A DISSERTATION ON

"SUB-CHRONIC EXPOSURE ASSESSMENT OF THE HISTOPATHOLOGICAL CHANGES IN THE LUNGS INDUCED BY ADMINISTRATION OF ACETAMINOPHEN IN MICE"

SUBMITTED TO THE
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
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DECLARATION FORM

I, **MOHD RAZI**, a student of **M. Tech Biotechnology** (II Year/ IV Semester), Integral University have completed my six months dissertation work entitled **"SUB-CHRONIC EXPOSURE ASSESSMENT OF HISTOPATHOLOGICAL CHANGES IN THE LUNG INDUCED BY ADMINISTRATION OF ACETAMINOPHEN IN MICE"** successfully from **CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH, CRK CAMPUS, SAROJNI NAGAR, LUCKNOW** under the able guidance of **DR. ANJANEYA AYANUR.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date
MOHD RAZI

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DR. SALMAN AKHTAR

CERTIFICATE

This is to certify that Mr. **MOHD RAZI** (Enrollment Number 1700100543) has carried out the research work presented in this thesis entitled **"SUB-CHRONIC EXPOSURE ASSESSMENT OF HISTOPATHOLOGICAL CHANGES IN THE LUNG INDUCED BY ADMINISTRATION OF ACETAMINOPHEN IN MICE"** for the award of **M. Tech Biotechnology** from **CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH, CRK CAMPUS, SAROJNI NAGAR, LUCKNOW** under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of his **M. Tech Biotechnology.**

I wish him good luck and bright future.

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I wish him good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish him good luck and bright future.

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MOHD RAZI

ABBREVIATIONS USED

COPD	Chronic obstructive pulmonary disease
PCBs	Polychlorinated biphenyls
ARDS	Acute respiratory distress syndrome
ILD	Interstitial lung disease
HP	Hypersensitivity pneumonitis
CWP	Coal worker's pneumoconiosis
COX	Cyclooxygenase
NSAIDs	Non-steroidal anti-inflammatory drugs
NAC	N-acetyl cysteine
IHC	Immune Histochemistry
H&E	Haematoxylin & Eosin
Mg	Milligram
Gm	Gram
Mm	Millimetre
Um	Micrometre
Ml	Milligram
L	Liter
e.g.,	For example,

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1 INTRODUCTION

The main respiratory organs in humans and most other animals are the lungs. They have a conical form with three surfaces, three boundaries, and an apex at the top of their structure (Suresh & Shimoda, 2016).

Human beings have two lungs. Within the thoracic cavity of the chest, humans have both a left and a right lung. These lungs are positioned on either side of the heart, next to the backbone. The right lung is divided into three lobes: upper, middle, and lower. The left lung, like the right, lacks a middle lobe. The left lung is made up of the upper and lower lobes. Male lungs typically weigh 155g to 720g in the right lung and 110g to 675g in the left lung, whereas female lungs weigh between 100g and 590g in the right and left lungs. (Weibel, 2017).

The fundamental functions of the lungs include two critical activities: eliminating carbon dioxide from the bloodstream and assimilating oxygen from the atmosphere. The complete procedure is referred to as gas exchange. Moreover, the lungs also play a crucial role in enabling human speech by facilitating the necessary airflow (Lalley, 2013).

The entire respiratory system, consisting of the trachea, bronchi, and bronchioles, is covered by a ciliated respiratory epithelium that houses mucus-secreting goblet cells.

Deep inside the lungs lie countless alveoli, small hollow chambers affectionately called air spaces or air sacs, serving as vital hubs for the exchange of oxygen and carbon dioxide. Making up roughly 90% of the total lung volume, the lung parenchyma forms the essential, functional fabric of the lungs (Gualtieri et al., 2019; Tawhai & Lin, 2010).

Inside the lungs, a vast diversity of microbial communities is found & interact with the airway epithelial cells and are crucial for preserving homeostasis. They include fungi, viruses, bacteria, and bacteriophages. *Streptococcus*, *Sphingomonas, Staphylococcus, Fusobacterium, Megasphaera, Acinetobacter, Veillonela, Pseudomonas,* and *Prevotella* are the main microorganisms. These bacteria interact with the airway epithelial cells and are crucial for preserving homeostasis. Aspergillus, Saccharomyces, Candida, and Malassezia are the most prevalent fungi identified in the lung (Wypych et al., 2019).

A substance is toxic if it has the potential to be harmful or have negative effects on health. Any chemical has the potential to be poisonous or destructive in specific situations.

Individuals often experience stress due to the presence of chemicals such as polychlorinated biphenyls (PCBs) and dioxin found in certain hazardous waste sites. Daily use items home cleaners, prescription and over-the-counter drugs, alcohol, petrol, pesticides, fuel oil, and cosmetics are all potentially hazardous. (Laskin et al., 2019; Yang et al., 2022). A single or brief exposure with a hazardous substance can have a detrimental influence on an organism, which is known as acute toxicity. Sub-chronic toxicity refers to a hazardous agent's ability to cause effects that persist more than a year but less than the lifetime of the exposed organism. Chronic toxicity refers to a chemical compound or a group of compounds' ability to induce detrimental effects over a lengthy period of time, often as a result of prolonged or continuous exposure, sometimes covering the entire lifespan of the afflicted organism.

Pulmonary toxicity is the damage of the lungs, lung tissues, arteries or veins that is also called lung toxicity

When lungs can return to normal stage after a short period when the toxicity resolved called acute pulmonary toxicity and when damage to the lungs is permanent or long-lasting called chronic pulmonary toxicity.

Acetaminophen was first of all invented in 1878 by H. N. Morse. In the United States and Japan, this drug is called Acetaminophen but in Europe and the majority of the world, it is known as paracetamol.

Although acetaminophen/paracetamol is taken in billions of dosages annually its route of action is still unknown. In the 1970s and again in the 2000s, it was thought that its mechanism had been found, but none of these hypotheses stood up to investigation (Botting & Ayoub, 2005; D'Arcy et al., 2021).

Acetaminophen is a pain reliever that is used to treat moderate to severe pain, as well as mild to moderate discomfort and fever. Among the common ailments treated are muscular pains, backaches, arthritis, sore throats, toothaches, flu, colds, headaches, and fevers. (Jacob et al., 2023).

Acetaminophen is both analgesic and antipyretic. According to the evidence, this medicine does not have anti-inflammatory qualities. Unlike the salicylate drug family, acetaminophen does not disrupt tubular secretion of uric acid when used at the

recommended doses, and it has no effect on acid-base balance. (Forrest et al., 1982; Macintyre et al., 2022).

The aim of this research is to investigate the histopathological changes in the lung resulting from chronic exposure to acetaminophen in mice. The study aims to elucidate the potential pulmonary effects of prolonged acetaminophen administration and contribute to a better understanding of its safety profile, particularly in terms of lung tissue alterations.

The objectives are the following that are the main purpose of this study.

1. To determine the histopathological alterations in the lung tissue of mice following sub-chronic exposure to acetaminophen.
2. To identify specific lung pathologies, such as inflammation, fibrosis, or cellular damage, resulting from prolonged acetaminophen exposure.

2 REVIEW OF LITRERATURE

2.1 Toxicity

Toxicity poses a significant threat to both the environment and public health. This study delves into its complexities, exploring different forms, sources, and impacts on humans and ecosystems. Toxicity refers to a substance's ability to harm living organisms, causing negative health effects and environmental damage. These harmful substances can come in various forms, such as chemicals, pollutants, and biological agents(Levin & Buccafusco, 2006).

The main sources of toxicity, including both natural factors like toxins from plants and animals, and human-induced factors like industrial processes and agriculture. It also covers emerging toxins like electronic waste and nanomaterials(Al-Mahayri et al., 2021).

Toxicity can harm human health in various ways, from acute poisonings to chronic illnesses, especially affecting vulnerable populations like children. It also has profound implications for ecosystems, affecting soil, water, air quality, and wildlife, with the potential for bioaccumulation and biomagnification in the food chain(Kanno, 2016).

The risk assessment process is crucial for managing toxicity, involving methods to determine substance toxicity and establish regulatory standards for protection. However, there are difficulties and controversies surrounding toxicity regulation. To combat toxicity effectively, several strategies have been developed, including prevention measures, waste management, pollution control technologies, and adopting greener alternatives. A precautionary approach is essential when dealing with potentially toxic substances(Birnbaum & Miller, 2015; Kanno, 2016).

The study presents case studies of toxicity incidents worldwide, analyzing causes, effects, and lessons learned from industrial accidents, chemical spills, and contamination outbreaks(Ellis, 1985).

Toxicity is a multifaceted problem requiring immediate attention. By understanding its various forms, sources, and effects, we can work together to create a safer and more sustainable environment for present and future generations. Responsible decision-making and collaboration are key to mitigating its negative impacts on the environment and human health (Levin & Buccafusco, 2006; Schep et al., 2009).

2.1.1 Chemical toxicity

Chemical toxicity is a crucial area of environmental and health sciences, studying how various chemical substances harm living organisms. These toxicants can include industrial chemicals, pesticides, heavy metals, pharmaceuticals, and natural toxins from plants and organisms. The impacts of chemical toxicity can be extensive, posing dangerous to human life and wildlife. Understanding how are toxicants interact with living systems is vital for assessing and mitigating hazards(Mackay et al., 2017; Zhu et al., 2014).

Toxicants enter the body by various routes, The effects is mainly depend in duration of exposure, genetics, and overall health. Chemical toxicity can lead to acute or chronic health issues, from skin irritations to organ damage, cancer, and neurological disorders. Certain chemicals can disrupt hormones and reproduction(Green et al., 2021; Kar & Leszczynski, 2019).

It also harms ecosystems and wildlife, reducing biodiversity and creating ecological imbalances in contaminated water, air, and soil. To protect public health and the environment, regulatory agencies and researchers assess chemical hazards, conduct studies, and seek safer alternatives. Risk assessment helps set safe exposure guidelines(Koontz et al., 2019; Lee et al., 2019). It's essential to adopt sustainable practices and policies to minimize exposure to harmful substances. By managing chemical hazards effectively, we can create a safer, healthier world for current and future generations(Mumtaz et al., 1998).

2.1.2 Environmental toxicity

Environmental toxicity is a crucial area of study focusing on chemicals' and pollutants' negative impact on the environment and living creatures (Wu et al., 2016). It aims to identify and mitigate threats to biodiversity, ecosystem health, and human well-being. Human activities have heavily impacted the environment by releasing pollutants from various sources like industries, agriculture, and waste disposal, making environmental toxicity a pressing concern at local and global levels(Chisti, 2004; Q. Yang et al., 2021).

