphology with significantly reduced abnormalities.

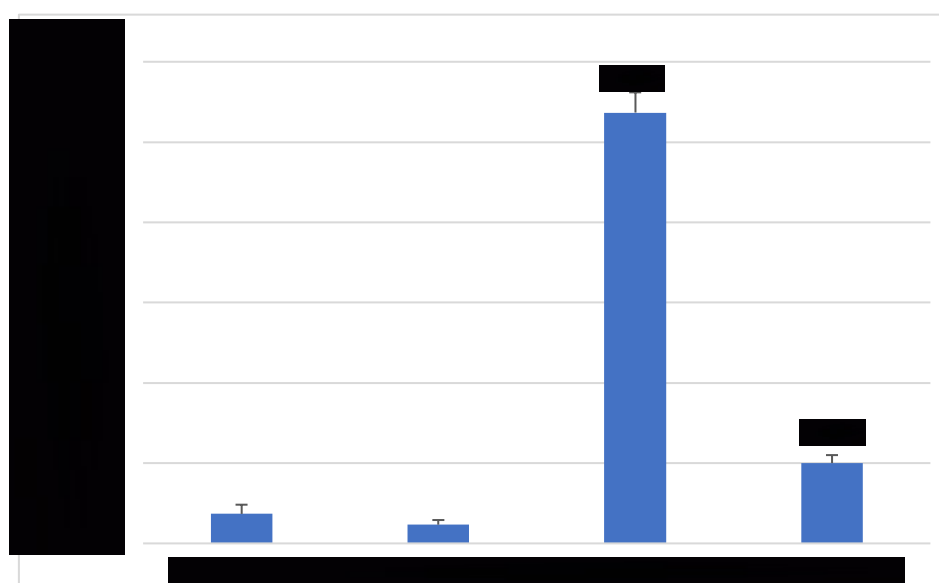


Figure 27. Effect of green tea extract and/or ibuprofen treatment on sperm head abnormalities after 45 days of experience.

a. Head shape and base abnormalities

The green tea extract group (GTE) preserved normal sperm head and base morphological patterns indistinguishable from untreated controls (Figure 28), reflecting intact spermatid differentiation. In contrast, the ibuprofen group (IBU) displayed severe disruption across all morphological parameters, with marked elevations in elongated, thinned, microcephalic, macrocephalic, multiple heads, and abnormal bases, indicating profound impairment of spermatogenesis. Co-administration of green tea with ibuprofen (IBU + GTE) yielded

substantial normalization of these anomalies, with counts approaching baseline values, evidencing the restorative action of tea polyphenols against NSAID-evoked teratogenic damage.

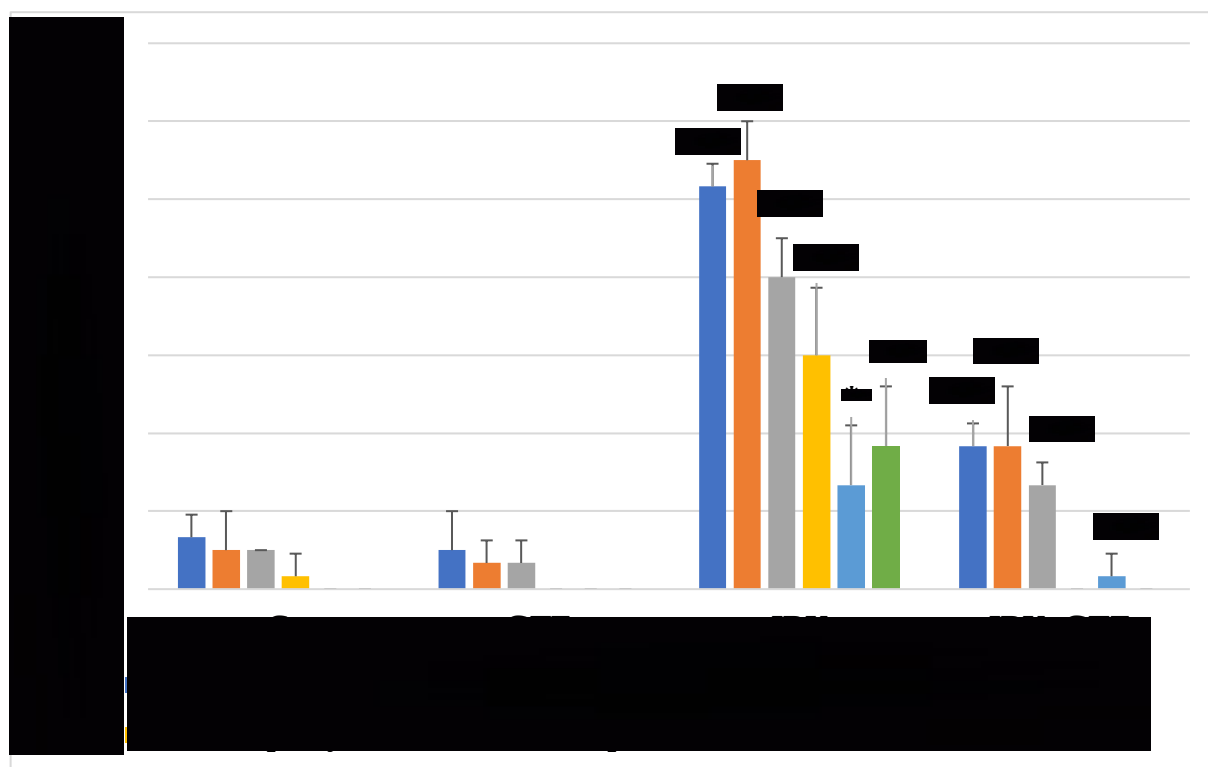


Figure 28. Effect of green tea extract and/or ibuprofen treatment on sperm head shape abnormalities (Count) after 45 days of experience.

b. Acrosomal defects

The control group (C) and green tea extract group (GTE) showed intact acrosomal structures with no detectable defects, reflecting normal sperm maturation. Conversely, the ibuprofen group (IBU) exhibited severe acrosomal damage, with marked elevations in both absent and malformed acrosomes (Figure 29), indicating disruption of the acrosome reaction essential for fertilization. The combined treatment group (IBU + GTE) restored acrosomal integrity.

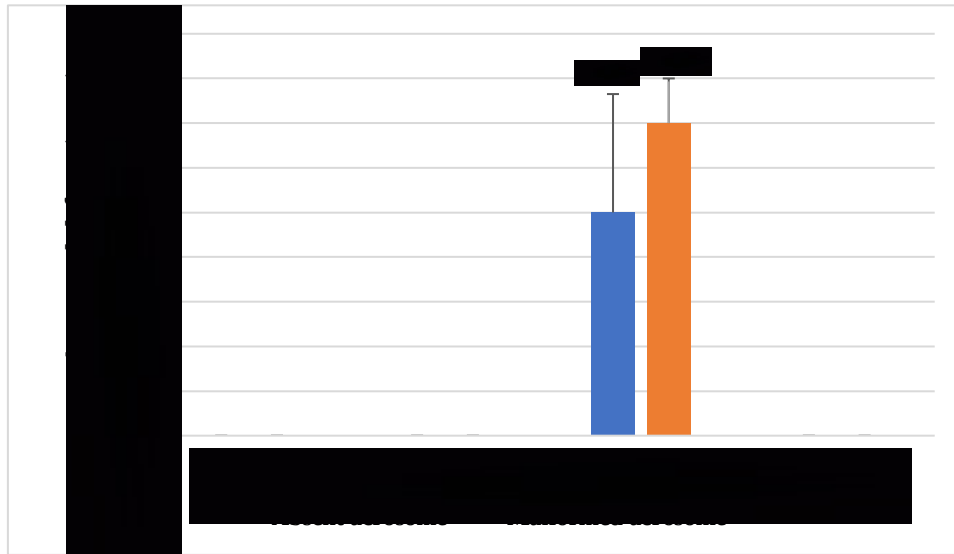


Figure 29. Effect of green tea extract and/or ibuprofen treatment on acrosomal defects after 45 days of experience.

5.2.3.2. Total midpiece abnormalities

The control group (C) and green tea extract group (GTE) maintained normal midpiece architecture with minimal total abnormalities, reflecting intact mitochondrial sheath formation (Figure 30). In contrast, the ibuprofen group (IBU) exhibited severe disruption in midpiece structure, with dramatic elevations in total midpiece abnormalities as well as specific defects including cytoplasmic droplets, thin midpiece, and angulation (bent midpiece) (Figures 30 and 31), indicating profound mitochondrial damage and impaired energy metabolism essential for sperm motility. The combined treatment group (IBU + GTE) showed substantial restoration of midpiece integrity with reduced total abnormalities and normalization across all defect categories, demonstrating the protective efficacy of green tea extract against ibuprofen-induced mitochondrial and structural defects.

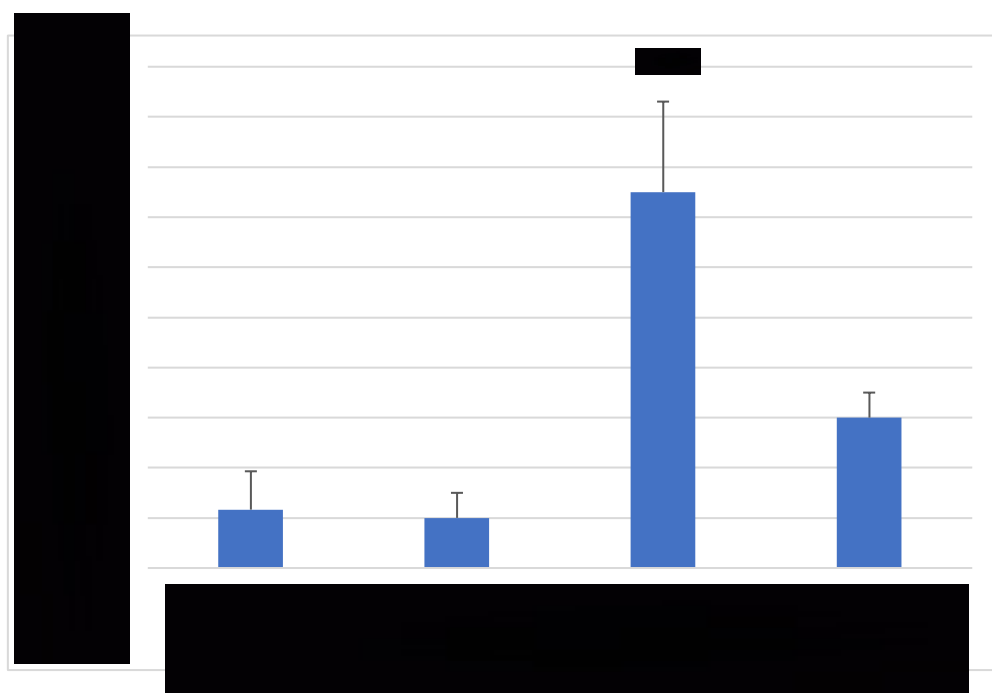


Figure 30. Effect of green tea extract and/or ibuprofen treatment on total midpiece abnormalities after 45 days of experience.