Toxicants can enter the environment through direct releases, spills, or atmospheric deposition and persist there for a long time. They can spread across vast distances, affecting even pristine areas far from the original contamination site. The consequences of environmental toxicity are numerous, including ecological imbalances, reduced

biodiversity, and contaminated water and air(L. Wang et al., 2021). Research in this field involves studying how toxicants behave in the environment, their effects on different species, and long-term impacts of chronic exposure(Lei et al., 2018; X. Wang et al., 2019). Addressing environmental toxicity requires a multidisciplinary approach involving toxicology, ecology, chemistry, and environmental science. Governments, international organizations, and advocacy groups play a crucial role in regulating and controlling pollutant emissions(Foster, 2003). Implementing sustainable practices, cleaner technologies, and efficient waste management are vital to minimize environmental toxicity and ensure the well-being of human life and ecosystems(Dobaradaran et al., 2021; Petriello et al., 2014).

2.1.3 Biological Toxicity

Biological toxicity, also known as toxicity, studies how various substances can harm living organisms, including microorganisms, plants, animals, and humans. It explores how toxic agents interact with biological systems, leading to harmful effects and health risks(L. E. Black et al., 1999).

Toxicity can arise from natural compounds found in plants and animals, as well as synthetic chemicals, heavy metals, pesticides, pharmaceuticals, and environmental pollutants. Understanding biological toxicity is crucial for protecting human health, biodiversity, and promoting safe practices(Hazelden, 2013; Rao et al., 2019).

Toxic substances can enter organisms through inhalation, ingestion, or skin absorption, causing damage to tissues, disrupting biological processes, and affecting cells. The severity of toxicity depends on factors such as dose, exposure duration, and individual susceptibility(Jing et al., 2022).

Acute toxicity results from immediate and severe effects due to high short-term exposure, while chronic toxicity occurs from prolonged exposure to lower levels, leading to cumulative damage and delayed health issues like cancer and neurological impairments(Ojulari et al., 2019; Patil et al., 2019).

Different organisms respond differently to toxicants due to variations in metabolism, physiology, and genetics. Researchers investigate species-specific responses to assess risks to humans and wildlife(Dasari et al., 2022).

The study of biological toxicity involves toxicology, pharmacology, biochemistry, and molecular biology. Scientists use experimental approaches, animal studies, in vitro tests, and computational models to evaluate toxicity and predict effects on living organisms(W. M. Wang & Jin, 2020).

2.1.4 Occupational Toxicity

Occupational toxicity, also called workplace toxicity, is a branch of occupational health that investigates potential chemical, physical, and biological substances have negative consequences encountered at work. It aims to protect employees' well-being by identifying, assessing, and controlling risks in various industries(Latza & Baur, 2005). Employees may encounter toxic substances like hazardous chemicals, dust, fumes, gases, and physical hazards such as noise and radiation. These substances can enter the body by inhalation, skin absorption, or ingestion, causing immediate or persistent health problems. (De Basea et al., 2011; Jensen et al., 2011).

The occupational toxicity involves understanding exposure routes and how toxic substances affect the body. Workers in different industries may experience a range of health effects, from mild irritations to severe conditions like cancer(Kim et al., 2020).

Certain occupations, like mining, construction, healthcare, and chemical production, face higher risks of exposure(Descatha et al., 2005). To mitigate these risks, governments set safety regulations, and employers must implement safety measures, provide protective equipment, and conduct risk assessments. Occupational health professionals assess workplace toxicity, conduct inspections, educate employees about hazards, and monitor their health. Advancements in technology and industry practices lead to safer alternatives and improved controls, reducing risks(McDiarmid & Gehle, 2006; D. J. Smith et al., 2022). By promoting safety measures and fostering a culture of workplace health, employers can protect employees' well-being and productivity in various industries(Donovan et al., 2012).

2.1.5 Behavioral Toxicity

Behavioral toxicity, also known as neurotoxicology, is a specialized field that investigates how chemical substances can harm the nervous system and behavior of living organisms, including humans. It explores the complex interactions between toxic agents and the brain, leading to various behavioral and neurological changes(Agues-Barbosa et al., 2023; P. Wang et al., 2019).

Toxic compounds causing behavioral effects include industrial chemicals, drugs, pesticides, heavy metals, and environmental pollutants. They can disrupt the nervous system, affecting neurotransmission and brain chemistry(Tilson, 1987). The behavioral changes resulting from toxicity vary based on the compound, dose, exposure duration, and individual vulnerability. Common effects include mood alterations, impaired memory and learning, reduced motor coordination, anxiety, depression, aggression, and even hallucinations(Driscoll et al., 2023; Hayase et al., 2000).

Research in neurobehavioral toxicity involves laboratory studies, animal experiments, and clinical observations in humans(Hindmarch & Kerr, 1992). Behavioral tests and neuroimaging help assess the impact of toxicants on the brain and behaviour. Epidemiological studies in vulnerable populations reveal links between toxic exposures and behavioral outcomes(Araújo & Blasco, 2019; Krzykwa & Sellin Jeffries, 2020).

2.1.6 Radiotoxicity

Radiotoxicity, also called radiation toxicity, the harmful effects of ionizing radiation on organisms that are alive. Ionizing radiation can be natural (cosmic rays) or man-made (X-rays, gamma rays, radioactive isotopes)(Kassis et al., 1987). The effects depend on radiation type, dose, exposure duration, and tissue sensitivity. High doses can cause immediate effects like radiation sickness and tissue damage, while lower doses over time may lead to cancer and genetic mutations(Jonkhoff et al., 1995; Osytek et al., 2021).

Radiation exposure can damage organs and DNA, increasing the risk of cancer. Nuclear accidents like Chernobyl and Fukushima have shown the severe health risks of large radioactive releases(Brower, 2007). To protect human health, regulatory agencies set safety limits for radiation exposure in various settings. Safety measures and protective guidelines are implemented for workers in radiation-exposed jobs. Medical technology advances aim to deliver therapeutic doses while minimizing harm to healthy tissues(Fernández et al., 2021; Maucksch et al., 2018).

2.1.7 Types of Toxicity

Toxicity can appear in different ways, each causing specific impacts on living organisms. Below are some common types of toxicity:

2.1.7.1 Acute Toxicity: This form of toxicity pertains to the immediate and severe adverse effects resulting from exposure to a toxic substance for a short period. It is

characterized by the quick onset of symptoms and can lead to serious health problems or fatalities(Ait Atmane et al., 2022; Mundy-Heisz et al., 2022).

2.1.7.2 Chronic Toxicity: Chronic toxicity is repeated exposure or prolonged to small amounts of toxic substances over an extended period. Unlike acute toxicity, its effects may not be immediately evident, but they can build up over time and result in long-term health issues(Hall, 2002; J. Yang et al., 2022).

2.1.7.3 Genotoxicity: Substances with genotoxic properties can harm the genetic material (DNA) of living cells, leading to mutations or abnormalities in chromosomes. This form of toxicity is strongly linked to cancer development and heritable genetic diseases (Oesch & Landsiedel, 2012).

2.1.7.4 Neurotoxicity: Neurotoxic chemicals have a negative impact on the neurological system., causing a waste of neurological issues. Typical appearance includes difficulties with cognitive function, motor skills, and sensory perception(H. Mochizuki, 2019).

2.1.7.5 Carcinogenicity: Carcinogens are substances that encourage or heighten the likelihood of cancer development. These toxic agents can damage DNA or interfere with cellular functions, triggering uncontrolled cell growth and the formation of tumors(Dekant et al., 2021).

2.1.7.6 Teratogenicity: Teratogens are substances that, when pregnant individuals are exposed to them, can lead to birth defects or abnormalities in developing fetuses(Vargesson, 2015).

2.1.7.7 Hepatotoxicity: Hepatotoxic substances can harm the liver, compromising its essential functions and potentially resulting in liver disease or failure(Le Daré et al., 2021).

2.1.7.8 Nephrotoxicity: Nephrotoxic substances target the kidneys, causing kidney damage and impairing their function(Santos et al., 2020).

2.1.7.9 Cardiotoxicity: Cardiotoxic substances can adversely affect the heart, leading to heart damage and various cardiovascular disorders(Lenneman & Sawyer, 2016).

2.1.7.10 Respiratory Toxicity: Respiratory toxicants impact the respiratory system, causing breathing difficulties, lung damage, and respiratory diseases(McKay, 2014).

2.1.7.11 Immunotoxicity: Immunotoxin substances weaken or suppress the immune system, increasing susceptibility to infections and diseases(Putman et al., 2003).

2.1.7.12 Endocrine Disruption: Endocrine disruptors interfere with the hormonal system, potentially causing various hormonal imbalances and related health problems(Marty et al., 2018).

2.1.7.13 Allergenicity: Allergens trigger allergic reactions in susceptible individuals, resulting in symptoms like skin rashes, respiratory issues, and anaphylaxis(Traidl-Hoffmann et al., 2009).

2.2 Lungs

Lungs are vital organs pertaining to the respiratory system that participate in the exchange of oxygen and carbon dioxide between the blood and the air. Their principal job is to make things easier. respiration, which is the process of breathing, enabling the organism to absorb oxygen and excrete carbon dioxide(Griese, 1999; Szpinda et al., 2015).

The lungs are crucial and complex organs that form an integral part of the respiratory system, enabling us to breathe and sustain life. These paired, spongy structures are found within the chest cavity and are surrounded and protected by a double-layered membrane known as the pleuradioxide membrane.(Fujino et al., 2011). Their primary function revolves around the exchange of oxygen and carbon dioxide, a process vital for cellular respiration and the overall functioning of the body. Through a series of intricate airways, the lungs facilitate the flow of air in and out of the body(Nichols et al., 2017; Yeates & Aspin, 1978a).

Figure 1: The 3D illustration of human respiratory organ, focusing on the location and structure of the lungs within the body. The lungs are a pair, located in the thoracic cavity of the chest. They flank the heart and are protected by the ribcage.

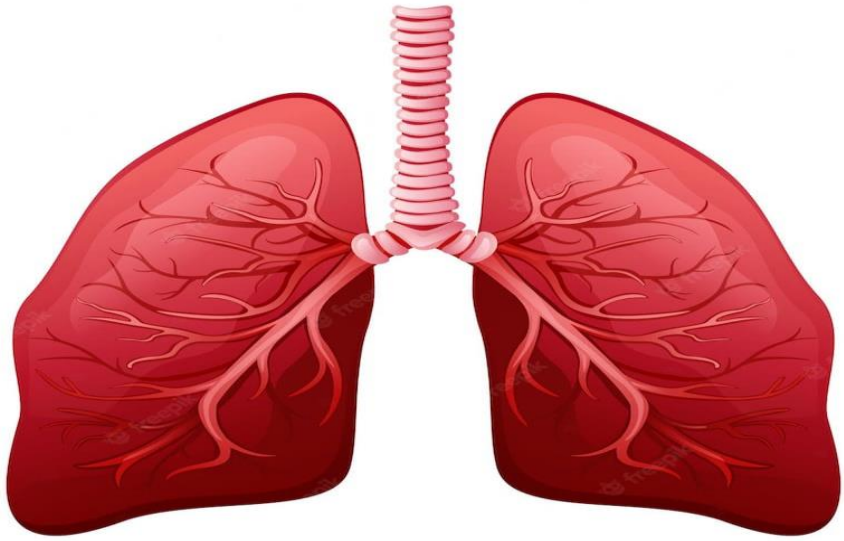

Figure 2: This figure presents an overview of the general structure of the lungs.