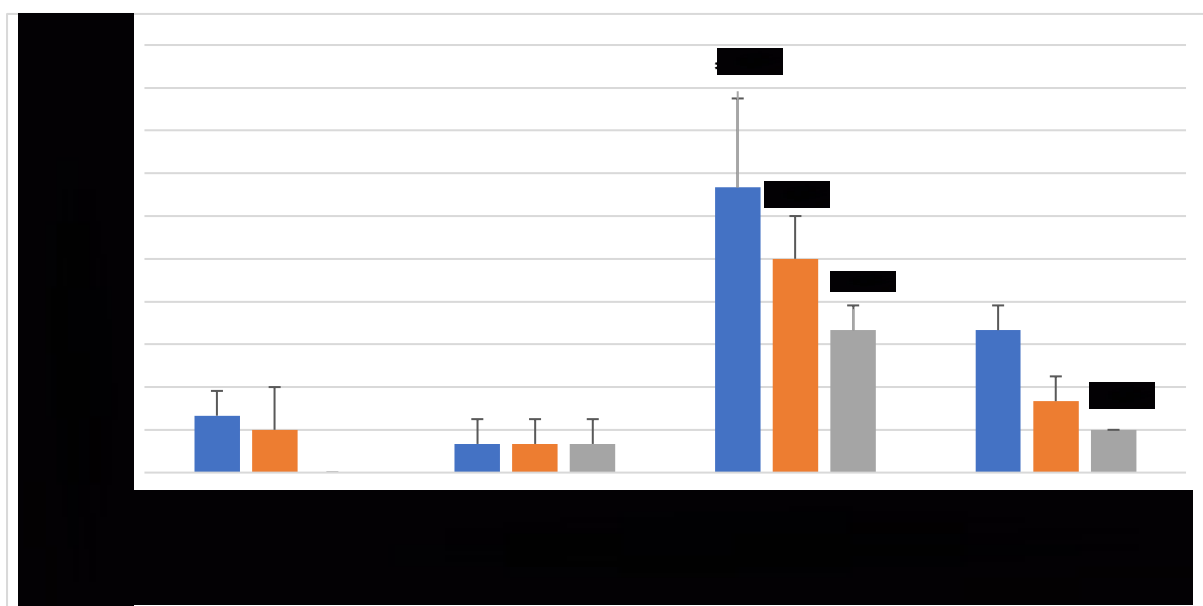


Figure 31. Effect of green tea extract and/or ibuprofen treatment on midpiece defects count after 45 days of experience.

5.2.3.3. Total flagellar abnormalities

Consistent with unimpaired spermiogenesis, the control group (C) and the green tea extract group (GTE) exhibited normal tail architecture with minimal overall flagellar abnormalities (Figure 32). The ibuprofen group (IBU) showed a sharp increase in total tail abnormalities with widespread pathology characterized by absent flagellum, short flagellum and irregular thickness (Figures 32 and 33), indicating widespread disruption of flagellar assembly and axonemal integrity, whereas these groups displayed intact flagella with almost no structural aberrations. These defects severely hinder the ability of sperm to move and fertilize. Total anomalies were significantly reduced and some defect categories returned to almost normal dimensions in the combined treatment group (IBU + GTE), confirming the restorative action of tea catechins against NSAID-induced tail damage and attesting to the wide protective scope of green tea polyphenols over the whole sperm tail.

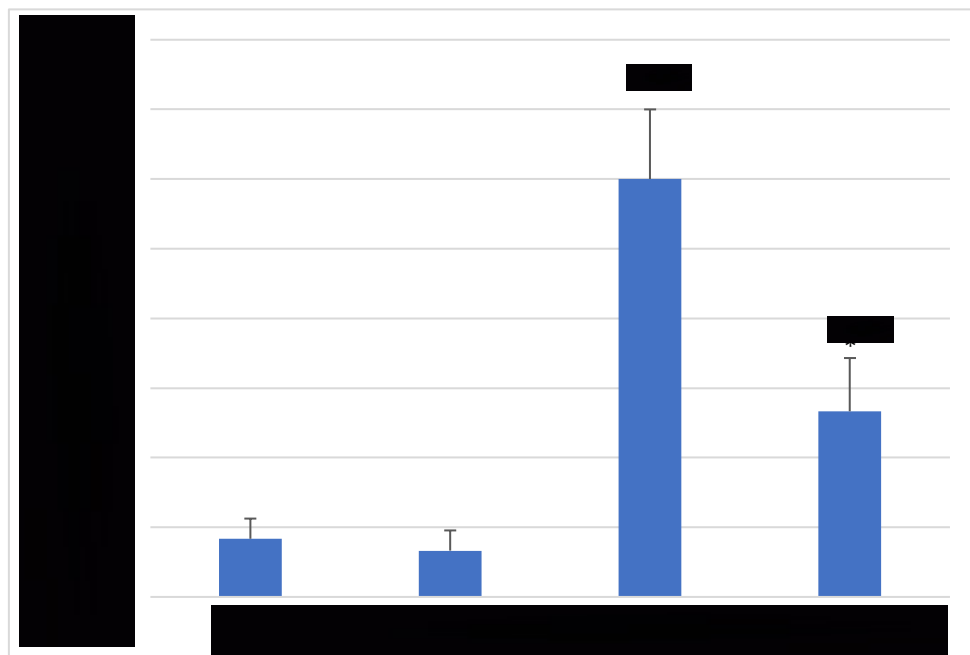


Figure 32. Effect of green tea extract and/or ibuprofen treatment on total flagellar abnormalities after 45 days of experience.