2.2.1 Structure of the Lungs:

The lungs are extraordinary organs that play a vital role in our respiratory system, enabling us to breathe and maintain life. Their intricate structure is cleverly designed to optimize the exchange of gases, ensuring a continuous supply of oxygen and efficient removal of carbon dioxide(Adams et al., 2023).

Located in the chest cavity, the lungs consist of a pair of spongy, cone-shaped organs protected by the pleura is a double-layered membrane. The right lung has three lobes, whereas the left lung has two lobes. allowing efficient use of space and optimal respiratory function(Yeates & Aspin, 1978b).

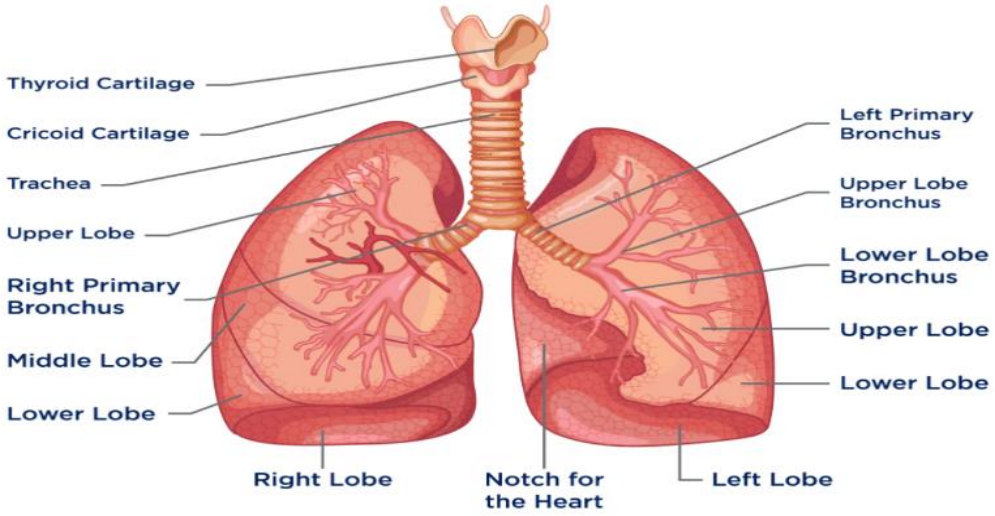

Figure 3: Anatomy of the Lungs

The airway system of the lungs begins with the trachea, a tube connecting the mouth and nose to the lungs. The trachea is divided into two major bronchi, each of which leads to one lung. The bronchi split deeper into the lungs into smaller bronchioles. creating an intricate tree-like structure that maximizes surface area and enables air to reach the tiniest air sacs called alveoli(Fehrenbach et al., 1994; Thurlbeck, 1977).

Gas exchange primarily occurs within the alveoli, which are thin-walled structures resembling grapes. It is encircled by a vast network of capillaries. During inhalation, Inhaled oxygen diffuses past the alveolar walls into the capillaries, where it binds to haemoglobin in red blood cells and is distributed throughout the body. Simultaneously,

carbon dioxide, a byproduct of cellular metabolism, travels from the circulation into the alveoli, where it is released during expiration.(DiFiore & Wilson, 1994; Hansen, 2013).

The function of the lungs is closely connected to the diaphragm and other respiratory muscles. When we inhale, the diaphragm contracts, expanding the lungs inflate with air as a result of the air entering the chest cavity. The diaphragm relaxes and the volume of the chest cavity decreases during exhale., leading to expulsion of air from the lungs(Agraval & Chu, 2022; Hofmann & Asgharian, 2003).

Additionally, the respiratory system has protective mechanisms in place to keep the lungs clean and free from harmful particles. Mucus-producing cells and cilia line the airways, capturing foreign particles and pathogens, which are then expelled from the body through coughing or sneezing(Lowery et al., 2013; Schreider & Raabe, 1981).

2.2.2 Function of the Lungs:

The lungs have a crucial part in the respiratory process, serving as essential organs responsible for exchanging gases between the air and our bloodstream. Their main purpose is to provide the body with vital oxygen, essential for cellular metabolism, while simultaneously removing carbon dioxide, a waste product of cellular respiration (Daggett et al., 1997; Ohno et al., 2022).

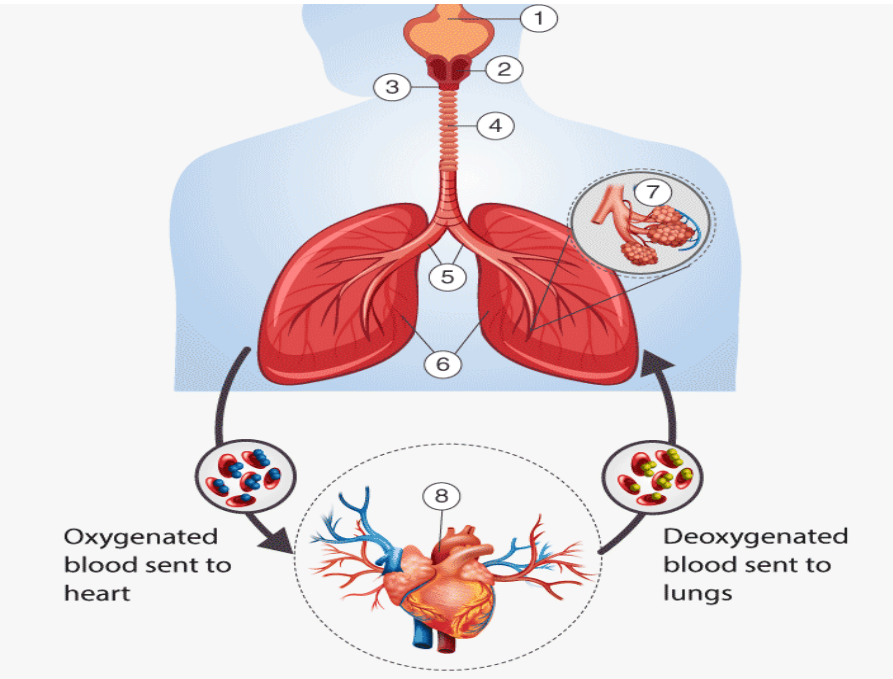

Figure 4: This figure illustrates the intricate mechanism of breathing.

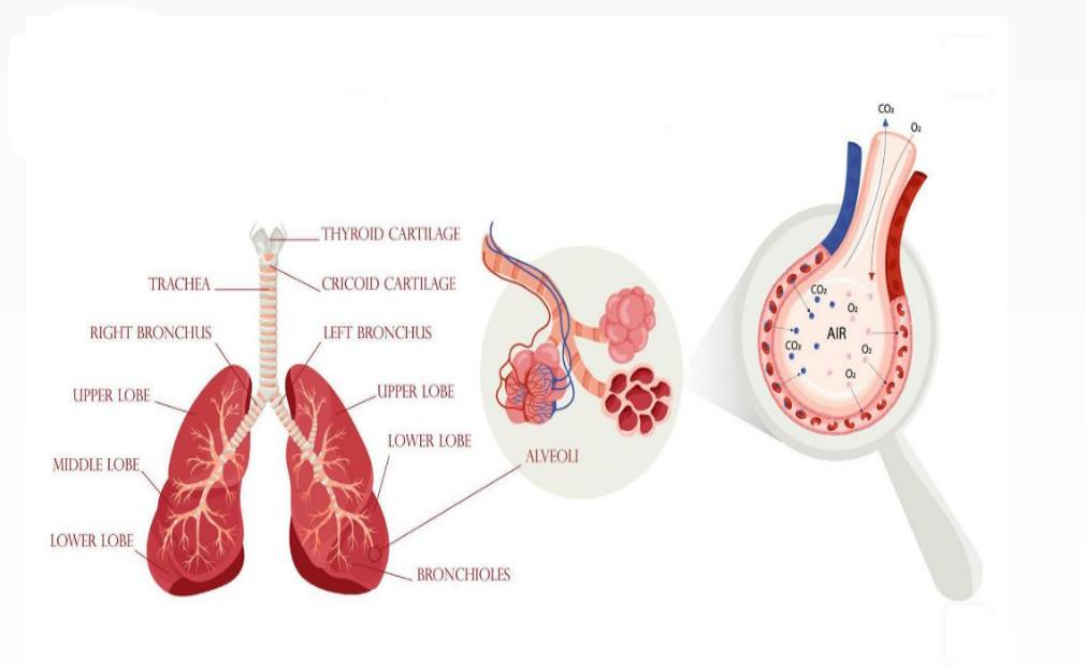

Figure 5: Alveolar Gas Exchange in Lungs

2.2.3 Gas Exchange: The main pulmonary function is enabling the gaseous exchange between the air & blood. During inhalation, inhaled air diffuses through alveoli (tiny air sacs) in the surrounding capillaries. Simultaneously, carbon dioxide relocates into the alveoli from the blood to be expelled during exhalation(West, 1987; Zieliński, 2002).

2.2.4 Oxygen Transport: When oxygen enters the bloodstream, it attaches to haemoglobin in red blood cells, resulting in the formation of oxyhemoglobin. The oxygen-rich blood is subsequently circulated throughout the body, supplying the oxygen required for cellular respiration and energy generation.(Leow, 2007).

2.2.5 Carbon Dioxide Removal: Carbon dioxide is the waste product of cellular metabolism that is carried back into the lungs by the blood. CO_2 moves into the alveoli from the blood during exhalation, ready to be expelled from the body(Parsons & Parsons, 1923).

2.2.6 Pulmonary Ventilation: The lungs aid in the process of breathing. The diaphragm and intercostal muscles flex during inhalation, enlarging the chest cavity and allowing air to rush in. Exhalation causes the diaphragm and intercostal muscles to relax, lowering the volume of the chest cavity and pushing air out of the lungs (J. Yang et al., 2020).

2.2.7 Acid-Base Balance: The lungs have a crucial function in controlling the body's acid-base balance. The lungs assist maintain the blood's ideal pH level by managing the quantities of carbon dioxide and bicarbonate ions in the blood. (Stickland et al., 2013).

2.3 Pulmonary toxicity

Pulmonary toxicity refers to the harmful effects that various substances or environmental factors can have on the respiratory system, particularly the lungs. When toxic substances are inhaled or enter the body, they can cause damage to lung tissues, impair the gas exchange process, and lead to various respiratory issues(Lucas et al., 2020). Understanding pulmonary toxicity is crucial for identifying potential hazards, preventing exposure, and implementing appropriate measures to protect respiratory health. This introduction provides a glimpse into the significance of pulmonary toxicity and its implications on overall well-being(Kovacic & Somanathan, 2009; B. R. Smith & Brian, 1991).