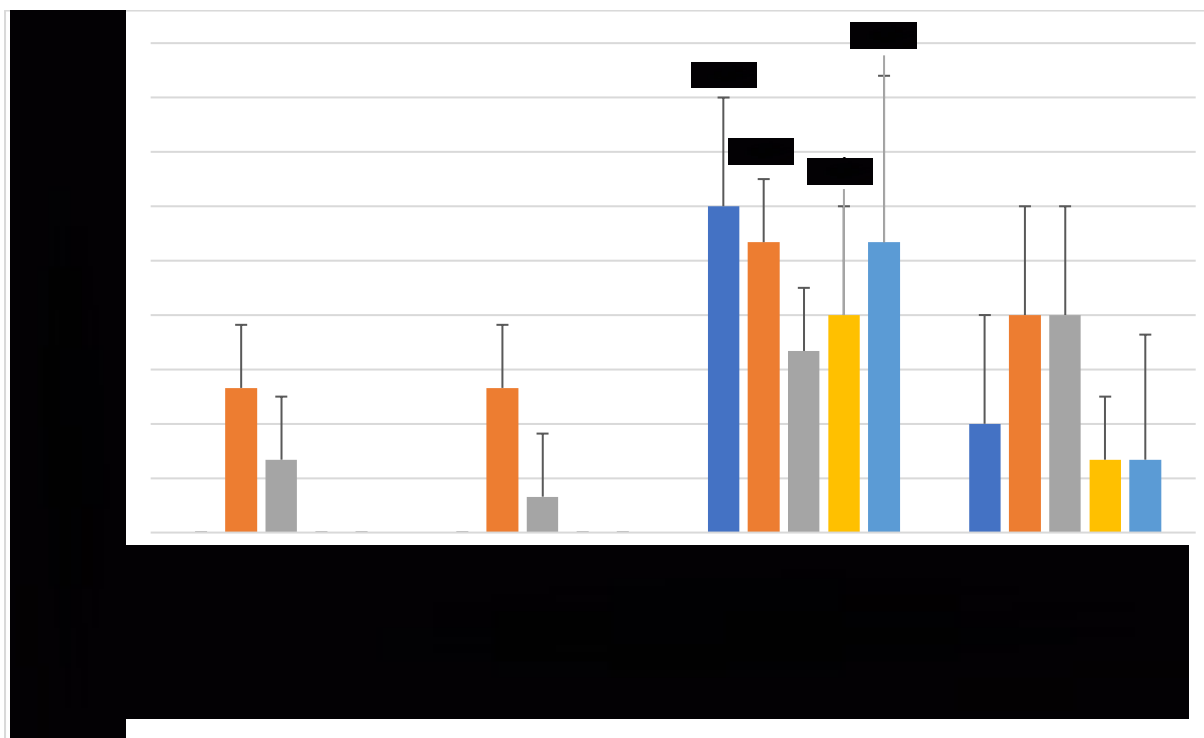


Figure 33. Effect of green tea extract and/or ibuprofen treatment on flagellar defect categories after 45 days of experience.



Figure 34. Microscopic examination of sperm morphology in the Control, GTE, IBU, and IBU+GTE groups A: Control, B: GTE, C: IBU, D: IBU+GTE

Sperm morphology constitutes a critical component of spermocytogramme analysis, referring to the systematic evaluation of spermatozoon structural integrity across three distinct regions: the head (containing the nucleus and acrosome), the midpiece (housing mitochondria for energy production), and the tail (flagellum responsible for propulsion). Normal morphology is defined by strict criteria including oval head shape with intact acrosome covering 40-70% of the head surface, slender midpiece of appropriate length and thickness, and elongated tail without bends or breaks. Any deviation from these criteria—such as microcephaly, macrocephaly, acrosomal defects, cytoplasmic droplets, or flagellar abnormalities—is classified as teratozoospermia, which directly compromises fertilizing capacity by impairing motility, oocyte recognition, and acrosome reaction (**World Health Organization, 2010**).

Disturbed spermatogenesis and faulty spermiogenesis are indicated by the significant increase in sperm head abnormalities seen in the IBU group. Anomalies that arise during nuclear remodeling and sperm maturation are reflected in morphological deformities such as elongated, tapering, microcephalic, macrocephalic, and numerous heads. **Barbosa et al. (2020)** showed reduced daily sperm production along with degradation in sperm quality and testosterone levels following ibuprofen use, indicating a similar reproductive impairment. These results point to decreased testicular function and poor germ cell growth after NSAID exposure.

These anomalies seem to be mostly caused by oxidative damage. According to **Nowicka-Bauer and Nixon (2020)**, elevated reactive oxygen species can target polyunsaturated fatty acids in sperm membranes, causing lipid peroxidation and cytotoxic aldehydes that damage sperm structure and function. Furthermore, according to **Dutta et al. (2019)**, oxidative imbalance has been closely linked to chromatin instability, sperm malfunction, and decreased fertilizing potential. Excessive ROS may impair proper head development and membrane integrity during spermiogenesis since spermatozoa have weak inherent antioxidant defenses.

Because the acrosome is crucial for oocyte penetration and zona pellucida recognition, the anomalies found in the IBU group are particularly concerning. Therefore, the chance for fertilization may be significantly decreased if this structure is deformed or absent. On the other hand, co-administration of green tea extract significantly decreased the incidence of acrosomal and head abnormalities, indicating reduction of oxidative damage caused by ibuprofen.

The IBU group had significantly more midpiece defects, such as cytoplasmic droplets, thinning, and angulation, which may indicate impaired sperm maturation and mitochondrial

function. The mitochondria in the sperm midpiece are in charge of producing the ATP needed for flagellar activity and sperm movement. Consequently, structural changes in this area may impair the ability of sperm to travel and metabolize energy. Similar findings have been linked to reproductive damage caused by oxidative stress. According to **Roodbari et al. (2015)**, oxidative imbalance can damage sperm structural integrity and mitochondrial function, resulting in abnormal sperm morphology and decreased motility. The cytoplasmic droplets seen in this study might be a sign of both poor clearance of leftover cytoplasm during spermiogenesis and insufficient epididymal maturation.

anomalies in the flagella. Sperm propulsive ability is completely eliminated by tail abnormalities, such as missing, short, and unevenly thickened flagella. The axoneme is made up of a distinctive "9+2" microtubule arrangement with dynein arms that use ATP-dependent sliding to produce bending motions. By causing tubulin depolymerization and dynein arm separation, ibuprofen exposure interferes with axonemal assembly (**Kristensen et al., 2018**). Regardless of other factors, spermatozoa cannot pass through the female reproductive system without functioning flagella. According to **Roy et al. (2025)**, EGCG continuously enhances tail integrity in all toxicant scenarios by preventing autophagic cell death and preserving the stability of cytoskeletal proteins. These preventive strategies are consistent with the significant decrease in both particular defect categories and overall flagellar abnormalities in our IBU+GTE group.

It's interesting to note that, in comparison to the IBU group, rats treated with both ibuprofen and green tea extract shown a noticeable improvement in flagellar morphology. **Salehi et al. (2025)** reported similar protective effects of antioxidant compounds against sperm structural damage, showing that antioxidant administration improved sperm morphology and reduced oxidative stress markers in models of toxicant-induced reproductive injury.