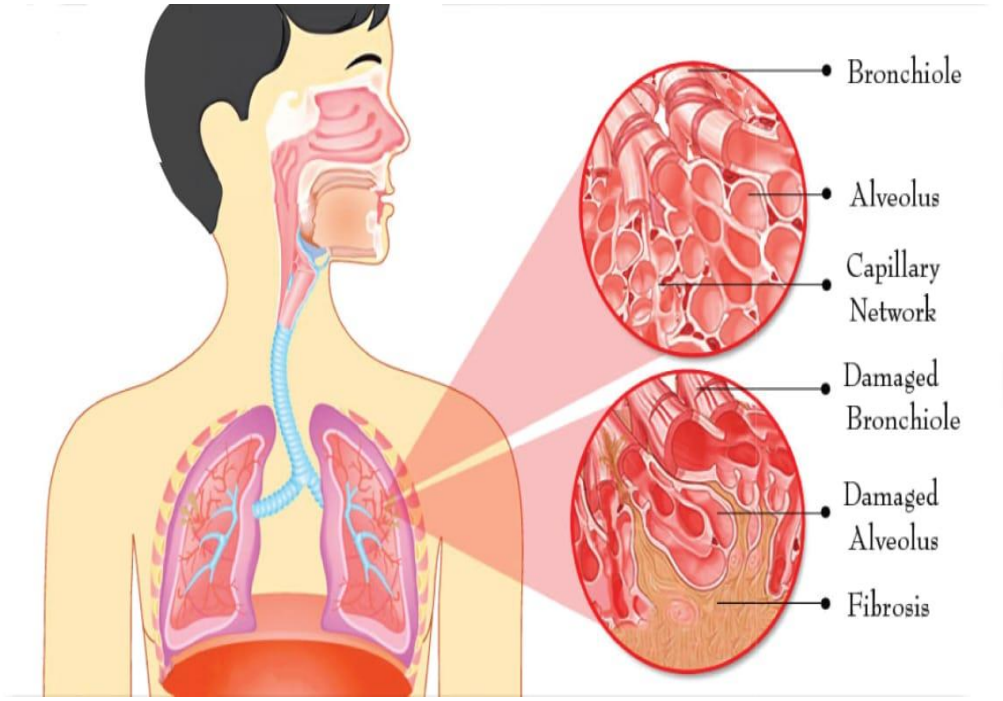

Figure 6: Pulmonary Toxicity of the lungs.

2.3.1 Causes:

Pulmonary toxicity can result from exposure to a diverse spectrum of substances, including gases, dust, chemicals, and particulate matter. Common sources of pulmonary toxicity include environmental pollutants, occupational hazards, certain medications, and recreational drug use(Card et al., 2008).

2.3.2 Mechanisms:

Pulmonary toxicity can occur through different mechanisms, such as inflammation, oxidative stress, fibrosis, and direct damage to lung tissues. Inflammatory responses triggered by toxic substances can lead to lung tissue damage and impair the gas exchange function of the alveoli, where oxygen is taken up and carbon dioxide is expelled(Jackson, 1985).

2.3.3 Symptoms:

Symptoms of pulmonary toxicity may vary depending on the specific toxic agent and the extent of exposure. Common Coughing, shortness of breath, and chest discomfort are all symptoms, wheezing, and increased making of mucus. Acute respiratory distress syndrome (ARDS) can occur in severe situations, pulmonary edema, and chronic lung disorder(Dong et al., 2020; Manke et al., 2013).

2.3.4 Prevention and Treatment:

Preventing pulmonary toxicity involves minimizing exposure to toxic substances, using protective equipment in occupational settings, and avoiding harmful environmental pollutants. Early recognition and prompt medical intervention are crucial for managing pulmonary toxicity. Treatment may involve supportive care, administering medications to alleviate symptoms, and managing complications arising from lung damage(KARPOVICH et al., 1951; Pitcher, 1992).

2.4 Effect of pulmonary toxicity on another organ

Lung toxicity can have significant effects on other organs in the body due to the interconnected nature of the circulatory and respiratory systems. When toxic substances or pollutants are inhaled and enter the lungs, they can be absorbed into the bloodstream and transported to other organs, leading to various adverse effects(Gentile et al., 2022).

2.4.1 Heart: The heart may be affected due to the reduced oxygen supply caused by lung toxicity. When the lungs fail to exchange gases efficiently, oxygen levels in the blood may drop, leading to an increased workload on the heart to pump oxygen-depleted blood. Over time, this can contribute to heart strain and potential cardiovascular complications(Forfia et al., 2013).

2.4.2 Liver: Toxic substances absorbed from the lungs can reach the liver through the bloodstream. The liver is responsible for metabolising and detoxifying toxic chemicals.. Prolonged exposure to lung toxins may lead to liver damage and impair its ability to function properly(Herrero et al., 2020).

2.4.3 Kidneys: The kidneys are responsible for filtering the blood and removing waste products from the body. Lung toxicity can impact kidney function indirectly by affecting blood oxygen levels and potentially contributing to kidney dysfunction(Dalgleish et al., 1984).

2.4.4 Brain: Inhaled toxins can reach the brain through the bloodstream and affect neural function. Reduced oxygen levels due to lung toxicity may lead to cognitive impairment and memory issues(Weiss et al., 1979).

2.4.5 Immune System: Lung toxicity may impair the immune system, rendering persons more prone to infections and diseases. Inflammatory responses triggered by toxic substances can also lead to systemic inflammation, affecting various body organ(Roberts et al., 2016; Weiss et al., 1979).

2.5 Disease due to Pulmonary Toxicity

One disease that can occur due to pulmonary toxicity is "Interstitial Lung Disease" (ILD). ILD is a set of lung illnesses characterized by lung tissue inflammation and scarring (fibrosis). which can be caused by exposure to certain toxic substances, environmental pollutants, medications, or occupational hazards(Fischer & Du Bois, 2012).

Pulmonary toxicity can trigger a pulmonary inflammatory reaction, leading to accumulation for scar tissue in the interstitial spaces between the air sacs (alveoli). As the fibrosis progresses, the lung's ability to expand and contract during breathing is compromised, resulting in symptoms such as persistent cough, shortness of breath, and reduced exercise tolerance (Basuita & Fidler, 2022; Ryerson et al., 2014).

Different types of ILD may arise from various toxic agents, and the severity can vary widely. Early diagnosis and appropriate management are crucial to prevent further lung damage and improve the quality of life for affected individuals. Identifying and avoiding the source of pulmonary toxicity is essential to prevent the development of ILD and other respiratory diseases linked to harmful exposures (England & Hershberger, 2020).

2.6 Types of ILD that can occur due to pulmonary toxicity

ILD (Interstitial Lung Disease) can be caused by exposure to various toxic agents. These toxic agents can lead to different types of ILD, depending on their specific characteristics and mechanisms of action. Here are some brief descriptions of ILD types that may arise from exposure to toxic agents (Ryerson & Collard, 2013).

2.6.1 Hypersensitivity pneumonitis: This type of ILD is caused by exposure to various organic dusts or chemicals, such as mold spores, bird droppings, or certain bacteria. HP occurs when the immune system overreacts to these substances, leading to inflammation in the lung's interstitial spaces(Girard & Cormier, 2010).

2.6.2 Asbestos-related ILD: Asbestos fibers, commonly found in construction materials, can cause ILD, particularly a subtype known as asbestosis. Prolonged inhalation of Scarring and fibrosis in the lung tissue are caused by asbestos fibres. (Spagnolo et al., 2015).

2.6.3 Silicosis: Silicosis is caused by the inhalation of crystalline silica dust, typically found in certain types of mining, construction, and sandblasting work. Silica particles trigger an inflammatory response, leading to lung tissue damage and fibrosis(Handra et al., 2023).

2.6.4 Coal worker's pneumoconiosis: Also known as "black lung disease," CWP develops in workers in coal mines exposed to dust over extended periods. Inhaling coal dust causes inflammation, accumulation of coal particles, and fibrosis in the lungs(Cui et al., 2020).

2.6.5 Drug-induced ILD: Some medications and drugs can cause ILD as an adverse reaction. These drugs may include certain antibiotics, chemotherapy agents, and anti-inflammatory medications. The mechanism of drug-induced ILD can vary based on the specific drug(Ng et al., 2023).

2.6.6 Radiation-induced pneumonitis: Exposure to high doses of radiation, such as during radiation therapy for cancer treatment, can cause inflammation and scarring in the lung tissue, leading to radiation-induced ILD(Xia et al., 2020).

2.6.7 Toxic fumes and gases: Inhalation of toxic fumes, such as ammonia, chlorine gas, or sulfur dioxide, can cause acute or chronic ILD, depending on the degree and length of exposure (Wada et al., 1994).

2.7 Acetaminophen

History of acetaminophen It was initially synthesised by a French scientist in the late nineteenth century Harmon Northrop Morse in 1877. However, Morse's discovery did not receive much attention at the time, and its medical applications were not explored(M. Black, 1984; Broughan & Soloway, 2000).

In 1950s, when research on pain relievers and fever-reducing medications was gaining momentum. Scientists were particularly interested in finding alternatives to salicylates like aspirin, which, though effective, caused stomach irritation and bleeding in some individuals(Josephy, 2005).

In 1953, a British pharmacologist named Maurice Brodie and his team rediscovered acetaminophen while investigating new analgesic compounds. They recognized its pain-relieving and fever-reducing properties, making it a promising alternative to aspirin without the same risk of gastrointestinal side effects(Salgia & Kosnik, 1999).

In 1955, acetaminophen was introduced in the United States as a prescription medication under the brand name Tylenol. It quickly became popular as a safer option for pain relief and fever reduction. Eventually, in 1959, Acetaminophen was approved for over-the-counter usage by the United States Food and Drug Administration (FDA), making it readily available without a prescription. (Edwards et al., 1986).

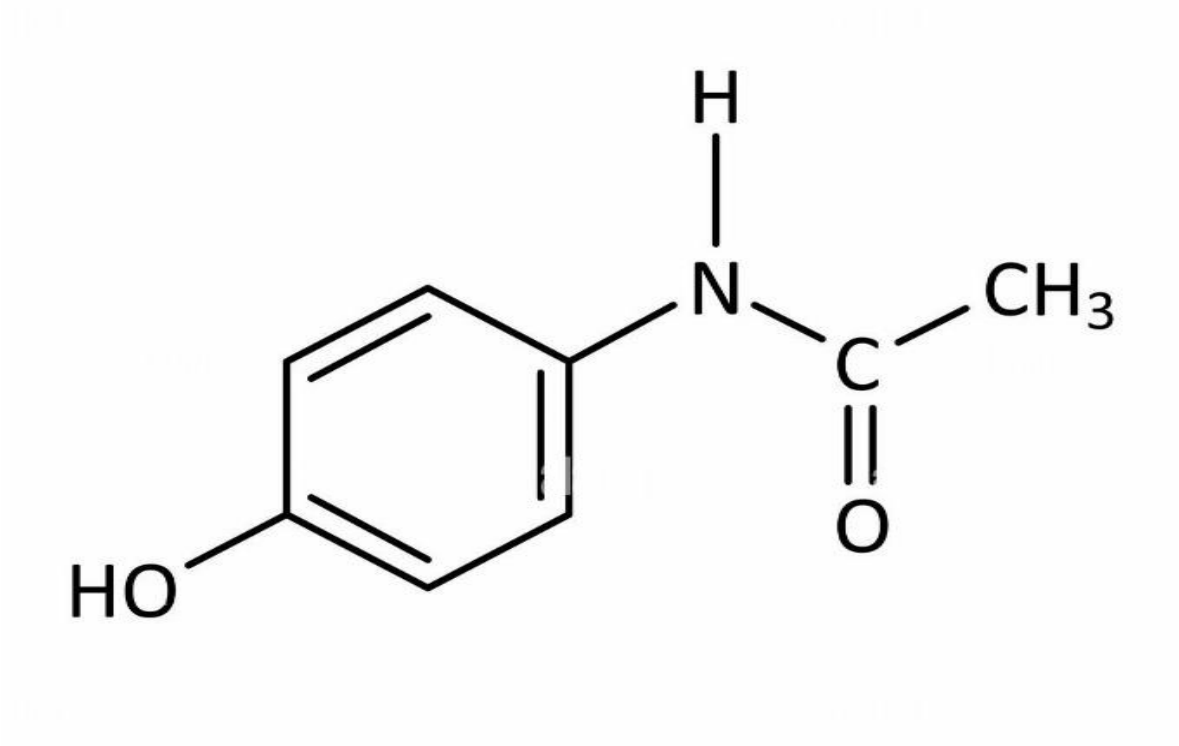

Figure 7: Chemical structure of Acetaminopehn.

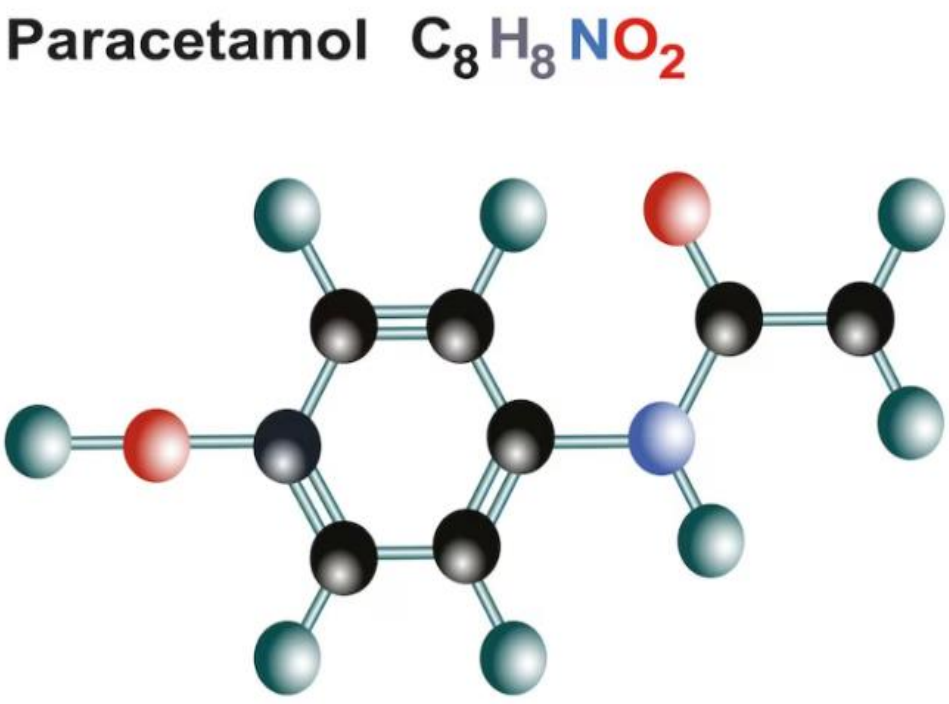

Figure 8: 3D structure of paracetamol/Acetaminophen.

Over the years, extensive research has been conducted to understand acetaminophen's mechanisms of action, safety profile, and proper dosing. It has become one of the most commonly used medications worldwide, available under various brand names and in combination with other drugs to address different symptoms(Lafrance et al., 2022).

Despite its widespread use and overall safety, acetaminophen can still pose risks if not used appropriately. As mentioned earlier, exceeding the recommended dosage or using it in combination with other medications containing acetaminophen can lead to liver damage. Therefore, it is crucial for individuals to follow the recommended dosing guidelines and seek advice from healthcare professionals when necessary(Mazer & Perrone, 2008).

The discovery and development of acetaminophen have had a significant impact on modern medicine, providing a valuable and widely available tool for pain management and fever reduction. However, caution must be exercised in its usage to ensure its safety and effectiveness. Always adhere to proper dosing instructions and consult healthcare professionals if there are any concerns(Bertolini et al., 2006; Mazer & Perrone, 2008).

2.7.1 Acetaminophen mechanism of action

The way acetaminophen, or paracetamol, works in the body is not completely understood, but it mainly involves the central nervous system and its impact on pain and fever regulation. Acetaminophen is not a nonsteroidal anti-inflammatory medication (NSAID) like aspirin or ibuprofen. It has limited anti-inflammatory properties and primarily acts as a pain reliever and fever reducer(Bonnefont et al., 2003; Jozwiak-Bebenista & Nowak, 2014).

While the precise mechanism of acetaminophen is believed to involve several processes, the most widely accepted explanation is that it affects specific areas of the brain. Here's a simplified version of how it's thought to work(Toussaint et al., 2010).

Inhibition of Prostaglandin Synthesis: Acetaminophen inhibits an enzyme in the brain known as cyclooxygenase (COX). COX is responsible for producing prostaglandins, which are chemical signals that promote pain, inflammation, and fever in response to injury or illness. Unlike NSAIDs, which target COX-1 and COX-2 enzymes are both present and thus reduce inflammation, acetaminophen primarily focuses on COX-2 in the brain while sparing COX-1 in other tissues. As a result, its anti-inflammatory effects are minimal(Gaudreault et al., 1988; Graham et al., 2005).

Nervous System Actions: Acetaminophen's main effects occur in the nervous system, including hypothalamus, which plays a function in body temperature regulation and initiating fever responses. By acting on these brain regions, it helps lower fever and alter the perception of pain(Krajčová et al., 2013).

It's essential to acknowledge that research on acetaminophen's exact mechanism of action is still ongoing, and there might be other factors contributing to its effects that haven't been fully understood yet. Nevertheless, the inhibition of COX-2 as well as its effect on the central nervous system are currently considered the primary factors responsible for its ability to relieve pain and reduce fever(Bozogluer et al., 2012; K. Mochizuki & Takayama, 2016).

As with any medication, it's crucial to use acetaminophen as directed and avoid exceeding the recommended dose to avoid probable side effects, especially on the liver (Miners et al., 1984).

2.8 Toxicity of Acetaminophen

Acetaminophen, generally known as paracetamol, is a popular over-the-counter pain reliever. medication to relieve pain and reduce fever. When taken as directed, it is generally considered safe for most individuals. However, like any medication, improper use or excessive consumption of acetaminophen can lead to toxicity. Here are some important points to understand about acetaminophen toxicity(Lewis & Paloucek, 1991; Shklovsky-Kordi et al., 2020).

2.8.1 Recommended Doses: The typical adult dose of acetaminophen ranges 325 to 1000 mg every 4 to 6 hours, with a maximum daily dosage of 4,000 mg (4 grammes). It is crucial to adhere to these dosing guidelines to avoid toxicity(Linden & Rumack, 1984).

2.8.2 Liver Toxicity: Acetaminophen is primarily metabolized in the liver. Taking excessive amounts or having pre-existing liver conditions, such as liver disease or alcohol use disorder, may overburden the liver's processing capabilities, resulting in liver injury or failure (Rubin et al., 2018).

2.8.3 Overdose: Acetaminophen overdose is a serious medical emergency. It can occur when a large amount of acetaminophen is taken at once or when smaller doses are consistently taken over several days, accumulating to a toxic level(Goodchild & Donaldson, 2022).

2.8.4 Symptoms of Overdose: Nausea, vomiting, sweating, and an overall sensation of malaise are early indicators of an acetaminophen overdose. More severe symptoms such as stomach discomfort, disorientation, jaundice (yellowing of the skin and eyes), and bleeding issues may emerge as the poisoning advances (Dogan et al., 2022)..

2.8.5 Liver Damage: Acetaminophen toxicity can cause severe liver damage. In severe situations, acute liver failure can be fatal and may necessitate a liver transplant. (Dart & Bailey, 2007).

2.8.6 Risk Groups: Certain groups are at higher risk of acetaminophen toxicity, including regular alcohol consumers, individuals with liver conditions, and those taking multiple medications containing acetaminophen (as this can unintentionally lead to overdose)(Last & Hulbert, 2009).

2.8.7 Antidote: In case of suspected acetaminophen overdose, immediate medical attention is essential. Treatment often involves N-acetylcysteine (NAC) is an antidote that, if administered early, can help avoid or minimise liver damage.

2.8.8 Combination Products: Acetaminophen is a common ingredient in many combination medications, such as cold and flu remedies or prescription painkillers. It's vital to carefully check the labels of all medications being used to avoid unintentional overdose(El-Farrash et al., 2019).

Always follow the dosing instructions on the medication label, and if you have any concerns or questions about acetaminophen or its potential toxicity, Consult a medical professional. They may provide you tailored recommendations based on your medical history and current health situation. Safety is paramount when using any medication, and responsible usage of acetaminophen can ensure its benefits while minimizing the risks.

3 METHODOLOGY

3.1 Reagent Setup

3.1.1 Normal salina

For 1L	
Sodium Chloride	**9 gm**
Distilled Water	**1000 ml**

3.1.2 Formal Saline

For 1L	
Formaldehyde	**100 ml**
Sodium Chloride	**9 gm**
Distilled Water	**900**

3.1.3 Acetaminophen or Paracetamol, a widely used analgesic and antipyretic medication, was procured from Sigma-Aldrich (St. Louis, MO, USA).

3.1.4 Potassium dihydrogen phosphate and **dibasic mono hydrogen phosphate**, commonly used as buffering agents, were purchased from Merck Chemical Private Limited.

3.1.5 The paraffin wax, an embedding medium used in histological processing, was obtained from Merck Chemical Private Limited as well.