5.2.4. Effect of a treatment on oxidative stress parameters

The GSH levels in the control group (C) remained normal, indicating a strong antioxidant defense system. Comparable results were displayed by the green tea extract group (GTE), suggesting that the extract does not negatively impact endogenous glutathione levels in physiological settings. GSH levels, on the other hand, significantly decreased in the ibuprofen group (IBU), indicating a serious depletion of the principal cellular antioxidant resource. The ability of green tea extract to replenish glutathione reserves depleted by ibuprofen-induced

oxidative stress was demonstrated by the combined treatment group (IBU + GTE), which recovered GSH levels to values similar with controls.

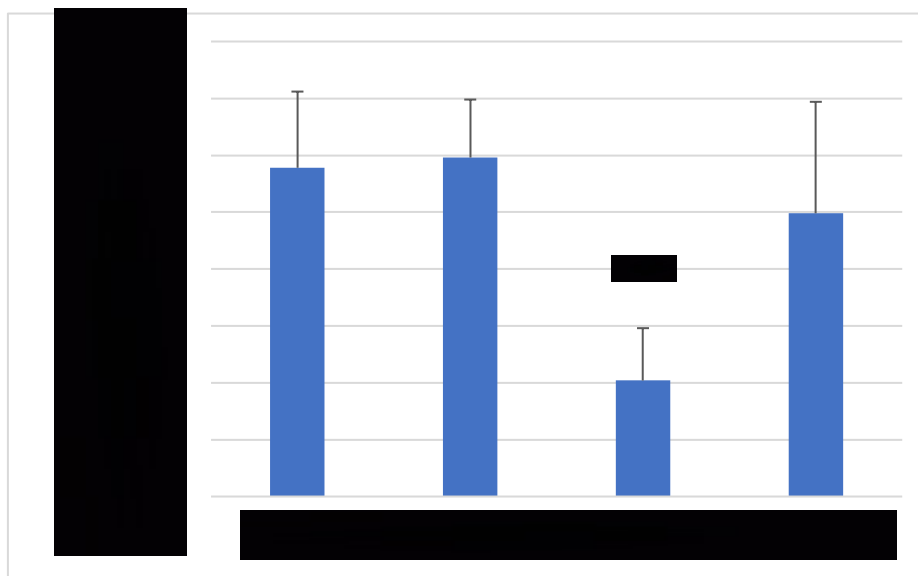


Figure 35. Effect of green tea extract and/or ibuprofen treatment on testicular GSH level after 45 days of experience.

Catalase (CAT) enzyme activity showed clear differences between the experimental groups. The control (C) and green tea extract (GTE) groups recorded similar catalase levels. In contrast, the ibuprofen (IBU) group showed a significant increase compared to the control group, while the ibuprofen + green tea extract (IBU + GTE) group showed a significant decrease compared to the ibuprofen group.

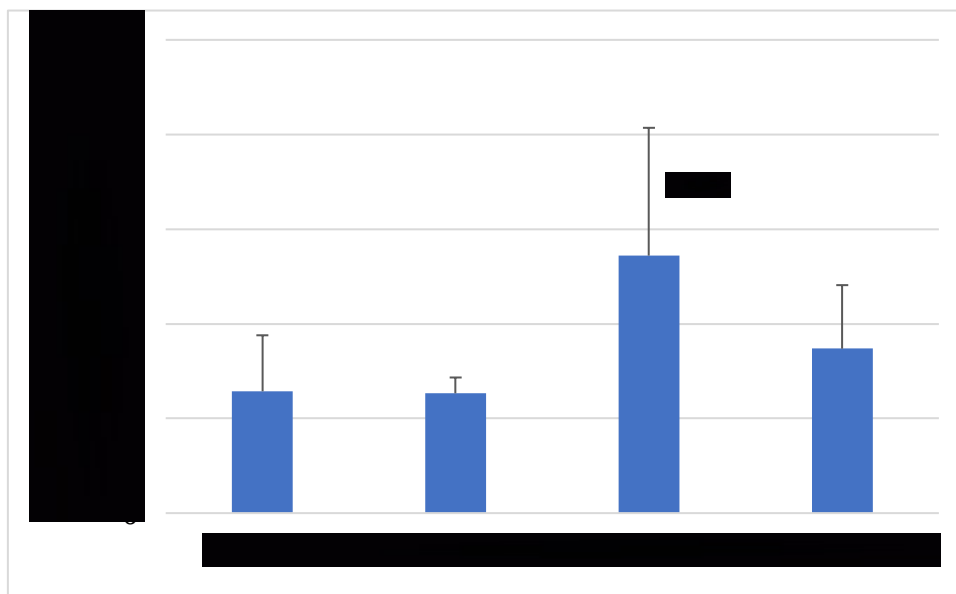


Figure 36. Effect of green tea extract and/or ibuprofen treatment on testicular CAT level after 45 days of experience.

Oxidative stress is a pathological condition resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the organism. Excessive ROS generation can damage lipids, proteins, and nucleic acids, leading to cellular dysfunction and tissue injury. Testicular tissue is particularly susceptible to oxidative stress because of its high rate of cell division, elevated oxygen consumption, and abundance of polyunsaturated fatty acids in sperm cell membranes (**Aitken and Roman, 2008**). Numerous studies have demonstrated that drug-induced reproductive toxicity is closely associated with oxidative stress and disruption of antioxidant defenses (**Noaishi et al., 2016**).

Glutathione (GSH) is one of the most important intracellular non-enzymatic antioxidants. It plays a crucial role in maintaining cellular redox homeostasis, detoxifying reactive oxygen species, and protecting cells against oxidative injury. In addition, GSH participates in the regulation of spermatogenesis and preservation of sperm membrane integrity (**Forman et al., 2009**). A reduction in GSH levels is considered a sensitive marker of oxidative stress and impaired antioxidant capacity in testicular tissue.