3.1.6 Xylene a clearing agent used in tissue processing, was acquired from Merck Chemical Private Limited. Xylene is commonly used in histology laboratories for clearing and dehydrating tissue samples.

3.2 Experimental Animal

Swiss albino mice weighing 20-25g are utilized for this study. The mice are housed in a controlled environment maintained at a temperature of 22±3°C. The room has an air conditioning system to ensure a comfortable and consistent temperature for the animals. The relative humidity is maintained at 50±5% to provide suitable conditions for the mice's well- being.

To establish a stable circadian rhythm, a 12-hour light/12-hour dark cycle is maintained in the animal facility. This lighting schedule mimicked the natural day and night cycles, allowing the mice to exhibit their normal behavior patterns.

Prior to the commencement of the study, the mice underwent a one-week acclimatization period.During this time, they were allowed to adapt to their new environment, ensuring that any initial stress due to transportation or housing changes was minimized.

The mice are provided with a certified pellet rodent diet obtained from Dayal Industries, located in Lucknow, India. This diet is specifically formulated to meet the nutritional requirements of rodents, providing them with essential nutrients for their growth and maintenance. Fresh water is available, ensuring the mice had unrestricted access to hydration.

The experimental protocol is followed as per the Institutional Animal Ethics Committee of CSIR-IITR (Council of Scientific and Industrial Research – Indian Institute of Toxicology and Research). The committee ensured that ethical guidelines and regulations for animal care and experimentation are followed strictly. The guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Government of India, were adhered to throughout the study. These guidelines aim to ensure the ethical treatment, welfare, and protection of animals involved in scientific research.

By adhering to these protocols and guidelines, the study aimed to ensure the well-being and ethical treatment of the Swiss albino mice throughout the experimental period, while also ensuring the validity and reliability of the

research findings.

3.2.1 Test System

Species	Mice
Strain	Swiss Albino Mice (In-house random bred)
Source	Animal Facility, CSIR-Indian Institute of Toxicology Research, CRK Campus, Sarojini Nagar Industrial Area,Kanpur Road, Lucknow-226008, India
Justification of Selection of Species	Mice is one of the standard test systems used to assesstoxicity and is acceptable to the regulatory authorities.
Justification for route of administration	The Oral is chosen because is the route of exposure oftarget population.
No and sex of Animals	80 Mice (40Male & 40Female)
Age at the start of the treatment	8-12 weeks
Body-weight at the start of the treatment	20-25 g
Identification	By mice accession numbers and cage cards.

3.3 Acclimatization

During the acclimatization phase of Swiss mice, their body weights in grams is measured and recorded daily for a period of seven days. The mice are placed in a controlled environment with appropriate temperature, humidity, and lighting conditions to ensure their adaptation to the new surroundings. Throughout this period, general observations are conducted daily, taking note of their behavior, activity levels, food and water consumption, and any signs of distress or abnormalities. The recorded data is crucial in establishing a baseline for the mice's health and behavior before the commencement of the main experimental procedures, ensuring accurate and reliable research outcomes.

3.4 Randomization

After the acclimatization period, the body weight of all mice are measured again just before the randomization process. This step is crucial to ensure that any potential changes in body weight during acclimatization are taken into account and that the mice are evenly distributed among the experimental groups. By obtaining their updated body weight measurements, we can ensure that the randomization process is fair and that any subsequent differences observed in the experimental groups can be attributed to the intended treatments rather than initial body weight variations. This practice helps to enhance the validity and reliability of the research findings.

3.5 Administration of Doses

Before dosing, animals should undergo fasting. For mice, food should be withheld for 3-4 hours, with water available. After the fasting period, animals are weighed and the test substance is administered. Following substance administration, food may be withheld for an additional 1-2 hours in mice. If the dose is given in fractions over time, provision of food and water may be necessary depending on the duration of the period.

The test substance is administered orally in a single dose using a stomach tube or suitable intubation canula. In exceptional cases where a single dose is not feasible, smaller fractions ofthe dose may be given within a 24-hour period.

3.6 Dose Levels: To determine the appropriate starting dose for toxicity testing, 60 animals are used for each step. The starting dose is selected fixed levels: 0, 125, 250 and 500 mg/kg body weight. The goal is to choose the dose level that is most likely to cause mortality in some of the animals. To calculate the dose for testing the toxicity of a drug orally in mice, several factors need to be taken into consideration, including the body weight of the mice and the desired dose per kilogram of body weight. In this case, we have

80 mice (40Male & 40Female) with body weights ranging from 20 to 25 grams, 60 of which will be exposed to the test substance at a dose of 125, 250 and 500 mg/kg. Here's how you can calculate the dose:

3.6.1 Determine the average body weight of the mice

To calculate the average body weight, sum up the body weights of all mice and then divide the total by the number of mice, which is 80.

3.6.2 Calculate the dose for each mouse:

Let's assume the average body weight is 22 grams:

3.6.3 Dose calculation:

Dose per mouse = Average body weight (in kg) x Desired dose per kg

Dose per mouse = 0.022 kg x 500 mg/kg = 11 mg

Dose per mouse = Each mouse in the exposed group should receive a dose of 11 mg of the drug orally.

Dosing should be regular 90 days as follows:

Group	**Dosing Level**	**No. Of Animal**	**Sex**		**Dose (mg)**
			M	**F**	
G1	Control	20	10	10	0.0 mg
G2	Low Dose	20	10	10	2.75 mg
G3	Mild Dose	20	10	10	5.50 mg
G4	High Dose	20	10	10	11 mg

When dosing period over then we will go for testing as following these steps:

3.7 Necropsy

Necropsy, also known as an autopsy or postmortem examination, is a scientific and medical procedure performed on a deceased individual to determine the cause of death and understand the underlying pathological conditions. The term "necropsy" is often used when

the examination is performed on animals, while "autopsy" is typically used when referring to humans.

The mice are sacrificed using Thiosol / Katamine drug,ensuring a humane and ethical approach. Organs of interest are carefully excised and immediately placed in normal saline to maintain their structural integrity.

3.8 Organ Examination and Sampling:

Carefully removed and examined each organ, one by one. Inspect the external appearance and note any visible abnormalities or diseases. This sample is then placed in a container with a preservative solution, such as formalin, to prevent degradation and maintain its structure.

3.9 Gross Examination: Once the tissue sample reaches the histopathology laboratory, it undergoes a gross examination. During this step, the pathologist or trained lab technician assesses the specimen's size, appearance, and orientation. The goal is to identify any abnormalities, locate the areas of interest, and determine the best way to process the tissue for further analysis.

3.10 Trimming

After the gross examination, the process of organ trimming begins. The pathologist or lab technician carefully selects representative sections from the tissue sample that provides valuable diagnostic information. These sections are cut (2-5mm) from the larger tissue block and placed onto a smaller cassette for further processing.

3.11 Tissue Processing

3.11.1 Dehydration

After fixation, the tissue needs to be dehydrated to remove water and replace it with a substance that provides support during sectioning. The tissue is processed through a series of increasing concentrations of alcohol (e.g., ethanol), starting from lower concentrations and gradually moving to higher concentrations. This dehydration step prepares the tissue for the next stage.

Transfer the fixed tissue samples to a series of increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100% ethanol). Each step should be at least 30 minutes to ensure adequate dehydration.

Figure 9: Tissue Processor used for dehydrate & clarification of tissues.

3.11.2 Clearing

The purpose of this step is to remove water from the tissue and replace it with alcohol, which prepares the tissue for the subsequent clearing and infiltration stages.

Transfer the dehydrated tissue samples to a container with xylene or a xylene substitute. Xylene is commonly used as a clearing agent.

Allow the tissue to stay in xylene for at least 30 minutes or until it becomes transparent. This step helps to remove residual alcohol from the tissue.

Once the tissue is dehydrated, it may still contain residual alcohol, which could interfere with the subsequent steps. To remove the alcohol completely, the tissue is placed in a clearing agent, typically xylene or similar chemicals. These agents are miscible with alcohol and help replace it entirely.

3.12 Wax Embedding

Place the tissue samples in a container with molten paraffin wax, which should be maintained at a temperature of around 56-58°C.

Ensure that the tissue is completely submerged in the molten wax, and let it stay in the paraffin for at least 1-2 hours to allow proper infiltration.

After infiltration, remove the tissue samples from the molten paraffin using forceps and orient them as desired.

Figure 10: Wax Embedding System used for making wax molds with tissues .

Carefully place the oriented tissue samples into tissue embedding molds, and fill the molds with more molten paraffin wax to cover the tissue completely.

Allow the wax to cool and solidify. The tissue is now embedded in a paraffin block.

3.13 Section Cutting

Once the paraffin has solidified, remove the paraffin block from the mold. The tissue should be well-embedded within the paraffin.

Trim the excess paraffin from the sides of the block, leaving a smooth surface.

Mount the paraffin block onto the microtome, and adjust the microtome settings to the desired thickness (usually 5-7 micrometers).

Figure 11: Microtome used for section cutting.

Cut thin sections from the paraffin block using the microtome. These sections will be mounted onto glass slides for further processing and staining.

3.14 Floating and Mounting:

Collect the thin tissue sections on the surface of a water bath set at around 40-45°C. The warmth of the water helps the sections to flatten and adhere to the surface.

Carefully pick up the sections using glass slides, and then allow the slides to dry thoroughly at room temperature or on a warm plate.

3.15 Staining

The tissue sections on the glass slides are now ready for further staining.

The Leica Auto Stainer is an automated staining system commonly used in histopathology laboratories for immunohistochemistry (IHC) and routine haematoxylin and eosin (H&E) staining.

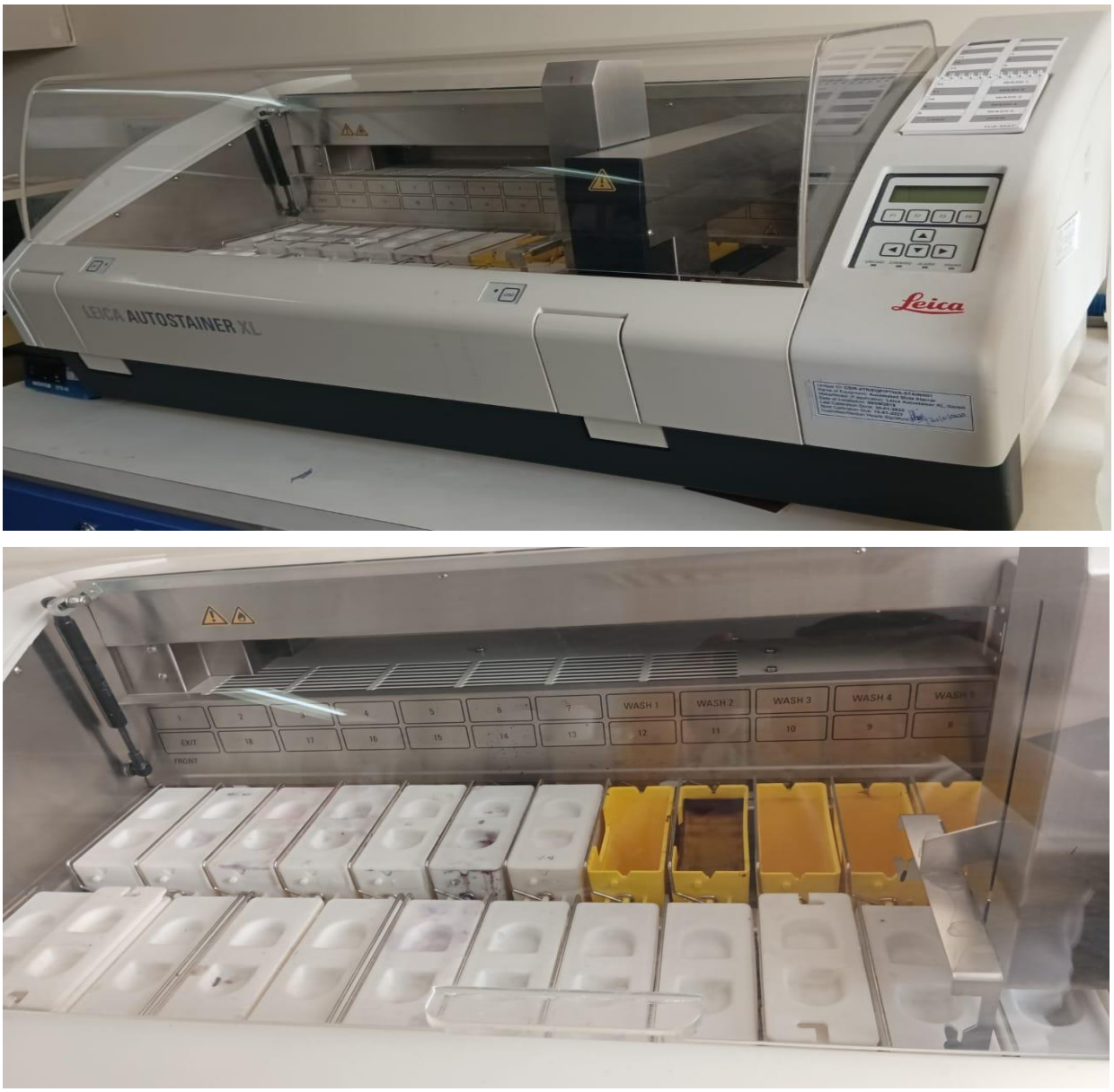

Figure 12: Auto Stainer machinery used for staining of slide for examination.

3.15.1 Preparation of Slides:

Ensure that tissue sections are properly prepared and mounted on glass slides. Proper sectioning and mounting are essential for consistent staining results.

3.15.2 Loading Slides:

Load the slides onto the slide racks of the Leica Auto Stainer, following the system's guidelines and capacity. Make sure the slides are arranged properly for the specific staining protocol.

3.15.3 Automated Staining:

The Leica Auto Stainer will automatically perform the various steps of the staining protocol, including deparaffinization, antigen retrieval (for IHC), staining with primary and secondary antibodies (for IHC), and the H&E staining steps for routine slides.

The system will also include appropriate rinsing steps using wash buffer to remove excess reagents between steps.

3.15.4 Drying:

After staining is complete, the system may include a drying step to remove excess moisture from the slides. This is particularly important for optimal mounting and slide preservation.

3.15.5 Mounting:

Remove the slides from the Leica Auto Stainer and carefully apply a coverslip using an appropriate mounting medium to preserve the stained tissue.

3.15.6 Cleaning and Maintenance:

After staining is complete, follow the Leica Auto Stainer’s cleaning and maintenance procedures to ensure the system is ready for observation under microscope.

4 RESULTS & DISCUSSION

4.1 RESULT

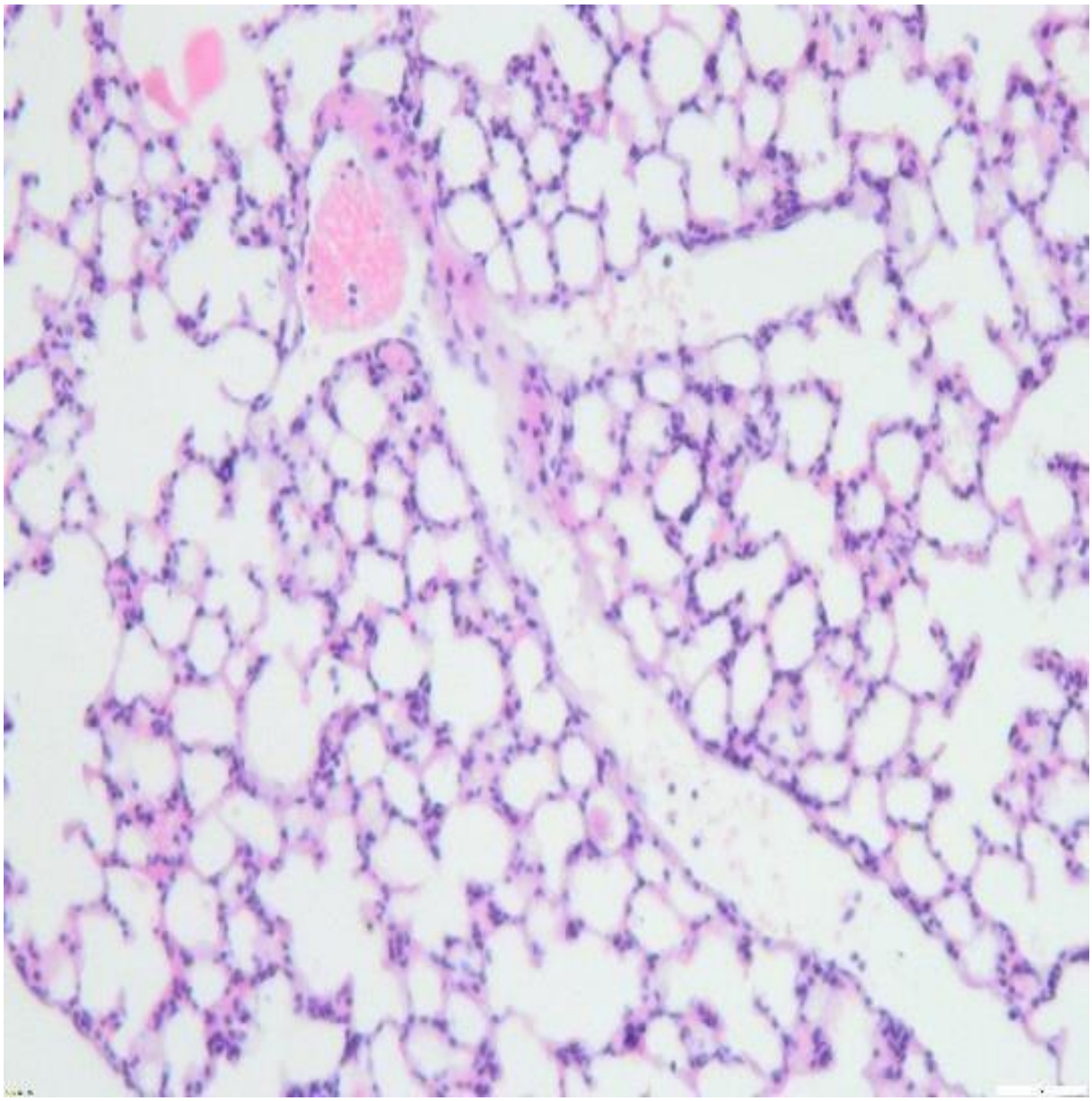

Figure A. Control group. Showing Normal architecture of lung paranchyma including normal alveoli wall and pulmonary capillaries. H&E Stain, X10.

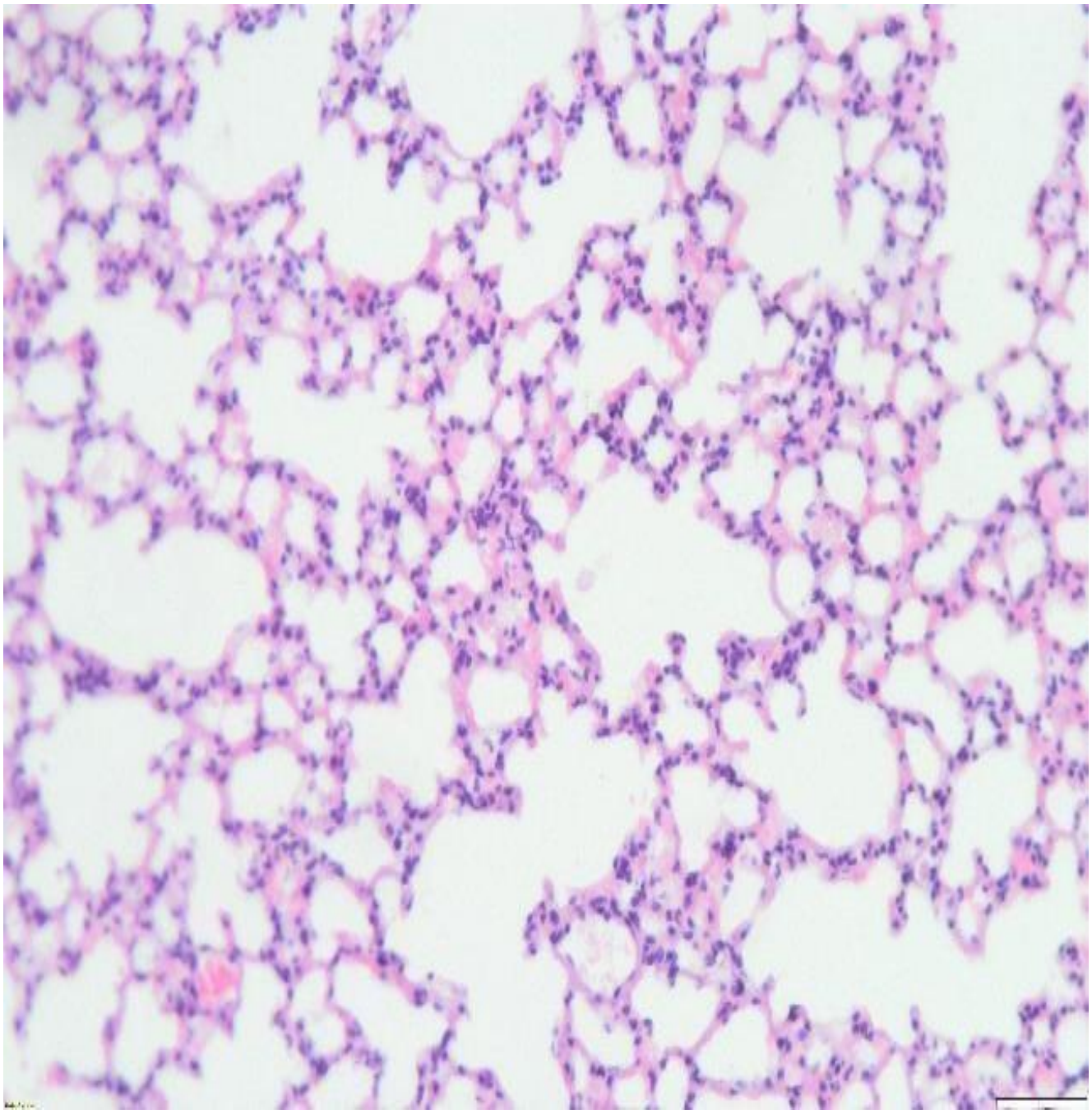

Figure B. *Low dose Acetaminophen treated group. Showed no significant tissue damage in lung paranchyma and alveolar walls.. H&E Stain, X10.*