In this study showed a significant decrease in GSH levels in the ibuprofen-treated group compared with the control group. These findings indicate that ibuprofen administration induced severe oxidative stress and depletion of intracellular antioxidant reserves. Similar findings were

reported by (Sharma et al., 2020), who demonstrated that ibuprofen exposure significantly reduced testicular antioxidant status and disrupted redox balance. According to Boizet-Bonhoure et al. (2022), the decrease in GSH levels following ibuprofen treatment may result from excessive utilization of glutathione in neutralizing reactive oxygen species and detoxifying lipid peroxidation products generated during oxidative stress.

The depletion of GSH observed in the ibuprofen-treated group may also be associated with increased lipid peroxidation and mitochondrial dysfunction in testicular tissue. Excessive reactive oxygen species can directly attack membrane phospholipids, proteins, and nucleic acids, leading to oxidative cellular damage and excessive consumption of glutathione reserves. Similar observations were reported by R. John Aitken and Shaun D. Roman (2008), who demonstrated that oxidative stress in the testes is strongly associated with lipid peroxidation, impaired antioxidant defenses, and disruption of spermatogenesis. Furthermore, oxidative stress-mediated depletion of glutathione may contribute significantly to testicular degeneration and reduced sperm quality.

Catalase (CAT) is a major enzymatic antioxidant that protects cells against oxidative stress by catalyzing the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. This enzyme plays a critical role in preventing the accumulation of H_2O_2 and limiting the formation of highly reactive hydroxyl radicals capable of inducing severe cellular damage (Nandi et al., 2019). Increased catalase activity is generally considered an adaptive cellular response against excessive reactive oxygen species production.

The elevation of catalase activity observed in the ibuprofen-treated group may also indicate enhanced oxidative metabolism and excessive accumulation of hydrogen peroxide (H_2O_2) in testicular tissue. Increased production of reactive oxygen species can stimulate antioxidant defense mechanisms as a compensatory response against oxidative damage. Similar findings were reported by Nandi et al. (2019), who demonstrated that catalase activity increases under oxidative stress conditions in order to eliminate excessive hydrogen peroxide and protect cells from oxidative injury. Furthermore, He et al. (2017), reported that excessive ROS production activates cellular signaling pathways involved in antioxidant enzyme expression, resulting in increased CAT activity during early oxidative stress conditions.

Conversely, co-administration of green tea extract with ibuprofen markedly restored GSH levels and ameliorate CAT activity compared with the ibuprofen-treated group, suggesting an attenuation of oxidative stress and improvement of antioxidant defense mechanisms. The

antioxidant potential of green tea is mainly related to its high concentration of catechins, especially epigallocatechin gallate (EGCG), known for their potent free radical scavenging activity and inhibitory effects on lipid peroxidation. Similar findings were reported by **Khan and Mukhtar (2019)**, who demonstrated that green tea polyphenols contribute to the maintenance of cellular redox balance through suppression of ROS production and enhancement of endogenous antioxidant defenses. Furthermore, **Forester and Lambert (2011)** indicated that green tea catechins help preserve intracellular glutathione levels and protect tissues against oxidative damage. Protective effects of green tea extract against reproductive oxidative stress were also described by **Ramadan et al. (2021)**, who reported improvement of antioxidant status and reduction of lipid peroxidation in testicular tissue.



Conclusion and Perspectives



In conclusion, the findings of the present study provide strong evidence that chronic exposure to ibuprofen induces significant testicular toxicity associated with oxidative stress and reproductive dysfunction in male Wistar rats. Ibuprofen administration resulted in marked disturbances in antioxidant status, as demonstrated by depletion of glutathione levels and elevation of catalase activity, indicating severe oxidative stress and disruption of cellular redox balance. In addition, ibuprofen adversely affected reproductive parameters, including testosterone secretion, sperm quality, reflecting impaired spermatogenesis and testicular dysfunction.

Co-administration of green tea extract remarkably attenuated most of the biochemical, oxidative, spermatic, hormonal induced by ibuprofen. The protective effect of green tea may be attributed to its richness in bioactive compounds, particularly catechins and polyphenols, known for their potent antioxidant, free radical scavenging, and cytoprotective activities. Green tea supplementation restored antioxidant balance, improved sperm parameters and testosterone levels, and preserved the structural integrity of testicular tissue.

These findings highlight the therapeutic potential of green tea as a natural antioxidant agent capable of reducing drug-induced reproductive toxicity and oxidative stress. The study also emphasizes the importance of natural phytotherapeutic compounds in protecting reproductive health against the harmful effects of commonly used pharmaceutical agents such as ibuprofen.

Future investigations should focus on identifying the precise molecular mechanisms involved in the protective effects of green tea extract on testicular tissue. Additional studies are also needed to isolate and characterize the specific bioactive compounds responsible for these beneficial effects and to evaluate their long-term safety and efficacy in clinical applications. Furthermore, exploring the potential synergistic effects between green tea polyphenols and other natural antioxidants may contribute to the development of effective therapeutic strategies against oxidative stress-related reproductive disorders.



Bibliographic References





A

- Aebi, H. (1984). Catalase in Vitro. In: Packer, L. (Ed.), *Methods in Enzymology*, Vol. 105, Academic Press, New York, pp. 121–126.
- Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M. K., & Shah, R. (2021). Male infertility. *The Lancet*, 397(10271), 319–333.
- Agarwal, A., Virk, G., Ong, C., & Du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health*, 32(1), 1–17.
- Aitken, R. J., & Baker, M. A. (2020). The role of oxidative stress in male infertility. *Andrology*, 8(4), 915–924.
- Aitken, R. J., & Roman, S. D. (2008). Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity*, 1(1), 15–24.
- Azizi, M., & Mehranjani, M. S. (2019). Protective effects of green tea extract on testicular damage induced by para-nonylphenol in adult rats. *International Journal of Reproductive BioMedicine*, 17(6), 409–418.
- Azizi, P., & Mehranjani, M. S. (2019). The effect of green tea extract on sperm parameters and histological changes of testis in rats exposed to para-nonylphenol. *International Journal of Reproductive BioMedicine*, 17(10), 717–726.



B

- Bagherpour, H., Karimpour Malekshah, A., Talebpour Amiri, F., & Azadbakht, M. (2019). Protective effect of green tea extract on deltamethrin-induced toxicity in mice testis: An experimental study. *International Journal of Reproductive BioMedicine*, 17(5), 337–348.
- Banihani, S. A. (2017). Effect of nonsteroidal anti-inflammatory drugs on male fertility. *Journal of Clinical Medicine Research*, 9(4), 275–284. <https://doi.org/10.14740/jocmr2937w>
- Barbosa, A.M., et al. (2020). Effects of ibuprofen on testicular development and sperm parameters in pre-pubertal rats. *Int. J. Mol. Sci.*, 21(18), 6789.

- Boizet-Bonhoure, B., Déjardin, S., Rossitto, M., Poulat, F., & Philibert, P. (2022). Using Experimental Models to Decipher the Effects of Acetaminophen and NSAIDs on Reproductive Development and Health. *Frontiers in Toxicology*, 4, 835360.
- Bushra, R., & Aslam, N. (2010). An overview of clinical pharmacology of ibuprofen. *Oman Medical Journal*, 25(3), 155–161.

C

- Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea: A review. *Journal of the American College of Nutrition*, 25(2), 79–99.
- Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: A literature review. *Chinese Medicine*, 5, 13. <https://doi.org/10.1186/1749-8546-5-13>
- Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182.
- Chang, K. (2015). World tea production and trade: Current and future development. Food and Agriculture Organization of the United Nations (FAO), Intergovernmental Group on Tea. Rome, Italy.

D

- Davies, N. M. (2021). Clinical pharmacokinetics of ibuprofen. *Clinical Pharmacokinetics*, 60(6), 679–690.
- Dayyih, W. A., et al. (2019). Evaluation of ibuprofen-induced reproductive toxicity in male rats. *Asian Journal of Pharmaceutical and Clinical Research*.
- Derry, C. J., Derry, S., & Moore, R. A. (2020). Single dose oral ibuprofen for acute postoperative pain in adults. *Cochrane Database of Systematic Reviews*, 2020(7), CD001548.

- Dutta, S., Majzoub, A., & Agarwal, A. (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab Journal of Urology*, 17(2), 87–97.

F

- Forester, S. C., & Lambert, J. D. (2011). The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention. *Molecular Nutrition & Food Research*, 55(6), 844–854.
- Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular Aspects of Medicine*, 30(1–2), 1–12.

G

- Guimarães-Ervilha, L. O., Ladeira, L. C. M., Carvalho, R. P. R., Bento, I. P. S., Bastos, D. S. S., Souza, A. C. F., Santos, E. C., Oliveira, L. L., Maldonado, I. R. S. C., & Machado-Neves, M. (2021). Green tea infusion ameliorates histological damages in testis and epididymis of diabetic rats. *Microscopy and Microanalysis*.

H

- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-Transferases. *Journal Of Biological Chemistry* / The α Journal of Biological Chemistry, 249(22), 7130–7139.
- Hassan, E., et al. (2019). The protective effect of epigallocatechin-3-gallate on testicular oxidative stress in lead-induced toxicity. *Toxicology*, 421, 152–163.
- He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*, 44(2), 532–553.
- Hughes, C. M., McElnay, J. C., & Fleming, G. F. (2016). Benefits and risks of self-medication. *Drug Safety*, 39(1), 45–55. <https://doi.org/10.1007/s40264-015-0368-6>

K

- Khan, N., & Mukhtar, H. (2013). Tea and health: Studies in humans. *Current Pharmaceutical Design*, 19(34), 6141–6147. <https://doi.org/10.2174/1381612811319340008>
- Kristensen, D. M., Desdoits-Lethimonier, C., Mackey, A. L., Dalgaard, M. D., De Masi, F., Munkbøl, C. H., Styris have, B., Antignac, J. P., Le Bizec, B., Platel, C., Hay-Schmidt, A., Jensen, T. K., Brunak, S., Skakkebaek, N. E., & Jégou, B. (2018). Ibuprofen alters human testicular physiology to produce a state of compensated hypogonadism. *Proceedings of the National Academy of Sciences of the United States of America*, 115(4), E715–E724.



- Liu, M., Ning, Z., Cheng, Y., Zheng, Z., Yang, X., Zheng, T., Li, N., & Wu, J. L. (2024). The key to 2,6-dichloro-1,4-benzoquinone reproductive toxicity and green tea detoxification: Covalent binding and competitive binding. *Ecotoxicology and Environmental Safety*, 286, 117239.



- Mahmoudi, R., Azizi, A., Abedini, S., Jahromi, V. H., Abidi, H., & Barnak, M. J. (2018). Green tea improves rat sperm quality and reduces cadmium chloride damage effect in spermatogenesis cycle. *Journal of Medicine and Life*, 11(4), 371–380.
- Mahmoudi, R., Fakhri, S., & Asadi, S. (2018). Protective effects of green tea on sperm quality and testicular oxidative stress in cadmium-exposed rats. *Andrologia*, 50(9), e13076.
- Matzkin, M. E., Mayerhofer, A., & Frungieri, M. B. (2012). The role of cyclooxygenase in testicular physiology and pathology. *Reproduction*, 144(2), 151–161.
- Musiał, C., Kuban-Jankowska, A., & Gorska-Ponikowska, M. (2020). Beneficial properties of green tea catechins. *International Journal of Molecular Sciences*, 21(5), 1744.



- Nandi, A., Yan, L. J., Jana, C. K., & Das, N. (2019). Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative Medicine and Cellular Longevity*, 2019, 9613090.
- Noaishi, M. A., & Abd alhafez, H. H. (2016). Hepatotoxicity and nephrotoxicity evaluation after repeated dose of acetamiprid in albino rats. *Egyptian Journal of Chemistry and Environmental Health*, 2(2), 439–452.
- Nowicka-Bauer, K., & Nixon, B. (2020). Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants*, 9(2), 134.



O

- O'Donnell, L., Stanton, P. G., & de Kretser, D. M. (2022). Endocrinology of the male reproductive system and spermatogenesis. *Endotext*.
- Onuora, I.J., et al. (2025). Green tea's impact on fertility hormones and oxidative stress markers in obese males with different gonadal statuses in Onitsha, Nigeria. *Ghana Med. J.*, 59(4), 1-10.