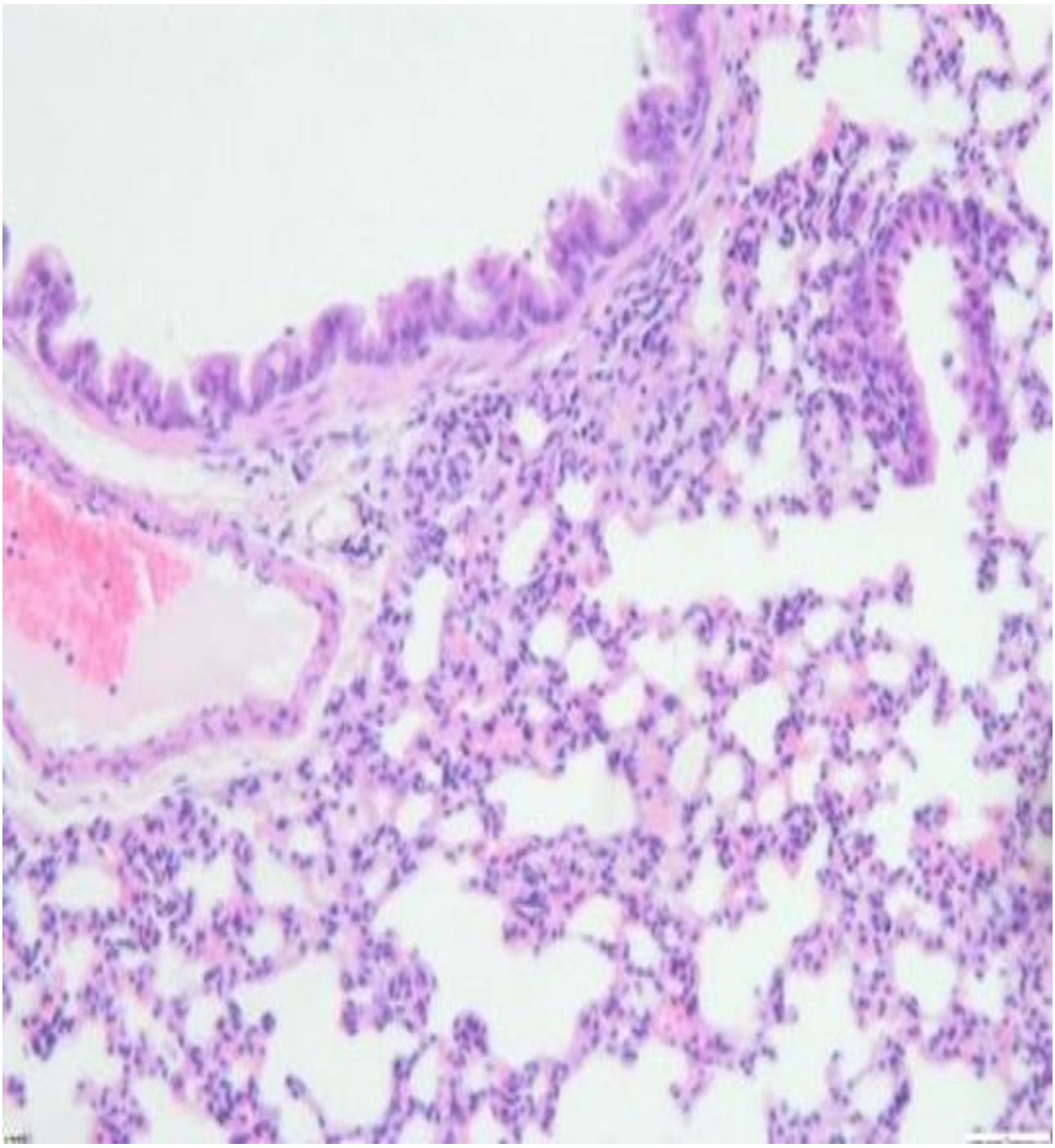

Figure C.

Mild dose Acetaminophen treated group. Showed no significant tissue damage in lung paranchyma and alveolar walls. H&E Stain, X10.

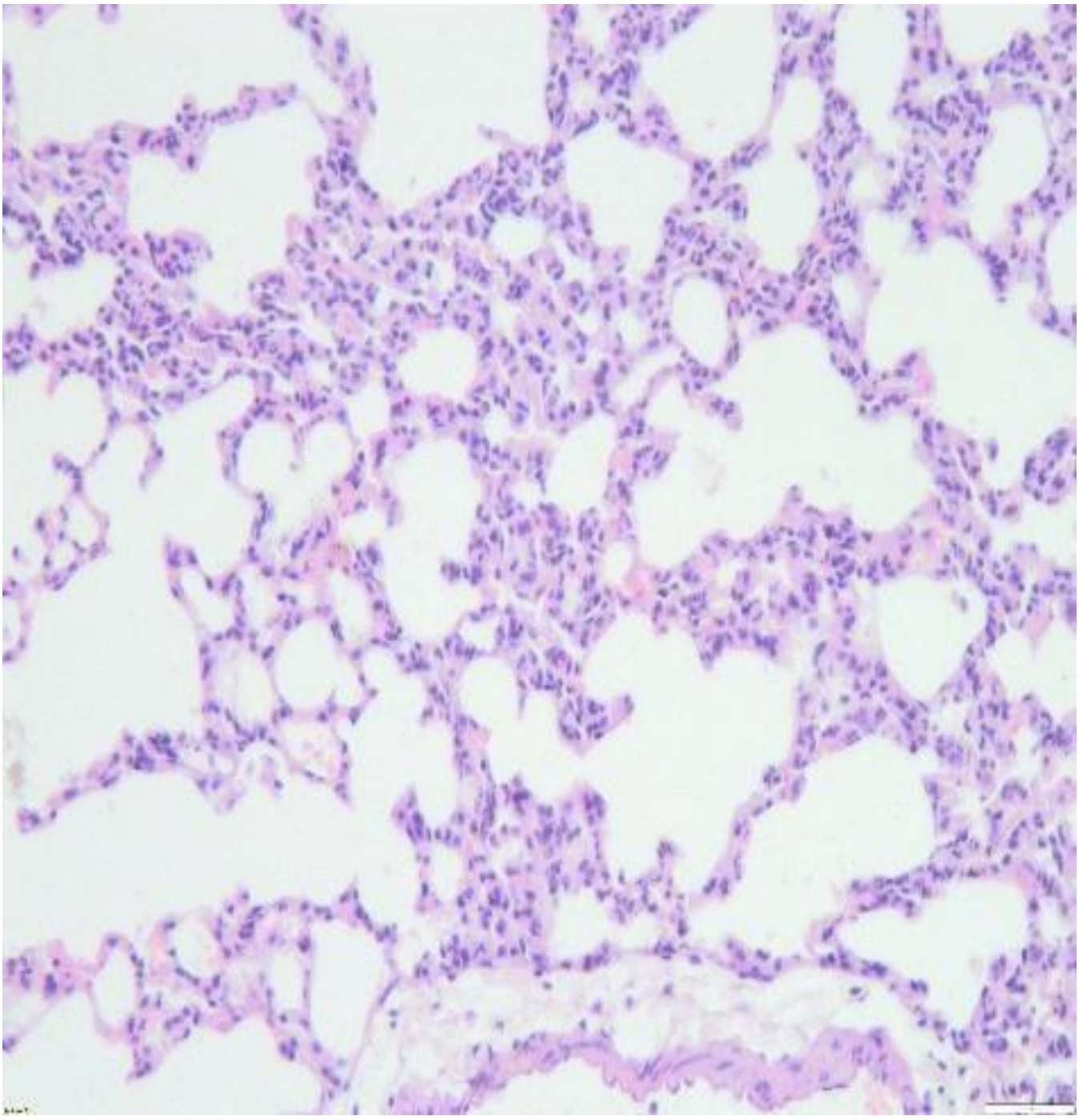

Figure D.

High dose Acetaminophen treated group. Showed no significant tissue damage in lung paranchyma and alveolar walls. H&E Stain, 10.

After performing all the procedure the slide stained with H&E Stain then observed under the microscope in 10x and found that there is no significant Showed tissue damage in lung paranchyma and alveolar walls. This work provides a standardized and integrated approach for assessing the effect of acetaminophen in mice by chronic exposure on specific mice organs.

When mice were treated with low, mild and high dose of acetaminophen in sub chronic study Showed no significant tissue damage in lung paranchyma and alveolar walls.

So found the given dose of acetaminophen in mice i.e., 2.75 mg, 5.50 mg and 11 mg was safe because in that dose there is no toxicity was seen.

This work can serve as a foundation for future investigations into the efficacy and safety of novel therapeutic interventions or the identification of potential toxicological concerns.

4.2 DISCUSSION

Moreover, the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), were within normal ranges in the lung tissues of the high-dose exposure group, suggesting the absence of an inflammatory response. The low-dose exposure group exhibited no significant changes in pulmonary function parameters, histopathological alterations, or inflammatory marker levels compared to the control group. Extrahepatic lesions induced by acetaminophen in the mouse. Based on these findings, we conclude that the substance does not induce pulmonary toxicity in mice. Our study provides reassurance regarding the safety of this substance and indicates no respiratory hazards associated with its exposure. These results are crucial for ensuring the well- being of individuals in both occupational and environmental settings. Nonetheless, furtherinvestigations are still needed to explore the underlying mechanisms and confirm these findings in human population.

5 CONCLUSION

This work provides a standardized and integrated approach for assessing the effects of drugs Acetaminophen on specific mice organs.
When mice were treated with low, mild and high dose of acetaminophen in sub chronic study Showed no significant tissue damage in lung paranchyma and alveolar walls.

This work can serve as a foundation for future investigations into the efficacy and safety of novel therapeutic interventions or the identification of potential toxicological concerns.
Further, it might be interesting to see effect of acetaminophen in mice by chronic exposure due to high dose of acetaminophen (Experimental investigation are on course in laboratory).

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