P

- Pérez-Aguirre, S.G., Viguera-Villaseñor, R.M., Bonilla-Jaime, H., Montes, S., Galván-Arzate, S., Hernández-Rodríguez, J., Marín de Jesús, S., Carrizales-Yañez, L., Rojas-Castañeda, J.C., & Arteaga-Silva, M. (2025). Epigallocatechin-3-Gallate Enhances Antioxidant Activity and Improves Testicular and Epididymal Histology in Cadmium-Exposed Prepubertal Rats. *International Journal of Molecular Sciences*, 26(17), 8264.



R

- Rainsford, K. D. (2009). Ibuprofen: Pharmacology, efficacy and safety. *Inflammopharmacology*, 17(6), 275–342.

- Ramadan, R., El-Sayed, Y., & Hassan, A. (2021). Protective effect of green tea extract against experimentally induced oxidative stress and reproductive toxicity. *Andrologia*, 53(3), e13912.
- Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31(5), 986–1000.
- Rivière, C., Richard, T., Quentin, L., Krisa, S., Mérillon, J. M., & Monti, J. P. (2017). Prostaglandins and male reproduction: Physiological roles. *Andrology*, 5(1), 11–23.
- Roodbari, F., Nikbakht, R., & Sahranavard, S. (2015). Effects of ibuprofen on sperm parameters in mice. *Iranian Journal of Reproductive Medicine*, 13(10), 629-634.
- Roy, N., Borle, S., & Singh, D. (2025). Modulatory role of epigallocatechin-3-gallate (EGCG) on male and female reproductive disorders. *Reprod. Toxicol.*, 138, 109049.

S

- Salehi, F., Rajabzadeh, O., Zarei, L., & Mokhayeri, Y. (2025). The Effect of Hydroalcoholic Extract of *Salvia miltiorrhiza* on Hormonal and Cellular Parameters of Spermatogenesis in Male Rats After the Consumption of Ibuprofen. *Food Sci. Nutr.*, 13(8), e70705.
- Sharma, P., Huq, A. U., & Singh, R. (2020). Ibuprofen-induced reproductive toxicity and oxidative stress in male rats. *Reproductive Toxicology*, 93, 1–10.
- Sharma, R., et al. (2020). Oxidative stress and reproductive toxicity associated with non-steroidal anti-inflammatory drugs. *Reproductive Toxicology*.
- Sies, H., Jones, D. P., & Bermudez Brito, M. (2022). Oxidative stress. *Annual Review of Biochemistry*, 91, 715–748. <https://doi.org/10.1146/annurev-biochem-032620-104801>
- Singh, B. N., Shankar, S., & Srivastava, R. K. (2011). Green tea catechins: Their role in health and disease. *Life Sciences*, 78(18), 2073–2080.

T

- Tiwari, S., Mishra, M., Salemi, M. R., Phinney, B. S., Newens, J. L., & Gomes, A. V. (2020). Gender-specific changes in energy metabolism and protein degradation as major

pathways affected in livers of mice treated with ibuprofen. *Scientific Reports*, 10(1), 3386.

- Tremellen, K. (2008). Oxidative stress and male infertility. *Human Reproduction Update*, 14(3), 243–258.

V

- Vane, J. R., & Botting, R. M. (2003). The mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs. *Thrombosis Research*, 110(5–6), 255–258.
- Venkatesh, S., Singh, A., Shamsi, M. B., Thilagavathi, J., Kumar, R., Mitra, D. K., & Dada, R. (2011). Clinical implications of oxidative stress in male infertility. *Urology*, 78(4), 809–814.

W

- Walker, W. H. (2021). Testosterone signaling and the regulation of spermatogenesis. *Biology of Reproduction*, 104(3), 496–509. <https://doi.org/10.1093/biolre/ioaa219>
- Wallace, J. L. (2008). Prostaglandins, NSAIDs, and gastric mucosal protection: Why doesn't the stomach digest itself? *Physiological Reviews*, 88(4), 1547–1565.
- Wang, Q., Su, Y., Yang, Q., Beta, T., Shen, F., & Liu, Q. (2025). EGCG attenuates BPA-induced male reproductive toxicity by regulating the blood-testis barrier by suppressing autophagy via AMPK/AKT/mTOR signaling pathway. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 242, 110111.
- Weckbercker G, Cory JG. Ribonucleotide reductase activity and growth of glutathione-dependent mouse leukaemia L 1210 cells in vitro. *Cancer* 1988;40:257- 264

Y

- Yang, C. S., & Wang, H. (2016). Cancer preventive activities of tea catechins. *Molecules*, 21(12), 1679.
- Yassa, H. A., George, S. M., Refaiy, A. E. R. M., & Abdel Moneim, E. M. (2013). *Camellia sinensis* extract attenuates acrylamide-induced testicular damage in albino rats. *Environmental Toxicology*.



Z

- Zadkhosh, N., Tavakol, H. S., Khosroshahi, A. G., Mosavi, L., Mehri, N., Felegari, H., & Ranjbar, A. (2016). Protective role of green tea on malathion-induced testicular oxidative damage in rats. *Asian Pacific Journal of Reproduction*, 5(1), 42–45.
- Zhao, T., Li, C., Wang, S., & Song, X. (2022). Green Tea (*Camellia sinensis*): A Review of Its Phytochemistry, Pharmacology, and Toxicology. *Molecules*, 27(12), 3909.

Webographic

- DrugBank Online. (2023). Ibuprofen. DrugBank database.
- International Tea Committee (ITC). (2021). Annual bulletin of statistics. London: International Tea Committee.
- PubChem. (2023). *Ibuprofen* (CID 3672). National Center for Biotechnology Information (NCBI).
- World Health Organization. (1992). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction (3rd ed.). Cambridge University Press, Cambridge.
- Food and Agriculture Organization (FAO). (2022). *FAOSTAT statistical database: Crops and livestock products (Tea production)*.

